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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 29, 2013, 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 = 27	Sample #	MS 291	MS 292	MS 293	MS 294	MS 295
	Gestational Age (weeks)	17.0	19.0	19.4	18.0	20.0
Maternal Race	Ethnic Group	White	White	Black	Hispanic	Asian
Maternal Weight	Pounds (lbs)	150	140	155	145	120
Maternal Age	Years	25	31	28	30	29
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	45.1 ± 3.5	93.5 ± 8.7	64.9 ± 4.9	26.4 ± 2.1	70.3 ± 4.8
	MOM ± Std. Dev.	1.18 ± 0.08	1.76 ± 0.16	1.13 ± 0.09	0.59 ± 0.05	1.04 ± 0.08
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	0.97 ± 0.11	0.76 ± 0.07	1.38 ± 0.10	0.58 ± 0.06	1.55 ± 0.09
	MOM ± Std. Dev.	1.05 ± 0.22	0.53 ± 0.09	0.90 ± 0.14	0.49 ± 0.09	0.83 ± 0.15
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	58.0 ± 7.0	30.2 ± 3.9	20.3 ± 1.9	45.2 ± 7.2	18.2 ± 2.5
	MOM ± Std. Dev.	2.46 ± 0.24	1.61 ± 0.18	1. 14 ± 0.10	2.19 ± 0.33	0.94 ± 0.14
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	359.3 ± 43.1	144.1 ± 17.2	209.8 ± 22.6	298.0 ± 33.7	221.5 ± 25.3
	MOM ± Std. Dev.	2.14 ± 0.25	0.79 ± 0.09	1.20 ± 0.13	1.76 ± 0.20	1.07 ± 0.15
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (92%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	G = 8% U = 8% A = 8%	NFA	NFA	NFA
	NTD Risk 1 in	6,200	1,090	5,600	10,000	5,000
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(-) (92%)	(-) (75%)	(-) (100%)	(+) (100%)	(-) (100%)
	Recommended Action**	G = 8% U = 8% A = 8%	G = 25% U = 17% A = 25%	NFA	G = 92% U = 58% A = 83%	NFA
	Risk Est. 1 in	1,500	484	3,300	30	3,650
2. Quad Test	Pos. (+) or Neg. (-)	(-) (88%)	(-) (100%)	(-) (100%)	(+) (100%)	(-) (100%)
	Recommended Action **	G = 13% U = 8% A = 13%	NFA	NFA	G = 92% U = 67% A = 88%	NFA
	Risk Est. 1 in	947	1,400	3,900	18	4,925
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	10,000	5,000	10,000	3,656	10,000

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

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

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:

N = 27 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 291 Wk 17.0	This specimen was obtained from a 25 year old white woman (Gravida = 1, Parity = 0) in her 17th week of gestation with a body weight of 150 lbs. A race correction was not indicated. She had no personal history of pregnancy loss. However, MShCG and MSDIA were both elevated in this sample. See critique for further discussion of this elevated MShCG and MSDIA sample. Her specimen was screen negative for NTD and for both Trisomies and all labs were in agreement. However, 12% of the labs using quad test assigned a positive T21 screen to this sample. This specimen had no amniotic fluid counterpart (see Critique).
MS 292 Wk 19.0	This specimen was obtained from a 31 year old White woman (Gravida = 3, Parity = 2) in her 19th week of gestation with a body weight of 140 lbs. She had a family (sibling) history of reproductive complications. Although her sample screened borderline negative for NTD, and her aneuploidy screens were negative for both Trisomy-18 and Trisomy-21 in the quad test, a T21 positive screen was generated in 25% of the labs using the triple test. The MS292 sample was paired to an amniotic fluid specimen (AF292) with elevated AFP (AFP MOM = 3.28). (See Critique.)
MS 293 Wk 19.4	This specimen was obtained from a 28 year old Black woman (Gravida = 2, Parity = 1) in her 19.4th week of gestation with a body weight of 155 lbs. She had a family reproductive history that was unremarkable. Her sample screened negative for NTD, as was her aneuploidy screen for Trisomies-21 and 18. This sample was not paired to an amniotic fluid specimen.
MS 294 Wk 18.0	This specimen was obtained from a 30 year old Hispanic woman (Gravida = 3, Parity = 1) in her 18th week of gestation with a body weight of 145 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100% by quad, 100% by triple) on the basis of low AFP and uE3, and moderately elevated hCG and inhibin-A levels. Recommendations for further action from labs reporting elevated T21 risks by quad screen were: genetic counseling, 92 %, ultrasound, 67 % and amniocentesis, 88 %; while by those using the triple tests were: genetic counseling, 92%; ultrasound, 58% and amniocentesis, 83%. Specimen MS294 resulted in a negative T18 screen in 100% of the participating labs. The sample was paired to an amniotic fluid specimen (AF294) which had a low AFP level (MOM = 0.21).
MS 295 Wk 20.0	This specimen was obtained from a 29 year old Asian woman (Gravida = 2, Parity = 1) in her 20th week of gestation with a body weight of 120 lbs. She had no personal history of pregnancy complications and her specimen resulted in a negative screen for NTD with no body weight but an ethnic correction indicated. The labs agreed that both Trisomy screens were negative. Specimen MS295 was not paired with an amniotic fluid specimen.

