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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 January 28, 2014, for Fetal Defect Markers, which included samples for first and second trimester screening, as well as amniotic fluids. Your laboratory's results and grades are printed on a separate sheet; also included are the grades from the previous two PT events. These will be mailed to you separately. Please review and sign your evaluation. Retain the signed evaluation in your files. You will need it for your next laboratory survey to demonstrate participation in the NYSPT program.

I. Graded Results Section: Table 1: Second Trimester Maternal Serum: Summary of All Lab Results

Samples *N = 26	Sample #	MS 306	MS 307	MS 308	MS 309	MS 310
	Gestational Age (weeks)	17.0	19.0	18.0	15.1	21.0
Maternal Race	Ethnic Group	White	Black	White	Asian	Hispanic
Maternal Weight	Pounds (lbs)	150	160	140	155	145
Maternal Age	Years	28	29	30	27	25
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	18.6 ± 1.4	55.0 ± 4.6	52.3 ± 3.6	29.5 ± 2.4	178.8 ± 12.8
	MOM ± Std. Dev.	0.48 ± 0.04	1.02 ± 0.10	1.13 ± 0.08	1.04 ± 0.09	2.54 ± 0.20
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	0.39 ± 0.06	1.14 ± 0.11	0.61 ± 0.06	0.26 ± 0.05	0.67 ± 0.08
	MOM ± Std. Dev.	0.41 ± 0.05	0.81 ± 0.09	0.50 ± 0.04	0.45 ± 0.07	0.30 ± 0.04
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	49.5 ± 5.6	14.4 ± 0.09	67.8 ± 9.7	25.3 ± 2.4	14.6 ± 1.3
	MOM ± Std. Dev.	2.07 ± 0.24	0.79 ± 0.11	3.17 ± 0.42	0.66 ± 0.08	0.91 ± 0.14
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	332.40 ± 21.5	213.1 ± 12.0	514.5 ± 21.0	161.6 ± 7.5	225.4 ± 10.6
	MOM ± Std. Dev.	1.95 ± 0.14	1.26 ± 0.10	2.90 ± 0.20	0.86 ± 0.07	1.05 ± 0.11
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (80%)
	Recommended Action**	NFA	NFA	NFA	NFA	G = 73% U = 69% A = 69%
	NTD Risk 1 in	10,000	10,000	7,170	5,000	123
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	G = 92% U = 58% A = 83%	NFA	NFA
	Risk Est. 1 in	18	4,700	61	2,823	1,060
2. Quad Test	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(+) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	G = 96% U = 60% A = 88%	NFA	G = 96% U = 60% A = 88%	NFA	NFA
	Risk Est. 1 in	11	4,831	15	10,000	3,700
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (92%)	(-) (92%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	1,099	10,000	5,150	249	433

*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.

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:

N = 26 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 306 Wk 17.0	This specimen was obtained from a 28 year old White woman (Gravida = 2, Parity = 0) in her 17th week gestation with a body weight of 150 lbs. She had no 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, 92%; ultrasound, 60%; amniocentesis, 88%; and from the triple test were: genetic counseling, 85%; ultrasound 58%; and amniocentesis, 83%. Specimen MS306 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 = 0.16).
MS 307 Wk 19.0	This specimen was obtained from a 29 year old Black woman (Gravida = 2, Parity = 1) in her 19th week of gestation with a body weight of 160 lbs. She had no personal history of pregnancy complications and her specimen resulted in a negative screen for NTD with no body weight or ethnic correction indicated. All the labs agreed that both Trisomy screens were negative. Specimen MS307 was not paired with an amniotic fluid specimen.
MS 308 Wk 18.0	This specimen was obtained from a 30 year old White woman (Gravida = 3, Parity = 0) in her 18th week of gestation with a body weight of 140 lbs. She had a family history of miscarriages. Her sample screened negative for NTD, but her aneuploidy screen was screen positive for Down syndrome. However her Trisomy-18 screen was negative. This sample was paired to an amniotic fluid specimen AFAFP level (MOM = 1.06). See Discussion for further detail.
MS 309 Wk 15.0	This specimen was obtained from a 27 year old Asian woman (Gravida = 3, Parity = 2) in her 15th week of gestation with a body weight of 155 lbs. She had no family history of reproductive complications. Her sample screened negative for NTD, and her aneuploidy screens were also consensus negative for both Trisomy-18 and Trisomy-21. The MS309 sample was not paired to an amniotic fluid specimen.
MS 310 Wk 21.0	This specimen was obtained from a 25 year old Hispanic woman (Gravida = 3, parity = 1) in her 21st week gestation with a body weight of 145 lbs. She had a personal history of pregnancy loss. Her sample was a positive screen for NTD (80% consensus; MOM = 2.54). Her screen was consensus negative for both Trisomies with all labs in agreement. Recommendations of further action from labs performing the NTD screen were: genetic counseling, 73%; ultrasound, 69%; and amniocentesis, 69%. The MS310 specimen had an amniotic fluid counterpart which was also elevated (MOM = 4.04).

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.

2) AMNIOTIC FLUID AFP (NTD-analysis):

N=19; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 306 Wk 17.0	AFP = 1.8 ± 0.2 µg/ml MOM = 0.16 ± 0.03	The AF306 sample was targeted as an NTD negative screen in the upper gestational age screening range. All labs categorized AF306 as a negative NTD screen specimen. This specimen had a maternal serum counterpart, MS306, which showed reduced levels of AFP (MOM = 0.48).
AF 307 Wk 19.0	AFP = 7.6 ± 0.9 µg/ml MOM = 1.00 ± 0.13	The AF307 sample was targeted for normal AFAFP value in the upper gestational age range. All labs called AF307 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 308 Wk 18.0	AFP = 10.1 ± 1.4 µg/ml MOM = 1.06 ± 0.18	The AF308 sample was targeted for a screen negative AFAFP value in the routine gestational age screening range. All labs reported this specimen as a screen negative AFAFP value. The AF308 specimen was paired MS308, which showed normal levels of AFP (MOM = 1.13).
AF 309 Wk 17.0	AFP = 6.8 ± 1.2 µg/ml MOM = 0.60 ± 0.10	The AF309 sample was targeted for a screen negative AFAFP value in the routine gestational age range. All labs reported this specimen as a screen negative AFAFP value. The AF309 specimen was not paired with a maternal serum sample.
AF 310 Wk 21.0	AFP = 20.4 ± 2.7 µg/ml MOM = 4.04 ± 0.57	The AF310 sample was targeted for an elevated AFAFP value in the upper gestational age range. All labs called AF310 a positive screen for AFAFP specimen. The AFAFP sample was matched to maternal serum specimen MS310 whose AFP was also elevated (MOM = 2.54).

II. Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 17	**Sample #**	**FT 306**	**FT 307**	**FT 308**	**FT 309**	**FT 310**
	Gestational Age (weeks)	11.1	11.5	12.0	12.5	13.0
Maternal Race	Ethnic Group	Asian	Hispanic	White	Black	White
Maternal Weight	Pounds (lbs)	120	145	140	150	135
Maternal Age	Years	28	23	25	30	21
Fetal Physical Measurements	Crown Rump Length (mm)	43	48	54	61	67
	NT Thickness (mm)	1.20	1.30	2.50	1.40	1.60
	NT – MOM ± Std. Dev.	1.09 ± 0.07	1.08 ± 0.06	1.86 ± 0.11	0.94 ± 0.06	0.98 ± 0.06
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	82.8 ± 13.0	81.1 ± 11.0	154.4 ± 26.0	70.5 ± 9.4	65.2 ± 8.4
	MOM ± Std. Dev.	0.89 ± 0.08	1.01 ± 0.09	2.00 ± 0.22	0.98 ± 0.10	0.96 ± 0.11
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	1323.8 ± 1061.1	1490.2 ± 1136.9	875.5 ± 701.1	2005 ± 1603.2	1530.8 ± 1287.4
	MOM ± Std. Dev.	1.88 ± 1.09	2.14 ± 1.23	1.02 ± 0.56	1.83 ± 1.19	1.19 ± 0.79
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(+) (88%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	G = 93% U = 40% A = 67% C = 53%	NFA	NFA
	Risk Estimate 1 in	12,400	16,200	49	11,600	10,000
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	6,400	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.

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:
N = 17 all-lab Consensus Values.

Sample#	Summary Comments:
FT 306 Wk 11.1	This specimen was obtained from a 28 year old Asian woman with a body weight of 120 lbs. Her gestational age at the time of screening was 11.1 weeks. She had no prior history of pregnancy complications or difficulties. This FT specimen was screen negative and all testing labs were in agreement. The FT306 risk estimate for Trisomy-21 was 1 in 12,400 and the Trisomy-18 risk was 1 in 10,000.
FT 307 Wk 11.5	This specimen was obtained from a 23 year old Hispanic woman of average body weight (145 lbs.). Her gestational age at the time of screening was 11.5 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative with an all-lab consensus of 100%. The FT307 risk estimate for Trisomy-21 was 1 in 16,200 (all lab median cutoff), and the Trisomy-18 risk was 1 in 10,000.
FT 308 Wk 12.0	This specimen was obtained from a 25 year old White woman of average body weight (140 lbs.). Her gestational age at the time of screening was 12.0 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 FT308 risk estimate for Trisomy-21 was 1 in 49, and the Trisomy-18 risk was 1 in 6,400. (See critique)
FT 309 Wk 12.5	This specimen was obtained from a 30 year old Black woman of average body weight (150 lbs.). Her gestational age at the time of screening was 12.5 weeks. She had no prior family history of pregnancy complications and adverse outcomes. This FT specimen was screen negative for Trisomy-21 and all testing labs were in agreement. The FT309 risk estimate for Trisomy-21 was 1 in 11,600, while the Trisomy-18 risk was 1 in 10,000.
FT 310 Wk 13.0	This specimen came from a 21 year old White woman with a body weight of 135 lbs. Her gestational age at the time of screening was 13.0 weeks. She reported no prior family history of pregnancy problems. This FT specimen was screen negative for Trisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT310 was 1 in 11,600, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.

III. Critique and Commentary:

A) Second Trimester Maternal Serum and Amniotic Fluid:

In general, the all-lab results were consistent with the targeted values for the NTD and the Trisomy Screens for risks and outcomes. The Caucasian maternal serum sample **MS310** was targeted as a screen positive specimen for NTD (Figs. 1, 2a and 3) and was matched to the **AF310** sample (Fig. 2b). 80% of the labs agreed that specimen **MS310** was screen positive for NTD and all labs agreed that the sample was negative for both Trisomy screens using both the triple and quad tests (Figs. 4-6). The **MS310** sample matched **AF310** exhibited elevated AFP levels strongly suggesting the presence of an NTD. As a follow-up, 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 **MS306** was obtained from a white woman with a prior sibling history of pregnancy complications. The fetal defect marker MOM values for specimen **MS306** (MSAFP-MOM = 0.48, MSuE3-MOM = 0.41, MShCG-MOM = 2.07, DIA-MOM = 1.95) presented the canonical profile of low MSAFP and low MSuE3 together with elevated MShCG and MSDIA, resulting in a T21 positive screen in which all labs agreed (100% by both triple and quad test) (Fig. 1) . In addition, the matched **AFAFP306** specimen had low AFP levels (MOM value = 0.16) (Fig. 2b). The T21 risk was 1 in 18 by triple test and 1 in 11 by quad test (Figs. 4, 5). The recommended further actions for the sample **MS306** were genetic counseling, 96%; ultrasound, 60%; 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, **MS307** and **MS309**, produced negative screens for NTD, T21, and T18, with no corrections for body weight or race being indicated.

The **MS308** specimen at 18 weeks presented an interesting case involving normal levels of MSAFP, low MSuE3, and both elevated MSHCG and MSDIA levels; this profile resulted in a negative screen for NTD but yielded a positive quad screen risk for T21 (positive risk 1 in 15) (Figs. 4-6). The T21 follow-up actions recommended for specimen **MS308** were genetic counseling, 96%; ultrasound, 60%; amniocentesis, 88%. Sample **MS308** was modeled after several literature case studies of pregnant women with autoimmune systemic lupus erythematosus (SLE) that exhibited aberrant levels of DS biomarkers (1-3). Prior to their current pregnancies, some of the SLE case study women had miscarriages and complicated pregnancies, but others had delivered normal term infants. Having been counseled on the effects of SLE during pregnancy, the women consented to continued gestation and underwent further tests which included ultrasound, 3-D scans, and SLE-related tests including serum autoantibody assays. Some of the patients in these studies of autoimmune SLE were also treated with low dose prednisone and aspirin prior to and during pregnancy. The women in these studies had known pre-existing SLE disease upon presentation at the first obstetrician's visit.

Similar to published reports, the maternal serum biomarkers of **MS308** revealed an MSAFP MOM of 1.13, MSuE3 MOM of 0.5, MSHCG MOM of 3.17, and MSDIA MOM of 2.90 (1, 2) resulting in a false positive screen for T21 despite normal AFP levels. Previous reports in the literature had also demonstrated that this biomarker profile often predicted other pregnancy complications and adverse outcomes (4, 5). These include miscarriage, low birth weight and stillbirth and increased thrombotic events (5-9). Although not true for specimen **MS308**, some pregnant women with SLE may also display elevated MSAFP values producing a false positive prenatal screen for NTD (3).

SLE is a chronic, multisystem autoimmune rheumatic-type disease of unknown etiology (6-8). As in other autoimmune diseases, the immune system attacks the body's cells and tissues resulting in inflammation and damage to multiple organ systems. SLE is characterized by the formation of antigen:antibody immune complexes which precipitate within blood vessels and interstitial spaces causing organ destruction (7). The organ/tissue damage encompasses multiple organs including the kidneys, lungs, brain, spinal cord, heart, and blood vascular system. Clinical manifestations in SLE patients include some of the following: skin and facial rashes, arthritis, hemolytic anemia, oral/nasal ulcers, seizures, psychotic symptoms, pericardial inflammations, leucopenia/thrombocytopenia, and kidney dysfunction (8). Gender differences in SLE patients are quite notable in that the disease occurs nine times more often in women than in men. There is no cure for SLE and the goal of treatment is to control symptoms. The laboratory diagnosis for SLE involves the use of one or more different serum autoantibody assays including antinuclear antibody, antiphospholipid antibodies, lupus anticoagulant, anti-RNA antibodies, and the anti-cardiolipin antibodies SS-A/Ro and SS-B/LA (9). The leading cause of death from SLE is cardiovascular disease due to accelerated progression of atherosclerosis and heart dysfunctions.

Patients with SLE display a multitude of physical and mental symptoms that may involve dermatological lesions/rashes, fever, malaise, joint pain, myalgia, fatigue, and temporary loss of cognitive abilities. SLE occurs mostly in women of child-bearing age between 15-40 years, and complicates the course of pregnancy. Although infertility can occur in lupus patients with kidney disease, normal rates of conception are observed in most women with SLE (11). The risk for Lupus disease is reported to be 1 in 1,500 to 1 in 3,000 pregnancies during a first gestation, and higher in subsequent pregnancies (12). The live birth rate in SLE pregnancies has been estimated at only 72% (13) because of spontaneous abortions, fetal death, and placental thrombosis due to the presence of the autoantibodies. The autoantibodies predispose women to thrombotic events leading to pulmonary emboli, deep vein thrombosis, and cardiovascular blockage (14).

The placenta of the SLE patient is highly sensitive to the immune complex deposits and inflammatory events occurring at the decidual-placental interfacial vasculature. Placental thrombotic damage can result in infarction, hematomas, fibrin deposits, trophoblast membrane thickening, and overall reduced placental size. As a result, spontaneous abortions, stillbirths, and IUGR are commonplace in SLE pregnancies (16). Pregnancies in women with SLE can further be affected at the renal, cardiovascular, pulmonary, and central nervous system levels (17). One such collateral damage of SLE is increased apoptosis or programmed cell death caused by the antigen-antibody complex deposition in the blood vessels and interstitial spaces of various organs (18). Renal damage caused by immune complex deposits occurs predominantly in the capillary walls of the glomerular filtration unit. As a result, a renal inflammatory response is triggered, leading to edema, hypertension, and proteinuria. SLE

diagnosis is complicated in the pregnant woman without case history because the symptoms of preeclampsia (PEC) are similar to the disease manifestations of pre-existing SLE or lupus nephritis. Also, central nervous system involvement leading to convulsions and seizures of SLE women are similar to those observed in pre-eclamptic women during pregnancy. The presence of thrombocytopenia and hemolysis may further confuse the diagnosis of SLE in pregnancy due to similarity with the H.E.L.L.P. (hemolysis, elevated liver enzymes, low platelets) syndrome and patients are managed as if they had PEC (19).

