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HEALTH

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Fetal Defect Marker Proficiency Test Mailout[1]
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Dear Laboratory Director,
Below you will find a summary and critique of the Proficiency Testing mail-out from May 6, 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: A. Table 1: Second Trimester Maternal Serum all-lab Results

Samples *N = 27	Sample #	MS 311	MS 312	MS 313	MS 314	MS 315
	Gestational Age (weeks)	18.0	15.0	16.0	16.0	20.0
Maternal Race	Ethnic Group	White	Asian	White	Black	Hispanic
Maternal Weight	Pounds (lbs)	150	130	140	180	160
Maternal Age	Years	26	30	31	29	25
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	31.7 ± 1.9	43.4 ± 2.7	101.1 ± 5.4	49.4 ± 3.8	210.8 ± 11.2
	MOM ± Std. Dev.	0.71 ± 0.04	1.37 ± 0.08	2.88 ± 0.17	1.49 ± 0.10	3.72 ± 0.27
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	0.53 ± 0.05	0.56 ± 0.06	0.63 ± 0.05	0.66 ± 0.06	1.20 ± 0.09
	MOM ± Std. Dev.	0.44 ± 0.05	0.88 ± 0.13	0.83 ± 0.17	0.95 ± 0.22	0.69 ± 0.09
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	43.9 ± 4.6	35.5 ± 3.3	34.7 ± 3.0	20.4 ± 2.1	15.5 ± 1.4
	MOM ± Std. Dev.	2.16 ± 0.24	0.85 ± 0.10	1.16 ± 0.10	0.74 ± 0.09	0.96 ± 0.14
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	543.9 ± 27.3	185.2 ± 12.2	293.7 ± 18.0	155.6 ± 7.1	241.6 ± 14.0
	MOM ± Std. Dev.	3.14 ± 0.20	0.90 ± 0.10	1.60 ± 0.10	0.97 ± 0.08	1.29 ± 0.13
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(+) (100%)	(-) (100%)	(+) (100%)
	Recommended Action**	NFA	NFA	G = 85% U = 92% A = 81% R = 12%	NFA	G = 85% U = 92% A = 88% R = 4%
	NTD Risk 1 in	10,000	3,000	90	4,730	15
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	G = 92% U = 58% A = 83%	NFA	NFA	NFA	NFA
	Risk Est. 1 in	50	7,500	5,850	9,250	7,500
2. Quad Test	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	G = 96% U = 68% A = 88%	NFA	NFA	NFA	NFA
	Risk Est. 1 in	10	16,600	19,500	20,000	20,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	3,713	10,000	10,000	10,000	10,000

*N = total numbers may vary since some labs do not test all analytes. The values represent the all-lab consensus based on the arithmetic mean ± Std. Dev.
(B) = borderline positive or negative, risk reflects central tendency (Median number for NTD/Down positive or negative/borderline screen). NFA = no further action; FA = further action; G = genetic counseling; U = ultrasound, A = amniocentesis, and R = repeat.**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.

I. 1) Second Trimester Maternal Serum Analytes:

B. Narrative Evaluation of Second Trimester Screening Results:

N = 27 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 311 Wk 18.0	This specimen was obtained from a 26 year old White woman (Gravida = 3, Parity = 1) in her 18th week of gestation with a body weight of 150 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, 68%; amniocentesis, 88%; while the triple tests were: genetic counseling, 92%; ultrasound 58%; and amniocentesis, 83%. Specimen MS311 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 = 0.64).
MS 312 Wk 15.0	This specimen was obtained from a 30 year old Asian woman (Gravida = 2, Parity = 1) in her 15th week of gestation with a body weight of 130 lbs. She had no personal history of pregnancy complications and her specimen resulted in a negative screen for NTD with no body weight but an ethnic correction indicated. The labs agreed that both Trisomy screens were negative. Specimen MS312 was not paired with an amniotic fluid specimen.
MS 313 Wk 16.0	This specimen was obtained from a 31 year old White woman (Gravida = 1, Parity = 0) in her 16th week of gestation with a body weight of 140 lbs. She had a family reproductive history of miscarriages. Her sample screened positive for NTD, and her aneuploidy screen was negative for Down syndrome and Trisomy-18 (see critique). Further action recommended was: genetic counseling 85%; ultrasound 92%; amniocentesis 81%; and repeat sample 12%. This sample was not paired to an amniotic fluid specimen.
MS 314 Wk 16.0	This specimen was obtained from a 29 year old Black woman (Gravida = 3, Parity = 2) in her 16th week of gestation with a body weight of 180 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 MS314 sample was not paired to an amniotic fluid specimen.
MS 315 Wk 20.0	This specimen was obtained from a 25 year old Hispanic woman (Gravida = 2, parity = 0) in her 20th week of gestation with a body weight of 160 lbs. She had a personal history of pregnancy loss. Her sample was a positive screen for NTD (100% consensus; MOM = 3.72). 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, 85%; ultrasound, 92%; amniocentesis, 88% and repeat sample, 4%. The MS315 specimen had an amniotic fluid counterpart which was also elevated (MOM = 2.72).

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

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.

I. 2) AMNIOTIC FLUID AFP (NTD-analysis):

N=20; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 311 Wk 18.0	AFP = 5.9 ± 0.8 µg/ml MOM = 0.64 ± 0.09	The AF311 sample was targeted as an NTD negative screen in the routine gestational age screening range. All labs categorized AF311 as a negative NTD screen specimen. This specimen had a maternal serum counterpart, MS311, which showed reduced levels of AFP (MOM = 0.71).
AF 312 Wk 17.0	AFP = 12.8 ± 1.7 µg/ml MOM = 1.14 ± 0.16	The AF312 sample was targeted for a normal AFAFP value in the routine gestational age range. All labs called AF312 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 313 Wk 19.0	AFP = 7.6 ± 1.2 µg/ml MOM = 1.01 ± 0.15	The AF313 sample was targeted for a screen negative AFAFP value in the upper gestational age screening range. All labs reported this specimen as screen negative for AFAFP. The AF313 specimen was not paired with a maternal serum sample.
AF 314 Wk 18.0	AFP = 11.3 ± 1.8 µg/ml MOM = 1.22 ± 0.16	The AF314 sample was targeted for a screen negative AFAFP value in the routine gestational age range. All labs reported this specimen as screen negative for AFAFP. The AF314 specimen was not paired with a maternal serum sample.
AF 315 Wk 20.0	AFP = 16.9 ± 1.9 µg/ml MOM = 2.72 ± 0.31	The AF315 sample was targeted for an elevated AFAFP value in the upper gestational age range. All labs called AF315 a positive screen for AFAFP specimen. The AFAFP sample was matched to maternal serum specimen MS315 whose AFP was also elevated (MOM = 3.72).

I. 3. First Trimester Maternal Serum Analytes:

A. Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 17	**Sample #**	**FT 311**	**FT 312**	**FT 313**	**FT 314**	**FT 315**
	Gestational Age (weeks)	13.1	11.2	11.9	11.5	11.3
Maternal Race	Ethnic Group	White	Asian	White	Hispanic	Black
Maternal Weight	Pounds (lbs)	120	145	140	150	135
Maternal Age	Years	21	27	25	30	29
Fetal Physical Measurements	Crown Rump Length (mm)	69	45	53	48	45
	NT Thickness (mm)	1.60	1.10	2.90	1.10	1.20
	NT – MOM ± Std. Dev.	0.96 ± 0.05	0.97 ± 0.06	2.22 ± 0.13	0.91 ± 0.05	1.03 ± 0.06
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	63.2 ± 11.0	87.5 ± 12.3	194.9 ± 34.4	88.8 ± 13.1	84.2 ± 11.7
	MOM ± Std. Dev.	0.87 ± 0.17	1.07 ± 0.13	2.47 ± 0.35	1.12 ± 0.11	0.94 ± 0.12
Pregnancy-Associated Plasma Protein–A (PAPP-A ng/mL or mIU/mL)	Mean ng/mL (N=14) ± Std. Dev.	3573.1 ± 603.0	2023.1 ± 357.5	1118.1 ± 178.7	2396.9 ± 418.0	1909.7 ± 316.0
	Mean mIU/mL (N=2) ± Std. Dev.	37.0 ± 1.9	20.8 ± 0.6	11.2 ± 0.2	24.1 ± 1.7	19.2 ± 0.7
	MOM ± Std. Dev.	3.48 ± 2.33	4.86 ± 2.47	2.00 ± 1.14	5.27 ± 2.68	3.50 ± 2.07
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(+) (94%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	G = 100% U = 40% A = 53% C = 60%	NFA	NFA
	Risk Estimate 1 in	20,000	19,500	39	14,000	16,000
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	5,000	10,000	10,000

*N = total numbers may vary since some labs do not test all analytes. (B) = borderline negative or positive; NFA = no further action; G = genetic counseling; U = ultrasound; A = amniocentesis; C = chorionic villus sampling; N = number of labs participating; FT = First Trimester. **This percentage is normalized to labs requesting further action. ***Results from methods that give IU/ml were converted to ng/ml as described in section D.1 below.

I. 3) First Trimester Maternal Serum Analytes:

B. Narrative Evaluation of First Trimester Screening Results:

N = 17 all-lab Consensus Values.

Sample#	Summary Comments:
FT 311 Wk 13.1	This specimen was obtained from a 21 year old White woman with a body weight of 120 lbs. Her gestational age at the time of screening was 13.1 weeks. She had no prior history of pregnancy complications. This FT specimen was screen negative and all testing labs were in agreement. The FT311 risk estimate for Trisomy-21 was 1 in 20,000 and the Trisomy-18 risk was 1 in 10,000.
FT 312 Wk 11.2	This specimen was obtained from a 27 year old Asian woman of normal body weight (145 lbs.). Her gestational age at the time of screening was 11.2 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen positive with an all-lab consensus of 100%. The FT312 risk estimate for Trisomy-21 was 1 in 19,500, and the Trisomy-18 risk was 1 in 10,000.
FT 313 Wk 11.9	This specimen was procured from a 25 year old White woman of average body weight (140 lbs.). Her gestational age at the time of screening was 11.9 weeks. She had no prior history of any pregnancy complications. This FT specimen was screen positive for Trisomy-21 and all testing labs were in agreement. The FT313 risk estimate for Trisomy-21 was 1 in 39, and the Trisomy-18 risk was 1 in 5,000.
FT 314 Wk 11.5	This specimen was procured from a 30 year old Hispanic woman of average body weight (150 lbs.). Her gestational age at the time of screening was 11.5 weeks. She had no prior family history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative for Trisomy-21 and all testing labs were in agreement. The FT314 risk estimate for Trisomy-21 was 1 in 14,000, while the Trisomy-18 risk was 1 in 10,000.
FT 315 Wk 11.3	This specimen came from a 29 year old Black woman with a body weight of 135 lbs. Her gestational age at the time of screening was 11.3 weeks. She reported no prior family history of pregnancy problems. This FT specimen was screen negative for bothTrisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT315 was 1 in 16,000, 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 **MS315** was targeted as a screen positive specimen for NTD (Figs. 2a and 3) and was matched to the elevated **AF315** sample (Fig. 2b). All labs (100%) agreed that specimen **MS315** was screen positive for NTD and negative for both Trisomy screens using both the triple and quad tests (Figs. 4-6). The **MS315** sample was matched to **AF315** (MSMOM = 3.72 vs AFMOM = 2.72), which also exhibited elevated AFP levels, strongly suggesting the presence of an NTD. As a follow-up, a polyacrylamide gel electrophoresis is indicated and should be performed to show the absence or presence of a diagnostic ACHE band that would indicate an NTD.

Sample **MS311** was obtained from a white woman with a prior sibling history of pregnancy complications. The fetal defect marker MOM values for specimen **MS311** (MSAFP-MOM = 0.71, MSuE3-MOM = 0.44, MShCG-MOM = 2.16, DIA-MOM = 3.14) presented the canonical 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). In addition, the matched **AF311** specimen had low AFP (MOM value = 0.64). The T21 risk was 1 in 50 by triple test and 1 in 10 by quad test (Figs. 5, 6). It is noteworthy that the T21 risk determined by Quad testing was 5 times greater than by triple testing. The recommended further actions for the sample **MS311** were genetic counseling, 96%; ultrasound, 68%; and amniocentesis, 88%, from labs performing the quad screen, and genetic counseling, 92%; ultrasound, 58% and amniocentesis, 83%, from labs performing the triple screen.

Two other specimens, **MS312** and **MS314**, produced negative screens for NTD, T21, and T18, with no corrections for body weight or race being indicated.

The **MS313** specimen at 16 weeks presented an interesting case involving elevated levels of MSAFP, and normal MSuE3, MSHCG, and MSDIA levels; this profile resulted in a positive screen for NTD (Risk = 1 in 90) and yielded a negative triple and quad screen risk for T21 (Figs. 4-6). The NTD follow-up actions recommended for specimen **MS313** were genetic counseling, 85%; ultrasound, 92%; amniocentesis, 81%. Sample **MS313** was modeled after eight literature case studies of pregnant women with autoimmune rheumatoid arthritis (RA) that exhibited aberrant levels of the AFP/NTD biomarker (1-2). Prior to their current pregnancies, most of the RA case study women had not experienced complicated pregnancies and had delivered normal term infants. After one such woman was counseled on the effects of medications for RA taken during pregnancy, she consented to continue gestation and underwent further tests, which included ultrasound, 3-D scans, and RA-related tests including serum autoantibody assays. Moreover, some of the patients in these studies of autoimmune RA were treated with low dose aspirin, prednisone and acetaminophen prior to and during pregnancy. All women in these studies had known pre-existing RA disease upon presentation at their first obstetrician's visit, and all delivered normal term infants with no signs of NTD.

Similar to the above published report, the maternal serum biomarkers of **MS313** revealed an MSAFP MOM of 3.10, MSuE3 MOM of 0.63, MSHCG MOM of 1.16, and MSDIA MOM of 1.60 (1) resulting in a false positive screen for NTD. Previous reports in the literature had also demonstrated that the AFP biomarker often predicted pregnancy complications and adverse outcomes in addition to NTD (3, 4). These included miscarriage, low birth weight, stillbirth, and increased thrombotic events (5, 6). Specimen **MS313** produced a false positive prenatal screen for NTD due to an elevated MSAFP that may have been caused by placental vasculitis leading to increased diffusion of AFP into the maternal bloodstream.

Rheumatoid arthritis (RA) is an autoimmune disease that results in a chronic, systemic disorder that affects many tissues and organs, especially flexible synovial joints (7). If not treated, RA leads to a disabling and painful condition causing substantial loss of function and mobility of the target tissue (i.e., synovial joints, etc.). The rheumatic process involves four developmental steps: 1) an inflammatory response in the capsule surrounding the joint; 2) an enlargement of synovial cells; 3) production of excess synovial fluid; and 4) deposition of fibrous tissue in the synovial joint environment (7, 8). Pathology of the disease process exhibits destruction of articular cartilage and ankylosis (fusion) of the joints. However, RA can further cause diffuse inflammation in the lungs, membranes surrounding the heart (pericardium), membranes of the lungs (pleura), white of the eyes (sclera) and various nodule lesions in subcutaneous tissues.