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

Instructional Note:

This notice regards the demographic data provided for the mock patients in the FEDM program. For the sake of this program, it will be understood that gravida indicates the pregnant status of a woman and parity is the state of having given birth to a completed term infant or infants. Thus, a gravida = n, indicates number (n) of times pregnant including the present one; a gravida = 2 indicates that the women was pregnant once before in addition to her present pregnancy. Parity = 1 indicates the patient already has one child; however, multiple birth is also considered as a single parity.

Example: A woman of gravida = 3, parity = 2 indicates that the pregnant woman has been pregnant twice before, and has two children.

2) AMNIOTIC FLUID AFP (NTD-analysis):

N=20; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 291 Wk 20.0	AFP = 8.4 ± 1.1 µg/ml MOM = 1.36 ± 0.19	The AF291 sample was targeted for a screen negative AFAFP value in the upper gestational age screening range. All labs reported this specimen as a screen negative AFAFP value. The AF291 specimen was not paired with maternal serum sample.
AF 292 Wk 19.0	AFP = 24.7 ± 3.7 µg/ml MOM = 3.28 ± 0.56	The AF292 sample was targeted for an elevated AFAFP value in the upper gestational age range. Most labs called AF292 a positive NTD screen for AFAFP specimen. The AF292 sample was matched to maternal serum specimen MS292, whose AFP was moderately elevated (MOM = 1.76). (See critique.)
AF 293 Wk 17.0	AFP = 10.4 ± 1.6 µg/ml MOM = 0.92 ± 0.16	The AF293 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 AF293 specimen was not paired with a maternal serum sample.
AF 294 Wk 18.0	AFP = 1.9 ± 0.2 µg/ml MOM = 0.21 ± 0.03	The AF294 sample was targeted for a low AFAFP value in the routine gestational age screening range. All labs called AF294 a non-elevated specimen for NTD. This AF sample was matched to maternal serum specimen MS294, whose AFP was also low (MOM = 0.59).
AF 295 Wk 20.0	AFP = 6.6 ± 1.0 µg/ml MOM = 1.04 ± 0.11	The AF295 sample was targeted for a negative NTD screen for AFAFP in the upper-gestational age screening window. All labs categorized this as an NTD screen negative specimen. This sample was not matched to a maternal serum specimen.