The musculoskeletal (MLS) and hematological systems of pregnant SLE patients are also negatively impacted. The most common MLS ailments are joint pain of the hands and wrist; however, most joints of the body are at risk. Unlike rheumatoid arthritis, deformities due to lupus do not form in the lower limbs and feet, and lupus arthritis is less disabling than rheumatoid arthritis; furthermore, SLE does not cause severe destruction in the afflicted joints. In comparison to lupus arthritis, anemia in SLE patients is more common by affecting about 50% of the patients (20). Unfortunately, a side effect of drug therapy for anemia in lupus patients is depressed platelet and white blood cell counts and moreover, patients with SLE may have anti-phospholipid antibody syndrome (PLAS), a thrombotic disorder (21). Abnormalities associated with PLAS may include a paradoxical prolonged thromboplastin clotting time together with a positive test for anti-phospholipid antibodies. These latter conditions commonly occur in the presence of hemorrhagic disorders. It is of interest that the most common autoantibody found in SLE patients is the anti-cardiolipin antibody, which contributes to the thrombophilia observed in pregnant SLE patients as well as in non-pregnant SLE patients.

In addition to the effects on the organ systems discussed above, cardiac, cerebral and pulmonary complications may also occur in the pregnant SLE patient. For example, the heart can be a target organ due to inflammation of various cardiac membranes, which causes pericarditis, myocarditis, endocarditis, and heart blockage, the latter resulting from damaged heart valves (22). Moreover, the pregnant SLE woman with lung and pleural inflammations can also manifest conditions of pleuritis, pleural effusions, pneumonitis, emboli, interstitial lung disease, pulmonary hypertension, and vascular lung hemorrhages (23). Immune complexes of SLE can further deposit within the central nervous system tissues and capillaries causing inflammation and destruction (24). Brain tissue destruction can result in delirium, mood swings, severe headaches, psychosis, depression, vascular damage, seizures, and cognitive dysfunction (25). Thus, pregnant SLE patients are normally monitored for elevated blood pressure, edema, and proteinuria as well as for decreased creatine breakdown and urine output. However, even though there exists a wide range of symptoms of varying severity in SLE, it is believed that pregnancy itself does not affect the long term prognosis of SLE in women who have displayed even severe disease manifestations.

B) Assay Kit Performance:

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in bar-graph format (Figs. 7-10). As shown in Figs. 7A-7D, AFP and AFPAFP mass measurements in serum among the individual kits mostly agreed. In contrast, when the kit specific uE3 MOMs and mass values were compared, MOM values from Siemens DPC Immulite 2000/2500 ranged 10% lower than those from Beckman (Fig. 8A and 8B), however, preliminary studies in our lab suggest this may derive from a matrix effect in our samples. 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. Finally, the method comparison for Inhibin-A displayed in Fig. 9A shows that the results from the Beckman Access/2 and UNICEL were similar, which is also reflected in the Inhibin MOM values (Fig. 9B).

Interestingly, when the AFP mass measurements in amniotic fluid were compared, there were small but noticeable differences among the various methods (Fig. 7C), while AFAFP MOM values (Fig.7D) were mostly the same except for a somewhat higher Siemens values. Since these specimens are derived from actual AF samples, these differences would be comparable to those seen in real patient testing**.**

C) Second Trimester Screening Software Utilized:

The alpha and Benetech PRA software packages were each used by 33.3% and 25.9%, of the labs, respectively; Robert Maciel (RMA) software was employed by 25.9%; and in-house and "other" softwares comprised 14.8%. 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. All laboratories that are **validation-approved** and presently perform first trimester Down syndrome screening are **REQUIRED** to test and report screen results. **Started with the mail-out in May 2013, first trimester results are graded.** Those laboratories not presently offering the test, nor planning to implement the test, can request that no further samples be sent to them. The FT sample (FT = first trimester) information provided to participating labs included maternal age, nuchal translucency (NT) in millimeters, last menstrual period (LMP), crown-rump length (CRL) in millimeters, race, maternal body weight, and date of blood draw.

Measurement of total hCG in the **FT306** specimen obtained from a woman in her 11th week of gestation resulted in an all lab mass mean of 82.8 $\pm$ 13.0 IU/ml, with a MOM of 0.89 $\pm$ 0.08; the all-lab mass mean for PAPP-A was 1323.8 $\pm$ 1061.1 ng/ml with a MOM of 1.88 $\pm$ 1.09, which together resulted in T21 negative screen assessment of 1 in 12,400 (Fig. 13). Further action was not indicated. Similarly, 100 % of labs considered the **FT306** specimen also screen negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Fig. 14).

For the **FT307** specimen from a 23 year old Hispanic woman, the all-lab mean gestational age was reported as 11.5 weeks. Analyte measurements for **FT307** resulted in an all-lab total hCG mass value of 81.1 $\pm$ 11.0 IU/ml (MOM = 1.01 $\pm$ 0.09), while the all-lab PAPP-A mass assessment was 1490.2 $\pm$ 1136.9 ng/ml (MOM = 2.14 $\pm$ 1.23). All labs agreed that the **FT307** sample was screen negative for T21 with a risk of 1 in 16,200 (Fig. 13). A negative screen was also achieved for T18 with a risk assessment of 1 in 10,000 (Fig. 14).

The **FT308** specimen was obtained from a 25 year old White woman with a gestational age of 12.0 weeks. Assay measurements resulted in an all-lab total hCG mass value of 154.4 $\pm$ 26.0 IU/ml (MOM = 2.00 $\pm$ 0.22); the all-lab PAPP-A mass value was 875.5 ± 701.1 ng/ml (MOM = 1.02 $\pm$ 0.56), which together resulted in an all-lab T21 positive screen consensus for **FT308** with a risk of 1 in 49 (Fig. 13). Further action was reported as: genetic counseling, 93%; ultrasound, 40%; amniocentesis, 67%; chorionic villi sampling, 53%. In contrast, the **FT308** specimen screened negative for T18 (1 in 6,400, Fig. 14).

Specimen **FT309** was obtained from a 30 year old (150 lbs) Black woman at 12.5 weeks gestation. The total hCG measurement resulted in a mass mean of 70.5 IU/ml $\pm$ 9.4, with a MOM of 0.98 $\pm$ 0.10, and an all-lab mass mean for PAPP-A was of 2005.4 $\pm$ 1603.2 ng/ml with a MOM of 1.83 $\pm$ 1.19. This resulted in an all-lab T21 negative risk assessment of 1 in 11,600 (Fig. 13), and a T18 negative risk assessment of 1 in 10,000 (Fig. 14).

For the **FT310** specimen from a 21 year old (135 lbs) White woman, the all-lab mean gestational age was reported as 13.0 weeks. Analyte measurements resulted in an all-lab total hCG concentration of 65.2 $\pm$ 8.4 IU/ml (MOM = 0.96 $\pm$ 0.11), and an all-lab PAPP-A concentration of 1530.8 $\pm$ 1287.4 ng/ml (MOM = 1.19 $\pm$ 0.79). The all-lab FT T21 risk assessment was 1 in 10,000 and all labs agreed that the **FT310** sample was negative for T21 (Fig. 13). Similarly, the **FT310** specimen was also screen negative for T18 with an all-lab risk assessment of 1 in 10,000 (Fig. 14).

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

In order to compare the Beckman UNICEL assays (53% users) for PAPP-A with those of the older Siemens Immulite and DSL assay platforms, a conversion factor was calculated from participating labs using data from the last seven PT mailouts (Note: this conversion factor may not be applicable to real patient samples because of potential matrix effects in the PT samples). Hence, Beckman UNICEL (y-axis) data for PAPP-A in MOM were plotted versus Siemens Immulite 2000 (x-axis) data in MOM yielding a linear correlation with an R^2 value of 0.9719, a slope of 0.4141 and a Y intercept of -0.0669 (Fig. 15). Using the respective correlation equation allowed us to convert mIU/ml values into ng/ml and to directly compare Beckman UNICEL PAPP-A mass units of ng/ml to the mIU/mL mass units generated by Siemens Immulite and DSL (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 ~15% higher than those by Beckman UNICEL, while the Siemens Immulite DPC instruments measured approximately 25% below the Beckman Access 2/UNICEL instruments. Overall, the hCG MoM values reflected the mass values but the differences were somewhat diminished (Fig. 11B). The results from the three PAPP-A kits, even when converted to the same mass units, were not consistent among one other (Fig. 12A)

with Siemens Immulite nearly 4 times greater than the other two kits. The Beckman UNICEL PAPP-A was comparable to the others, while Anshlite was 20% lower than Beckman UNICEL. Thus, when the PAPP-A kit MOMs were compared, Siemens Immulite 2000 were 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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2.) AFP-Peptide Epitope Characterization

The 9 to 10 amino acid (AA) peptides that constitute the immunodominant and the subdominant AFP peptide epitopes of the HLA-A and HLA/DRs and their positions are displayed in Tables 2 and 3. A detailed analysis of the composition, traits, and characterization of the constituent AAs of the AFP epitope was reported by Meng and associates using HLA-A2.1 MHC class I molecules on T cells pulsed with AFP peptides [30]. All peptides showed varying degrees of potency in the induction/activation of an immune response in T-cells (Table-4). These researchers had previously demonstrated that the binding of AFP peptides to the HLA-A2.1 complex exhibited slow on-rate and off-rate kinetics [6] which resulted in a more prolonged antigen presentation to APCs. The HLA-A2.1 molecules preferentially bound peptide epitopes containing Ile(I), Leu(L), or Met(M) at AA position #2 (P2) and Val(V)and L at position #9 (P9). The P2 and P9 positions on the AA sequence were found to be the main anchor positions for complexing to the MHC molecules (class Ia). The substitution, replacement, or mutation at the AFP P9 location followed by binding analysis revealed that L, V, and Ala(A) substitutions were favored over Gln(Q) at P9. At the P3 location, an A residue (neutral for binding) was mutated to either V or L while the P9 contained the cannonical L residue. With the double combination of V and L at the P3 and P9 positions, respectively, AFP-peptides bound to the MHC complex at higher levels than parental peptides.

In priming of the immune T-cell response, replacements at AFP P9 followed by a binding analysis showed that L, V, and A substitutions were favored over Q at P9 (Table-4). Although a V or L at P3 in combination with L at P9 revealed enhanced binding characteristics and the induction of IFNgamma-producing cells, the magnitude of immune response induction (priming) was not improved from the parent peptide. Thus, the P3 location is not a critical site for priming. The effect on immune response priming was then analyzed at locations P1, P4, P5, P6, P7, and P8. Although substitutions of A from P4 to P8 did not alter peptide binding, replacement at P7 and P8 did lower immune priming; however, L-to-Gly(G) and L-to-A at P4 both showed that L was critical for priming. Thus, L at P4 and P9 were both essential for priming (Table-4).

Concerning T-cell recognition and re-stimulation, the anchor positions of P2 and P9 were again sensitive to mutation. Five of six peptide variations which were mutated at P2, P9, or P3/P9 showed improved binding to the HLA molecule, but only the Q-to-L replacement resulted in optimal AFP-specific immune recognition and/or stimulation (Table-4). A substitution of A at P9 had an adverse effect on both immune priming and recognition. Four other substituted peptides (V-to-A at P2; Q-to-V at P9; Val-to-L at P9; and L P3 with L P9) had no effect on AFP-specific immune stimulation. In further T-cell stimulation studies, all analogs at P2, P4, P5 and P8 were recognized to at least some degree by the AFP-primed splenocytes. Recognition by T-cells with A substitutions at P1, P6, P7, P8 had little or no effects on the induction of IFN gamma-producing cells. At both P4 and P5, A substitutions were slightly lowered as were the double substitution of A at P4 and P7 and A at P4 and P9. Finally, G substitution at P4 resulted in low levels of IFN gamma-producing cells (Table-4).

The characteristics and traits of the 9-10 amino acid of the AFP peptide epitopes are displayed in Tables-3 and 4, while the sequence identity and similarity homologies are displayed in Table-2. It can be observed that all P2 and P9 sites displayed hydrophobic, non-polar amino acids. The P9 position favored L, V, I, and Phe(F), while the P2 position showed L, V, M, F, and Tyr(Y). Thus, the type of amino acid at P2 and P9 was largely of the hydrophobic, non-polar type in the immunodominant epitopes (Table-3). In comparison, the AAs at P2 n the sub-dominant sites were mostly polar uncharged and aromatic types. Positions P3 through P7 consisted of more variable types of AAs than did AAs at P2 and P9. This was mostly true at position P1 and P8 although the polar uncharged AA type was favored at P8, while P1 displayed a non-polar majority (Table-3). Thus, the type of amino acid residues and their hydrophobicity/hydrophilicity traits appeared to display common patterns in the AFP-derived peptide epitopes.

Peptide-MHC binding to the TCR binding groove has been studied on x-ray crystal structures using the HLA-A2/ TCR molecular complex [28]. The peptide-binding groove formed by the MHC alpha1/alpha2 domains is structured to bind peptides 8–10 AAs long in an extended confirmation (Fig-3). Eight Beta strands establish the platform floor of the groove, with two anti-parallel alpha-helices serving as its walls [31] (Fig-4). The crystallographic data showed that bulky side-chain of L at P9 bound to the MHC complex interacting with residues in the binding portion of the TCR groove; interestingly, the AAs at the peptide N-terminus (P2) did the same. These areas could interact with the hydroxyl group of a TCR groove-positioned tyrosine that formed a hydrogen bond linkage with the MHC/peptide complex. These and other studies showed that the L bulky side-chain at P4 was oriented upward toward the TCR surface that could interface with the MHC/peptide cluster [17]. If a Q were

substituted at P9 (in place of L), the Q side-chain pointed away from the TCR-binding groove, which proved unfavorable. The residues in the HLA-A2 molecular complex (L-81, Y-116, and Y-123) accommodates the L-P9 binding which provided a bulky side-chain for hydrophobic interaction. As compared to L, Q at P9 resulted in poor binding characteristics due to unfavorable association kinetics. As a trade-off, however, a Q at P9 was able to induce higher levels of T cell priming and recognition. A L bulky side-chain would also point upward at the P4 location suggesting that L-P4 also contributes to the MHC-to-TCR contact points (Fig-4); thus, an A replacement at P4 was found to result in the loss of both priming and recognition effects. However, a substitution at both P4 and P9 was recognized to some degree by the primed AFP-specific cells, suggesting less stringent requirements for activation in primed compared to naïve T-cells. These results suggested that AFP peptide epitopes with weak MHCs might enhance immunogenicity in a clinical setting where primed T-cells might cross-recognize peptide epitopes and lyse AFP-expressing tumor cells presenting the same epitope [30]. In fact, some AFP subdominant epitopes showed similar or higher avidity for T-cell binding and activation; these could be detected and expanded in HCC patients [26].

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

(1) Source: J Med Screen. 2013 Jun;20(2):57-65

Title: Detection of trisomy 18 and trisomy 13 using first and second trimester Down's syndrome screening markers

Authors: Bestwick JP, Huttly WJ, Wald NJ

Abstract: OBJECTIVE: To estimate the detection rates (DRs) and false-positive rates (FPRs) in the incidental identification of trisomy 18 (T18) and trisomy 13 (T13) as part of antenatal screening for Down's syndrome (DS) using the Combined, Quadruple and Integrated test markers. METHODS: Screening marker levels on 224 T18 and 67 T13 pregnancies screened for DS were evaluated. Estimated means, standard deviations and correlation coefficients were used with published estimates for unaffected pregnancies to derive detection algorithms for the two disorders. DRs and FPRs of the algorithms were estimated using Monte Carlo simulation. RESULTS: In T18 and T13 pregnancies first trimester nuchal translucency was raised, free β-human chorionic gonadotrophin (hCG) and pregnancy associated plasma protein-A reduced. In T18 pregnancies second trimester alphafetoprotein, unconjugated oestriol and free β-hCG were reduced. In T13 pregnancies second trimester inhibin-A was raised. These markers specified T18 and T13 algorithms. The DS Combined test algorithm detected 42% of T18 and 59% of T13 (2.00% FPR); 88% and 74% by adding the T18 Combined test algorithm (2.17% FPR) and 89% and 75% by further adding the T13 Combined test algorithm (2.19% FPR). The corresponding detection rates for the Quadruple test were: 2% and 17% (2.00% FPR), 55% and 17% (2.16% FPR) and 55% and 19% (2.28% FPR), and for the Integrated test were: 40% and 64% (2.00% FPR), 92% and 65% (2.12% FPR) and 92% and 72% (2.18% FPR). CONCLUSIONS: Antenatal screening for DS detects about 40% of T18 and about 60% of T13 pregnancies. The addition of a T18 algorithm substantially increases the detection of both trisomies with a small increase in the FPR. The further addition of a T13 algorithm results in a small increase in the detection of T13.