Although the cause of RA is not known, it is of autoimmune etiology in which cellular and humoral immune responses are mounted against one's own body. Autoimmune diseases produce a range of disorders from organ-specific (thyroiditis) to systemic disorders with multi-organ involvement. Disorders that largely, but not exclusively, affect joints and muscles are grouped as the autoimmune rheumatic diseases, and include rheumatoid arthritis, systemic lupus erythematosus, primary Sjogren syndrome, systemic sclerosis (scleroderma), idiopathic inflammatory myositis, and systemic vasculitis (9). These multisystem autoimmune rheumatic diseases are heterogeneous disorders associated with substantial morbidity and mortality. Although such diseases can present the classical rheumatic symptoms making rapid diagnosis possible, they also share symptoms common to other disorders including arthralgia (joint pain), myalgia (muscle pain), dry eye, and pulmonary, renal, and neurological involvement; hence, differentiation between the various autoimmune diseases can sometimes be difficult (10).

While the world adult population with RA is approaching 1.0%, women with RA present three times as often as men in middle age. Thus, RA affects mainly adults in the USA and 5 to 50 per 100,000 people per year show newly developed disease (11). In the year 2010, RA resulted in nearly 50,000 deaths globally (12). The age of onset in women is between 40 to 50 years, while men are somewhat later. Although onset of RA disease is relatively uncommon in women under the age 20 years , late onset can occur until the age of 80. Some Native American Indian groups have higher prevalence rates (5 – 6%), and people from the Caribbean region display lower rates of RA prevalence than the overall population (12).

RA is a chronic disease and although rare spontaneous remissions may occur, one of the most striking examples of temporary improvement in disease activity is that induced by pregnancy. It has been known for many years that about 70% of patients with RA remit during pregnancy; however, the cause for this beneficial effect is not known (13). Moreover, the mechanism of remission of RA in pregnancy is still not understood but has been proposed as an induction of an immunosuppressive state by soluble factors yet unknown (14). Suggested immunosuppressive factors have included quad test biomarkers and pregnancy-associated glycoproteins. Thus, many RA patients are prescribed immunosuppressive drugs such as azathioprine and glucocorticoids.

The marked improvement of RA in pregnant women has long been observed by obstetricians and perinatologists. It is believed that the pathogenic mechanisms in RA are a consequence of immune processes and that the pregnant state induces the emergence of systemic immunosuppressive factors in the mother (14). Local non-specific immunosuppressive soluble factors produced by the mother, fetus, and/or placenta are thought to play a major role in retaining the fetus as an allograft within the mother. These soluble factors possess immunomodulatory properties and are produced in excess at the

fetoplacental interface in order to prevent fetal rejection (13). Such candidate factors have been identified as AFP, hCG, PAPP-A, Inhibin-A, pregnancy-specific B1 glycoprotein, cytokines (IL-6, IL-10, INF-alpha), tumor necrosis factor-alpha (TNF-alpha), prostaglandins, and sex steroids (hydroxyl-estrones) (15). For example, hCG has been shown to inhibit mixed human lymphocyte cultures and to suppress lectin-induced lymphoblast cell transformation when added to cell cultures (16, 17). Together with uromodulin, hCG has further been shown to inhibit T-cell and monocyte function when administered at low concentrations (18). In addition, studies in pregnant animals treated with hCG in models of bacterial cell wall-induced arthritis have shown a dose dependent reduction in the clinical symptoms (19). In this report, hCG therapy reduced the inflammatory cell infiltrates and suppressed the overproduction of TNF-alpha, IL-6 and IL-1B, all of which contribute to bone and cartilage degradation. Thus, hCG was found to exert a protective effect by modulation of inflammatory mediators in experimental arthritis.

Alpha-fetoprotein (AFP) is another gestational-age-dependent biomarker that has exhibited immunomodulatory properties. Human AFP has been shown to suppress the mitogenic stimulatory response produced by various lectins (Con-A, LPS) as well as to inhibit the mixed leukocyte reaction (MLR) by inducing the production of suppressor T-cells (20, 21). It was further demonstrated the AFP suppressed Class-II major histocompatibility complex (MHC) expression on macrophages, which may allow the fetus to acquire tolerance to self-antigens during development in utero. AFP has also been implicated in the masking of Class II MHC antigen expression by human myelocyte macrophages (22). Recent studies have further shown that AFP can inhibit natural killer cell activity (23) and enhance cytokine, chemokine, and growth factor activity (24). It was also demonstrated that AFP can shield target cells from TNF-alpha induced apoptosis (25, 26) and can protect cells from cytotoxic lymphocyte attack by blocking the caspase apoptotic pathway (27, 28).

It has also been demonstrated in a study of 60 patients with various rheumatic diseases (RA, SLE, and osteoarthritis) that all patients demonstrated significantly higher serum and synovial fluid levels of both Activin-A and Inhibin-A compared to healthy controls (29). In addition, serum levels of Activin-A and Inhibin-A showed positive correlations with disease activity in RA patients. It has been demonstrated that Inhibin-A affects several immune parameters in RA patients such as decreasing interferon-gamma production, and stimulating IL-1B secretion by cytokines. Both systemic and local immune responses are influenced by Inhibin-A and it was proposed that Inhibin-A influences immune cell differentiation during monocyte and lymphocyte proliferation (30). Inhibin-A production has been reported to be stimulated by pro-inflammatory mediators such IL-1beta, TNF-alpha, and lipopolysaccharide (LPS), and that Inhibin-A can facilitate the TGF-beta mediated immune suppression in thymocytes (31). Thus, Inhibin-A together with TGF-beta may play important roles in the immune response. Finally, Inhibin-A is capable of stimulating cartilage formation in joints as indicated by increased expression of type-II collagen in RA and synthesis of proteoglycan in animal models (32). It appears that the RA disease remission during pregnancy may be of a multifactorial etiology that may prove difficult to sort out.

RA is a form of autoimmunity whose cause is not completely understood. Although physical stress and emotional effects may be involved, possible triggers of the autoimmune response are not known. At least part of the risk of RA disease has been ascribed to a genetic basis (7). RA is strongly associated with the inherited tissue type major histocompatibility complex (MHC) antigen HLA-DR4 (especially DR0401 and 0404) and with the gene products "Protein Tyrosine Phosphatase Non-receptor type 22" (PTPN22) and "Peptidyl Arginine Deiminase Type IV" (PAD14) (33, 34). Hence, family history is an important risk factor in RA. Inheriting the PTPN22 gene has been shown to double a person's susceptibility to RA. The PAD14 gene has been identified as a major risk factor in people of Asian descent, but less so in European descent. First degree relatives' prevalence rate is 2 – 3% and disease-genetic concordance in monozygotic twins is approximately 15 – 20% (35). Smoking is the most significant non-genetic risk factor with RA being 3 times more common in smokers versus non-smokers (36). A potential association was found between RA and Epstein-Barr virus (EBV) and human herpes virus-6; thus, individuals with RA also exhibit an abnormal response to EBV and have high levels of anti-EBV antibodies (37).

Women with RA during pregnancy appear to be at little risk for increased obstetric complications. Pregnancy loss may be slightly increased in women with RA; however, the vast majority of women with RA are successful in childbearing (10). Moreover, women with RA do not appear to be at significant risk for preterm birth, pre-eclampsia, or fetal growth restriction; thus, special obstetric monitoring for these adverse outcomes is not warranted (13, 38). The exception occurs in cases of severely deformed RA patients where the mechanics of vaginal delivery pose a problem. Concern about RA drugs taken during pregnancy arise due to possible adverse fetal effects. Glucocorticoids are administered to patients whose RA does not improve or remit during pregnancy. Relatively low doses of prednisone are usually adequate to manage RA during pregnancy. Intra-articular (into joints) steroid injections are used if necessary in pregnancy (10). Non-steroidal anti-inflammatory drugs (aspirin, Tylenol, Motrin) can be taken as needed in minimum doses to control the inflammations (39). Methotrexate cannot be administered in the first trimester and only rarely in other trimesters (7). The safety of anti- TNF-alpha inhibitors is unknown in pregnancy and they are to be avoided. Likewise, Leflunomide is teratogenic in animal models and is not used in pregnant women.

Clinical diagnosis of the patient is based on symptoms, physical exam, radiographs, and autoimmune antibody (blood) assays. Imaging procedures such as x-rays of the hands and feet are performed on patients with polyarthritis (40).

The x-rays can demonstrate articular osteopenia (low bone density), soft tissue swelling, loss of joint fluid and space, bone erosions, and joint dislocations. The other medical imaging techniques used for RA patients are magnetic resonance imaging and Doppler ultrasound. The blood tests performed for suspected RA patients are for antibodies directed against Rheumatoid Factor (RF), a non-specific and denatured protein present in several autoimmune disorders (41). Another more specific antibody is produced against fibrin and vimentin connective tissue proteins in which the amino acid arginine is replaced by the amino acid citrulline, not one of the 20 standard amino acids (34). This event results in misfolding of the proteins causing a change in structure and function. The body produces antibodies against these "non-recognized" mutated proteins. Other tests for RA patients include red blood cell sedimentation rates, C-reactive protein, anti-nuclear antibody, neural function tests, liver enzymes, and serum ferritin levels. While RF antibodies are present in 65% of patients, the anti-citrulline antibodies are present in 95% of RA patients (42).

The drug treatments to manage RA patients encompasses three main classes of medications; A) nonsteroidal anti-inflammatory drugs (aspirin, Motrin, Aleve, acetaminophen); B) disease modifying drugs (methotrexate, sulfasalazine, leflunomide, tumor necrosis factor inhibitors such as infliximab, IL-1 blockers, rituximab , and abatacept); and C) anti-inflammatory agents (COX-2 inhibitors) such as Celecoxib, glucocorticoids, corticosteroids such as prednisone and hydrocortisone, and azathioprine, an immunosuppressive purine analog (9). Alternative medicine drugs have also been employed for the treatment of pain in RA and have included acupuncture, bee venom extracts, dietary supplements, and pulsed electromagnetic field therapy (39). The beneficial dietary supplements for RA include various herbal remedies (curcumin), omega-3-medications, and gamma binotenic acid. The omega-3-polyunsaturated fatty acids found in fish oils have been consistently found effective for RA treatments in reducing pain and tender joint stiffness. RA is known to reduce the lifespan of patients from three to twelve years. However, the use of the new biologic drug therapies may extend the life of patients with RA and possibly reduce the risk and progression of atherosclerosis common in such patients.

Aside from connective tissue involvement, RA can also cause diffuse inflammation in multiple organs and tissues throughout the body. Even though the joints exhibit inflammation at the synovial membranes, the entire joints themselves become swollen, tender, and stiff, which limits their movements. Cutaneous rheumatoid nodules in the skin are another feature characteristic of RA exhibiting a type of inflammatory reaction known as a fibrinoid necrotizing granuloma (44). Several forms of dermal vasculitis also occur in RA which manifest as purplish discoloration of the skin. Fibrosis of the lungs occurs in rheumatoid disease as a consequence of drug treatments (methotrexate), and pleural effusions and rheumatoid lung disease are also associated with RA (45). People with RA are also prone to vascular sclerosis events, and the risks of heart attack and strokes can likewise be increased. RA may affect the kidney glomerulus through vascular pathology events and renal amyloid deposits which occur as a consequence of chronic inflammation caused by mesangial infiltrates (46). Other symptoms of organs/tissues affected by RA include: 1) ocular sclerosis, dry eye, and conjunctivitis of the eye; 2) increased hepatic secretion of acute phase proteins and enzymes; 3) RBC anemia, WBC neutropenia, and thrombocytosis; 4) neurological signs include peripheral neuropathy, nerve compression and erosion leading to clumsiness; 5) osteoporosis; 6) and generalized symptoms of malaise, fatigue, low grade fever, loss of appetite, and reduced body weight.

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

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in bar-graph format (Figs. 7-10). As shown in Figs. 7A-7D, MS AFP and AFAFP mass measurements among the individual kits mostly agreed. When the kit specific uE3 MOMs and mass values were compared, MOM values from Siemens DPC Immulite 2000/2500 were approximately 20% higher than those from Beckman kits despite there being little difference in the mass values measured (Fig. 8A and 8B). Regarding the hCG kits (Fig. 10A), the Beckman Access 2 instrument results were about 5% higher than those from Beckman UNICEL, while the Siemens Immulite 2000 results were 10% lower than those from the other assay platforms. These differences were, however, not reflected in the MOM values (Fig. 10B). Finally, the method comparison for Inhibin-A displayed in Fig. 9A shows that the results from the Beckman Access/2 and UNICEL were essentially identical, which was also reflected in the Inhibin MOM values (Fig. 9B).

When the AFP mass measurements and MOM values in amniotic fluid were compared (Fig. 7C and 7D), those from Siemens Immulite were noticeably higher. Since these specimens are derived from actual AF samples, these differences would be comparable to real patient testing**.**

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

The alpha and Benetech PRA software packages were each used by 34.6% and 23.1%, of the labs, respectively; Robert Maciel (RMA) software was employed by 26.9%; and in-house and "other" softwares comprised 15.4%. Programs classified as "other" are presumably proprietary software packages.

D) First Trimester Screen:

Five first trimester maternal serum mock samples were provided in the present mailout. However, laboratories not presently offering the test, nor planning to implement the test, can request that no further samples be sent to them. The FT sample (FT = first trimester) information provided to participating labs included maternal age, nuchal translucency (NT) in millimeters, last menstrual period (LMP), crown-rump length (CRL) in millimeters, race, maternal body weight, and date of blood draw.

The all lab measurement of specimen **FT311** (GA=13.1 weeks) was obtained from a 21 year old white woman. The total hCG resulted in a mass mean of 63.2 ± 11.0 IU/ml, with a MOM of 0.87 ± 0.17. The mass mean for PAPP-A users employing ng/ml was 3573.1 ± 603.0 (N=14); while those employing mIU/ml was 35.6 ± 1.9 (N=2) with an overall MOM of 3.48 ± 2.33. The **FT311** sample displayed a T21 negative screen result with a risk assessment of 1 in 20,000 (Fig. 13). Further action was not indicated. Finally, 100 % of labs considered the **FT311** specimen screen negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Fig. 14).

For the **FT312** specimen from a 27 year old asian woman, the gestational age all-lab mean was reported as 11.2 weeks. Assay measurements for **FT312** resulted in an all-lab total hCG mass measurement of 87.5 ± 12.3 IU/ml (MOM = 1.09 ± 0.13). The PAPP-A mass assessment for users employing ng/ml was 2023.1 ± 357.5 (N=14), while those employing mIU/ml was 20.8 ± 0.6 (N=2) and the all-lab MOM was 4.86 ± 2.47. All labs agreed that the **FT312** sample was screen negative for T21 with a risk of 1 in 19,500 (Fig. 13). A negative screen was achieved for T18 with a risk assessment of 1 in 10,000 (Fig. 14).