II. Non-Graded Results Section (will be graded in the next PT):

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 17	**Sample #**	**FT 291**	**FT 292**	**FT 293**	**FT 294**	**FT 295**
	Gestational Age (weeks)	12.4	13.0	10.9	11.9	11.5
Maternal Race	Ethnic Group	Asian	Hispanic	Black	White	Hispanic
Maternal Weight	Pounds (lbs)	130	160	155	150	145
Maternal Age	Years	35	21	32	25	26
Fetal Physical Measurements	Crown Rump Length (mm)	59	67	41	53	48
	NT Thickness (mm)	1.40	1.60	1.10	2.90	1.10
	NT – MOM ± Std. Dev.	0.96 ± 0.06	0.98 ± 0.06	1.04 ± 0.06	2.21 ± 0.12	0.91 ± 0.05
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	62.7 ± 9.3	65.5 ± 10.8	84.5 ± 14.4	145.7 ± 32.0	65.2 ± 12.4
	MOM ± Std. Dev.	0.82 ± 0.10	1.03 ± 0.12	0.97 ± 0.15	1.91 ± 0.27	0.81 ± 0.10
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	4057.9 ± 2782.8	10452.6 ± 7363.7	5900.4 ± 4066.0	3239.1 ± 2221.5	3691.7 ± 2588.6
	MOM ± Std. Dev.	3.38 ± 1.91	8.36 ± 4.90	9.29 ± 5.38	3.78 ± 2.17	4.74 ± 2.68
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (75%)	(-) (100%)
	Recommended Action **	NFA	NFA	NFA	G = 80% U = 33% A = 47% C = 47%	NFA
	Risk Estimate 1 in	10,000	20,000	10,000	177	20,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	NFA
	Risk Estimate 1 in	10,000	10,000	10,000	5,250	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 291 Wk 12.4	This specimen was obtained from a 35 year old Asian woman with a body weight of 130 lbs. Her gestational age at the time of screening was 12.4 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 FT291 risk estimate for both Trisomy-21 and Trisomy-18 was 1 in 10,000.
FT 292 Wk 13.0	This specimen was procured from a 21 year old Hispanic woman of average body weight (160 lbs.). Her gestational age at the time of screening was 13.0 weeks. She had no prior history of any pregnancy complications. This FT specimen was screen negative for Trisomy-21 and all testing labs were in agreement. The FT292 risk estimate for Trisomy-21 was 1 in 20,000, and the Trisomy-18 risk was 1 in 10,000.
FT 293 Wk 10.9	This specimen was obtained from a 32 year old white woman of average body weight (155 lbs.). Her gestational age at the time of screening was 10.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative with all testing labs in agreement. The FT293 risk estimate for Trisomy-21 was 1 in 10,000, and the Trisomy-18 risk was also 1 in 10,000.
FT 294 Wk 11.9	This specimen was procured from a 25 year old white woman of average body weight (150 lbs.). Her gestational age at the time of screening was 11.9 weeks. She had a prior family history of pregnancy complications and adverse outcomes. This FT specimen was screen positive for Trisomy-21 but only 75% of testing labs were in agreement (see Critique). The FT294 risk estimate for Trisomy-21 was 1 in 177, while the Trisomy-18 risk was 1 in 5,250 with 100% of testing labs in agreement that the T18 screen was negative.
FT 295 Wk 11.5	This specimen came from a 26 year old Hispanic woman with a body weight of 145 lbs. Her gestational age at the time of screening was 11.5 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 FT295 was 1 in 20,000, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.

III. Critique and Commentary:

A) Second Trimester Maternal Serum and Amniotic Fluid:

In general, the all-lab results were consistent with the targeted values for the NTD and the Trisomy Screens for risks and outcomes. The Caucasian maternal serum sample **MS292** was targeted as a borderline negative specimen for NTD (Figs. 1 and 3) and was matched to the elevated **AF292** sample (Fig. 2). All but two labs (92%) agreed that specimen **MS292** was screen negative for NTD and negative for both Trisomy screens using the quad test. However, 25% of triple test users called MS292 positive for T21 with further action recommended as genetic counseling 25%; ultrasound, 17%; and amniocentesis, 25%. Possibly, the combination of a moderately elevated MShCG (MOM=1.61) and a low MSuE3 (MOM=0.53) that was not counter-balanced by low DIA (MOM=0.79) may have accounted for this screen assignment. This **MS292** sample was matched with **AF292,** which exhibited elevated AFP, suggesting NTD. A possible explanation for the discrepancy between the MS and AF sample could be that there was a fetal-maternal bleed in the AF sample. To eliminate this possibility, a polyacrylamide gel electrophoresis should be done to show the absence or presence of a diagnostic ACHE band.