(2) Source: Prenat Diagn. 2013 Nov 13. [Epub ahead of print]

Title: First trimester maternal serum alpha-fetoprotein is not raised in pregnancies with open spina bifida

Authors: Spencer K, Khalil A, Brown L, Mills I, Horne H

Abstract: Two recent studies have suggested that maternal serum AFP levels are increased in the first trimester of pregnancies in which the fetus has an open spina bifida. This is contrary to previously published studies. This study assesses further whether maternal serum AFP is elevated in the first trimester in cases with open spina bifida. METHODS: Cases with open spina bifida were identified from our fetal database and corresponding first trimester screening samples were retrieved and analysed for maternal serum AFP. A control group was selected by taking 3 samples matched for gestational age (exact day), ethnicity and smoking status and received in the laboratory on the same day. AFP was measured with the Kryptor platform and Free β-hCG and PAPP-A results were available from the fetal database. RESULTS: 39 Open spina bifida cases were identified with a control group of 126 cases. The median MoM AFP in the cases were not significantly different from the controls (0.92 v 1.06 p = 0.3511) as was the case for Free β-hCG (0.87 v 0.95 p = 0.7146) and PAPP-A (1.04 v 1.04 p = 0.261). CONCLUSION: Our results confirm that maternal serum biochemical markers in the first trimester are unable to distinguish cases in which the fetus has open spina bifida.

(3) Source: Orv Hetil. 2013 Jun 30;154(26):1026-30

Title: [Screening of trisomy 21 nowadays. Is maternal age so important?]

Authors: Szabó A, Alasztics B, Bánhidy F, Valent S.

Abstract: INTRODUCTION: Trisomy 21 is the most common chromosomal abnormality, therefore, screening and diagnosis of this disorder is in the centre of attention worldwide. An efficient screening method is the combined test based on maternal age, ultrasound signs, biochemical markers, and a risk ratio can be calculated based on these data. AIM: The aim of the authors was to determine the causes of missed prenatal diagnosis of Down's syndrome at the 2nd Department of Obstetrics and Gynecology, Semmelweis University. METHOD: A retrospective study was carried out by collecting data from medical records of mothers who had delivered a newborn with Down's syndrome in the Department between 2008 and 2012. Each medical record was analyzed individually. RESULTS: In most cases the missed diagnosis of Down's syndrome occurred when the expectant mother failed to attend the first trimester screening or did not take the risk of invasive diagnostic procedures needed for fetal karyotyping. CONCLUSIONS: Analysis of fetal DNA circulating in maternal plasma can be a solution for those who refuse invasive fetal diagnostics. This test has high sensitivity and very low false positive rate. It has become available since the end of 2011 in the United States and, since the autumn of 2012, in Hungary, too. The test, however, is not reimbursed by national health insurance.

(4) Source: Ultrasound Obstet Gynecol. 2013 Oct;42(4):409-15

Title: Biparietal diameter at 11-13 weeks' gestation in fetuses with open spina bifida

Authors: Khalil A, Coates A, Papageorghiou A, Bhide A, Thilaganathan B

Abstract: OBJECTIVE: To ascertain the reported association between reduced biparietal diameter (BPD) at 11-13 weeks' gestation and open spina bifida and to investigate its predictive value in a single-center study. METHODS: This was a retrospective study of fetuses in which BPD was measured at 11-13 weeks' gestation, including 27 fetuses with isolated open spina bifida subsequently diagnosed at 16-24 weeks and 7775 unaffected controls. BPD values were converted into multiples of the expected median (MoM) after adjustment for crown-rump length and maternal

characteristics. Multivariable logistic regression analysis was used to determine the maternal characteristics significantly associated with spina bifida. The performance of screening was determined by receiver-operating characteristics curve analysis. BPD values at 11-13 weeks' gestation were compared with those measured in the second trimester using Z-scores. RESULTS: BPD values at 11-13 weeks' gestation were below the 5(th) centile in 44.4% of cases of open spina bifida. In these fetuses, the median BPD MoM value was significantly smaller than that in the control group (0.930 vs 0.998 MoM; P < 0.0001). Multivariable logistic regression analysis showed a significant contribution from maternal age (P = 0.008) and BMI (P = 0.028) to the association between BPD MoM and spina bifida. The detection rate using BPD measurements in the first trimester was 55.6% with a false-positive rate of 11.6%. In fetuses with open spina bifida, the BPD Z-scores were significantly lower at 16-24 weeks compared to those recorded at 11-13 weeks (median, -1.71 (range, -3.98 to -0.20) vs -1.30 (-3.75 to 2.61); P = 0.006). CONCLUSION: Fetuses with open spina bifida have a smaller BPD in the first trimester. This observation may be useful in early screening. It is likely that a combination of maternal characteristics such as age and BMI, fetal BPD and maternal serum alpha-fetoprotein measured in the first trimester would provide a clinically useful screening test for open spina bifida.

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

(1) Source: Clin Nucl Med. 2013 Oct 22. [Epub ahead of print]

Title: Carcinosarcoma of the uterine corpus on 18F-FDG PET/CT in a postmenopausal woman with elevated AFP

Authors: Chen F, Yu C, You X, Mi B, Wan W

Abstract: Uterine carcinosarcoma (termed malignant mixed müllerian tumor) is a rare neoplasm of the uterus with a poor prognosis. There have been very few cases in the literature describing the PET/CT findings of uterine carcinosarcoma. We report a case of tissue-proven carcinosarcoma of the uterine corpus in a 65-year-old woman with elevated serum alpha-fetoprotein (AFP), whose F-FDG PET/CT showed a 10.3-cm mass in the uterus with uneven high FDG uptake. The SUVmax was 12.8. After surgery, the patient received 6 courses of chemotherapy, and the serum levels of AFP decreased to reference range.

(2) Source: Pediatr Surg Int. 2013 Jul;29(7):709-13

Title: Diagnostic accuracy of preoperative alpha-fetoprotein as an ovarian tumor marker in children and adolescents: not as good as we thought?

Authors: Loh AH, Gee KW, Chua JH

Abstract: PURPOSE: To evaluate the diagnostic accuracy of preoperative serum alpha-fetoprotein (AFP) levels in predicting malignancy risk in children and adolescents presenting with ovarian neoplasms. METHODS: In 110 girls aged 18 and below diagnosed with ovarian neoplasms, we retrospectively correlated preoperative serum AFP levels with histological diagnosis of germ cell tumor or immature teratoma (GCT/IT) versus non-GCT/IT, and benign versus non-benign. We determined area under receiver-operating characteristic curves (AUC), sensitivity, specificity, and likelihood ratios. RESULTS: Twenty patients (18.2 %) had non-benign ovarian neoplasms, of which 12 had GCT/IT (10.9 %). In diagnosing GCT/IT versus non-GCT/IT, specificity of preoperative serum AFP was 87.8 %, sensitivity 66.7 %, and AUC 0.853. Excluding infants to remove the effects of increased variance in AFP in this group, specificity improved (92.0 %), but not sensitivity (66.7 %); AUC was 0.926. Increasing AFP cutoff to two times upper normal limit improved specificity (94.9 %), but not sensitivity (66.7 %). For benign versus non-benign tumors,

AFP specificity was only 88.9 % and sensitivity 50.0 %. CONCLUSION: The diagnostic accuracy of preoperative serum AFP for detecting GCT/IT in girls was limited by poor sensitivity and positive predictive value. Excluding infants and raising cutoff levels improved specificity marginally. Clinicians should be aware of these limitations when using AFP in the preoperative evaluation of childhood ovarian neoplasms.

(3) Source: Hinyokika Kiyo. 2013 Jun;59(6):373-6

Title: [A case of alpha-fetoprotein-producing female urethral adenocarcinoma]. [Article in Japanese]

Authors: Mimura Y, Mizusawa H, Saito T, Hirabayashi N

Abstract: We report a rare case of alpha-fetoprotein (AFP)-producing female urethral adenocarcinoma. A 52- year-old woman had urinary frequency. Ultrasonography showed a mass near the bladder. Therefore, she was referred to our hospital. Magnetic resonance imaging showed an approximately 4 cm mass at the urethra. Computed tomography did not show any lymphnode metastasis or distant metastasis. High serum levels of AFP were revealed. Carcinoembryonic antigen (CEA) and prostate specific antigen (PSA) were within the normal range. A transvaginal needle biopsy suggested adenocarcinoma. Radical cystourethrectomy and ileal conduit formation were performed. Histopathological diagnosis was adenocarcinoma. Immunohistochemical staining was positive for AFP and CEA, and negative for PSA. Serum AFP normalized immediately postoperatively. Adjuvant chemotherapy or radiotherapy was not performed. Eleven years postoperatively, the patient showed no evidence of tumor recurrence. To our knowledge, this is the first reported case of AFP producing female urethral adenocarcinoma.

(4) Source: Indian J Pathol Microbiol. 2013 Jan-Mar;56(1):54-6.

Title: Alfa-fetoprotein secreting ovarian sex cord-stromal tumor.

Authors: Jashnani KD, Hegde CV, Munot SP

Abstract: Ovarian sex cord-stromal tumors are relatively infrequent neoplasms that account for approximately 8% of all primary ovarian tumors. They are a heterogeneous group of neoplasms composed of cells derived from gonadal sex cords (granulosa and Sertoli cells), specialized gonadal stroma (theca and Leydig cells), and fibroblasts. They may show androgenic or estrogenic manifestations. We report such a tumor associated with markedly raised serum alpha-fetoprotein (AFP) levels in a young female presenting with a mass and defeminising symptoms. Serum AFP levels returned to normal on removal of tumor.

C) News of Note: Abstracts of New Markers:

(1) Source: Springerplus. 2013 Sep 29;2:490

Title: The importance of rapid aneuploidy screening and prenatal diagnosis in the detection of numerical chromosomal abnormalities

Authors: Elsayed GM, El Assiouty L, El Sobky ES.

Abstract: OBJECTIVES: Evaluation of Fluorescent in situ hybridization (FISH) as a tool for rapid aneuploidy screening (RAS) of high risk pregnancies, before its approval in the national antenatal screening and genetic diagnosis program in Egypt. METHODS: The cytogenetic data of prenatal specimens, and results of FISH of 100 patients performed between, January 2009 and December 2009, at the Medical Genetics Center (MGC) laboratory were retrieved and reviewed. AneuVysion

Assay kit was used for detection of 13, 21, X, Y, 18 aneuploidies. RESULTS: Maternal age varied from 21 to 44 years (mean was 35.6 year). Ninety percent of pregnancies had normal chromosomes and 10% of the cases had numerical chromosomal abnormalities. Trisomy 21 was the most frequent chromosomal disorder across all indications (5%), followed by Turner syndrome (2%), trisomy 18 (2%), and trisomy 13 (1%). When comparing the FISH data with karyotype results for chromosomes 13, 18, 21, X, and Y in the 83 individual tested, no false positive or negative results were detected by the FISH assay. The result obtained by FISH and the banding cytogenetic were in complete accordance. CONCLUSION: This study supports the integration of amniotic fluid (AF) FISH as a RAS test, in to routine antenatal practice for identification of chromosome aneuploidies. There are trends towards delayed childbearing and most cases of Down Syndrome (DS) are currently detected post-nataly in the Egyptian population. Consequently, the live birth prevalence of DS has increased, which might lead to a serious negative public health effects.

(2) Source: Semin Fetal Neonatal Med. 2013 Nov 6. pii: S1744-165X(13)00095-4. [Epub ahead of print]

Title: Non-invasive prenatal testing for Down syndrome

Authors: Twiss P, Hill M, Daley R, Chitty LS

Abstract: Prenatal screening and diagnosis of Down syndrome and other major aneuploidies may be transformed following the identification of cell-free fetal DNA in maternal plasma at the end of the last millennium. Next generation sequencing has enabled the development of tests that accurately predict the presence of fetal trisomies by analysis of cell-free DNA in maternal blood from as early as 10 weeks of gestation. These tests are now widely available in the commercial sector but are yet to be implemented in publicly led health services. In this article we discuss the technical, social, and ethical challenges that these new tests bring.

(3) Source: Fetal Diagn Ther. 2013 Nov 13. [Epub ahead of print]

Title: Patient's choice between a non-invasive prenatal test and invasive prenatal diagnosis based on test accuracy

Authors: Chan YM, Leung TY, Chan OK, Cheng YK, Sahota DS

Abstract: OBJECTIVE: To assess how pregnant women choose between a non-invasive DNA test (NIDT) and an invasive prenatal test (IPD) based on the accuracy of the test. MATERIALS AND METHODS: Pregnant women who attended for first-trimester combined screening assessment of risk of Down syndrome were invited to participate in an interviewer-administered survey. Women were asked to choose between NIDT (variable detection rate but no miscarriage risk) and IPD (~100% detection rate but 0.5-1% miscarriage risk) if their screening test was positive for Down syndrome using the standard gamble technique. RESULTS: 358 women were approached of which 106 (29.6%) were unwilling to participate in the study as it had already been decided in advance which additional test they would have if they were screened positive. Of these 106 women, 70 (19.6%) would only choose IPD whereas 36 (10%) would only choose NIDT. Among those who agreed to undertake the gamble and participate in the study (n = 252), 50% were willing to accept NIDT as an alternative to IPD provided that NIDT had a detection rate of 95%. CONCLUSION: The majority can accept NIDT as an alternative to IPD provided that the test is 95% accurate in the diagnosis of Down syndrome. Current evidence indicates that the detection rate of NIDT will be higher than this level. Health professionals should consider NIDT as an alternative to IPD when counseling women with a positive screening test.

(4) Source: Fetal Diagn Ther. 2013 Oct 26. [Epub ahead of print]

Title: First-trimester contingent screening for trisomies 21, 18 and 13 by biomarkers and maternal blood cell-free DNA testing

Authors: Nicolaides KH, Syngelaki A, Poon LC, Gil MM, Wright D

Abstract: OBJECTIVE: To examine potential performance of screening for trisomies by cell-free (cf) DNA testing in maternal blood contingent on results of first-line testing by combinations of fetal translucency thickness (NT), fetal heart rate (FHR), ductus venosus pulsatility index (DV PIV), and serum-free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A), placental growth factor (PLGF) and α-fetoprotein (AFP). METHODS: Performance was estimated for firstly, screening by cfDNA in all pregnancies and secondly, cfDNA testing contingent on results of first-line testing by combinations of ultrasound and biochemical markers. RESULTS: In first-line screening by cfDNA testing, the detection rate for trisomy 21 and trisomies 18 or 13 would be 99 and 96%, respectively, after invasive testing in 1% of the population. In contingent screening, a detection rate of 98% for trisomy 21 and 96% for trisomy 18 or 13, at an invasive testing rate of 0.7%, can be achieved by carrying out cfDNA testing in about 35, 20 and 11% of cases identified by first-line screening with the combined test alone (age, NT, FHR, β-hCG, PAPP-A), the combined test plus PLGF and AFP and the combined test plus PLGF, AFP and DV PIV, respectively. CONCLUSIONS: Effective first-trimester screening for trisomies can be achieved by contingent screening incorporating biomarkers and cfDNA testing.

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

(1) Source: J Perinatol. 2013 Oct;33(10):754-8

Title: Abnormal analyte preeclampsia: do the second-trimester maternal serum analytes help differentiate preeclampsia subtypes?

Authors: Critchfield AS, Paulus JK, Farez R, Urato AC

Abstract: OBJECTIVE: To determine if serum screen analytes identify preeclamptic patients at risk for small-for-gestational age newborns, maternal laboratory abnormalities and preterm delivery (<37 weeks gestation). STUDY DESIGN: Using a retrospective cohort of 102 preeclamptic patients, associations between serum screen analytes and newborn birth-weight percentile, gestational age (GA) at delivery and maternal pre-delivery laboratory abnormalities were evaluated using correlation coefficients and local polynomial regression. RESULT: Inhibin-A and maternal serum alpha fetoprotein were inversely correlated with newborn birth-weight percentile (-0.27, P=0.006; -0.35, P=0.00004) and delivery GA (r=-0.42, P<0.0001; r=-0.26, P=0.008) and positively correlated with pre-delivery aspartate aminotransferase (r=0.22, P=0.03; r=0.21, P=0.04) and lactate dehydrogenase (r=0.33, P=0.0007; r=0.29, P=0.004). A positive correlation was noted between both second-trimester beta human chorionic gonadotropin and estriol and maternal pre-delivery creatinine (0.28, P=0.004; 0.4, P<0.0001, respectively). Hundred percent of patients with ≥ 2 abnormal analytes delivered before 37 weeks gestation. CONCLUSION: Preeclamptic patients with abnormal serum screen analytes are more likely to have small-for-gestational age newborns, deliver preterm and have pre-delivery laboratory abnormalities.