The **FT313** specimen was obtained from a 25 year old white woman with a gestational age of 11.9 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 194.9 ± 34.4 IU/ml (MOM = 2.47 ± 0.35). The PAPP-A mass measurement of users employing ng/ml was 1118.1 ± 178.7 (N=14); while those employing mIU/ml was 11.2 ± 0.2 (N=2) (all-lab MOM = 2.00 ± 1.14). The all-lab T21 screen consensus for **FT313** was positive with a risk assessment of 1 in 39 (Fig. 13). Further action was reported as: genetic counseling, 100%; ultrasound, 40%; amniocentesis, 53%; chorionic villi sampling, 60%. Finally, the **FT313** specimen screened negative for T18 (1 in 5,000, Fig. 14).

The all lab total hCG measurement of the 11.5 week specimen **FT314** obtained from a 30 year old (150 lbs.) hispanic woman was 88.8 IU/ml ± 13.1, with a MOM of 1.12 ± 0.11. In addition, the mass measurement of PAPP-A users employing ng/ml was 2396.9 ± 418.0 (N=14), while those employing mIU/ml was 24.1 ± 1.7 (N=2) with an overall MOM of 5.27 ± 2.68. This resulted in an all-lab negative T21 risk assessment of 1 in 14,000 for the **FT314** specimen (Fig. 13) and a negative screen assessment for T18 of 1 in 10,000 (Fig. 14).

For the **FT315** specimen obtained from a 29 year old (135 lbs) black woman, the gestational age all-lab mean was reported as 11.3 weeks. Analyte measurements resulted in an all-lab total hCG concentration of 84.2 ± 11.7 IU/ml (MOM = 0.94 ± 0.12). The mass measurement of PAPP-A users employing ng/ml was 1909.7 ± 316.0 (N=14) while those employing mIU/ml was 19.2 ± 0.1 (N=2) (MOM = 3.50 ± 2.07). The all-lab FT T21 risk assessment was 1 in 16,000 and all labs agreed that the **FT315** sample was negative for T21 (Fig. 13). Similarly, the **FT315** specimen was also screen negative for T18 with an all-lab risk assessment of 1 in 10,000 (Fig. 14).

D. 1.) First Trimester Assay Kit Performance:

In order to compare the older Siemens Immulite assay platforms to the Beckman UNICEL and AnshLabs/Anshlite assays (53% users) for PAPP-A, a conversion factor given in the AnshLabs/Anshlite package insert of 0.00256 mIU/ml = 1ng/ml was used (Fig. 12A). However, for grading purposes, each lab's results were compared to their own peer group without conversion.

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 Access/2 were ~5% higher than those by Beckman UNICEL, 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 deviations from the median were somewhat diminished for DPC and elevated for Beckman Access/2 (Fig. 11B). The results from the three PAPP-A kits, even when converted to the same mass units, were not consistent among each other (Fig. 12A) with Siemens Immulite nearly 4 times higher than Beckman while Anshlite was 25% lower than Beckman UNICEL. Similarly, when the PAPP-A kit MOMs were compared, Siemens Immulite 2000 was nearly triple those from Anshlite and Beckman (Fig. 12B).

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" which are proprietary software packages.

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Teachings on Alpha-fetoprotein

Vol. 6, Part 4

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

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

Therapeutic use of AFP in liver tumors

It was once thought that high concentrations of soluble pro- teins contributed to the maintenance of peripheral tolerance to self-proteins. That concept has now been challenged in clinical immunotherapy trials using MHC class I-restricted peptide epitopes derived from AFP (Table 2). AFP is thought to be a self-protein expressed by the fetal liver at high levels, which is transcriptionally repressed at birth. However, AFP is transcriptionally unmasked in many HCCs and such patients display AFP plasma levels in the high ug/ml range. One investigative team that had previ- ously identiWed four immunodominant HLA-A*0201- restricted peptides derived from human AFP (Table 2), found that these AFP epitopes could stimulate speciWc T cell responses in peripheral blood lymphocytes obtained from normal volunteers [7]. These researchers further reported that AFP peptide-reactive T cells could be expanded in vivo in HCC patients immunized with four diVerent AFP derived peptides [9]. A pilot phase I clinical trial was undertaken in which HLA-A*0201 patients with AFP-positive HCCs were immunized with intradermal vac- cinations of four AFP peptides (100 ugm or 500 ugm each) emulsified in incomplete Freund's adjuvant (Table 2). All of the patients ($n = 6$) generated T cell responses to most if not all peptides as measured by direct IFN gamma enzyme- linked and MHC class I tetramer Xow-cell assays. The investigators demonstrated that the human T cell repertoire was indeed capable of recognizing AFP-peptides presented as MHC class I antigens even in the face of high circulating levels of tumor-derived AFP [9].

Even though breaking immunologic tolerance towards hepatoma-associated AFP peptide antigens appears now possible, the use of this concept for the treatment of immu- nocompromised HCC patients has been minimal to date. In one study, investigators analyzed whether dendritic cells (DCS) from HCC patients transduced with a HAFP- expressing adenovirus and co-cultured with cytokine- induced killer (CIK) cells could induce a strong speciWc immune response against hepatoma cells [18]. An HAFP- encoding adenovirus (Ad-HAFP) was generated and DCs from healthy donors or patients were transduced at high eYciencies. DCs were further co-cultured with autologous CIK-cells, and studied for their ability to lyse HCC tumor cells. AFP-transduced DCs strongly activated CIK cells to lyse 70% of AFP-expressing HCC cells, however, cytotox- icity was signiWcantly higher when lymphocytes were co- cultured with Ad-HAFP-transduced DCs rather than with Ad-mock-transduced DCs. These data indicated that an AFP-speciWc immune response had been mounted against the HCC cells. Interestingly, CIK cells from patients with AFP-positive hepatomas could be stimulated to lyse AFP-expressing HCC cells as eVectively as CIK cells from healthy individuals; the response was stronger than with CIK cells from patients without AFP-secreting HCCs. These data showed that patient-derived DCs transduced with an AFP-expressing adenovirus and co-cultured with autologous CIK cells could induce a strong AFP-speciWc immune response against hepatoma cells. These authors advanced the view that this approach could serve as an adoptive and/or DC-based immunotherapy for hepatoma patients [18].

Even though AFP-peptides could act as a possible target for a hepatocellular carcinoma (HCC)-speciWc vaccination as previously shown, some studies have demonstrated that dendritic cells (DCs) treated with AFP become dysfunc- tional. In a previous study, researchers were able to trans- fect AFP mRNA into DCs and observe the ability of DCs to induce AFP-speciWc CD4(+) and CD8(+) T cells [36]. It was hoped that AFP could be processed and presented by DCs directly, rather than just released into culture media, so there would be no AFP negative eVect on the function of DCs. The investigators employed immature DCs generated from peripheral blood mononuclear cells (PBMCs) of HLA-A2(+) HCC patients which were transfected with AFP mRNA. The transfected, matured DCs were then used to stimulate autologous T cells. Their results showed that the expression of cell membrane proteins of DCs following transfection were strongly increased, and that interleukin- 12 (p70) release into the supernatant was signiWcantly ele- vated with only a slight AFP release into the transfected DC supernatants. CTLs induced by the transfected DCs speciW- cally recognized an HLA-matched AFP positive HepG2 cell line; in addition, AFP-speciWc proliferative T cell responses could be induced. This study related that an AFP mRNA

transfection strategy could generate fully functional DCs and induce speciWc T cells to recognize AFP(+) hepa- toma cells [40].

Table 3 The alpha-fetoprotein (AFP) derived MHC Class I T cell restricted epitopes HLA-A*0201 and HLA-A*2402/HLA-DR are listed accord- ing to their aYrmed immunodominant and subdominant epitope amino acid (AA) sequences

T-Cell Epitopes

HLA-A2.1 Immunodominant Site

AFP AA * Sequence #	Amino Acid Sequence								
137	P	L	F	Q	V	P	E	P	V
325	G	L	S	P	N	L	N	R	F/L
**235	F	Q	A	I	T	V	T	K	L
158	F	M	N	K	F	L	Y	E	I
542	G	V	A	L	Q	T	M	K	Q
364	Q	L	A	V	S	V	I	L	R/V
‡ H/H Index	(+) 0.13	(+) 0.28	(±) 0.07	(±) 0.06	(-) 0.10	(+) 0.36	(±) 0.04	(-) 0.67	(+) 1.03

HLA-A*2402/HLA-DR Immunodominant Site

AFP AA Sequence #	Amino Acid Sequence								
414	R	S	C	G	L	F	Q	K	L
403	K	Y	I	Q	E	S	Q	A	L
357	E	Y	S	R	R	H	P	Q	L
434	A	Y	T	K	K	A	P	Q	L
424	E	Y	Y	L	Q	N	A	F	L
249	K	V	N	F	T	E	I	Q	K/L
‡ H/H Index	(-) 0.89	(+) 0.36	(+) 0.06	(-) 0.37	(-) 0.52	(-) 0.18	(-) 0.11	(-) 0.39	(+) 0.29

Identity Similarity

Subdominant Site

AFP AA Sequence #	Amino Acid Sequence								
218	L	L	N	Q	H	A	C	A	V
547	T	M	K	Q	E	F	L	I	N/L
555	N	L	V	K	Q	K	P	Q	I
485	C	I	R	H	E	M	T	P	V
507	N	R	R	P	C	F	S	S	L/V
492	P	V	N	P	G	V	G	Q	C
‡ H/H Index	(-) 0.16	(+) 0.14	(-) 0.89	(-) 0.27	(-) 0.36	(+) 0.20	(+) 0.22	(-) 0.12	(+) 0.39

Subdominant Site

AFP AA * Sequence #	Amino Acid Sequence								
7	I	F	L	I	F	L	L	N	F
591	C	F	A	E	E	G	Q	K	L
150	A	Y	E	E	D	R	E	T	F
504	S	Y	A	N	R	R	P	C	F
322	K	P	E	G	L	S	P	N	L
307	T	L	E	R	G	Q	C	I	I
‡ H/H Index	(-) 0.09	(+) 0.29	(-) 0.14	(-) 0.46	(-) 0.30	(-) 0.63	(-) 0.15	(-) 0.29	(+) 0.60

The AFP stretches of 9–10 AAs are grouped and listed according to their sequence identity/similarity matching rather than their numerical order on the AFP molecule. The HLA-A*0201 restricted T cell epitopes are largely involved with T cell priming, recognition, and IFN-gamma secretion, while the HLA-A*2402 sites generate cytotoxic T cells which secrete IFN-gamma. Both sites involve AFP peptide binding to a T cell receptor via a MHC interaction

‡ Consensus H/H Index (+, ¡, §) = Hydrophobicity/hydrophilicity numerical index assigned values (Eisenberg, D. et al Faraday Sym. Chem. Doc. 17:109, 1982)

* AFP amino acid numbering includes the N-terminus signal sequence. ** Proposed Immunodominant candidate site

‡ Data were collected and collated from the References [7, 33]

Table 4 Amino acid mutation analysis[a] of MHC Class 1 Epitopes from AFP-derived peptides following interaction with the T cell receptor. Following peptide insertion into the TCR binding groove, the following list of cell responses can be observed

Amino acid position no.	Anchor position	AFP peptide binding	T Cell receptor contact sites	Conformational change induction	Priming of immune response	T cell response (cytokines)
1			§		§	§
2	++	++			++	+
3		++				++
4		+	++		++	++
5			§		§	+
6			+		§	+
7		+	+	++	§	+
8			§		§	+
9	++	++	++	++	++	++

[a] The eVect of mutation at the amino acid (AA) position indicated above is coded in the following manner: § = minor eVect; + = moderate eVect; + + = major eVect. Data was collated and summarized from AA mutational modeling studies using the 9-mer peptide AFP 542–550 G V A L Q T M K Q (HLA-A*0201) derived from the references [7, 15, 26]

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

(1) 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.

(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: J Reprod Med 59:76-80, 2014.

Title: Effect of underlying infertility factors on second trimester serum screening results.

Authors: Kosus N, Kosus A, Duran M, Turhan NO.

Abstract: OBJECTIVE: To determine whether assisted reproductive technologies (ART) alone or an underlying cause of infertility has any effect on second trimester serum screening results. STUDY DESIGN: Second trimester serum screening results of ART pregnancies of women with polycystic ovary syndrome (IVF-P group) were compared with those of women who underwent

ART due to malefactor infertility (IVF-M group) and of women who conceived spontaneously. RESULTS: Comparison of the groups for a-fetoprotein, beta-hCG, and beta-hCG multiples of the median (MoM) revealed a significant difference between the IVF-M and control groups. Comparison of groups for unconjugated estriol (uE3) and uE3 MoM levels revealed a statistically significant difference between the IVF-P versus the control groups. CONCLUSION: It seems advisable to use a population of ART pregnancies, preferably divided by type of treatment and the etiology of the infertility, when establishing median curves for second trimester serum screening markers.

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

(1) Source: J Pediatr Hematol Oncol, 2014.

Title: Hepatoblastoma in Children With Beckwith-Wiedemann Syndrome: Does it Warrant Different Treatment?

Authors: Trobaugh-Lotrario AD, Venkatramani R, Feusner JH.

Abstract: Patients with Beckwith-Wiedemann Syndrome (BWS) are predisposed to developing hepatoblastoma. Clinical data were reviewed in all cases of hepatoblastoma in patients with BWS reported in the literature and from personal cases. Patients were identified by literature review using PubMed and by a search of the authors' local tumor registries. Fifty-six patients were identified. The median age of presentation with hepatoblastoma was 6 months (range birth-30 mo). Thirteen of 26 patients were born prematurely. Of 31 evaluable patients, 19 exhibited hemihypertrophy. Thirty-two of 33 patients with alpha-fetoprotein data reported had elevated levels at diagnosis. Overall survival was 75% (27 of 36 patients). Of 25 patients with data who survived, 24 were treated with chemotherapy and surgery (vs. only 2 of 8 who did not survive). All 9 patients with hepatoblastoma detected by routine screening with outcomes reported were surviving at the time of the reports. Overall survival was high in patients with BWS and hepatoblastoma, especially given lower stage at presentation and when treated with surgery and chemotherapy. Future prospective trials should evaluate if BWS is independently associated with outcome and if the outcome is improved by routine screening.

(2) Source: Int Urol Nephrol, 2014.

Title: AFP-producing urothelial carcinoma of the bladder: a case report.

Authors: Ye J, Xu X, Fan M, Xue D, Zhuang Q.

Abstract: We report a case of urothelial carcinoma of the urinary bladder with concurrent alpha-fetoprotein (AFP) elevation. A 60-year-old male was admitted for gross hematuria. Subsequent analyses revealed elevated serum AFP levels (970.20 ng/ml). He had no history of hepatitis, and hepatobiliary disease was not detected on computed tomography or ultrasound. Carcino-embryonic antigen was normal. The patient underwent radical cystectomy and was found to have a high-grade urothelial carcinoma of bladder on pathology. In addition, immunohistochemical staining of the tumor cells showed strong AFP positivity. Postoperatively, serum AFP levels decreased gradually to normal. In summary, urothelial carcinoma of the urinary bladder with AFP elevation is rare, and the mechanism and prognosis require further exploration.

(3) Source: Fetal Pediatr Pathol, 2014.

Title: Sinonasal Pure Yolk Sac Tumor: A Case Report and Literature Review.

Authors: Chuang HC, Kang CJ, Lee LY.