Sample **MS294** was obtained from a white woman with a prior sibling history of pregnancy complications. The fetal defect marker MOM values for specimen **MS294** (MSAFP-MOM = 0.59, MSuE3-MOM = 0.49, MShCG-MOM = 2.19, DIA-MOM = 1.76) presented the canonical profile of low MSAFP and low MSuE3, together with elevated MShCG and MSDIA and thus resulted in a T21 positive screen with all labs in agreement (100% by both triple and quad test). The T21 risk was 1 in 30 by triple test and 1 in 18 by quad test (Figs. 5, 6). The recommended further actions for the sample **MS294** were genetic counseling, 92%; ultrasound, 58%; and amniocentesis, 83% from

labs performing the triple screen, and genetic counseling, 92%; ultrasound, 67% and amniocentesis was 88% from labs performing the quad screen.

Two other specimens, **MS293** and **MS295**, produced negative screens for NTD, T21, and T18, with no corrections for body weight or race being indicated.

The **MS291** specimen at 17 weeks was an interesting case involving high levels of MShCG and MSDIA, but normal levels of AFP and uE3, which resulted in a negative screen for NTD, but in a borderline negative screen for T21 and negative for T18. The T21 follow-up actions recommended for specimen **MS291** were genetic counseling, 13%; ultrasound, 8%; amniocentesis, 13%; and repeat testing, 0%. The sample **MS291** was modeled after several literature case studies of pregnant women with breast cancer who exhibited elevated levels of both MShCG and MSDIA (see below Ref #1-3). The patients from these case studies had experienced breast cancer diagnosis and treatment during pregnancy. Several years prior to the present pregnancy, most of the women had completed uncomplicated pregnancies with spontaneous delivery of normal infants of average birth weight. Following the present diagnosis of breast cancer, the women had decided to continue their pregnancies and received treatments including radiation, chemotherapy, and sometimes surgery during the gestation period.

In the above case report of the patient MS291 providing the 2nd trimester specimens, the biomarker test results confirmed the presence of high HCG/DIA in the 17th week of pregnancy. Previous studies had demonstrated that elevated levels of MShCG together with MSDIA often predicted pregnancy complications which could include placental dysfunction, threatened miscarriage, preterm birth, and pre-eclampsia (1, 2, 3, 4). In the case of MS291, the patient had no prior history of pregnancy loss or complications and a paired amniotic fluid was not available for AFAFP and chromosome analysis at time of specimen collection. Although the combination of two elevated analytes plus advanced age (MS291, 35 years) in the triple/quad tests can generate a Down syndrome positive screen (5, 6), this was not the case since AFP and uE3 generated normal MOM values. Thus, elevated MShCG and MSDIA, together with low or normal MOM MSAFP (1.18) and MSuE3 (1.05) could generate a false positive screen for T21 and did so in 8-12% of the screening labs.

Breast cancer (BC) during pregnancy is an uncommon, event, but has been found to occur in 3.8% of all breast cancers in women (7, 8). This type of pregnancy-associated cancer is referred to as Gestational Breast Cancer (GBC). It can occur from 1 in 3,000 to 1 in 10,000 pregnancies; and aside from cervical cancer, GBC is the most common cancer seen during pregnancy (9). Although BCs can occur in young women and premenopausal women, most women with GBC range in age from 30 to 38 years (5). Historically, women with GBC have experienced poor survival rates due to delays in diagnosis and treatment and have a risk of recurrence of 75% (10). GBC has increased in recent years due to: 1) women delaying childbearing to 30 to 40 years of age in order to pursue personal or professional goals; 2) an increased use of mammography; and 3) hormone replacement therapy (5). As expected, pregnancy coincident with BC could lead to additional stress, anxiety, and emotional crisis to the mother; however, no evidence exists that GBC can become more neoplastic during pregnancy. Furthermore, a patient with GBC can often become pregnant again after receiving various therapeutic treatment regimens and future fertility of the woman is not affected (11, 12).