(2) Source: Early Hum Dev. 2013 Sep;89(9):777-80

Title: Evaluation of the impact of maternal smoking on ultrasound and endocrinological markers of first trimester placentation

Authors: Jauniaux E, Suri S, Muttukrishna S

Abstract: OBJECTIVES: To study the effect of maternal smoking on 2D ultrasound measurements and maternal serum (MS) levels of endocrinologic markers of placentation. STUDY DESIGN: Prospective population-based cohort study of 32 smokers and 96 non-smoking controls with a normal pregnancy outcome. MAIN OUTCOME MEASURES: Placental thickness and 2D-volume and MS levels of pregnancy-associated plasma protein A (PAPP-A) and free-beta human chorionic gonadotrophin (fβhCG) at 11-13(+6)weeks of gestation and mid-trimester MS α-fetoprotein (AFP), unconjugated estriol (uE3) and inhibin A levels. RESULTS: The MS levels of fβhCG and PAPP-A were significantly ($P < 0.01$ and $P < 0.001$, respectively) lower in the serum and the level of inhibin A significantly ($P < 0.001$) higher in the smokers than in controls. There was no significant difference for the MSAFP, MSuE3 placental thickness, basal plate surface and volume between the groups. CONCLUSION: The placental morphological alterations secondary to maternal smoking are mainly at the level of the villous trophoblast and are not associated with changes in the placental size or utero-placental interface during the first trimester of pregnancy.

(3) Source: Am J Obstet Gynecol. 2013 Sep;209(3):223.e1-5

Title: Combined screening for open spina bifida at 11-13 weeks using fetal biparietal diameter and maternal serum markers

Authors: Bernard JP, Cuckle HS, Bernard MA, Brochet C, Salomon LJ, Ville Y

Abstract: OBJECTIVE: Screening at 11-13 weeks with ultrasound biparietal diameter (BPD) can detect half of open spina bifida cases. Maternal serum α-fetoprotein (AFP) levels at 15-19 weeks are increased 3- to 4-fold, in open spina bifida. We assessed whether combined screening using BPD, AFP, and other serum markers at 11-13 weeks would increase detection. STUDY DESIGN: Maternal AFP levels were measured on serum stored at 11-13 weeks in 44 open spina bifida and 182 unaffected pregnancies, and results were expressed in multiples of the median (MoM) for gestational age. All samples had been measured for free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein (PAPP)-A. A multivariate Gaussian model was used to predict screening performance from the serum data and BPD measurements on 80 cases, including 36 previously published. RESULTS: The median AFP level in cases was 1.201 MoM, significantly higher than in unaffected pregnancies ($P < .01$, 1 tail). The median free β-hCG was significantly reduced to 0.820 MoM ($P < .02$), but the median PAPP-A was similar in cases and controls. Modeling predicted the following: BPD alone would detect 50% of cases for a 5% false-positive rate or 63% for 10%; adding AFP increases detection by 2%; and a combined test with BPD, AFP, and free β-hCG detects 58% for 5% or 70% for 10%. CONCLUSION: Combining AFP and BPD with free β-hCG as part of first-trimester aneuploidy screening would also allow early detection about two-thirds of cases with open spina bifida.

(4) Source: Reprod Sci. 2013 Oct 31. [Epub ahead of print]

Title: First-trimester combined screening is effective for the detection of unbalanced chromosomal translocations at 11 to 12 weeks of gestation

Authors: Huang S, Chang C, Cheng P, Hsiao C, Soong Y, Duan T

Abstract: The first trimester combined screening, which analyzes fetal nuchal translucency and levels of free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal serum, is routinely used to detect abnormal pregnancies associated with Down syndrome and other trisomy aneuploidies. Based on the hypothesis that major chromosomal translocations could lead to similar biochemical and developmental outcomes during early embryo development, we compared these markers among pregnancies with normal, balanced, or unbalanced fetal karyotypes. Among the parents, 71 (73%) carry balanced reciprocal translocation and 26 (27%) have Robertsonian translocation. Of the 97 pregnancies tested, 39 (40%), 37 (37%),

and 22 (23%) fetuses had normal karyotype, balanced chromosomal translocations, and unbalanced chromosomal translocations, respectively. Importantly, we found that pregnancies with an unbalanced translocation had significantly higher free β-hCG multiple of the median (MoM) and larger nuchal translucency thickness than those with normal karyotype or balanced translocations. Analysis showed that the area under a receiver operating characteristic curve (AUC) is 0.716, 0.820, and 0.936 for free β-hCG MoM, PAPP-A MoM, and fetal nuchal translucency, respectively. When these 3 independent factors were combined, the AUC reached 0.976. In addition, logistic regression showed that the most optimal model for predicting an unbalanced chromosomal translocation is a combination of PAPP-A and nuchal translucency with an AUC of 0.980. Therefore, the first trimester combined screening is not only effective in the screening of Down syndrome and other trisomy abnormalities but also has high sensitivity for the detection of unbalanced chromosomal translocations in fetuses.

E) Abstracts of New Assay Methodologies:

(1) Source: Biosens Bioelectron. 2013 Oct 15;53C:324-329

Title: Platinum porous nanoparticles hybrid with metal ions as probes for simultaneous detection of multiplex cancer biomarkers

Authors: Wang Z, Liu N, Ma Z

Abstract: In this work, platinum porous nanoparticles (PtPNPs) absorbed metal ions as electrochemical signals were fabricated. Clean-surface PtPNPs were prepared by a surfactant-free method and decorated with amino groups via 2-aminoethanethiol. Amino capped PtPNPs complexation with Cd^{2+} and Cu^{2+} to form PtPNPs-Cd^{2+} and PtPNPs-Cu^{2+} hybrids, respectively. Anti-CEA and Anti-AFP separately labeled with PtPNPs-Cd^{2+} and PtPNPs-Cu^{2+} were used as distinguishable signal tags for capturing antigens. The metal ions were detected in a single run through differential pulse voltammetry (DPV) without acid dissolution, electric potentials and peak heights of which reflected the identity and concentrations of the corresponding antigen. Ionic liquid reduced graphene oxide (IL-rGO) modified glassy carbon electrode (GCE) was used as a substrate, which was rich in amino groups to immobilize antibodies by glutaraldehyde through cross-link between aldehyde groups and amino groups. Using the proposed probes and platform, a novel sandwich-type electrochemical immunosensor for simultaneous detecting carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) was successfully developed. This immunoassay possessed good linearity from 0.05ngmL^{-1} to 200ngmL^{-1} for both CEA and AFP. The detection limit of CEA was 0.002ngmL^{-1} and that of AFP was 0.05ngmL^{-1} (S/N=3). Furthermore, analysis of clinical serum samples using this immunosensor was well consistent with the data determined by the enzyme-linked immunosorbent assay (ELISA). It suggested that the proposed electrochemical immunoassay provided a potential application of clinical screening for early-stage cancers.

(2) Source: Biosens Bioelectron. 2013 Oct 15;53C:305-309

Title: Label-free immunosensor based on Pd nanoplates for amperometric immunoassay of alpha-fetoprotein

Authors: Wang H, Li H, Zhang Y, Wei Q, Ma H, Wu D, Li Y, Zhang Y, Du B

Abstract: In this paper, Pd nanoplates were used as a kind of electrode materials for fabrication of an electrochemical immunosensor, which was applied for detection of cancer biomarker alpha-fetoprotein (AFP). Thanks to the unique structure and properties of Pd nanoplates, the antibody of AFP (Ab) was effectively immobilized onto the surface of the Pd nanoplates modified glassy carbon electrode (GCE). Moreover, the good electrochemical properties of Pd nanoplates greatly improved the electronic transmission rate and enhanced the electrochemical signal, which led to an increase of the detection sensitivity. Based on the specific antibody-antigen interaction, a label-

free immunosensor based on Pd nanoplates was developed for sensing of AFP. The current method allows us to detect AFP over a wide concentration range from 0.01 to 75.0ng/mL with a detection limit of 4pg/mL. The proposed immunosensor has been used to determine AFP in human serum with satisfactory results.

(3) Source: Biosens Bioelectron. 2013 Sep 25;53C:65-70

Title: Triple signal amplification using gold nanoparticles, bienzyme and platinum nanoparticles functionalized graphene as enhancers for simultaneous multiple electrochemical immunoassay

Authors: Jia X, Chen X, Han J, Ma J, Ma Z

Abstract: Here we demonstrated an ultrasensitive electrochemical immunoassay employing graphene, platinum nanoparticles (PtNPs), glucose oxidase (GOD) and horseradish peroxidase (HRP) as enhancers to simultaneously detect carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP). This immunosensor is based on the observation that multiple-labeled antibodies (thionine-labeled anti-CEA and ferrocene-labeled anti-AFP) recognition event yielded a distinct voltammetric peak through "sandwich" immunoreaction, whose position and size reflected the identity and level of the corresponding antigen. Greatly enhanced sensitivity for cancer markers is based on a triple signal amplification strategy. Experimental results revealed that the immunoassay enabled simultaneous determination of CEA and AFP in a single run with wide working ranges of 0.01-100ngmL^{-1}. The detection limits reached 1.64pgmL^{-1} for CEA and 1.33pgmL^{-1} for AFP. No obvious cross-talk was observed during the experiment. In addition, through the analysis of clinical serum samples, the proposed method received a good correlation with ELISA as a reference. The signal amplification strategy could be easily modified and extended to detect other multiple targets.

(4) Source: Biosens Bioelectron. 2013 Sep 30;53C:160-166

Title: A label-free immunosensor based on graphene nanocomposites for simultaneous multiplexed electrochemical determination of tumor markers.

Authors: Jia X, Liu Z, Liu N, Ma Z

Abstract: Here we prepared a label-free electrochemical immunosensor employing indium tin oxide (ITO) sheets as working electrodes and graphene nanocomposites as supporting matrix for simultaneous determination of carcinoembryonic antigen (CEA) and α-fetoprotein (AFP). Reduced graphene oxide/thionine/gold nanoparticles nanocomposites were synthesized and coated on ITO for the immobilization of anti-CEA while reduced graphene oxide/Prussian Blue/gold nanoparticles were used to immobilize anti-AFP. The immunosensor determination was based on the fact that due to the formation of antibody-antigen immunocomplex, the decreased response currents of thionine and Prussian Blue were directly proportional to the concentrations of corresponding antigens. Experimental results revealed that the multiplexed immunoassay enabled the simultaneous determination of CEA and AFP with linear working ranges of 0.01-300ngmL^{-1}. The limit of detections for CEA is 0.650pgmL^{-1} and for AFP is 0.885pgmL^{-1}. In addition, the methodology was evaluated for the analysis of clinical serum samples and received a good correlation with the enzyme linked immunosorbent assay.

F) Special Abstract Selection:

(1) Source: Fetal Diagn Ther. 2013 Oct 26. [Epub ahead of print]

Title: Validation of correction factors for serum markers for first-trimester Down syndrome screening in singleton pregnancies conceived with assisted reproduction

Authors: Engels MA, Pajkrt E, Groot DT, Schats R, Twisk JW, van Vugt JM

Abstract: OBJECTIVE: To validate previously computed correction factors for free β-human chorionic gonadotrophin (fβ-hCG) and pregnancy-associated plasma protein-A (PAPP-A) in in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) pregnancies with hormone treatment and to determine the effect on false-positive rate (FPR). METHODS: Retrospective study on 249 IVF and 250 ICSI cases and 20,190 controls. Correction factors 1.42 (PAPP-A), 1.17 (fβ-hCG) in IVF; 1.56 (PAPP-A) in ICSI were applied on the absolute serum concentrations. Analysis was done on $\log_{10}$-transformed multiples of medians (MoMs). RESULTS: In the controls, mean PAPP-A and fβ-hCG MoM were 1.004 and 1.062. Before correction, mean PAPP-A MoM was significantly lower in IVF (0.757; $p < 0.001$) and in ICSI (0.671; $p < 0.001$) and after correction comparable (1.071; $p = 0.053$ in IVF; 1.048; $p = 0.178$ in ICSI). Before correction, mean fβ-hCG MoM was comparable (1.054; $p = 0.59$ in IVF and 1.051; $p = 0.56$ in ICSI) and after correction significantly higher in IVF (1.241; $p < 0.001$). After correction the likelihood for receiving a false-positive result was 1.03 in IVF pregnancies (95% CI 0.98-1.09; $p = 0.248$) and 1.02 in ICSI pregnancies (95% CI 0.97-1.07; $p = 0.448$). CONCLUSIONS: After correction the FPR in IVF and ICSI pregnancies with hormone treatment reduces to the observed FPR in the controls.

(2) Source: Eur J Pediatr. 2013 Aug 23. [Epub ahead of print]

Title: Association of trisomy 18 with hepatoblastoma and its implications.

Authors: Tan ZH, Lai A, Chen CK, Chang KT, Tan AM

Abstract: Hepatoblastoma is a highly malignant embryonal liver tumor that occurs almost exclusively in infants and toddlers. Trisomy 18 is the second most common autosomal trisomy after trisomy 21 and is generally considered a lethal disorder. Ten cases of hepatoblastoma in children with trisomy 18 have been published to date. Here, we report on two female patients with trisomy 18 and pretreatment extent of disease (PRETEXT) stage 1 hepatoblastoma, which support the presence of a nonrandom association between hepatoblastoma and trisomy 18. Both patients underwent primary surgical resection without any neoadjuvant or adjuvant chemotherapy. The histologies returned as pure fetal epithelial type, and combined fetal and embryonal epithelial type. There was no evidence of recurrence on serial abdominal ultrasound and serum alpha-fetoprotein levels on follow-up. Conclusion: Primary surgical resection is a treatment approach that can be considered in children with trisomy 18 and PRETEXT stage 1 tumor. However, in view of the overall prognosis for trisomy 18, the decision on the optimal treatment is a delicate one and has to be individualized in the context of the best interests of the child.

(3) Source: Prenat Diagn. 2013 Oct 28. doi: 10.1002/pd.4256. [Epub ahead of print]

Title: Placental methylation markers in normal and trisomy 21 tissues

Authors: Yin YZ, She Q, Zhang J, Zhang PZ, Zhang Y, Lin JW, Ye YC

Abstract: OBJECTIVE: The objective of this study is to combine multiplex ligation-dependent probe amplification (MLPA) and bisulfite sequencing to determine DNA methylation markers for noninvasive prenatal diagnosis of Down syndrome. METHODS: DNA methylation ratios (MR) of four fragments (CGI149, CGI045, HLCS-1, and HLCS-2) on chromosome 21 were evaluated in blood cells from 13 nonpregnant women, 15 euploidies, and 11 Down Syndrome (DS) placentae. Ratios were measured by bisulfite sequencing and methylation-specific (MS)-MLPA. RESULTS: The MS-MLPA and bisulfite sequencing results were concordant. CGI149, CGI045, and HLCS-2

were unmethylated in all nonpregnant blood cells. CGI149, CGI045, HLCS-1, and HLCS-2 were methylated in most of the euploid (13, 11, 15, and 15, respectively) and DS placentae (10, 11, 11, and 11, respectively). The median placental DNA MR in CGI149 was 0.4578 (interquartile range, 0.3568-0.5169) and 0.5918 (interquartile range, 0.5618-0.6659) in euploid and DS placentae, respectively ($p = 0.001$). Using placental MR at 0.5390 as a threshold, we detected DS at 90.9% sensitivity and 93.3% specificity. CONCLUSION: The MS-MLPA is an effective alternative to bisulfite sequencing in assessing placental MR. CGI149 is a potential marker for the noninvasive diagnosis of Down syndrome.

(4) Source: Turk J Pediatr. 2013 Mar-Apr;55(2):152-7

Title: Serum alpha-fetoprotein levels in neonatal cholestasis

Authors: Demir H, Hızal G, Uslu Kızılkan N, Gürakan F, Talim B, Coskun T, Kale G, Yüce A

Abstract: Alpha-fetoprotein (AFP) is used as a tumor marker for hepatocellular carcinoma, hepatoblastoma and germ cell tumors. It may also be elevated in infants with some hepatobiliary disorders. The mechanism of AFP elevation in neonatal cholestasis is not known. We retrospectively evaluated serum AFP levels in 53 infants with neonatal cholestasis. Thirty patients (56.6%) had elevated AFP, and the ratio of patients with elevated AFP was significantly high in both the metabolic diseases and idiopathic neonatal hepatitis groups ($p=0.021$). Serum aspartate aminotransferase (AST) levels increased significantly in patients with elevated AFP ($p=0.004$). Steatosis was the distinctive histopathological finding of the patients with high AFP. The patients with steatosis had significantly higher standard deviation (SD) score of AFP than the patients without steatosis ($p=0.001$). We have shown AFP elevation in neonatal cholestasis due to metabolic disorders and idiopathic neonatal hepatitis and its association with steatosis and AST elevation.