Abstract: Extragonadal pure yolk sac tumor of sinonasal origin is very rare. We report herein a case with sinonasal yolk sac tumor in a 1 year and 3 months old girl. The initial complaint was persistent nasal bleeding for about 2 months. Computed tomography (CT) and magnetic resonance imaging (MRI) revealed a lobulated soft tissue mass in paranasal sinus that extended to oral cavity, nasopharynx, and oropharynx. The histology showed typical features of yolk sac tumor and the positive immunohistochemical staining of SALL4 and alpha-fetoprotein. After tumor excision, adjuvant chemotherapy of JEB regimen was prescribed. After the follow-up for 13-months, alpha-fetoprotein was normal and neither tumor progression nor metastasis was found. We review the previous literature and discuss the etiology, histology, treatment, and the prognosis of the rare sinonasal yolk sac tumor.

C) News of Note: Abstracts of New Markers:

(1) Source: Clin Biochem, 2014.

Title: Human chorionic gonadotropin and alpha-fetoprotein in cerebral spinal fluid: Method validation and retrospective review.

Authors: Mitsios JV, McClellan A, Brown S, Gronowski AM.

Abstract: OBJECTIVE: Measurement of human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) in cerebrospinal fluid (CSF) can aid in the diagnosis of germ cell tumors (GCTs). Matrix effects can influence test results when alternative sample types are used, therefore, alternative sample types should always be validated before clinical use. Here we have validated the Advia(R) Centaur total hCG and AFP methods for use with CSF. We also performed a retrospective review of 5years of CSF hCG and AFP measurements sent out from our institution. DESIGN AND METHODS: Both hCG and AFP concentrations were measured using the ADVIA Centaur(R) total hCG or AFP assay. RESULTS: The Centaur hCG and AFP assays, performed on CSF, had intra- and inter-assay imprecisions <10.2% CV. The assays were linear over a dynamic range of 10-1000IU/L for hCG and 10-1000mug/L for AFP. Retrospective chart review confirmed that GCTs have a male predominance and are diagnosed most frequently in the second decade of life. The data also illustrate the importance of measuring both serum and CSF concentrations as CSF can be elevated in the absence of serum elevations. CONCLUSIONS: The Centaur total hCG and AFP methods accurately quantify hCG and AFP in CSF.

(2) Source: Biomed Res Int 2014:164081, 2014.

Title: Biochemical markers of spontaneous preterm birth in asymptomatic women.

Authors: Chan RL.

Abstract: Preterm birth is a delivery that occurs at less than 37 completed weeks of gestation and it is associated with perinatal morbidity and mortality. Spontaneous preterm birth accounts for up to 75% of all preterm births. A number of maternal or fetal characteristics have been associated with preterm birth, but the use of individual or group biochemical markers have advanced some of the understanding on the mechanisms leading to spontaneous preterm birth. This paper provides a summary on the current literature on the use of biochemical markers in predicting spontaneous preterm birth in asymptomatic women. Evidence from the literature suggests fetal fibronectin, cervical interleukin-6, and alpha-fetoprotein as promising biochemical markers in predicting spontaneous preterm birth in asymptomatic women. The role of gene-gene and gene-environment interactions, as well as epigenetics, has the potential to further elucidate and improve understanding of the underlying mechanisms or pathways of spontaneous preterm birth. Refinement in study design and methodology is needed in future research for the development and validation of individual or group biochemical marker(s) for use independently or in conjunction

with other potential risk factors such as genetic variants and environmental and behavioral factors in predicting spontaneous preterm birth across diverse populations.

(3) Source: Clin Biochem, 2014.

Title: Down syndrome screening: Suitability of a WHO 5 standardized total hCG assay.

Authors: Palomaki GE, Lambert-Messerlian G.

Abstract: OBJECTIVE: To determine how the adoption of the new WHO 5th International Standard (IS 07/364) will affect the suitability of total betahCG as a marker for second trimester Down syndrome screening, compared to the current WHO 3rd IS (75/537). DESIGN AND METHODS: Assays employing both standards were evaluated on the Beckman Coulter DxI platform. Matched betahCG results from 232 fresh second trimester maternal serum samples were compared. In addition, stored samples from 51 Down syndrome and 251 matched control sera were also tested with both assays and results converted to weight-adjusted multiples of the median (MoMs). These results were combined with maternal age and the existing alpha-fetoprotein, unconjugated estriol and inhibin-A MoM levels to compute patient-specific Down syndrome risk. RESULTS: Correlation between the two sets of results on fresh samples was high (r=0.993), but showed a proportional increase of 33% (95% CI 31% to 35%) in results using the new versus old assay across the range of measurements. betahCG results in the case/control dataset were also highly correlated (0.994) and showed a similar proportional increase (34%). After computing assay-specific MoMs, the resulting 'triple' and 'quadruple' Down syndrome risks were highly correlated, and resulted in no difference in either of the two detection rates (78% and 88%, respectively) or false positive rates (6.4%, 6.8%). CONCLUSIONS: Laboratories using the DxI platform with the new total betahCG 5th IS assay will need to compute new reference (medians), but can expect no impact on the clinical validity of the associated Down syndrome screening programs.

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

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

Title: Routine measurement of amniotic fluid alpha-fetoprotein and acetylcholinesterase: the need for a reevaluation.

Authors: Flick A, Krakow D, Martirosian A, Silverman N, Platt LD.

Abstract: OBJECTIVE: The objective of the study was to evaluate whether the current screening regimen of measuring amniotic fluid alpha-fetoprotein (AF-AFP) at the time of amniocentesis and reflex acetylcholinesterase testing vs ultrasound alone to detect neural tube and ventral wall defects offers improved diagnostic accuracy and cost benefit. STUDY DESIGN: A retrospective chart review on all patients who had amniocentesis performed at 1 center over the past 11 years was performed. Those with an elevated AF-AFP were compared with those whose AF-AFP was within normal limits. Ultrasound findings and outcomes were reviewed in all cases to assess whether neural tube defects (NTDs) or ventral wall defects (VWDs) were missed by AF-AFP or ultrasound screening. A cost-benefit analysis was then performed. RESULTS: Of 6232 women who underwent amniocentesis between January 2002 and December 2012, 81 had an elevated AF-AFP with or without a positive acetylcholinesterase (AChE). Of these 81 women, 13 had NTDs and 5 had VWDs. The sensitivity of the detailed ultrasound was 100% in detecting NTDs and VWDs, whereas that of the AF-AFP ranged from 22% to 77%, with the inclusion of AChE. The total expenditure for AF-AFP in our sample set (n = 6232 amniocentesis at $76.00 per AF-AFP) was $473,632, and all NTDs and VWDs were detected by ultrasound. Translated to a national laboratory (>42,447 samples/year), the cost savings in 2011 alone would be $3,225,972. CONCLUSION: Given the accuracy of high-resolution ultrasound in the detection of both NTDs

and VWDs, measuring AF-AFP and AChE as a reflex-screening test is not a cost-effective approach.

(2) Source: Am J Obstet Gynecol, 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.

(3) Source: BMC Pregnancy Childbirth 14:35, 2014.

Title: Screening models using multiple markers for early detection of late-onset preeclampsia in low-risk pregnancy.

Authors: Park HJ, Kim SH, Jung YW, Shim SS, Kim JY, Cho YK, Farina A, Zanello M, Lee KJ, Cha DH.

Abstract: BACKGROUND: Our primary objective was to establish a cutoff value for the soluble fms-like tyrosine kinase 1(sFlt-1)/placental growth factor (PlGF) ratio measured using the Elecsys assay to predict late-onset preeclampsia in low-risk pregnancies. Our secondary objective was to evaluate the ability of combination models using Elecsys data, second trimester uterine artery (UtA) Doppler ultrasonography measurements, and the serum fetoplacental protein levels used for Down's syndrome screening, to predict preeclampsia. METHODS: This prospective cohort study included 262 pregnant women with a low risk of preeclampsia. Plasma levels of pregnancy-associated plasma protein-A (PAPP-A) and serum levels of alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin, and inhibin-A were measured, and sFlt-1/PlGF ratios were calculated. All women underwent UtA Doppler ultrasonography at 20 to 24 weeks of gestation. RESULTS: Eight of the 262 women (3.0%) developed late-onset preeclampsia. Receiver operating characteristic curve analysis showed that the third trimester sFlt-1/PlGF ratio yielded the best detection rate (DR) for preeclampsia at a fixed false-positive rate (FPR) of 10%, followed by the second trimester sFlt-1/PlGF ratio, sFlt-1 level, and PlGF level. Binary logistic regression analysis was used to determine the five best combination models for early detection of late-onset preeclampsia. The combination of the PAPP-A level and the second trimester sFlt-1/PlGF ratio yielded a DR of 87.5% at a fixed FPR of 5%, the combination of second and third trimester sFlt-1/PlGF ratios yielded a DR of 87.5% at a fixed FPR of 10%, the combination of body mass index and the second trimester sFlt-1 level yielded a DR of 87.5% at a fixed FPR of 10%, the

combination of the PAPP-A and inhibin-A levels yielded a DR of 50% at a fixed FPR of 10%, and the combination of the PAPP-A level and the third trimester sFlt-1/PlGF ratio yielded a DR of 62.5% at a fixed FPR of 10%. CONCLUSIONS: The combination of the PAPP-A level and the second trimester sFlt-1/PlGF ratio, and the combination of the second trimester sFlt-1 level with body mass index, were better predictors of late-onset preeclampsia than any individual marker.

E) Abstracts of New Assay Methodologies:

(1) Source: Biosens Bioelectron 58C:338-344, 2014.

Title: An integrated giant magnetoimpedance biosensor for detection of biomarker.

Authors: Wang T, Yang Z, Lei C, Lei J, Zhou Y.

Abstract: A Dynabeads-labeled magnetic immunoassay (MIA) has been developed by using an integrated giant magnetoimpedance (GMI) biosensor for the detection of alpha-fetoprotein (AFP). The GMI biosensor (Cr/Cu/NiFe/Cu/NiFe/Al2O3/Cr/Au films) integrated magnetic sensing elements and a biomolecular immunoplatform. Au film was modified with 11-Mercaptoundecanoic acid (11-MUA) for the immobilization of AFP monoclonal antibody. Double antibody sandwich immunoassay was used to specifically capture and label AFP antigen. Functionalized Dynabeads were conjugated to AFP antigen by streptavidin-biotin binding assay. GMI responses were measured for sensitive detection of AFP from 1 to 10ng/ml. Our results revealed that the presence of AFP on the biosensor improved the GMI effect owing to the induced magnetic dipole of superparamagnetic Dynabeads, and the GMI ratio was greatly increased at high frequency. Specificity of MIA was tested through the use of 1% bovine serum albumin (BSA). The underlying biophysical mechanisms responsible for the enhanced GMI effect in the detection of AFP were discussed. This work provides a complex lab-on-chip MIA for the detection of biomarker, which may open up a new way for the development of GMI-based MIA in clinical trials.

(2) Source: Clin Chim Acta 431:113-117, 2014.

Title: Chemiluminescence immunoassay based on microfluidic chips for alpha-fetoprotein.

Authors: Fan F, Shen H, Zhang G, Jiang X, Kang X.

Abstract: BACKGROUND: Conventional immunoassays are labor intensive, time consuming, expensive and require large pieces of equipment for detection. In an effort to overcome these shortcomings, this study established an immunoassay method of alpha fetoprotein (AFP) in serum in combination with the microfluidic chip technology. METHODS: A sandwich immunoassay approach was applied to detect AFP based on microfluidic chips and the chemiluminescence as detection signal. The chip used in this method was composed of a polydimethylsiloxane (PDMS) microchannel layer over a PDMS base layer. RESULT: AFP concentration and chemiluminescence intensity were linearly correlated over the concentration ranging from 12.5 to 200ng/ml, and a detection limit as low as 1.5ng/ml using this method. The coefficients of variation were 9.91% and 11.4% for the within- and between-run assays, respectively. More than 50 clinical samples were tested and the results obtained for this method strongly correlated with Roche's electrochemiluminescence (ECL) kit. CONCLUSIONS: The proposed method offers a reliable, simple, reagent safe and inexpensive analytical platform for the determination of AFP in serum, and promotes the development of high throughput screening and point-of-care testing (POCT) diagnostics in clinical practice.

(3) Source: Small 10:705, 2014.

Title: Nanocomposites: Graphene-Ruthenium(II) Complex Composites for Sensitive ECL Immunosensors (Small 4/2014).

Authors: Xiao FN, Wang M, Wang FB, Xia XH.

Abstract: Ru-PTCA/G nanocomposites are synthesized via a one step synthesis procedure under moderate conditions followed by covalent attachment of an amine derivative Ru(II) complex through amide bond formation. As X.-H. Xia and co-workers report on page 706, the product shows good electrochemical activity and ca. 21 times higher luminescence quantum efficiency than the adsorbed Ru(II) complex. A solidstate electrochemiluminescent (ECL) immunosensor is developed based on the Ru-PTCA/G nanocomposites for sensitive detection of alpha-fetoprotein (AFP). The ECL peak intensity in the presence of AFP is much lower than that in the absence of AFP due to the insulating effect of the formed antigen-antibody complex, which inhibits the electron transfer and the mass transfer of tripropylamine to the surface of the Ru-PTCA/G modified electrode.

F) Special Abstract Selection:

(1) Source: J Gynecol Obstet Biol Reprod (Paris) 43:5-11, 2014.

Title: [Interpretation of atypical values of maternal serum markers].

Authors: Geyl C, Subtil D, Vaast P, Coulon C, Clouqueur E, Deruelle P, Debarge V.

Abstract: OBJECTIVES: Prenatal screening was set up to identify patients at high-risk of chromosome 21 trisomy based on maternal serum markers measurement. However, the risk of trisomy 21 should not be the only result considered by obstetricians. In fact, abnormal marker values can be associated with other fetal diseases and used to improve maternal and fetal follow-up. Our objective was therefore to study other predictive values of maternal serum markers. MEANS AND METHODS: A search through publications was conducted using the PubMed(R) or Cochrane(R) databases. RESULT: In case of high PAPP-A there is no link with any complications. Second trimester high hCG or first trimester low hCG are associated with an increased vascular risk. High alpha-fetoprotein level is a marker of neural tube defects or abdominal wall defect. Persistence of high alpha-fetoprotein with normal echography can suggest other rare fetal diseases. Low maternal serum markers suggests 18 trisomy. Oestriol reflects the fetal hypothalamo-hypophyseal axis and can be used as a diagnosis tool. CONCLUSION: Serum markers could be interesting tools for the identification of high-risk pregnancy and the prevention of neonatal complications. They also appear as a potential help to diagnose certain congenital malformations.

(2) 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.

(3) Source: Am J Obstet Gynecol 210:172, 2014.

Title: The role of maternal serum alpha-fetoprotein in screening for open spina bifida at 11-13 weeks.

Authors: Spencer K.