Nulliparous women have been reported to exhibit an increased BC risk proposed to be due to the presence of undifferentiated cells (stem cells) in the adult breast tissue (see below). Moreover, there is an effect of parity and ethnicity reflected in pregnancy biomarker profile studies (hCG, IGF) across ethnic groups exhibiting various incidences of breast cancer (13). Overall, pregnancy hormone levels were found to be higher in Hispanic/Black than in White/Asian women (14). In general, bearing children prior to age 28 has been shown to have a “protective” effect against BC, thought to be due to exposure to hCG, which serves to promote differentiation of breast progenitor (stem) cells. Clinical epidemiological studies have shown that such protection can confer a 40% reduction of lifetime risk of getting BC (15). Interestingly, mothers giving birth to a Down Syndrome baby (elevated hCG and Inhibin (DIA), decreased AFP and uE3 display no such protection from risk of BC; the reason for this is not known (5, 6). Also, no protection against BC is observed if the pregnancy is aborted, or terminated early (16). In a study of 2,216 cases, the risk of getting BC was further correlated with placental size following two full-term completed pregnancies (17). It was found that the larger the placental size, the greater the risk of BC possibly due to the increased breast vascularization and angiogenesis (18). In this regard, it is noteworthy that hCG has been reported to be an angiogenic factor in breast growth during normal pregnancy by hCG inducing increased synthesis of Vascular Endothelial Growth Factor (VEGF); this might also apply to BC cell growth during pregnancy (18, 19).

The biomarkers studied during pregnancy include hCG and prolactin which can affect cell differentiation, while AFP, IGF-I, and IGF-II can influence cell proliferation (1). Cell surface receptors for hCG in the placenta are also increased during normal pregnancy as they do in epithelial cells of breast carcinomas during pregnancy (16, 20). In relation to other steroid and peptide hormone receptors and proteins, GBC cells have been reported to be both estrogen receptor (ER) and progesterone receptor (PR) negative, while levels of c-erbB2 and E-cadherin were noted to increase in GBC tissues. These receptor and growth factor increases occurred together with elevated levels of Inhibin-A (DIA), Mucin-1, IGF-1, IGF-BP, cAMP, and Protein Kinase-A; in contrast, decreased levels of Transforming Growth Factor (TGF) were reported (16, 20, 21, 22).

The proposed mechanism of BC protection by hCG and Inhibin has been extensively studied and described (16, 23, 24). Several investigator groups, using clinical cases and human BC cell culture lines, have proposed that hCG confers a protective effect against BC in that it prevents and/or reduces normal-to-cancer cell transformation by increasing differentiation of adult breast cells (16, 25). It has been reported that hCG promotes lobular, ductal, and interlobular breast cell differentiation, reduces breast cell proliferation, and increases the process of BC cell apoptosis, while inducing the synthesis and secretion of ovarian inhibin A and B (23, 24, 25). HCG was already known to stimulate the ovary to increase expression and production of Inhibins, which have a powerful differentiating effect on breast tissue (23). In the overall protection against BC in women, hCG reduces breast cell proliferation, promotes the synthesis of new gene product (e.g. Inhibin), and initiates the differentiation of the mammary epithelium during pregnancy; these actions result in the accumulation of cells in the G1 phase of the cell cycle and a reduction in S-phase activity (23). As discussed above, once stimulated by hCG, hCG receptor concentrations increase in the placenta and the hCG receptor stimulates cAMP and protein Kinase A production. This action induces the immediate early phase response genes c-fos/c-jun (i.e. AP-1 complex), which regulate target gene expression. HCG further induces the production of p53 and bcl-2, which increases the apoptotic process in abnormal breast cells (23, 24).