G) Alpha-fetoprotein Specific Abstracts:

(1) Source: Cancers (Basel). 2011 Jun 20;3(2):2709-33

Title: Mechanism of cancer growth suppression of alpha-fetoprotein derived growth inhibitory peptides (GIP): comparison of GIP-34 versus GIP-8 (AFPep). Updates and prospects

Authors: Mizejewski GJ

Abstract: The Alpha-fetoprotein (AFP) derived Growth Inhibitory Peptide (GIP) is a 34-amino acid segment of the full-length human AFP molecule that inhibits tumor growth and metastasis. The GIP-34 and its carboxy-terminal 8-mer segment, termed GIP-8, were found to be effective as anti-cancer therapeutic peptides against nine different human cancer types. Following the uptake of GIP-34 and GIP-8 into the cell cytoplasm, each follows slightly different signal transduction cascades en route to inhibitory pathways of tumor cell growth and proliferation. The parallel mechanisms of action of GIP-34 versus GIP-8 are demonstrated to involve interference of signaling transduction cascades that ultimately result in: (1) cell cycle S-phase/G2-phase arrest; (2) prevention of cyclin inhibitor degradation; (3) protection of p53 from inactivation by phosphorylation; and (4) blockage of K+ ion channels opened by estradiol and epidermal growth factor (EGF). The overall mechanisms of action of both peptides are discussed in light of their differing modes of cell attachment and uptake fortified by RNA microarray analysis and electrophysiologic measurements of cell membrane conductance and resistance. As a chemotherapeutic adjunct, the GIPs could potentially aid in alleviating the negative side effects of: (1) tamoxifen resistance, uterine hyperplasia/cancer, and blood clotting; (2) Herceptin antibody resistance and cardiac (arrest) arrhythmias; and (3) doxorubicin's bystander cell toxicity.

(2) Source: Eur J Paediatr Neurol. 2013 Sep 29. pii: S1090-3798(13)00140-2

Title: Alpha-fetoprotein, a fascinating protein and biomarker in neurology

Authors: Schieving JH, de Vries M, van Vugt JM, Weemaes C, van Deuren M, Nicolai J, Wevers RA, Willemsen MA

Abstract: Alpha-fetoprotein (AFP) is present in fetal serum in concentrations up to 5,000,000 μg/l. After birth, AFP gene expression is turned down with a subsequent fall of the serum concentrations of this albumin-like protein to 'adult values' of circa 0.5-15 μg/l from the age of 2 years onwards. Irrespective of its assumed important functions, individuals with AFP deficiency appear fully healthy. The other way around, the presence of AFP in the circulation after the first years of life doesn't seem to harm, since individuals with 'hereditary persistence of AFP' are also without clinical abnormalities. During pregnancy, AFP (in maternal serum) has long been recognized as a marker for congenital anomalies of the fetus. Equally well known is AFP as biomarker for hepatocellular carcinoma and some other malignancies. There are at least four neurodegenerative disorders, all inherited as autosomal recessive traits and characterized by the presence of cerebellar ataxia, abnormal ocular movements, and neuropathy, for which an elevated concentration of serum AFP is an important diagnostic biomarker. The availability of a reliable biomarker is not only important during screening or diagnostic processes, but is also relevant for objective follow-up during (future) therapeutic interventions.

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

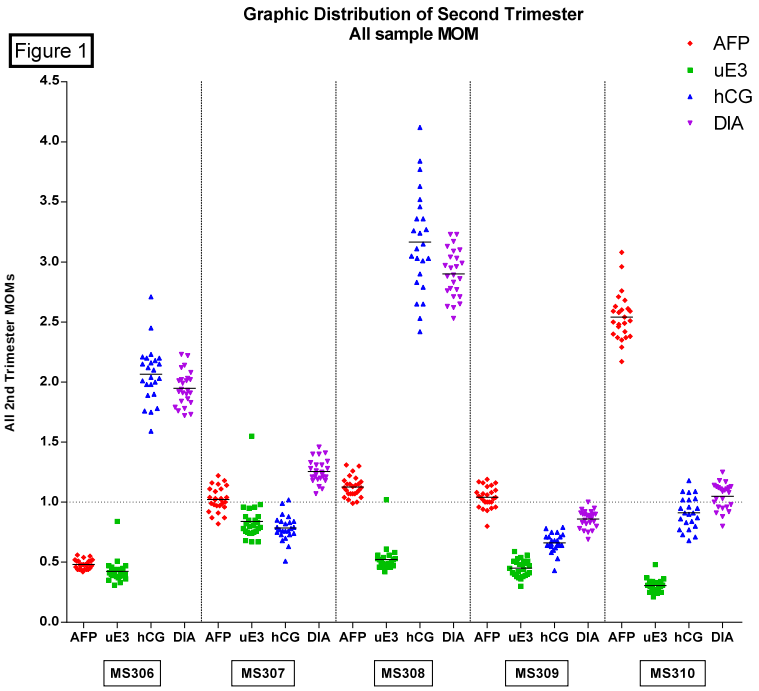


Figure 2a

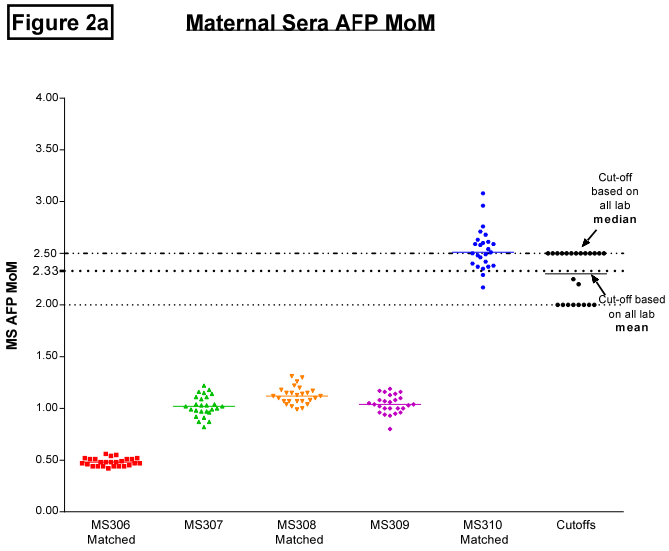


Figure 2b

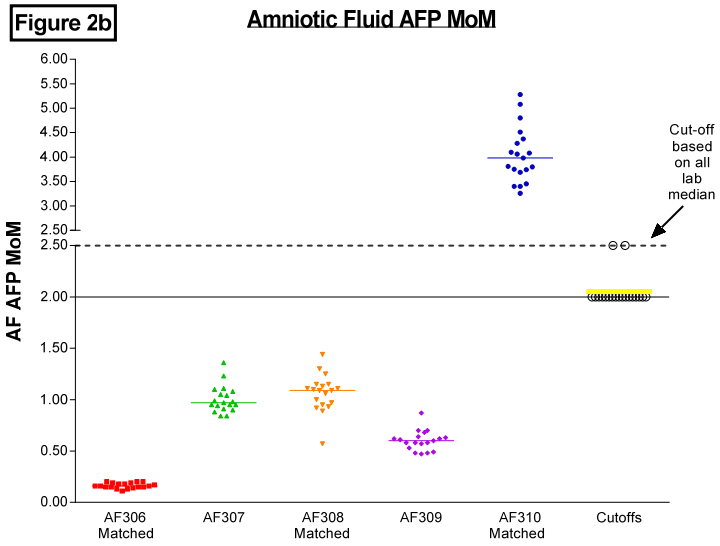


Figure 3

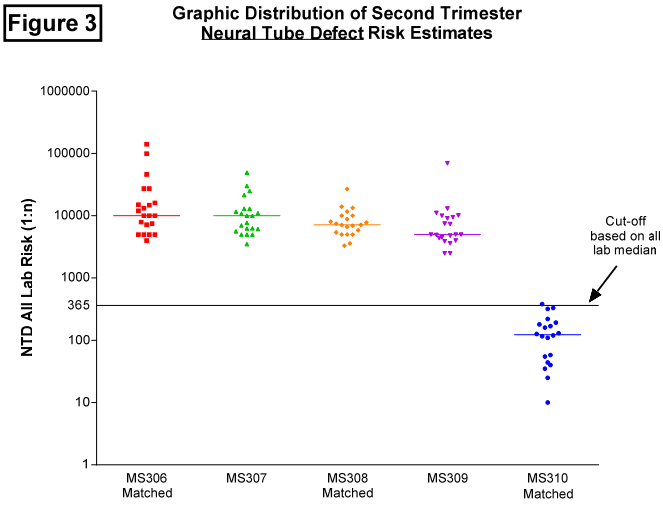


Figure 4

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

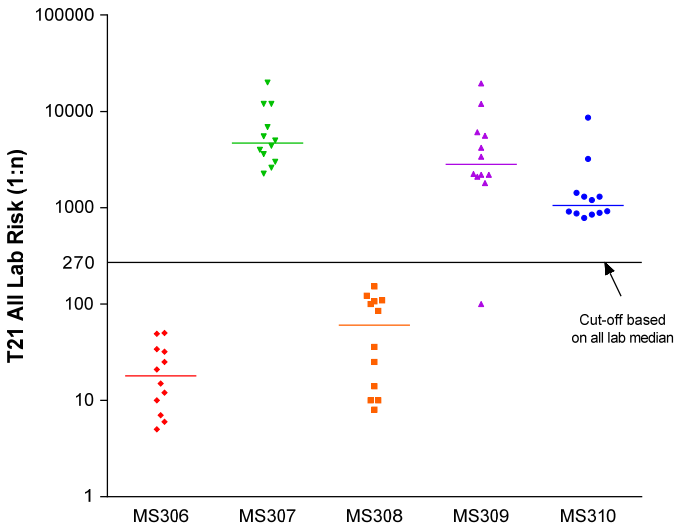


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

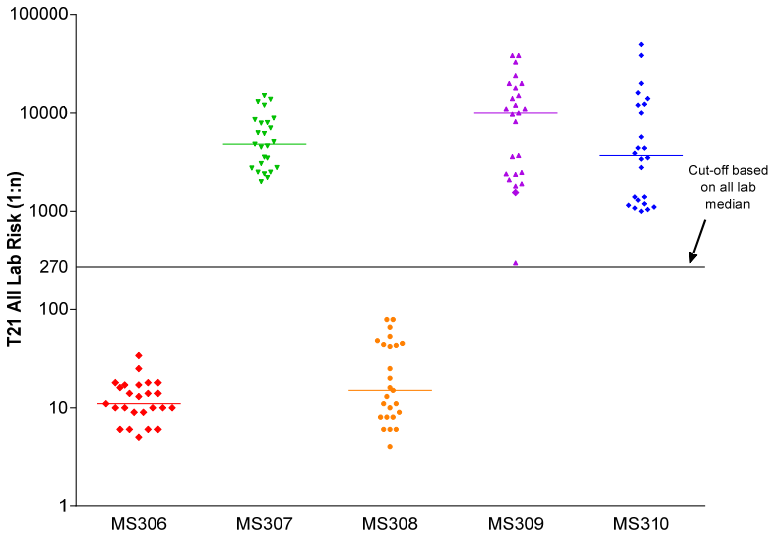
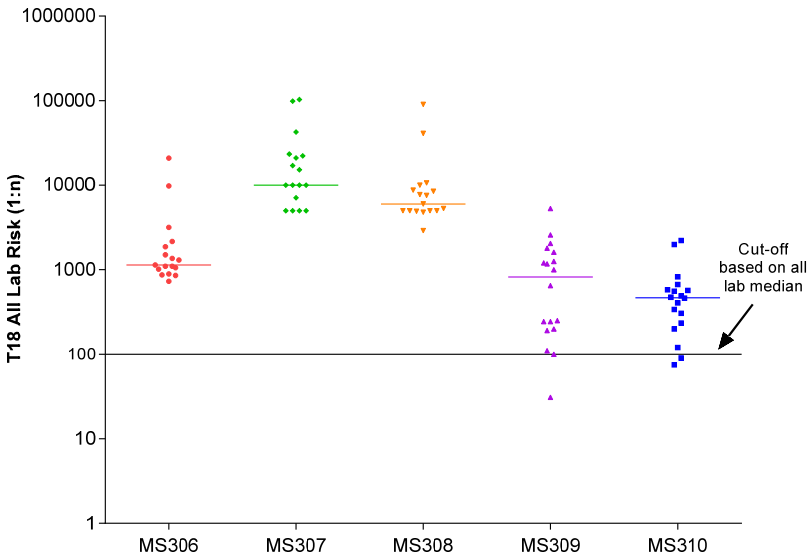


Figure 6

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates



NYS FEDM PT 1/14
Second Trimester

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

Figure 7A

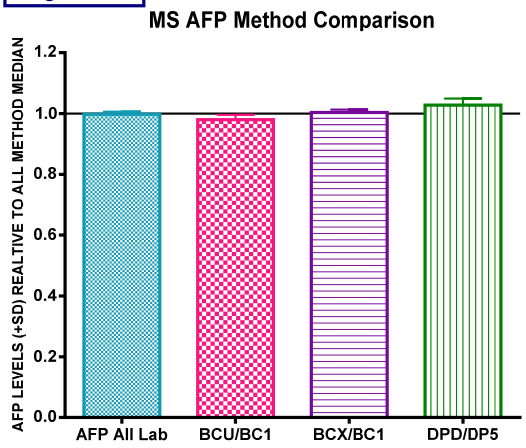


Figure 7B

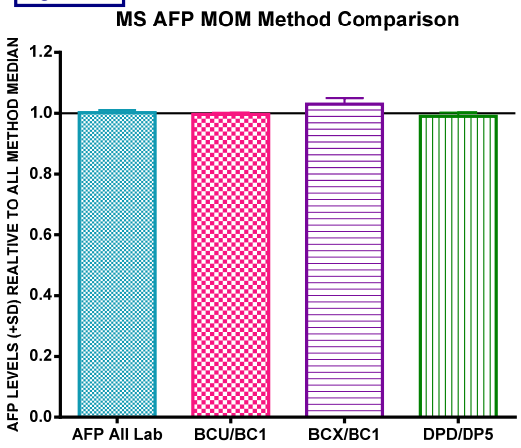


Figure 7C

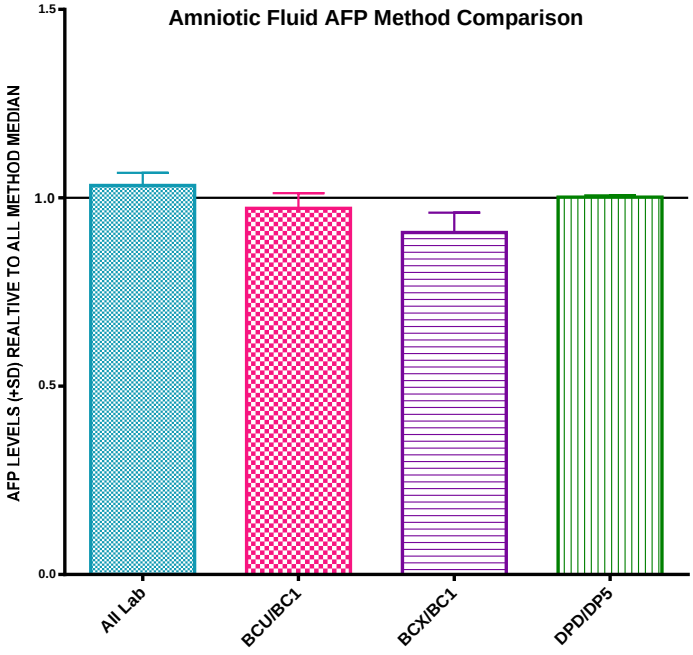
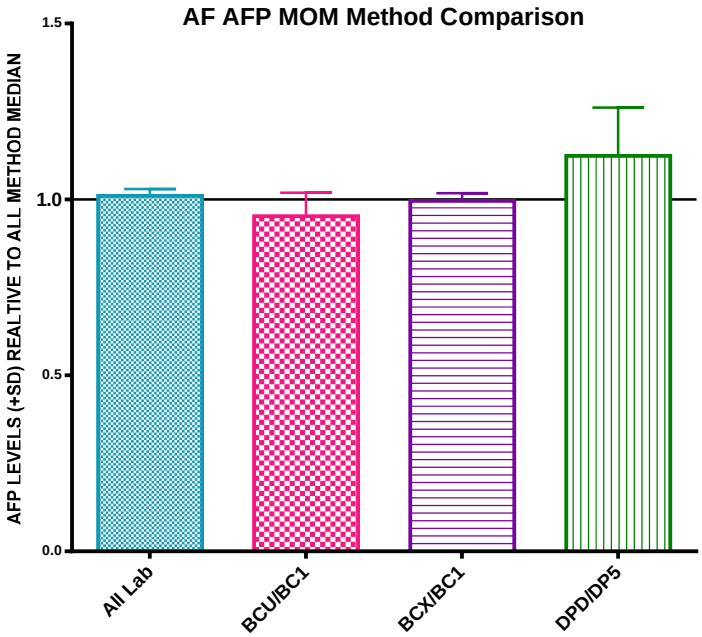