Abstract: Comment in

Reply: To PMID 23673229. [Am J Obstet Gynecol. 2014]

Comment on

Combined screening for open spina bifida at 11-13 weeks using fetal biparietal diameter and maternal serum markers. [Am J Obstet Gynecol. 2013]

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

Figure 1

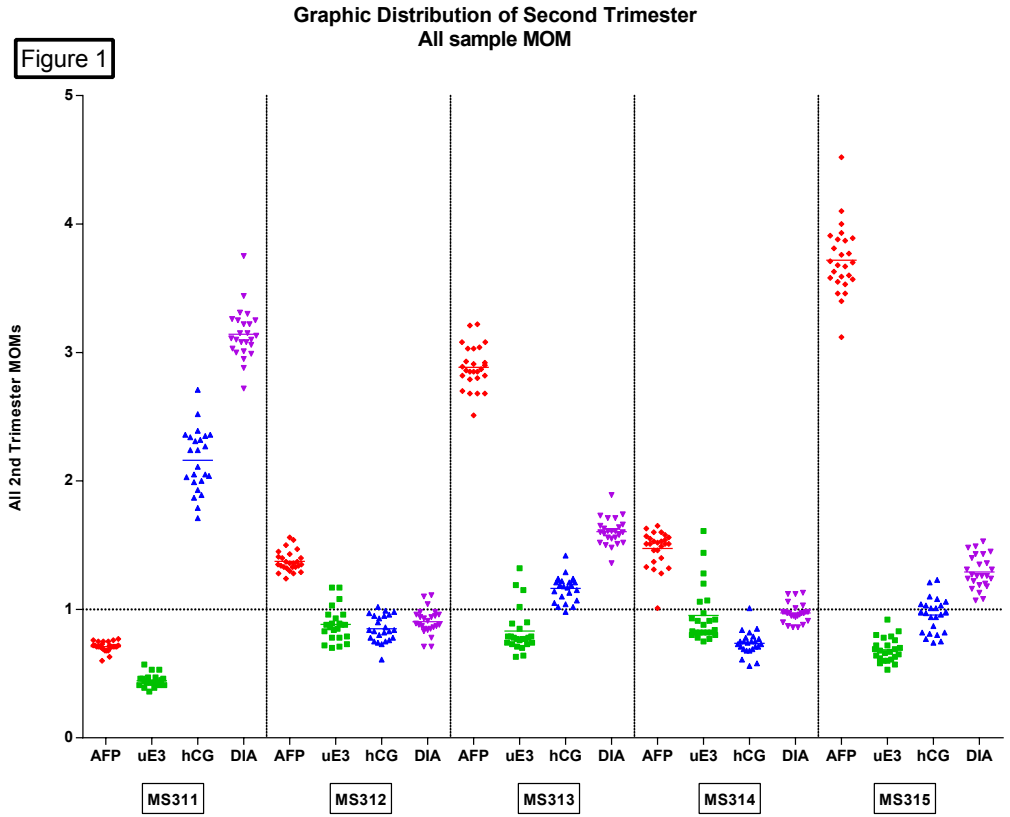


Figure 2a

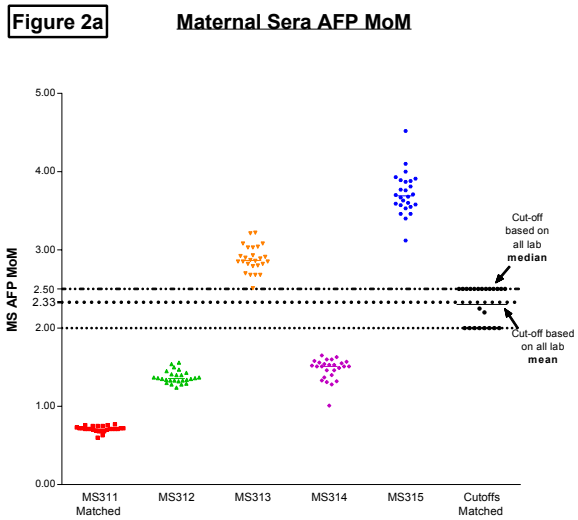


Figure 2b

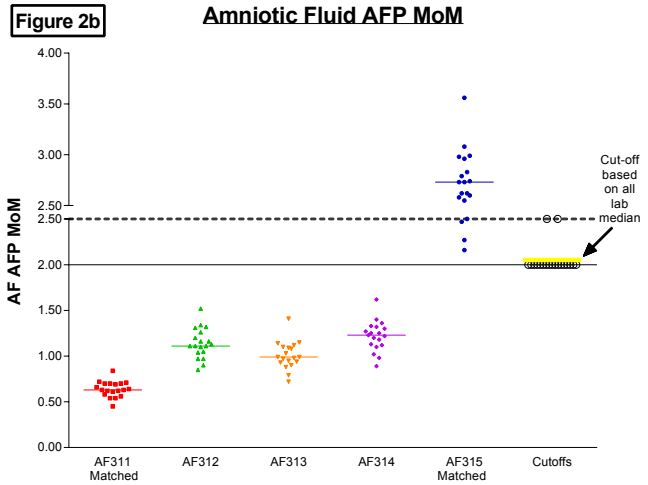


Figure 3

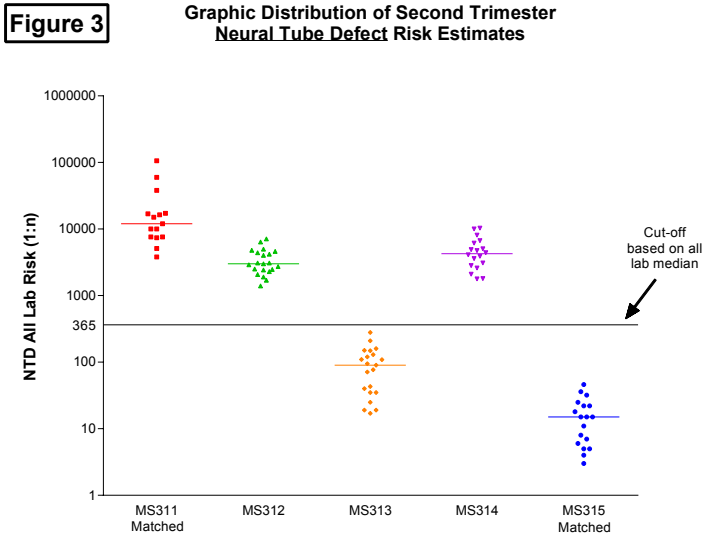


Figure 4

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

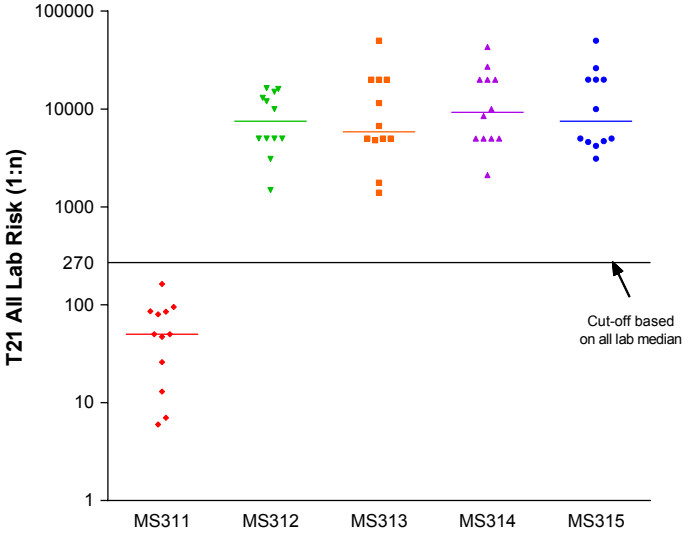


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

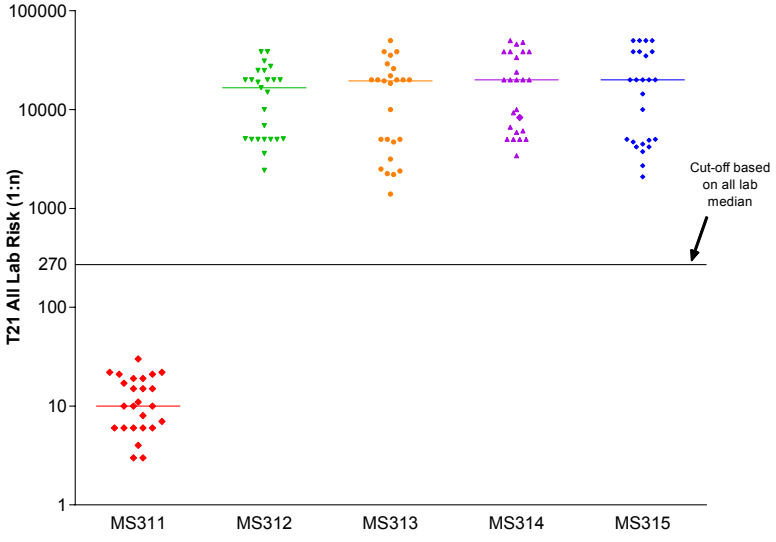
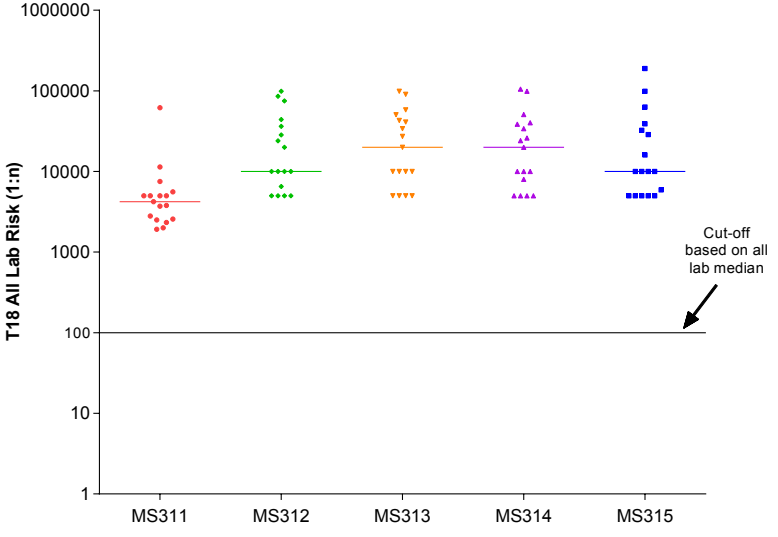


Figure 6

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

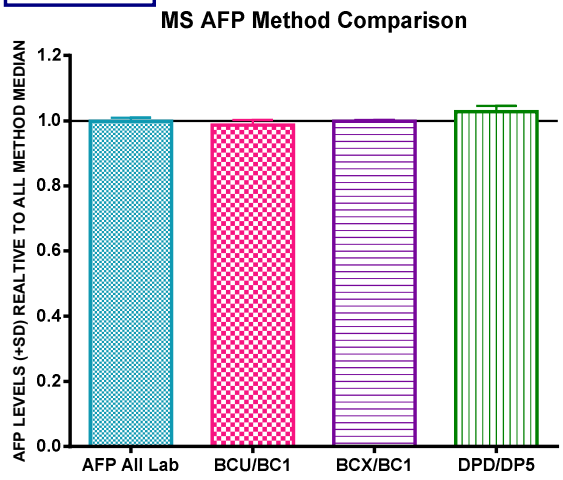


Figure 7B

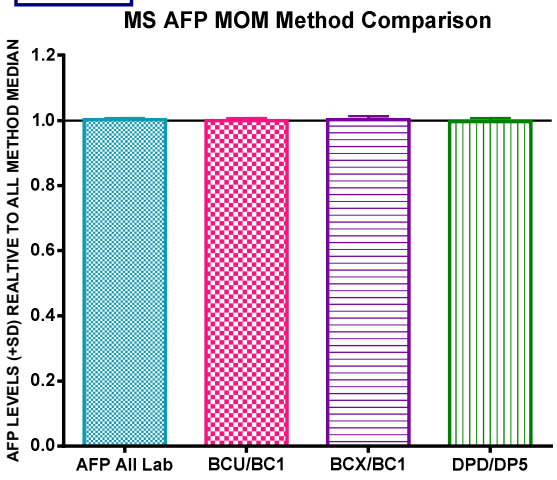


Figure 7C

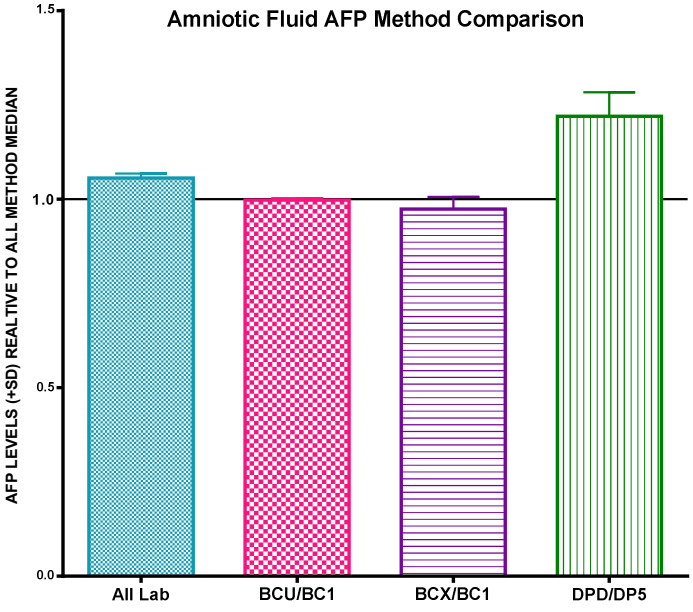
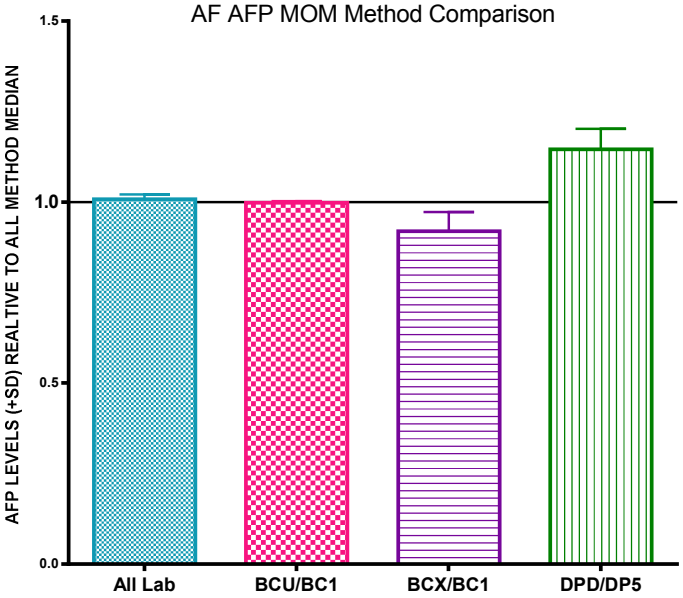