As discussed above, the inhibition of mammary tumorigenesis by hCG has been ascribed to an increased expression of Inhibins (26). Inhibins are a family of heterodimeric proteins produced by ovarian granulosal cells and have structural homology to the Transforming Growth Factor family and to the Mulherian Inhibiting Substance (MIS) (16, 23). The physiological primary role of Inhibin-A is to inhibit FSH secretion at the level of the pituitary; such action contributes to preventing ovulation during pregnancy (27). In animal models, the incidence of interductal proliferations, ductal cell carcinomas in situ, and palpable tumors are markedly reduced in the presence of high levels of Inhibins A and B. Treatment with hCG elevates the levels of Inhibin A and B mRNAs as well as c-myc and c-jun in mouse models. It was found that hCG, accompanied by Inhibin production, induced histone acetylation in human breast epithelial cells in culture by controlling gene transcription (i.e. TRPM2, ICE) (28). Using MCF-10F breast cells in culture, the authors detected elevated levels of Histone-H3 and H4 after exposure to hCG and Inhibins and such action is predicted to occur in patients' breast cancer cells. Overall, the above study ascertained that the hCG-induced production of inhibins and subsequent gene activation was associated with both mammary cell differentiation and subsequent breast tumor cell regression.

Concerning histopathology, no differences were found in GBC patients versus non-pregnant patients (19). Stage-II and III, rather than Stage-I are more commonly observed in GBC patients due to the delays in diagnosis described above (10, 21). In cases of GBC, 79% of the breast cancer cells were found localized in lobular regions versus only 15% in non-pregnant BC patients; moreover 80% were of Grade-III versus 33% in non-pregnant breast cancers (22). Often, GBC exhibits more advanced stages (i.e. Stages II and III) than in non-pregnant patients because BC growth time has already been several years in duration (9, 21).

In the therapeutic treatment of women with GBC, much is knowledge and experience has been gained. Within a few weeks of the onset of pregnancy, certain interductal epithelial cells begin to multiply and form intralobular ducts and lobules (29). Such breast tissue (germinal) areas are more susceptible to hormonal stimulation than other cells within the lobular/ductal systems. This proliferation phase occurs largely during the first half of pregnancy, after which time the mature breast cells are maintained until parturition (8). It is during this "hormone sensitive" time period in early pregnancy that GBC cells receive their enhanced growth signals to proliferate. However, drug therapy during the first trimester once thought to be preferred, is now recommended to the second and third trimesters. Chemotherapy in these periods has been reported to result in stillbirth, preterm birth, IUGR, and low birth weight infants (21, 30, 32). Chemotherapy drugs such as doxorubicin have long been known to cause cardiovascular damage along with methotrexate but, drugs such as tamoxifen, fluorouracil, adriamycin, and cytoxan

have been employed during pregnancy (21, 30). The aim of present medical care is to preserve the integrity of the breast during pregnancy. Since the GBCs are ER and PR negative, pregnancy hormones do not further stimulate growth of the BC, i.e., the breast tumor is hormone insensitive; however, the germinal "stem" breast cells could still respond to hormones such as hCG, Inhibin, TGF, and others (31).

Concerning other treatment and diagnostic modalities, mammographies have been reported to be difficult to interpret due to increased fluid accumulations in the breast during pregnancy. However, fine needle biopsies can be performed after the first trimester (31), but radiation is avoided and should be recommended after a birth has occurred. It has also been recommended that chemotherapy be administered during the second and third trimesters if necessary (9, 32). Chemotherapy is largely not dangerous, but has been shown to result, on occasions, in adverse pregnancy outcomes such as intra-uterine growth retardation, pre-term birth, fetal death, and low birth weight (32). Treatments utilizing surgery (modified radical mastectomy) followed by regional radiation (avoiding chest wall irradiation) has been successfully reported (21, 32, 33). Surgery during pregnancy can be performed if pregnancy is near term (3, 4). Overall, cancer management has been found to be no different than in non-pregnant patients (32). Finally, removal of the ovary is no longer recommended since no benefit in outcome has been derived (8, 33).