Figure 7D



NYS FEDM PT 1/14
Second Trimester

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

Figure 8A

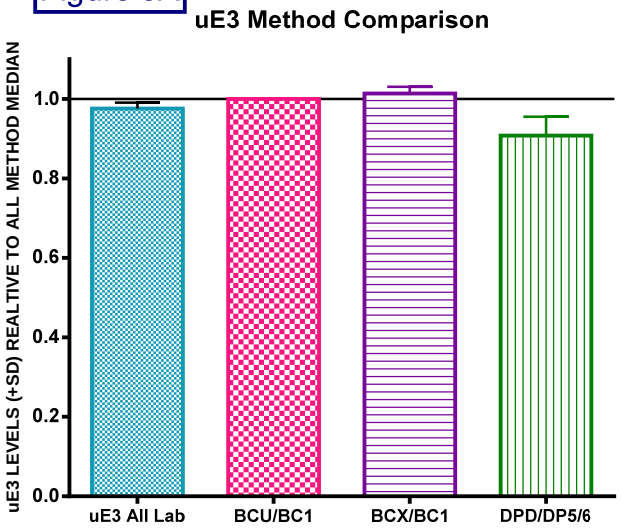


Figure 8B

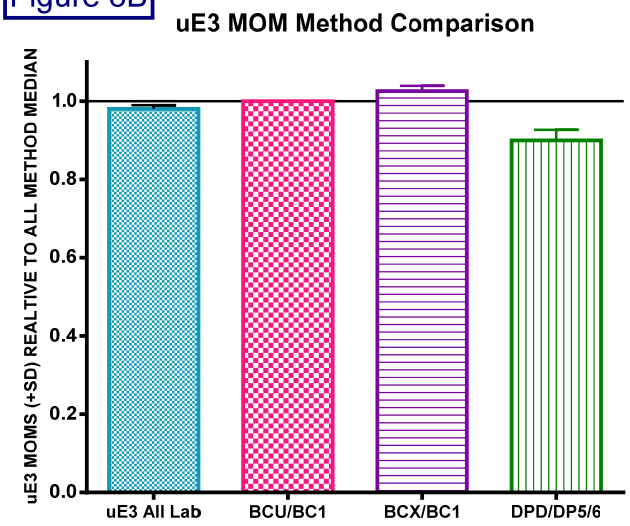


Figure 9A

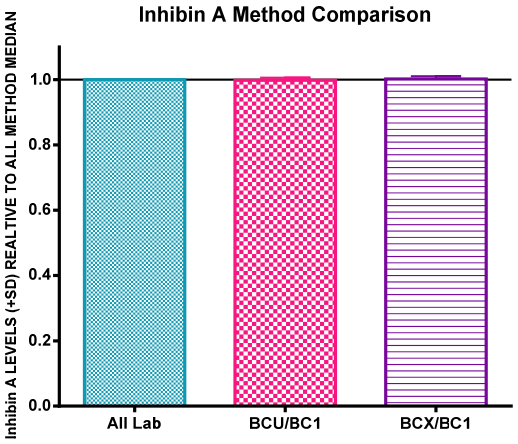


Figure 9B

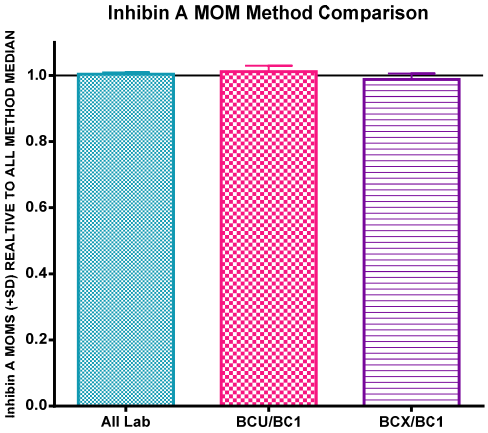


Figure 10A

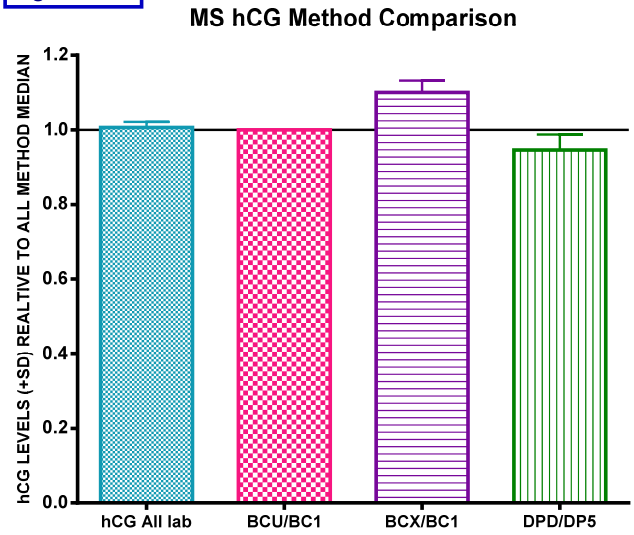
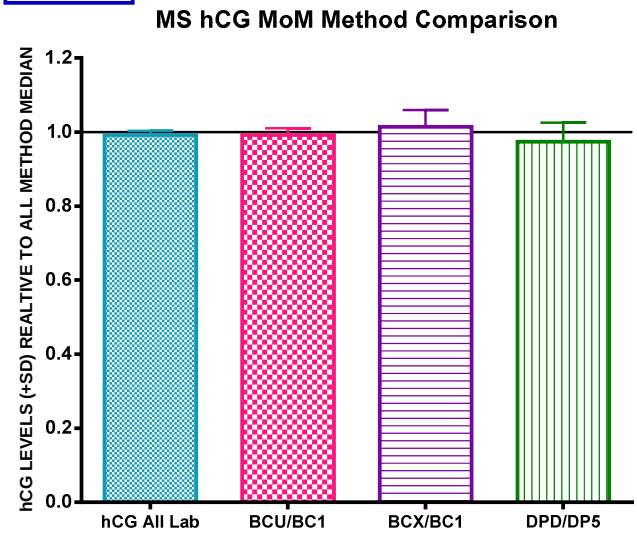


Figure 10B



NYS FEDM PT 1/14
First Trimester

Figure 11A

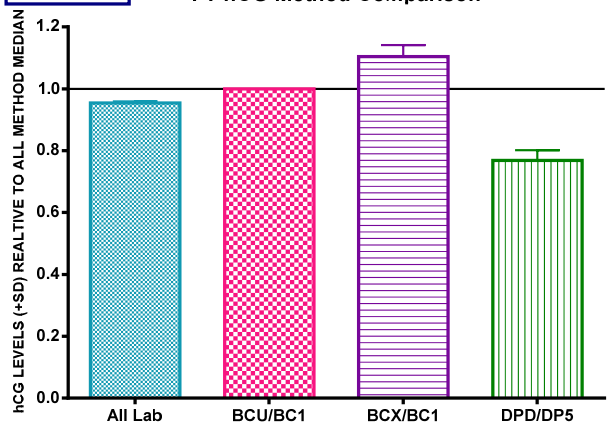


Figure 11B

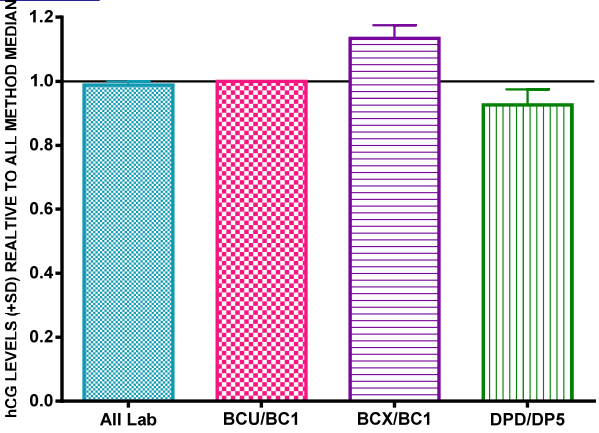


Figure 12A

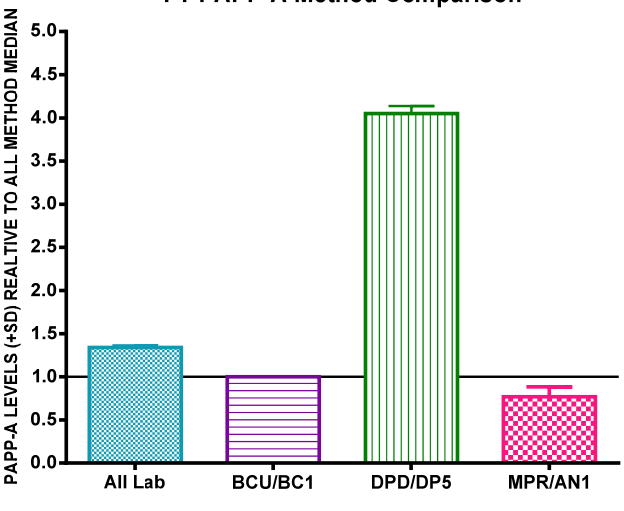
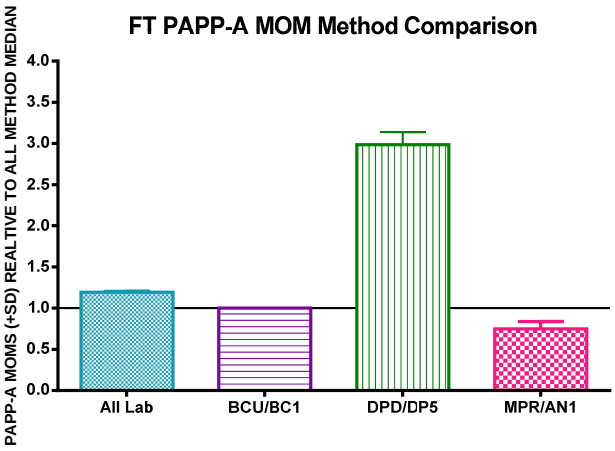


Figure 12B



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

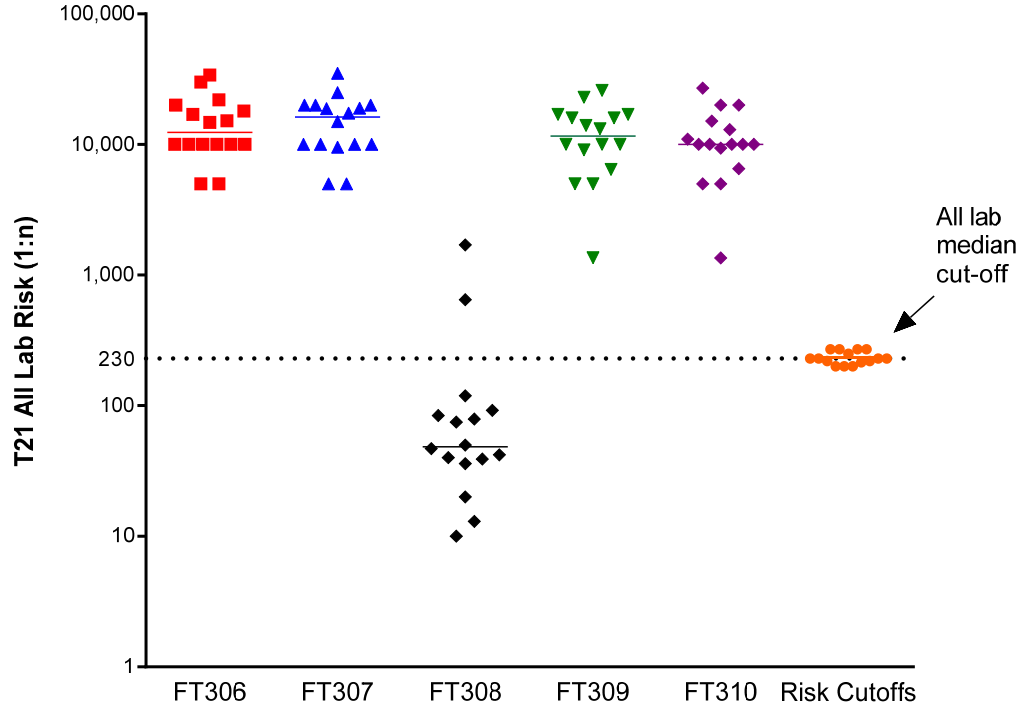
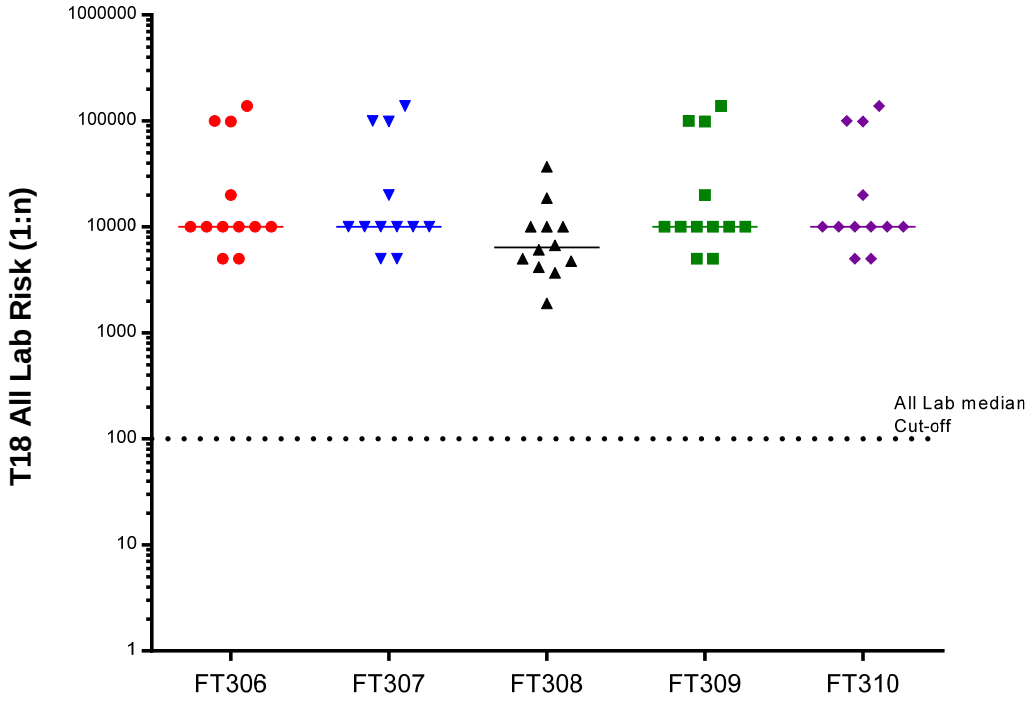


Figure 14

Graphic Distribution of First Trimester Trisomy 18 Risk Estimates



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

	FT306	FT307	FT308	FT309	FT310
FT Gestational Age All Lab Mean:					
Mean	11.1	11.5	12.0	12.5	13.0
SD	0.10	0.13	0.04	0.14	0.05
%CV	0.9%	1.1%	0.4%	1.1%	0.4%
mean+3*SD	11.4	11.9	12.1	12.9	13.1
mean-3*SD	10.8	11.1	11.8	12.1	12.8
N	17	17	17	17	17

	FT306	FT307	FT308	FT309	FT310
FT NT MoM All Lab Mean:					
Mean	1.09	1.08	1.86	0.94	0.98
SD	0.07	0.06	0.11	0.06	0.06
%CV	6.5%	5.3%	6.1%	6.0%	5.8%
mean+3SD	1.30	1.25	2.20	1.11	1.15
mean- 3SD	0.88	0.91	1.52	0.77	0.81
N	16	16	16	16	16
All Median	1.09	1.08	1.86	0.94	0.98