Figure 7D



NYS FEDM PT 5/14
Second Trimester

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

Figure 8A

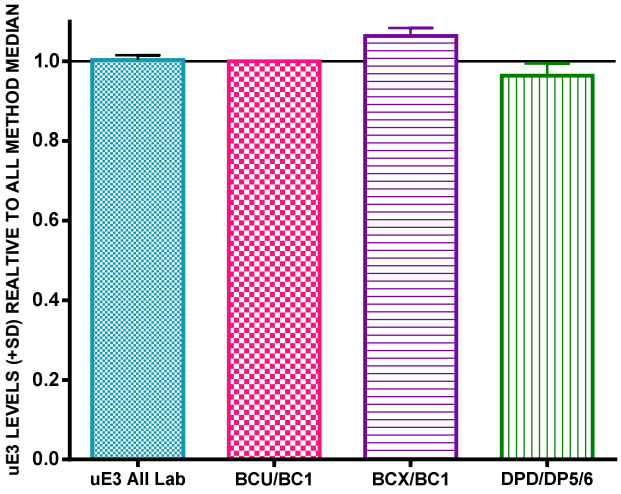


Figure 8B

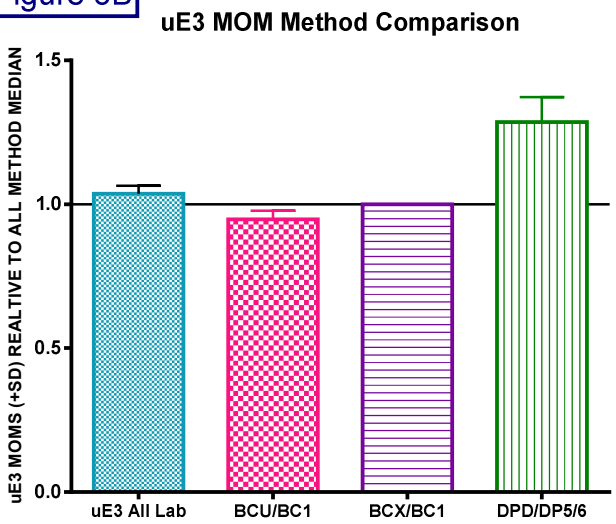


Figure 9A

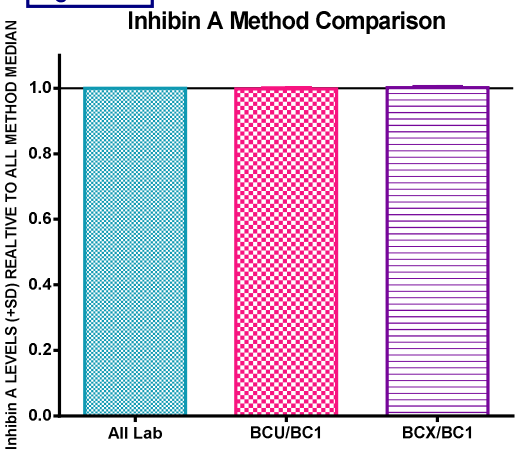


Figure 9B

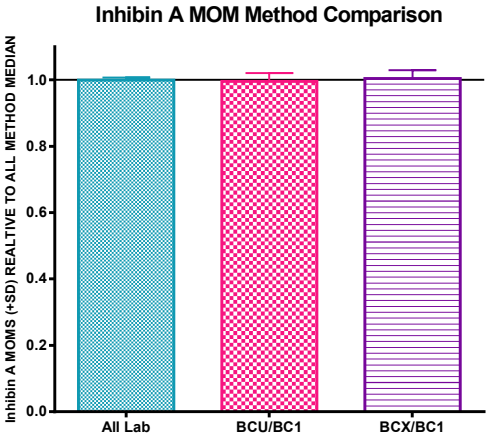


Figure 10A

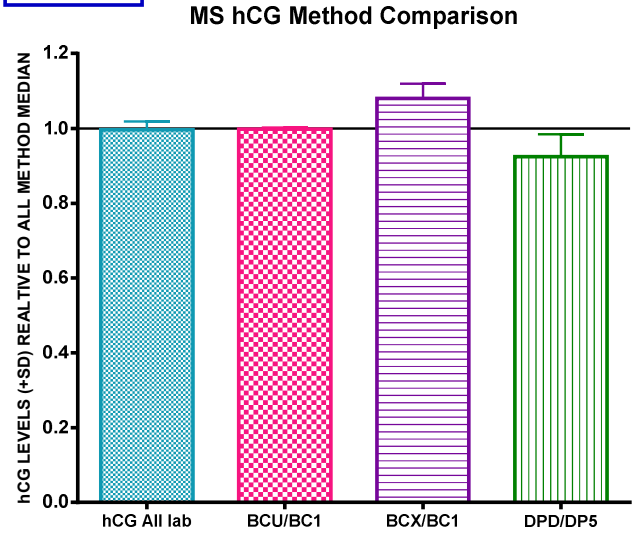
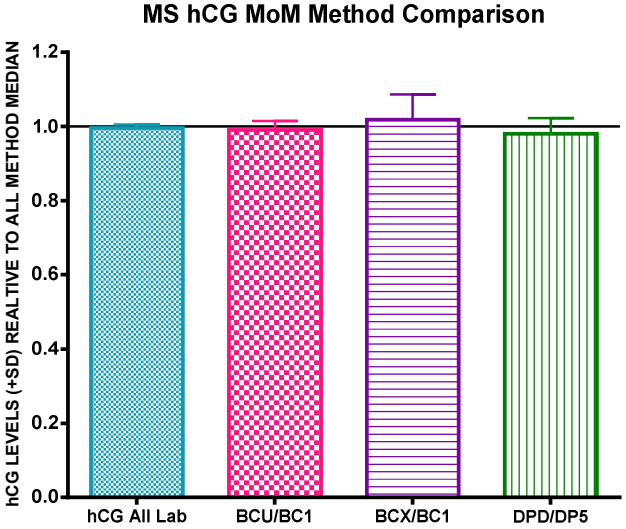


Figure 10B



NYS FEDM PT 5/14
First Trimester

Figure 11A

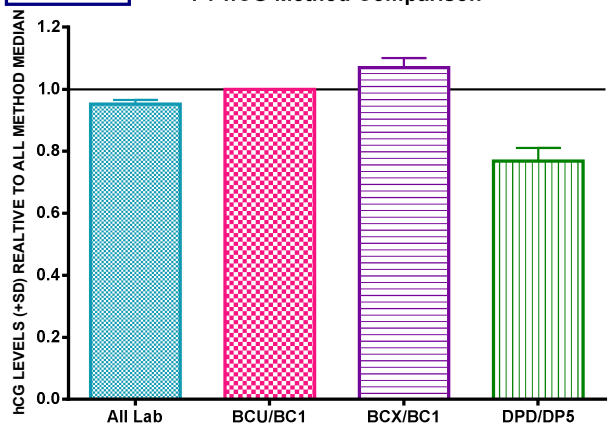


Figure 11B

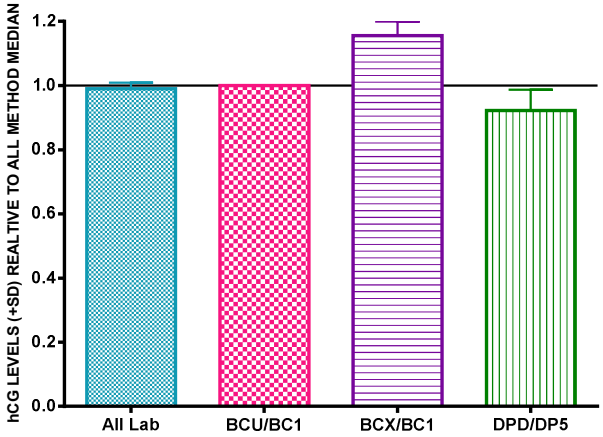


Figure 12A

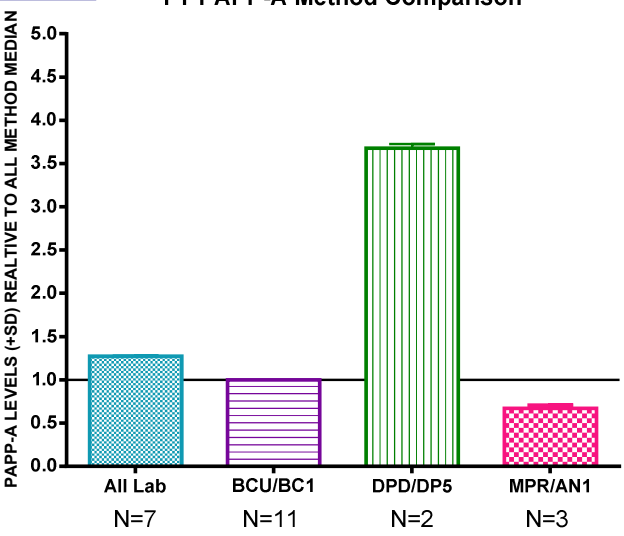
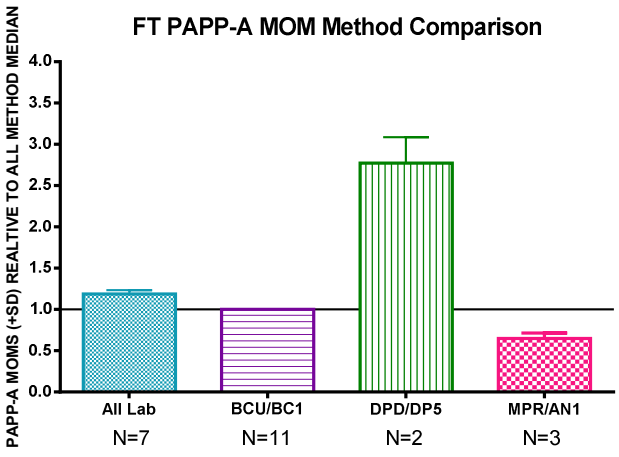


Figure 12B



BCX/BC1 = Beckman Access/2
BCU/BC1 = Beckman Unicel
DPD/DP5 = Siemens Immulite 2000
MPR/AN1 = AnshLite Reagents

Figure 13

Graphic Distribution of First Trimester Trisomy 21 Risk Estimates

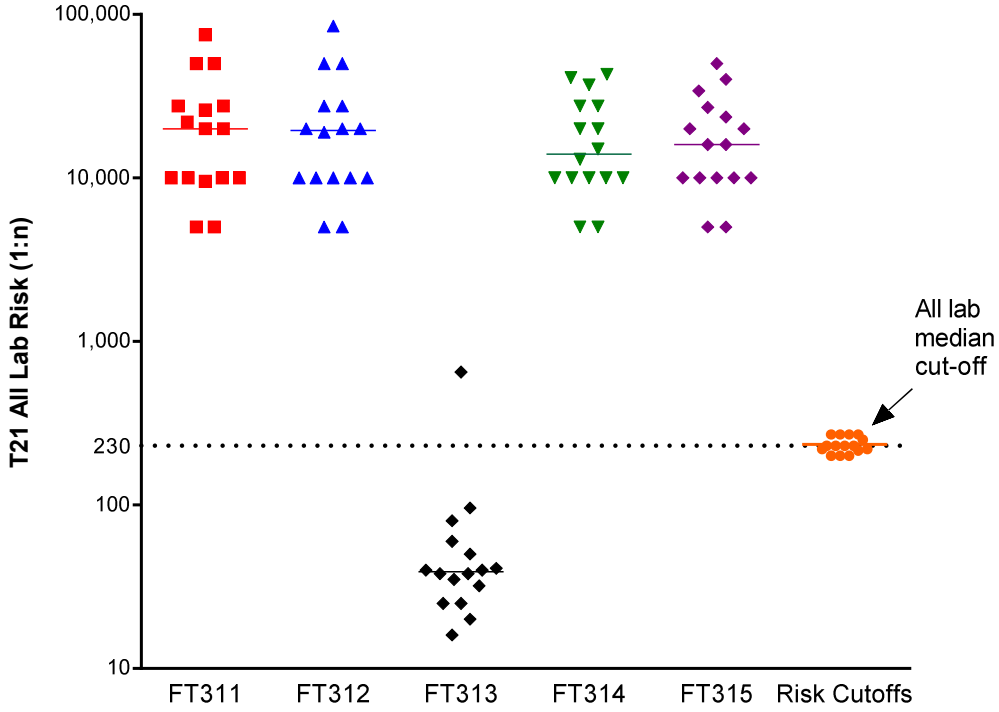
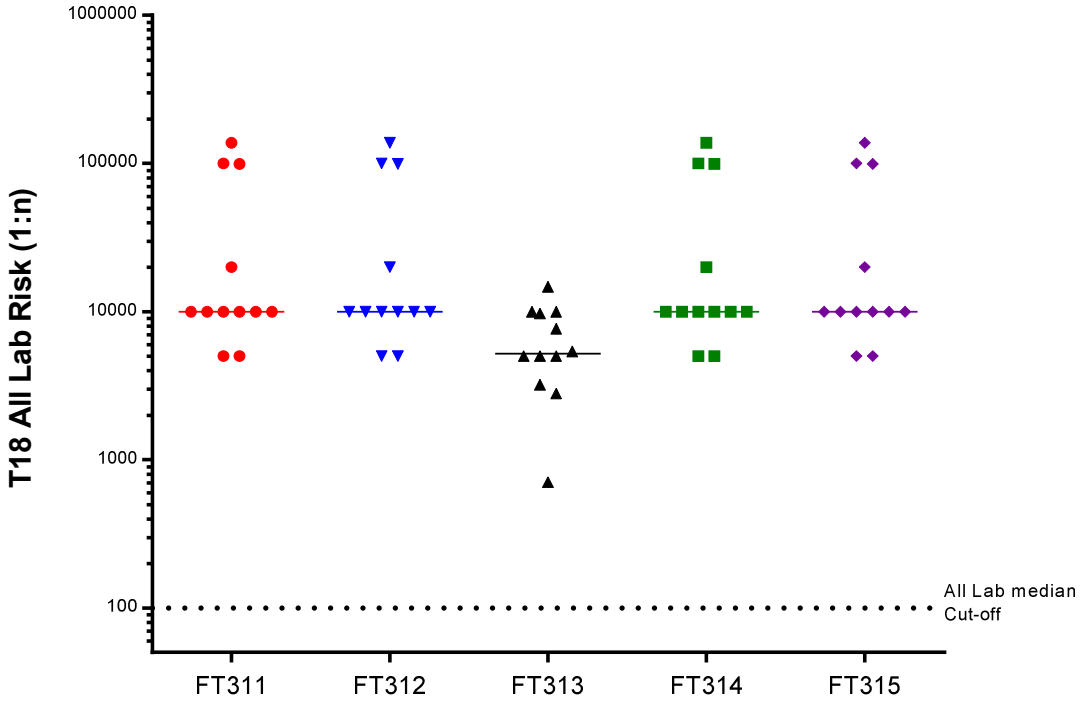


Figure 14

Graphic Distribution of First Trimester Trisomy 18 Risk Estimates



New York State Fetal Defect Markers Proficiency Test,
May 2014
Summary of Results

	MS 311	MS 312	MS 313	MS 314	MS 315
Gestational Age All Lab Mean:					
Mean	18.0	15.0	16.0	16.0	20.0
SD	0.00	0.00	0.00	0.00	0.00
%CV	0.0%	0.0%	0.0%	0.0%	0.0%
mean+3*SD	18.0	15.0	16.0	16.0	20.0
mean-3*SD	18.0	15.0	16.0	16.0	20.0
N	26	26	26	26	26