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

	FT306	FT307	FT308	FT309	FT310
FT hCG All Lab Mean:					
mean	82.8	81.1	154.4	70.5	65.2
SD	13.0	11.0	26.0	9.4	8.4
%CV	15.7%	13.5%	16.8%	13.4%	12.9%
mean+3SD	121.8	114.1	232.4	98.7	90.4
mean- 3SD	43.8	48.1	76.5	42.2	40.0
N	16	16	16	16	16
All lab median	84.4	83.6	156.0	72.7	67.4
mean/All kit median	0.95	0.95	0.95	0.96	0.96
FT hCG Beckman Unicel (BCU/BC1) mean:					
mean	87.5	85.5	162.0	73.3	67.7
SD	7.0	7.3	16.0	5.9	6.1
%CV	8.0%	8.6%	9.8%	8.1%	9.1%
mean+3SD	108.4	107.5	209.9	91.1	86.1
mean- 3SD	66.5	63.4	114.2	55.5	49.2
N	10	10	10	10	10
median	88.0	86.8	158.6	73.4	70.0
mean/All kit median	1.00	1.00	1.00	1.00	1.00
FT hCG Beckman Access (BCX/BC1) mean:					
mean	97.3	90.0	186.8	81.8	73.5
N	2	2	2	2	2
mean/All kit median	1.11	1.05	1.15	1.12	1.09
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	64.0	65.8	119.4	57.9	54.9
SD	3.8	3.6	8.2	3.7	5.5
%CV	5.9%	5.5%	6.9%	6.3%	10.1%
mean+3SD	75.3	76.7	143.9	68.9	71.5
mean- 3SD	52.6	54.9	94.8	46.9	38.2
N	4	4	4	4	4
median	64.1	64.5	119.7	58.2	54.7
mean/All kit median	0.73	0.77	0.74	0.79	0.81
FT hCG kit average:					
mean	82.9	80.4	156.0	71.0	65.3
SD	17.1	12.8	34.1	12.1	9.5
all kit median	87.5	85.5	162.0	73.3	67.7

	FT306	FT307	FT308	FT309	FT310
FT hCG MoM All Lab Mean:					
Mean	0.89	1.01	2.00	0.98	0.96
SD	0.08	0.09	0.22	0.10	0.11
%CV	8.6%	8.4%	11.1%	10.6%	11.9%
mean+3*SD	1.11	1.27	2.66	1.30	1.30
mean - 3*SD	0.66	0.76	1.33	0.67	0.61
N	15	15	15	15	15
All lab Median	0.88	0.99	1.97	0.96	0.97
mean/All kit Median	1.00	0.99	0.99	0.97	0.99
MS hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	0.88	1.02	2.02	1.02	0.96
SD	0.07	0.09	0.21	0.07	0.10
%CV	7.6%	8.7%	10.4%	7.1%	10.8%
mean+3SD	1.08	1.29	2.66	1.23	1.28
mean-3SD	0.68	0.75	1.39	0.80	0.65
N	10	10	10	10	10
median	0.87	1.01	2.06	1.01	0.97
mean/All kit median	1.00	1.00	1.00	1.00	1.00
MS hCG MoM Beckman Access (BCX/BC1) mean:					
mean	1.04	1.10	2.36	1.14	1.08
N	1	1	1	1	1
mean/All kit median	1.18	1.08	1.17	1.12	1.12
MS hCG MoM DPC Immulite2000 (DPD/DP5) mean:					
mean	0.86	0.98	1.83	0.87	0.90
SD	0.07	0.08	0.12	0.08	0.14
%CV	7.9%	8.0%	6.4%	8.8%	15.1%
mean+3SD	1.06	1.21	2.19	1.10	1.31
mean-3SD	0.65	0.74	1.48	0.64	0.49
N	4	4	4	4	4
median	0.86	0.97	1.84	0.87	0.94
mean/All kit median	0.97	0.96	0.91	0.85	0.94
FT hCG MoM kit average:					
mean	0.93	1.03	2.07	1.01	0.98
SD	0.10	0.06	0.27	0.14	0.09
all kit median	0.88	1.02	2.02	1.02	0.96

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

	FT306	FT307	FT308	FT309	FT310
FT PAPP-A All Lab Mean:					
Mean	1323.8	1490.2	875.5	2005.4	1530.8
SD	1061.1	1136.9	701.1	1603.2	1287.4
%CV	80.2%	76.3%	80.1%	79.9%	84.1%
mean + 3SD	4507.0	4901.0	2978.9	6814.9	5392.9
mean- 3SD	-1859.5	-1920.6	-1227.9	-2804.2	-2331.3
N	16	16	16	16	16
All Lab Median	962.9	1107.4	653.0	1537.2	1154.5
mean/All kit median	1.37	1.36	1.33	1.31	1.33
FT PAPP-A Beckman Unicel(BCU/BC1) Mean:					
Mean	969.7	1096.6	656.8	1533.1	1152.9
SD	72.5	69.5	45.2	127.5	100.0
%CV	7.5%	6.3%	6.9%	8.3%	8.7%
mean + 3SD	1187.2	1305.2	792.4	1915.6	1452.9
mean - 3SD	752.2	888.1	521.3	1150.7	852.9
N	11	11	11	11	11
Kit Median	964.0	1098.0	664.0	1542.6	1167.0
mean/All kit median	1.00	1.00	1.00	1.00	1.00
***FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:**					
Mean	4016.1	4384.7	2649.6	6041.1	4778.1
N	2	2	2	2	2
mean/All kit median	4.14	4.00	4.03	3.94	4.14

***Note: The above table contains converted values (mIU/ml->ng/ml) from equation obtained based on in house correlation data. (see critique)**

FT PAPP-A AnshLite (SMR, MPR or APM/AN1) Mean:					
Mean	827.1	1003.6	494.8	1046.4	751.7
SD	126.0	224.5	27.5	275.1	13.3
%CV	15.2%	22.4%	5.6%	26.3%	1.8%
mean + 3SD	1205.1	1677.2	577.4	1871.6	791.6
mean - 3SD	449.2	330.0	412.3	221.3	711.7
N	3	3	3	3	3
Kit Median	871.4	1106.7	495.5	991.0	745.0
mean/All kit median	0.85	0.92	0.75	0.68	0.65
FT PAPP-A kit average:					
mean	1937.7	2161.6	1267.1	2873.5	2227.5
SD	1801.4	1925.8	1200.0	2753.9	2217.9
all kit median	969.7	1096.6	656.8	1533.1	1152.9

	FT306	FT307	FT308	FT309	FT310
FT PAPP-A MoM All Lab Mean:					
Mean	1.88	2.14	1.02	1.83	1.19
SD	1.09	1.23	0.56	1.19	0.79
%CV	58.0%	57.6%	55.4%	65.0%	65.9%
mean + 3SD	5.16	5.83	2.71	5.41	3.55
mean- 3SD	-1.39	-1.55	-0.67	-1.74	-1.17
N	16	16	16	16	16
All Lab Median	1.50	1.72	0.85	1.60	0.97
mean/ All kit median	1.20	1.21	1.17	1.18	1.21
FT PAPP-A MoM Beckman Unicel(BCU/BC1) Mean:					
Mean	1.57	1.77	0.87	1.55	0.98
SD	0.27	0.22	0.09	0.32	0.12
%CV	17.0%	12.3%	11.0%	20.8%	12.2%
mean + 3SD	2.37	2.42	1.15	2.52	1.34
mean - 3SD	0.77	1.12	0.58	0.58	0.62
N	11	11	11	11	11
Kit Median	1.49	1.69	0.85	1.61	0.99
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	4.60	5.21	2.43	4.73	3.15
N	2	2	2	2	2
mean/All kit median	2.93	2.95	2.80	3.04	3.21

FT PAPP-A MoM (SMR or APM/AN1) Mean:					
Mean	1.30	1.51	0.64	1.02	0.68
N	2	2	2	2	2
Kit Median	1.30	1.51	0.64	1.02	0.68
mean/ All kit median	0.83	0.85	0.73	0.65	0.69
FT PAPP-A MoM kit average:					
mean	2.49	2.83	1.31	2.43	1.60
SD	1.83	2.07	0.97	2.01	1.35
all kit median	1.57	1.77	0.87	1.55	0.98

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Summary of Results

	MS 306	MS 307	MS 308	MS 309	MS 310
Gestational Age All Lab Mean:					
Mean	17.0	19.0	18.0	15.0	21.0
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	17.0	19.0	18.0	15.0	21.0
mean-3*SD	17.0	19.0	18.0	15.0	21.0
N	26	26	26	25	26

	MS 306	MS 307	MS 308	MS 309	MS 310
MS AFP All Lab Mean:					
mean	18.6	55.0	52.3	29.5	178.8
SD	1.4	4.6	3.6	2.4	12.8
%CV	7.7%	8.3%	7.0%	8.1%	7.2%
mean+3SD	22.9	68.7	63.2	36.7	217.2
mean-3SD	14.3	41.4	41.3	22.4	140.4
N	26	26	26	26	26
median	18.55	54.1	52.1	29.3	177
mean/all kit median	1.00	0.99	1.00	0.99	1.01
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	18.3	53.9	51.5	28.8	176.0
SD	1.3	4.7	4.1	2.1	14.4
%CV	7.0%	8.8%	7.9%	7.2%	8.2%
mean + 3SD	22.1	68.1	63.7	35.0	219.1
mean - 3SD	14.5	39.6	39.3	22.6	132.9
N	14	14	14	14	14
Median	18.2	53.2	50.8	28.2	171.3
mean/All kit median	0.98	0.97	0.99	0.96	1.00
MS AFP Beckman Access/2 (BCX/BC1) mean:					
mean	19.0	55.4	52.1	30.0	176.5
SD	1.9	5.2	4.0	3.2	9.2
%CV	9.9%	9.4%	7.6%	10.7%	5.2%
mean+3SD	24.7	71.0	64.0	39.6	204.0
mean-3SD	13.4	39.7	40.2	20.4	149.0
N	5	5	5	5	5
median	19.0	53.1	52.2	28.6	175.6
mean/all kit median	1.02	1.00	1.00	1.00	1.00
MS AFP Siemens Immulite 2000 (DPD/DP5) mean:					
mean	18.7	57.0	53.5	30.7	187.7
SD	1.3	3.6	1.8	2.3	9.6
%CV	7.1%	6.4%	3.4%	7.4%	5.1%
mean+3SD	22.6	67.9	58.9	37.5	216.5
mean-3SD	14.7	46.1	48.1	23.8	158.9
N	6	6	6	6	6
median	18.7	58.3	54.2	31.6	188.0
mean/all kit median	1.00	1.03	1.03	1.02	1.06
MS AFP kit average:					
mean	18.7	55.4	52.4	29.8	180.1
SD	0.4	1.5	1.1	1.0	6.6
all kit median	18.7	55.4	52.1	30.0	176.5

	MS 306	MS 307	MS 308	MS 309	MS 310
MS AFP MoM All Lab Mean:					
mean	0.48	1.02	1.13	1.04	2.54
SD	0.04	0.10	0.08	0.09	0.20
%CV	7.9%	9.9%	7.5%	8.6%	7.9%
mean+3SD	0.60	1.33	1.38	1.31	3.14
mean-3SD	0.37	0.72	0.87	0.77	1.94
N	26	26	26	26	25
All Median	0.48	1.02	1.12	1.04	2.51
mean/all kit median	1.01	1.00	1.01	0.99	1.00
MS AFP MoM Beckman Unicel (BCU/BC1) mean:					
Mean	0.48	1.02	1.12	1.05	2.52
SD	0.03	0.11	0.09	0.08	0.21
%CV	7.0%	11.1%	8.1%	7.8%	8.2%
mean + 3SD	0.58	1.37	1.39	1.29	3.14
mean - 3SD	0.38	0.68	0.84	0.80	1.90
N	14	14	14	14	14
Median	0.48	1.03	1.09	1.02	2.50
mean/all kit median	1.00	1.00	1.00	0.99	0.99
MS AFP MoM Beckman Access/2 (BCX/BC1) mean:					
Mean	0.50	1.05	1.16	1.06	2.64
SD	0.05	0.12	0.11	0.09	0.31
%CV	9.8%	11.3%	9.6%	8.3%	11.7%
mean + 3SD	0.65	1.40	1.50	1.32	3.56
mean - 3SD	0.36	0.69	0.83	0.80	1.72
N	5	5	5	5	4
Median	0.51	0.99	1.17	1.03	2.56
mean/all kit median	1.05	1.02	1.04	1.00	1.04
MS AFP MoM Siemens Immulite 2000 (DPD/DP5) mean:					
Mean	0.46	1.01	1.11	1.06	2.54
SD	0.04	0.07	0.04	0.07	0.11
%CV	7.6%	7.4%	3.7%	6.5%	4.2%
mean + 3SD	0.57	1.23	1.23	1.26	2.86
mean - 3SD	0.36	0.79	0.98	0.85	2.21
N	6	6	6	6	6
Median	0.46	1.03	1.11	1.07	2.56
mean/all kit median	0.97	0.99	0.99	1.00	1.00
MS AFP MoM kit average:					
mean	0.48	1.03	1.13	1.05	2.57
SD	0.02	0.02	0.03	0.01	0.07
all kit median	0.48	1.02	1.12	1.06	2.54

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	MS 306	MS 307	MS 308	MS 309	MS 310
MS uE3 All Lab Mean:					
mean	0.39	1.14	0.61	0.26	0.67
SD	0.06	0.11	0.06	0.05	0.08
%CV	14.4%	9.8%	9.6%	17.7%	12.0%
mean+3SD	0.55	1.47	0.78	0.40	0.91
mean-3SD	0.22	0.80	0.43	0.12	0.43
N	24	24	24	24	24
mean/all kit median	0.95	0.98	0.98	0.98	1.00
MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	0.40	1.16	0.62	0.27	0.67
SD	0.03	0.08	0.04	0.02	0.06
%CV	7.9%	6.7%	6.0%	7.6%	8.6%
mean+3SD	0.50	1.39	0.73	0.33	0.84
mean-3SD	0.31	0.92	0.51	0.21	0.50
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	0.43	1.24	0.66	0.32	0.76
SD	0.03	0.04	0.02	0.02	0.05
%CV	6.8%	3.2%	2.6%	7.7%	6.9%
mean+3SD	0.52	1.36	0.71	0.39	0.92
mean-3SD	0.34	1.12	0.61	0.25	0.60
N	5	5	5	5	5
mean/all kit median	1.07	1.07	1.06	1.20	1.13
MS uE3 Siemens Immulite/2000 (DPD/DP6) mean:					
Mean	0.31	1.01	0.53	0.20	0.58
SD	0.02	0.11	0.04	0.02	0.05
%CV	7.6%	10.4%	7.2%	11.9%	8.5%
mean+3SD	0.38	1.33	0.64	0.27	0.73
mean-3SD	0.24	0.70	0.42	0.13	0.43
N	6	6	6	6	6
mean/all Kit Median	0.76	0.87	0.85	0.75	0.87
MS uE3 kit average:					
mean	0.38	1.14	0.60	0.26	0.67
SD	0.07	0.11	0.07	0.06	0.09
all kit median	0.40	1.16	0.62	0.27	0.67

	MS 306	MS 307	MS 308	MS 309	MS 310
MS uE3 MoM All Lab Mean:					
Mean	0.41	0.81	0.50	0.45	0.30
SD	0.05	0.09	0.04	0.07	0.04
%CV	11.6%	11.1%	8.9%	15.8%	13.8%
mean+3SD	0.55	1.08	0.64	0.66	0.42
mean-3SD	0.27	0.54	0.37	0.24	0.17
N	24	24	24	24	23
mean/all kit Median	0.98	0.98	0.95	0.94	0.98
MS uE3 MoM Beckman Unicel (BCU/BC1) Mean:					
Mean	0.39	0.77	0.48	0.41	0.29
SD	0.03	0.06	0.02	0.04	0.03
%CV	7.7%	7.3%	5.1%	9.6%	11.2%
mean+3SD	0.48	0.93	0.55	0.53	0.38
mean-3SD	0.30	0.60	0.40	0.29	0.19
N	13	13	13	13	13
mean/all kit Median	0.95	0.92	0.91	0.87	0.95
MS uE3 MoM Beckman Access/2 (BCX/BC1) Mean:					
Mean	0.44	0.83	0.53	0.50	0.32
SD	0.06	0.10	0.06	0.07	0.06
%CV	14.3%	12.4%	10.8%	13.8%	17.1%
mean+3SD	0.62	1.14	0.70	0.71	0.49
mean-3SD	0.25	0.52	0.36	0.29	0.16
N	5	5	5	5	4
mean/all kit Median	1.05	1.00	1.00	1.05	1.06
MS uE3 MoM Siemens Immulite/2000 (DPD/DP6) Mean:					
Mean	0.41	0.89	0.54	0.48	0.30
SD	0.06	0.09	0.04	0.09	0.05
%CV	14.2%	10.0%	8.0%	19.1%	15.8%
mean+3SD	0.59	1.16	0.66	0.75	0.45
mean-3SD	0.24	0.62	0.41	0.20	0.16
N	6	6	6	6	6
mean/all kit Median	1.00	1.08	1.02	1.00	1.00
MS uE3 MoM kit average:					
mean	0.41	0.83	0.51	0.46	0.30
SD	0.02	0.06	0.03	0.05	0.02
all kit median	0.41	0.83	0.53	0.48	0.30