	MS 311	MS 312	MS 313	MS 314	MS 315
MS AFP All Lab Mean:					
mean	31.7	43.4	101.1	49.9	210.8
SD	1.9	2.7	5.4	3.0	11.2
%CV	6.1%	6.2%	5.3%	6.1%	5.3%
mean+3SD	37.5	51.5	117.3	58.9	244.6
mean-3SD	25.9	35.3	84.9	40.8	177.1
N	26	26	26	25	26
median	31.5	43.2	101.8	49.4	209.85
mean/all kit median	1.00	0.98	1.00	1.01	1.01
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	31.7	42.5	99.9	48.5	208.8
SD	2.1	2.2	6.7	4.3	13.6
%CV	6.7%	5.3%	6.7%	8.9%	6.5%
mean + 3SD	38.1	49.3	119.9	61.4	249.6
mean - 3SD	25.3	35.8	79.8	35.5	168.0
N	14	14	14	14	14
Median	31.5	42.8	99.6	48.6	208.6
mean/All kit median	1.00	0.96	0.99	0.98	1.00
MS AFP Beckman Access/2 (BCX/BC1) mean:					
mean	31.5	44.2	101.0	49.5	207.4
SD	1.4	2.4	2.1	2.5	2.6
%CV	4.5%	5.3%	2.1%	5.1%	1.2%
mean+3SD	35.7	51.3	107.2	57.1	215.2
mean-3SD	27.3	37.1	94.7	41.9	199.7
N	5	5	5	5	5
median	31.0	43.1	100.4	48.8	207.3
mean/all kit median	1.00	1.00	1.00	1.00	0.99
MS AFP Siemens Immulite 2000 (DPD/DP5) mean:					
mean	31.9	44.8	103.8	51.5	219.2
SD	2.2	3.7	3.6	3.3	5.9
%CV	6.9%	8.2%	3.4%	6.4%	2.7%
mean+3SD	38.5	55.9	114.6	61.4	236.9
mean-3SD	25.2	33.8	93.1	41.6	201.4
N	6	6	6	6	6
median	32.5	43.5	103.5	52.0	220.5
mean/all kit median	1.01	1.01	1.03	1.04	1.05
MS AFP kit average:					
mean	31.7	43.8	101.6	49.8	211.8
SD	0.2	1.2	2.0	1.5	6.4
all kit median	31.7	44.2	101.0	49.5	208.8

	MS 311	MS 312	MS 313	MS 314	MS 315
MS AFP MoM All Lab Mean:					
mean	0.71	1.37	2.88	1.49	3.72
SD	0.04	0.08	0.17	0.10	0.27
%CV	5.3%	5.8%	5.8%	6.9%	7.2%
mean+3SD	0.82	1.61	3.38	1.80	4.52
mean-3SD	0.60	1.13	2.38	1.18	2.92
N	26	26	26	25	26
All Median	0.71	1.36	2.87	1.51	3.69
mean/all kit median	1.00	1.00	1.00	1.01	1.00
MS AFP MoM Beckman Unicel (BCU/BC1) mean:					
Mean	0.72	1.37	2.90	1.48	3.73
SD	0.04	0.08	0.20	0.16	0.33
%CV	5.3%	5.5%	7.0%	10.9%	8.9%
mean + 3SD	0.83	1.59	3.51	1.96	4.73
mean - 3SD	0.60	1.14	2.29	1.00	2.73
N	14	14	14	14	14
Median	0.72	1.37	2.88	1.52	3.69
mean/all kit median	1.01	0.99	1.00	1.00	1.00
MS AFP MoM Beckman Access/2 (BCX/BC1) mean:					
Mean	0.71	1.40	2.89	1.48	3.69
SD	0.01	0.10	0.14	0.13	0.18
%CV	1.7%	6.8%	4.7%	8.7%	5.0%
mean + 3SD	0.75	1.69	3.30	1.87	4.24
mean - 3SD	0.67	1.11	2.48	1.10	3.14
N	5	5	5	5	5
Median	0.71	1.35	2.90	1.52	3.60
mean/all kit median	1.00	1.02	1.00	1.00	0.99
MS AFP MoM Siemens Immulite 2000 (DPD/DP5) mean:					
Mean	0.70	1.38	2.86	1.48	3.78
SD	0.05	0.09	0.12	0.10	0.13
%CV	7.4%	6.6%	4.2%	7.1%	3.4%
mean + 3SD	0.85	1.65	3.22	1.79	4.16
mean - 3SD	0.54	1.10	2.50	1.16	3.40
N	6	6	6	6	6
Median	0.71	1.36	2.85	1.51	3.82
mean/all kit median	0.98	1.00	0.99	1.00	1.01
MS AFP MoM kit average:					
mean	0.71	1.38	2.88	1.48	3.73
SD	0.01	0.02	0.02	0.00	0.04
all kit median	0.71	1.38	2.89	1.48	3.73

New York State Fetal Defect Markers Proficiency Test,
May 2014
Summary of Results

	MS 311	MS 312	MS 313	MS 314	MS 315
MS uE3 All Lab Mean:					
mean	0.53	0.56	0.63	0.66	1.20
SD	0.05	0.06	0.05	0.06	0.09
%CV	9.5%	10.8%	8.4%	8.7%	7.6%
mean+3SD	0.68	0.74	0.79	0.83	1.47
mean-3SD	0.38	0.38	0.47	0.49	0.92
N	24	24	24	24	24
mean/all kit median	1.00	0.99	1.01	1.02	1.00
MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	0.53	0.56	0.62	0.65	1.20
SD	0.03	0.05	0.03	0.04	0.09
%CV	6.4%	8.5%	4.8%	5.9%	7.8%
mean+3SD	0.63	0.71	0.71	0.76	1.48
mean-3SD	0.43	0.42	0.53	0.53	0.92
N	13	13	13	13	13
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS uE3 Beckman Access/2 (BCX/BC1) mean:					
mean	0.57	0.60	0.66	0.70	1.24
SD	0.03	0.06	0.05	0.05	0.05
%CV	6.1%	9.5%	8.0%	6.9%	4.4%
mean+3SD	0.67	0.77	0.82	0.85	1.40
mean-3SD	0.46	0.43	0.50	0.55	1.08
N	5	5	5	5	5
mean/all kit median	1.07	1.07	1.07	1.08	1.03
MS uE3 Siemens Immulite/2000 (DPD/DP5 or 6) mean:					
Mean	0.51	0.52	0.61	0.65	1.16
SD	0.08	0.07	0.08	0.09	0.11
%CV	15.3%	13.5%	13.4%	13.4%	9.3%
mean+3SD	0.74	0.73	0.85	0.91	1.48
mean-3SD	0.27	0.31	0.36	0.39	0.83
N	6	6	6	6	6
mean/all Kit Median	0.95	0.92	0.98	1.00	0.97
MS uE3 kit average:					
mean	0.53	0.56	0.63	0.66	1.20
SD	0.03	0.04	0.03	0.03	0.04
all kit median	0.53	0.56	0.62	0.65	1.20

	MS 311	MS 312	MS 313	MS 314	MS 315
MS uE3 MoM All Lab Mean:					
Mean	0.44	0.88	0.83	0.95	0.69
SD	0.05	0.13	0.17	0.22	0.09
%CV	10.8%	15.1%	20.8%	23.4%	13.5%
mean+3SD	0.59	1.28	1.35	1.62	0.96
mean-3SD	0.30	0.48	0.31	0.28	0.41
N	23	23	24	24	24
mean/all kit Median	1.00	1.01	1.05	1.06	1.06
MS uE3 MoM Beckman Unicel (BCU/BC1) Mean:					
Mean	0.42	0.83	0.73	0.83	0.65
SD	0.03	0.10	0.05	0.08	0.07
%CV	6.7%	11.9%	7.2%	9.6%	11.1%
mean+3SD	0.50	1.12	0.89	1.07	0.86
mean-3SD	0.33	0.53	0.58	0.60	0.43
N	13	13	13	13	13
mean/all kit Median	0.94	0.94	0.93	0.93	1.00
MS uE3 MoM Beckman Access/2 (BCX/BC1) Mean:					
Mean	0.45	0.87	0.79	0.90	0.65
SD	0.02	0.06	0.06	0.06	0.04
%CV	4.1%	7.4%	7.5%	6.7%	6.2%
mean+3SD	0.50	1.07	0.97	1.08	0.77
mean-3SD	0.39	0.68	0.61	0.72	0.53
N	5	5	5	5	5
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	0.53	1.11	1.07	1.25	0.80
SD	0.07	0.20	0.18	0.25	0.08
%CV	12.9%	18.3%	16.9%	19.9%	9.6%
mean+3SD	0.74	1.71	1.61	2.00	1.03
mean-3SD	0.33	0.50	0.53	0.51	0.57
N	6	6	6	6	6
mean/all kit Median	1.19	1.26	1.35	1.40	1.23
MS uE3 MoM kit average:					
mean	0.47	0.93	0.87	0.99	0.70
SD	0.06	0.15	0.18	0.23	0.09
all kit median	0.45	0.87	0.79	0.90	0.65

New York State Fetal Defect Markers Proficiency Test,
May 2014
Summary of Results

	MS 311	MS 312	MS 313	MS 314	MS 315
MS hCG All Lab mean:					
mean	43.9	35.5	34.7	20.4	15.5
SD	4.6	3.3	3.0	2.1	1.4
%CV	10.5%	9.2%	8.7%	10.1%	8.7%
mean+3SD	57.7	45.3	43.7	26.5	19.6
mean-3SD	30.1	25.7	25.7	14.2	11.5
N	24	24	24	24	24
mean/all kit median	0.97	0.99	0.99	1.03	1.00
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	45.0	35.9	35.1	19.6	15.5
SD	4.0	3.0	2.8	2.1	1.4
%CV	8.9%	8.5%	8.1%	10.6%	9.2%
mean+3SD	57.0	45.1	43.6	25.9	19.8
mean-3SD	33.0	26.8	26.5	13.4	11.2
N	13	13	13	13	13
median	45.40	36.20	35.90	20.40	15.60
mean/All kit median	1.00	1.00	1.00	0.99	1.00
MS hCG Beckman Access/2 (BCX/BC1) mean:					
mean	47.5	38.3	37.3	22.9	16.4
SD	1.7	1.7	1.6	0.9	0.9
%CV	3.6%	4.4%	4.2%	4.1%	5.5%
mean+3SD	52.6	43.4	42.1	25.7	19.1
mean-3SD	42.4	33.2	32.6	20.0	13.8
N	5	5	5	5	5
median	47.5	38.5	37.3	22.5	16.4
mean/all kit median	1.06	1.07	1.06	1.15	1.06
MS hCG Siemens Immulite 2000 (DPD/DP5) mean:					
mean	38.4	32.3	31.7	19.8	15.0
SD	2.2	2.0	1.4	0.7	1.3
%CV	5.7%	6.1%	4.5%	3.5%	8.7%
mean+3SD	44.9	38.2	36.0	21.9	18.8
mean-3SD	31.9	26.4	27.4	17.7	11.1
N	6	6	6	6	6
median	38.2	31.8	31.7	19.8	14.9
mean/all kit median	0.85	0.90	0.90	1.00	0.97
MS hCG kit average:					
mean	43.6	35.5	34.7	20.8	15.6
SD	4.7	3.0	2.8	1.8	0.8
all kit median	45.0	35.9	35.1	19.8	15.5

	MS 311	MS 312	MS 313	MS 314	MS 315
MS hCG MoMs All Lab Mean:					
mean	2.16	0.85	1.16	0.74	0.96
SD	0.24	0.10	0.10	0.09	0.14
%CV	11.3%	12.2%	8.6%	12.5%	14.2%
mean+3SD	2.89	1.16	1.46	1.01	1.36
mean-3SD	1.43	0.54	0.86	0.46	0.55
N	24	24	24	24	24
mean/All Kit Median	0.99	1.01	0.99	1.00	0.99
MS hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	2.23	0.84	1.15	0.71	0.97
SD	0.22	0.11	0.10	0.09	0.13
%CV	9.7%	12.5%	8.7%	12.1%	13.3%
mean+3SD	2.88	1.16	1.45	0.97	1.35
mean-3SD	1.58	0.53	0.85	0.45	0.58
N	13	13	13	13	13
median	2.32	0.84	1.18	0.71	0.98
mean/All kit median	1.02	1.00	0.97	0.96	1.00
MS hCG MoM Beckman Access/2 (BCX/BC1) mean:					
mean	2.19	0.90	1.19	0.81	0.89
SD	0.32	0.10	0.14	0.13	0.14
%CV	14.5%	11.4%	11.9%	16.0%	16.0%
X+3SD	3.15	1.20	1.62	1.19	1.32
X-3SD	1.24	0.59	0.77	0.42	0.46
N	5	5	5	5	5
median	2.11	0.95	1.19	0.77	0.82
mean/All kit median	1.00	1.06	1.01	1.10	0.92
MS hCG MoM Siemens Immulite 2000 (DPD/DP5) mean:					
mean	1.99	0.82	1.18	0.74	0.99
SD	0.18	0.11	0.06	0.04	0.15
%CV	8.9%	12.9%	5.4%	5.1%	15.5%
X+3SD	2.52	1.14	1.37	0.85	1.44
X-3SD	1.46	0.50	0.99	0.62	0.53
N	6	6	6	6	6
median	2.02	0.78	1.20	0.75	1.00
mean/All kit median	0.91	0.97	1.00	1.00	1.02
MS hCG MoM kit average:					
mean	2.1	0.9	1.2	0.8	0.9
SD	0.1	0.0	0.0	0.1	0.1
all kit median	2.2	0.8	1.2	0.7	1.0

New York State Fetal Defect Markers Proficiency Test,
May 2014
Summary of Results

	MS 311	MS 312	MS 313	MS 314	MS 315
MS Inhibin A all lab mean:					
Mean	543.9	185.2	293.7	155.6	241.6
SD	27.3	12.2	18.0	7.1	14.0
%CV	5.0%	6.6%	6.1%	4.6%	5.8%
mean + 3SD	625.8	221.7	347.8	176.9	283.7
mean- 3SD	461.9	148.6	239.6	134.3	199.4
N	25	25	25	25	25
All Lab Median	547.0	185.0	291.6	157.0	243.4
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	542.8	183.7	294.5	155.2	241.0
SD	28.5	13.0	18.8	6.4	15.1
%CV	5.2%	7.1%	6.4%	4.1%	6.2%
mean + 3SD	628.3	222.8	351.0	174.4	286.2
mean- 3SD	457.4	144.6	238.0	136.1	195.9
N	17	17	17	17	17
kit median	543.1	184.0	292.7	155.6	242.9
mean/all kit median	1.00	0.99	1.00	1.00	1.00
MS Inhibin A Beckman Access/2 (BCX/BC1) mean:					
Mean	546.0	188.2	292.1	156.4	242.8
SD	26.4	10.2	17.3	8.8	12.5
%CV	4.8%	5.4%	5.9%	5.6%	5.1%
mean + 3SD	625.1	218.8	344.0	182.9	280.2
mean- 3SD	466.9	157.6	240.2	129.9	205.3
N	8	8	8	8	8
kit median	548.9	189.4	290.1	158.8	245.3
mean/All kit median	1.00	1.01	1.00	1.00	1.00
MS Inhibin A kit average:					
mean	544.4	186.0	293.3	155.8	241.9
SD	2.2	3.2	1.7	0.9	1.2
all kit median	544.4	186.0	293.3	155.8	241.9

	MS 311	MS 312	MS 313	MS 314	MS 315
MS Inhibin A MoM All Lab mean:					
mean	3.12	0.90	1.60	0.97	1.29
SD	0.15	0.10	0.10	0.08	0.13
%CV	5.0%	10.9%	6.5%	7.8%	10.0%
mean+3SD	3.58	1.20	1.92	1.20	1.68
mean-3SD	2.65	0.61	1.29	0.75	0.91
N	24	25	25	25	25
mean/all kit median	0.99	0.99	1.00	1.00	1.01
MS Inhibin A MoM Beckman Unicel (BCU/BC1) mean:					
Mean	3.14	0.88	1.59	0.97	1.32
SD	0.22	0.10	0.11	0.08	0.14
%CV	6.9%	11.2%	7.0%	8.0%	10.6%
mean + 3SD	3.79	1.18	1.93	1.21	1.74
mean- 3SD	2.49	0.59	1.26	0.74	0.90
N	17	17	17	17	17
Kit Median	3.11	0.90	1.60	0.97	1.26
mean/all kit median	1.00	0.96	0.99	1.00	1.03
MS Inhibin A MoM Beckman Access (BCX/BC1) mean:					
Mean	3.15	0.95	1.63	0.97	1.24
SD	0.16	0.09	0.09	0.08	0.08
%CV	5.1%	9.1%	5.5%	7.8%	6.8%
mean + 3SD	3.64	1.21	1.90	1.19	1.49
mean- 3SD	2.66	0.69	1.36	0.74	0.98
N	8	8	8	8	8
Kit Median	3.11	0.90	1.60	0.97	1.26
mean/all kit median	1.00	1.04	1.01	1.00	0.97
MS Inhibin A MoM kit average:					
mean	3.14	0.92	1.61	0.97	1.28
SD	0.01	0.05	0.03	0.00	0.06
all kit median	3.14	0.92	1.61	0.97	1.28

New York State Fetal Defect Markers Proficiency Test,
May 2014
Summary of Results

	AF311	AF312	AF313	AF314	AF315
AF AFP All Lab mean :					
mean	5.9	12.8	7.6	11.3	16.9
SD	0.8	1.7	1.2	1.8	1.9
%CV	14.2%	13.6%	15.3%	16.0%	11.6%
mean+3SD	8.4	18.0	11.1	16.7	22.7
mean-3SD	3.4	7.5	4.1	5.9	11.0
N	19	19	19	19	19
All kit median	5.6	12.1	7.2	10.6	16.3
mean/all kit mean	1.06	1.05	1.06	1.07	1.04
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	5.6	12.1	7.2	10.5	16.2
SD	0.6	1.5	1.0	1.4	1.9
%CV	11.2%	12.6%	14.4%	13.3%	11.5%
X+3SD	7.4	16.7	10.4	14.6	21.9
X-3SD	3.7	7.5	4.1	6.3	10.6
N	12	12	12	12	12
median	5.5	12.1	7.2	10.6	16.4
mean/all kit median	1.00	1.00	1.00	0.99	1.00
AF AFP Beckman Access/2 (BCX/BC1) mean:					
mean	5.2	12.0	6.9	10.6	16.3
N	2	2	2	2	2
median	5.2	11.95	6.85	10.55	16.25
mean/all kit median	0.93	0.99	0.95	1.00	1.00
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	6.9	14.4	9.0	13.7	18.4
SD	0.6	1.2	0.6	0.6	1.6
%CV	8.1%	8.1%	6.7%	4.5%	8.9%
mean+3SD	8.6	17.9	10.7	15.5	23.3
mean-3SD	5.2	10.9	7.2	11.8	13.5
N	4	4	4	4	4
median	7	14.4	8.8	13.75	18.75
mean/all kit median	1.24	1.19	1.24	1.30	1.13
AF AFP kit average:					
mean	4.4	9.6	5.8	8.7	12.7
SD	0.9	1.4	1.1	1.8	1.3
all kit median	5.6	12.1	7.2	10.6	16.3

	AF311	AF312	AF313	AF314	AF315
AF AFP MoM All Lab Mean:					
mean	0.64	1.14	1.01	1.22	2.72
SD	0.09	0.16	0.15	0.16	0.31
%CV	13.5%	14.5%	14.9%	13.5%	11.5%
mean+3SD	0.90	1.63	1.46	1.71	3.66
mean-3SD	0.38	0.64	0.56	0.73	1.79
N	19	19	19	19	19
All median	0.63	1.11	0.99	1.23	2.73
mean/all kit median	1.01	1.02	1.02	0.99	1.00
AF AFP MoM Beckman Unicel(BCU/BC1) mean:					
Mean	0.62	1.12	0.98	1.16	2.66
SD	0.06	0.14	0.12	0.09	0.23
%CV	10.0%	12.5%	12.6%	8.1%	8.7%
X+3SD	0.81	1.54	1.34	1.44	3.35
X-3SD	0.43	0.70	0.61	0.88	1.97
N	12	12	12	12	12
median	0.62	1.12	0.96	1.19	2.68
mean/all kit median	1.00	1.00	1.00	1.00	0.99
AF AFP MoM Beckman Access (BCX/BC1) mean:					
Mean	0.54	0.98	0.89	1.10	2.68
N	2	2	2	2	2
median	0.54	0.98	0.89	1.10	2.68
mean/all kit median	0.87	0.88	0.91	0.94	1.00
AF AFP MoM DPC Immulite 2000 (DPD/DP5) mean:					
Mean	0.72	1.23	1.15	1.42	2.88
SD	0.08	0.20	0.18	0.14	0.46
%CV	11.1%	16.0%	16.0%	9.8%	15.9%
X+3SD	0.96	1.82	1.69	1.84	4.25
X-3SD	0.48	0.64	0.60	1.00	1.50
N	4	4	4	4	4
median	0.70	1.16	1.09	1.37	2.68
mean/all kit median	1.16	1.10	1.17	1.22	1.08
AF AFP MoM kit average:					
mean	0.63	1.11	1.00	1.23	2.74
SD	0.09	0.13	0.13	0.17	0.12
all kit median	0.62	1.12	0.98	1.16	2.68

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

	FT311	FT312	FT313	FT314	FT315
FT Gestational Age All Lab Mean:					
Mean	13.1	11.2	11.9	11.5	11.3
SD	0.06	0.13	0.11	0.13	0.12
%CV	0.5%	1.2%	0.9%	1.1%	1.1%
mean+3*SD	13.2	11.6	12.2	11.9	11.7
mean-3*SD	12.9	10.8	11.6	11.1	10.9
N	17	17	17	17	17

	FT311	FT312	FT313	FT314	FT315
FT NT MoM All Lab Mean:					
Mean	0.96	0.97	2.22	0.91	1.03
SD	0.05	0.06	0.13	0.05	0.06
%CV	5.7%	5.9%	6.0%	5.5%	5.6%
mean+3SD	1.12	1.14	2.62	1.06	1.20
mean- 3SD	0.79	0.79	1.82	0.76	0.86
N	16	16	16	16	16
All Median	0.96	0.95	2.20	0.91	1.02

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

	FT311	FT312	FT313	FT314	FT315
FT hCG All Lab Mean:					
mean	63.2	87.5	194.9	88.8	84.2
SD	11.0	12.3	34.4	13.1	11.7
%CV	17.4%	14.1%	17.7%	14.8%	13.9%
mean+3SD	96.3	124.5	298.2	128.1	119.4
mean- 3SD	30.2	50.5	91.5	49.5	49.0
N	16	16	16	16	16
All lab median	65.0	92.4	208.8	92.2	88.3
mean/All kit median	0.96	0.96	0.93	0.96	0.95
FT hCG Beckman Unicel (BCU/BC1) mean:					
mean	65.7	91.5	209.6	92.8	88.9
SD	11.2	9.3	22.4	8.1	6.6
%CV	17.1%	10.1%	10.7%	8.8%	7.4%
mean+3SD	99.4	119.3	276.8	117.2	108.6
mean- 3SD	32.0	63.8	142.5	68.3	69.1
N	10	10	10	10	10
median	68.5	93.9	215.4	96.5	89.2
mean/All kit median	1.00	1.00	1.00	1.00	1.00
FT hCG Beckman Access (BCX/BC1) mean:					
mean	69.6	99.5	216.2	103.2	94.1
N	2	2	2	2	2
mean/All kit median	1.06	1.09	1.03	1.11	1.06
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	54.0	71.5	147.3	71.6	67.8
SD	5.8	2.9	17.9	8.7	7.8
%CV	10.7%	4.0%	12.2%	12.1%	11.4%
mean+3SD	71.3	80.1	201.1	97.7	91.0
mean- 3SD	36.6	62.8	93.5	45.6	44.5
N	4	4	4	4	4
median	54.0	72.4	155.8	73.2	67.2
mean/All kit median	0.82	0.78	0.70	0.77	0.76
FT hCG kit average:					
mean	63.1	87.5	191.0	89.2	83.6
SD	8.1	14.4	38.0	16.1	13.9
all kit median	65.7	91.5	209.6	92.8	88.9

	FT311	FT312	FT313	FT314	FT315
FT hCG MoM All Lab Mean:					
Mean	0.87	1.07	2.47	1.12	0.94
SD	0.17	0.13	0.35	0.11	0.12
%CV	19.3%	12.6%	14.0%	10.1%	12.5%
mean+3*SD	1.37	1.47	3.51	1.46	1.29
mean - 3*SD	0.37	0.66	1.44	0.78	0.59
N	15	15	15	15	15
All lab Median	0.88	1.10	2.36	1.13	0.92
mean/All kit Median	1.01	1.01	0.96	1.00	0.97
MS hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	0.86	1.06	2.57	1.12	0.97
SD	0.18	0.14	0.33	0.09	0.08
%CV	20.4%	13.6%	12.7%	7.7%	7.9%
mean+3SD	1.39	1.49	3.55	1.38	1.19
mean-3SD	0.34	0.63	1.59	0.86	0.74
N	10	10	10	10	10
median	0.89	1.09	2.47	1.13	0.93
mean/All kit median	1.00	1.00	1.00	1.00	1.00
MS hCG MoM Beckman Access (BCX/BC1) mean:					
mean	1.06	1.22	2.86	1.30	1.09
N	1	1	1	1	1
mean/All kit median	1.23	1.15	1.11	1.16	1.13
MS hCG MoM DPC Immulite2000 (DPD/DP5) mean:					
mean	0.84	1.05	2.15	1.07	0.84
SD	0.16	0.12	0.13	0.15	0.16
%CV	18.9%	11.5%	6.3%	13.9%	18.5%
mean+3SD	1.31	1.41	2.55	1.51	1.31
mean-3SD	0.36	0.69	1.74	0.62	0.37
N	4	4	4	4	4
median	0.81	1.06	2.10	1.10	0.85
mean/All kit median	0.97	0.99	0.84	0.95	0.87
FT hCG MoM kit average:					
mean	0.92	1.11	2.52	1.16	0.97
SD	0.12	0.10	0.36	0.12	0.12
all kit median	0.86	1.06	2.57	1.12	0.97

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

	FT311	FT312	FT313	FT314	FT315
FT PAPP-A All Lab Mean:					
Mean	4930.7	2785.8	1522.8	3274.0	2606.1
SD	2817.3	1585.2	838.7	1827.9	1444.7
%CV	57.1%	56.9%	55.1%	55.8%	55.4%
mean + 3SD	13382.7	7541.5	4039.0	8757.8	6940.1
mean- 3SD	-3521.3	-1969.8	-993.4	-2209.8	-1728.0
N	16	16	16	16	16
All Lab Median	3840.8	2150.3	1182.3	2585.1	2010.5
mean/All kit median	1.27	1.27	1.27	1.26	1.29
FT PAPP-A Beckman Unicel(BCU/BC1) Mean:					
Mean	3868.3	2188.1	1201.5	2598.0	2025.2
SD	143.5	116.3	73.3	128.1	172.4
%CV	3.7%	5.3%	6.1%	4.9%	8.5%
mean + 3SD	4298.9	2536.8	1421.5	2982.4	2542.4
mean - 3SD	3437.7	1839.3	981.5	2213.7	1508.1
N	11	11	11	11	11
Kit Median	3860.0	2173.5	1215.0	2599.0	2013.9
mean/All kit median	1.00	1.00	1.00	1.00	1.00
***FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:**					
Mean	14433.6	8125.0	4355.5	9414.1	7480.5
N	2	2	2	2	2
mean/All kit median	3.73	3.71	3.62	3.62	3.69

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

FT PAPP-A AnshLite (SMR, MPR or APM/AN1) Mean:					
Mean	2490.7	1418.2	812.3	1659.4	1486.1
SD	154.1	254.4	46.2	124.5	397.3
%CV	6.2%	17.9%	5.7%	7.5%	26.7%
mean + 3SD	2952.9	2181.3	951.0	2032.8	2678.1
mean - 3SD	2028.5	655.2	673.6	1286.0	294.1
N	3	3	3	3	3
Kit Median	2422.0	1479.0	806.9	1655.0	1379.0
mean/All kit median	0.64	0.65	0.68	0.64	0.73
FT PAPP-A kit average:					
mean	6930.9	3910.4	2123.1	4557.2	3663.9
SD	6533.9	3670.2	1943.1	4232.3	3316.2
all kit median	3868.3	2188.1	1201.5	2598.0	2025.2

	FT311	FT312	FT313	FT314	FT315
FT PAPP-A MoM All Lab Mean:					
Mean	3.48	4.86	2.00	5.27	3.50
SD	2.33	2.47	1.14	2.68	2.07
%CV	67.0%	50.8%	56.9%	50.7%	59.2%
mean + 3SD	10.47	12.27	5.42	13.30	9.72
mean- 3SD	-3.51	-2.54	-1.41	-2.75	-2.71
N	15	15	15	15	15
All Lab Median	2.81	4.05	1.69	4.29	2.49
mean/ All kit median	1.25	1.13	1.20	1.16	1.21
FT PAPP-A MoM Beckman Unicel(BCU/BC1) Mean:					
Mean	2.78	4.30	1.67	4.54	2.90
SD	0.17	0.67	0.17	0.69	0.75
%CV	6.0%	15.5%	10.0%	15.2%	25.9%
mean + 3SD	3.29	6.30	2.18	6.61	5.15
mean - 3SD	2.28	2.30	1.17	2.47	0.64
N	11	11	11	11	11
Kit Median	2.81	4.05	1.69	4.29	2.48
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	9.00	10.38	4.74	11.55	8.20
N	2	2	2	2	2
mean/All kit median	3.23	2.41	2.83	2.54	2.83

FT PAPP-A MoM (SMR or APM/AN1) Mean:					
Mean	1.77	2.43	1.09	3.03	2.14
N	2	2	2	2	2
Kit Median	1.77	2.43	1.09	3.03	2.14
mean/ All kit median	0.63	0.56	0.65	0.67	0.74
FT PAPP-A MoM kit average:					
mean	4.51	5.70	2.50	6.37	4.41
SD	3.91	4.16	1.96	4.55	3.30
all kit median	2.78	4.30	1.67	4.54	2.90