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	MS 306	MS 307	MS 308	MS 309	MS 310
MS hCG All Lab mean:					
mean	49.5	14.4	67.8	25.3	14.6
SD	5.6	0.9	9.7	2.4	1.3
%CV	11.3%	6.5%	14.4%	9.4%	8.8%
mean+3SD	66.3	17.3	97.0	32.4	18.5
mean-3SD	32.6	11.6	38.6	18.2	10.8
N	24	24	24	24	24
mean/all kit median	0.98	1.00	0.98	1.02	1.01
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	50.4	14.4	69.3	24.9	14.2
SD	4.1	0.9	6.3	1.6	1.3
%CV	8.2%	6.1%	9.1%	6.6%	8.8%
mean+3SD	62.78	17.07	88.15	29.86	17.91
mean-3SD	38.08	11.79	50.40	20.03	10.40
N	13	13	13	13	13
median	50.10	14.40	68.20	24.20	14.00
mean/All kit median	1.00	1.00	1.00	1.00	0.98
MS hCG Beckman Access/2 (BCX/BC1) mean:					
mean	55.1	15.0	78.4	28.7	16.0
SD	3.9	0.7	4.6	1.8	0.5
%CV	7.0%	4.8%	5.9%	6.1%	3.0%
mean+3SD	66.7	17.2	92.2	34.0	17.4
mean-3SD	43.4	12.8	64.5	23.4	14.6
N	5	5	5	5	5
median	55.6	14.5	78.8	28.6	16.0
mean/all kit median	1.09	1.04	1.13	1.15	1.11
MS hCG Siemens Immulite 2000 (DPD/DP5) mean:					
mean	42.7	14.0	55.7	23.4	14.5
SD	1.6	1.1	5.6	0.8	1.1
%CV	3.7%	8.1%	10.0%	3.4%	7.4%
mean+3SD	47.4	17.4	72.5	25.8	17.7
mean-3SD	37.9	10.6	39.0	21.0	11.3
N	6	6	6	6	6
median	42.8	13.7	54.8	23.2	14.3
mean/all kit median	0.85	0.97	0.80	0.94	1.00
MS hCG kit average:					
mean	49.4	14.5	67.8	25.7	14.9
SD	6.3	0.5	11.4	2.7	1.0
all kit median	50.4	14.4	69.3	24.9	14.5

	MS 306	MS 307	MS 308	MS 309	MS 310
MS hCG MoMs All Lab Mean:					
mean	2.07	0.79	3.17	0.66	0.91
SD	0.24	0.11	0.42	0.08	0.14
%CV	11.4%	13.6%	13.4%	12.0%	14.9%
mean+3SD	2.77	1.11	4.44	0.90	1.32
mean-3SD	1.36	0.46	1.89	0.42	0.50
N	24	24	24	24	23
mean/All Kit Median	1.01	1.00	0.99	1.03	1.04
MS hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	2.05	0.80	3.20	0.64	0.88
SD	0.21	0.08	0.41	0.08	0.12
%CV	10.1%	10.2%	12.9%	12.0%	13.2%
mean+3SD	2.68	1.04	4.44	0.88	1.23
mean-3SD	1.43	0.55	1.96	0.41	0.53
N	13	13	13	13	13
median	2.01	0.78	3.26	0.65	0.87
mean/All kit median	1.00	1.02	1.00	1.00	1.00
MS hCG MoM Beckman Access/2 (BCX/BC1) mean:					
mean	2.21	0.75	3.44	0.73	0.86
SD	0.34	0.19	0.43	0.07	0.19
%CV	15.2%	25.4%	12.4%	9.4%	22.5%
X+3SD	3.22	1.33	4.72	0.94	1.43
X-3SD	1.20	0.18	2.16	0.52	0.28
N	5	5	5	5	4
median	2.20	0.75	3.36	0.75	0.83
mean/All kit median	1.08	0.96	1.07	1.13	0.97
MS hCG MoM Siemens Immulite 2000 (DPD/DP5) mean:					
mean	1.98	0.78	2.86	0.64	1.02
SD	0.17	0.07	0.30	0.07	0.08
%CV	8.5%	9.5%	10.5%	10.9%	8.3%
X+3SD	2.48	1.01	3.77	0.85	1.27
X-3SD	1.47	0.56	1.96	0.43	0.77
N	6	6	6	6	6
median	2.04	0.78	2.91	0.64	1.03
mean/All kit median	0.96	1.00	0.89	0.99	1.16
MS hCG MoM kit average:					
mean	2.1	0.8	3.2	0.7	0.9
SD	0.1	0.0	0.3	0.1	0.1
all kit median	2.1	0.8	3.2	0.6	0.9

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	MS 306	MS 307	MS 308	MS 309	MS 310
MS Inhibin A all lab mean:					
Mean	332.4	213.1	514.5	161.6	225.4
SD	21.5	12.0	21.0	7.5	10.6
%CV	6.5%	5.6%	4.1%	4.6%	4.7%
mean + 3SD	396.9	249.2	577.6	184.0	257.1
mean- 3SD	267.9	177.0	451.4	139.3	193.7
N	25	25	25	25	25
All Lab Median	330.5	214.7	514.0	161.0	224.0
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	330.6	212.6	513.9	161.9	224.6
SD	19.5	12.2	19.4	7.7	11.9
%CV	5.9%	5.7%	3.8%	4.8%	5.3%
mean + 3SD	389.2	249.1	572.2	185.0	260.2
mean- 3SD	272.0	176.1	455.6	138.8	189.0
N	17	17	17	17	17
kit median	329.5	210.4	512.1	159.5	224.0
mean/all kit median	0.99	1.00	1.00	1.00	0.99
MS Inhibin A Beckman Access/2 (BCX/BC1) mean:					
Mean	336.3	214.0	515.7	161.1	227.2
SD	26.3	12.5	25.5	7.4	7.5
%CV	7.8%	5.9%	4.9%	4.6%	3.3%
mean + 3SD	415.1	251.5	592.2	183.2	249.6
mean- 3SD	257.5	176.4	439.2	138.9	204.7
N	8	8	8	8	8
kit median	334.7	216.5	520.1	162.2	225.9
mean/All kit median	1.01	1.00	1.00	1.00	1.01
MS Inhibin A kit average:					
mean	333.4	213.3	514.8	161.5	225.9
SD	4.0	0.9	1.3	0.6	1.8
all kit median	333.4	213.3	514.8	161.5	225.9

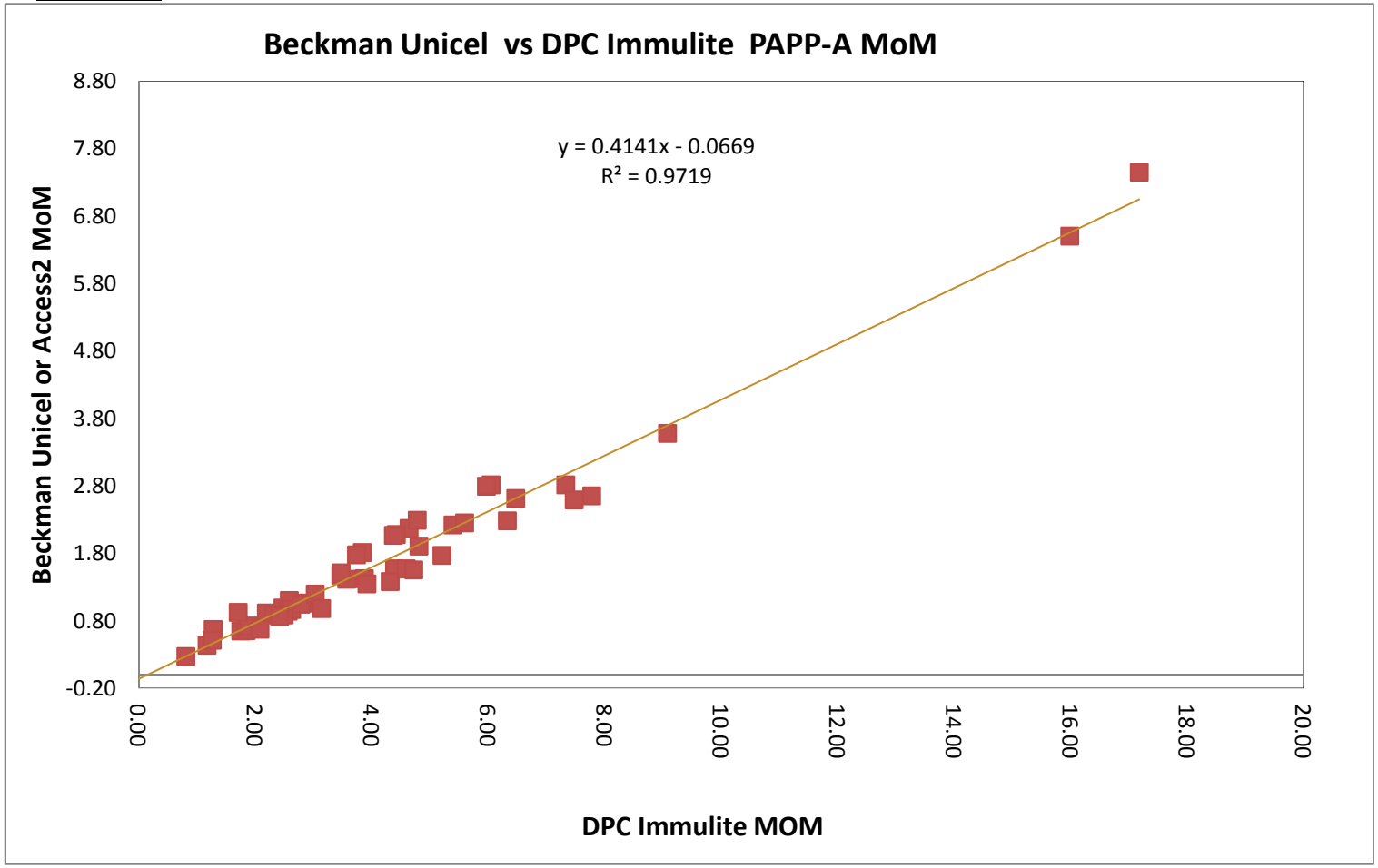
	MS 306	MS 307	MS 308	MS 309	MS 310
MS Inhibin A MoM All Lab mean:					
mean	1.95	1.26	2.90	0.86	1.05
SD	0.14	0.10	0.20	0.07	0.11
%CV	7.3%	7.7%	6.9%	8.5%	10.5%
mean+3SD	2.38	1.55	3.50	1.08	1.38
mean-3SD	1.52	0.96	2.30	0.64	0.72
N	25	25	25	25	24
mean/all kit median	1.00	1.01	1.00	1.00	1.01
MS Inhibin A MoM Beckman Unicel (BCU/BC1) mean:					
Mean	1.94	1.27	2.90	0.85	1.06
SD	0.14	0.10	0.20	0.08	0.11
%CV	7.5%	8.1%	7.0%	9.5%	10.8%
mean + 3SD	2.37	1.58	3.50	1.09	1.40
mean- 3SD	1.50	0.96	2.29	0.61	0.72
N	17	17	17	17	17
Kit Median	1.93	1.23	2.90	0.89	1.08
mean/all kit median	0.99	1.02	1.00	0.99	1.02
MS Inhibin A MoM Beckman Access (BCX/BC1) mean:					
Mean	1.97	1.22	2.91	0.88	1.02
SD	0.15	0.08	0.21	0.05	0.10
%CV	7.5%	6.2%	7.1%	6.1%	9.6%
mean + 3SD	2.41	1.45	3.53	1.03	1.31
mean- 3SD	1.53	0.99	2.29	0.72	0.73
N	8	8	8	8	7
Kit Median	1.93	1.23	2.89	0.89	1.07
mean/all kit median	1.01	0.98	1.00	1.01	0.98
MS Inhibin A MoM kit average:					
mean	1.95	1.25	2.90	0.86	1.04
SD	0.02	0.04	0.01	0.02	0.03
all kit median	1.95	1.25	2.90	0.86	1.04

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

	AF306	AF307	AF308	AF309	AF310
AF AFP All Lab mean :					
mean	1.8	7.6	10.1	6.8	20.4
SD	0.2	0.9	1.4	1.2	2.7
%CV	9.0%	11.5%	14.0%	17.3%	13.1%
mean+3SD	2.3	10.2	14.3	10.3	28.4
mean-3SD	1.3	4.9	5.9	3.3	12.4
N	18	19	19	19	19
All kit median	1.8	8.2	9.8	7.0	21.8
mean/all kit mean	0.99	0.93	1.03	0.97	0.94
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	1.8	7.0	9.4	6.1	18.9
SD	0.1	0.5	1.0	0.6	2.2
%CV	7.7%	7.7%	10.6%	10.4%	11.6%
X+3SD	2.2	8.7	12.4	8.0	25.5
X-3SD	1.4	5.4	6.4	4.2	12.4
N	11	12	12	12	12
median	1.8	7.0	9.4	5.9	19.2
mean/all kit median	0.97	0.86	0.96	0.88	0.87
AF AFP Beckman Access/2 (BCX/BC1) mean:					
mean	1.9	8.2	9.8	7.0	21.8
N	2	2	2	2	2
median	1.9	8.15	9.8	6.95	21.75
mean/all kit median	1.01	1.00	1.00	1.00	1.00
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	1.8	8.7	12.1	8.7	23.4
SD	0.1	0.6	0.8	0.4	0.7
%CV	5.2%	6.6%	6.6%	4.3%	3.1%
mean+3SD	2.1	10.4	14.5	9.8	25.5
mean-3SD	1.5	6.9	9.7	7.5	21.2
N	4	4	4	4	4
median	1.85	8.6	12.4	8.7	23.5
mean/all kit median	1.00	1.06	1.23	1.24	1.07
AF AFP kit average:					
mean	1.8	7.9	10.4	7.2	21.4
SD	0.0	0.8	1.4	1.3	2.2
all kit median	1.8	8.2	9.8	7.0	21.8

	AF306	AF307	AF308	AF309	AF310
AF AFP MoM All Lab Mean:					
mean	0.16	1.00	1.06	0.60	4.04
SD	0.03	0.13	0.18	0.10	0.57
%CV	16.1%	13.1%	17.1%	16.0%	14.0%
mean+3SD	0.24	1.40	1.61	0.89	5.74
mean-3SD	0.08	0.61	0.52	0.31	2.34
N	19	19	19	19	19
All median	0.16	0.97	1.09	0.60	3.98
mean/all kit median	1.02	1.03	0.98	1.00	1.02
AF AFP MoM Beckman Unicel(BCU/BC1) mean:					
Mean	0.16	0.96	1.01	0.56	3.81
SD	0.02	0.09	0.17	0.06	0.39
%CV	12.6%	9.1%	16.6%	10.5%	10.3%
X+3SD	0.22	1.22	1.51	0.74	4.98
X-3SD	0.10	0.70	0.50	0.39	2.63
N	12	12	12	12	12
median	0.16	0.95	1.03	0.58	3.77
mean/all kit median	1.00	0.90	1.00	1.00	0.86
AF AFP MoM Beckman Access (BCX/BC1) mean:					
Mean	0.16	1.07	1.01	0.57	4.52
N	2	2	2	2	2
median	0.16	1.07	1.01	0.57	4.52
mean/all kit median	0.96	1.00	1.00	1.00	1.02
AF AFP MoM DPC Immulite 2000 (DPD/DP5) mean:					
Mean	0.17	1.11	1.25	0.74	4.44
SD	0.03	0.18	0.16	0.09	0.60
%CV	19.7%	16.2%	12.6%	12.0%	13.5%
X+3SD	0.27	1.64	1.72	1.00	6.24
X-3SD	0.07	0.57	0.77	0.47	2.64
N	4	4	4	4	4
median	0.17	1.05	1.23	0.70	4.43
mean/all kit median	1.04	1.04	1.23	1.31	1.00
AF AFP MoM kit average:					
mean	0.16	1.04	1.09	0.62	4.25
SD	0.01	0.08	0.14	0.10	0.39
all kit median	0.16	1.07	1.01	0.57	4.44

Fig. 15


Beckman Unicel vs DPC Immulite PAPP-A MoM
y = 0.4141x - 0.0669
R² = 0.9719
Beckman Unicel or Access2 MoM
8.80
7.80
6.80
5.80
4.80
3.80
2.80
1.80
0.80
-0.20
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
DPC Immulite MOM