Although GBC was once thought to be an incurable disease of the pregnant women, this outlook has changed in recent times in the clinical community (10). It is true, however, that pregnancy can impede cancer detection, resulting in low 5-year and 10-year survival times (19); further a risk of recurrence of 75% is seen due to delays in cancer diagnosis, staging, and treatments (8, 10, 29). Unfortunately, changes in breast anatomy and physiology during pregnancy (i.e. tenderness, increased size and vascularization, nodule formation) can mask breast lump formation, and fibroadenomas can enlarge because they rapidly respond to increases in circulating pregnancy hormone levels (35). The adverse effects of the delays in diagnosis, staging, and imaging have been seen to decrease due to more aggressive patient management of GBC with biopsies, surgery (lumpectomy), radiation, and chemotherapy being practiced during the second and third trimesters (36). However, it was observed that women with GBC were 2.5 times more likely to exhibit metastatic disease than non-pregnant women with BC, have a higher risk of late stage BC, because they were often not diagnosed until the 5^{th} to 7^{th} month of pregnancy. Although pregnancy can have an adverse effect on BC, the prognosis for women with GBC is no different than in non-pregnant BC patients (10). The risk of mortality of women with GBC is only greater if an enlarged tumor mass (diameter) is found and when a large number of metastatic auxiliary lymph nodes are involved (32, 37). In most studies, it was determined that 1) the pregnancies of GBC patients should not be medically terminated, but that early birth could be induced at 37 weeks; 2) pregnancy has not been found to stimulate enhanced growth of the tumor; 3) therapeutic treatment measures should not be delayed or altered; and 4) therapy during pregnancy does not affect the future fertility of the woman (11, 38). Finally, it has been reported that birth defects and pregnancy complications did not differ between GBC and non-pregnant patients.

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

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in a bar-graph format (Figs. 7- 10). As shown in Figs. 7A and 8A, AFP and uE3 mass measurements in serum among the individual kits mostly agreed. In contrast, when the kit specific uE3 MOMs were compared, values from Siemens DPC Immulite 2000/2500 ranged from 20 to 30% higher than those from Beckman (Fig. 8B); however preliminary studies in our lab suggest this may derive from a matrix effect in our samples. Regarding the hCG kits (Fig. 10), the Beckman Access 2 instrument results were about 5% higher than those from Beckman UNICEL, while the Siemens Immulite 2000 results were 15-20% lower than those from the other assay platforms. These differences were also reflected in the MOM values. Finally, the method comparison for Inhibin-A displayed in Fig. 9A shows that the results from the Beckman Access/2 or UNICEL were similar whereas the Diagnostic Systems Lab (DSL) assay platform results were 20-25% lower, which is also reflected in the Inhibin MOM values (Fig. 9B).

Interestingly, when the AFP mass measurements in amniotic fluid were compared, the differences among the various methods appeared somewhat variable (Fig. 7C), while AFAFP MOM values (Fig.7D) were mostly the same throughout. In particular, AF-mass value results from the Abbott Axsym and Siemens Immulite 2000 were 10-20% higher than those from the Beckman UNICEL and Access 2 instruments. Since these specimens are derived from actual AF samples, these differences would be comparable to real patient testing**.**

C) Second Trimester Screening Software Utilized:

The alpha and Benetech PRA software packages were each used by 26.9% and 23.1%, of the labs, respectively; Benetch was 3.8%, Robert Maciel (RMA) software was employed by 30.8%; and in-house and "other" softwares comprised 15.4%. Labs using 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; however, the laboratory results will **not** be graded at this time. **Starting with the next mail-out in May 2013 first trimester results will be 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.

The all lab measurement of the 12.4 week 35 year old Asian **FT291** specimen for total hCG resulted in a mass mean of 62.7 $\pm$ 9.3 IU/ml, with a MOM of 0.82 $\pm$ 0.10; the all-lab mass mean for PAPP-A was 4057.9 $\pm$ 2782.8 ng/ml with a MOM of 3.38 $\pm$ 1.91. The **FT291** sample displayed a T21 negative screen assessment. As a result, the all-lab T21 risk assessment for **FT291** was 1 in 10,000 (Fig. 13). Further action was not indicated. Finally, 100 % of labs considered the **FT291** specimen screen negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Fig.14).

The **FT292** specimen was obtained from a 21 year old Hispanic woman with a gestational age of 13.0 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 65.5 $\pm$ 10.8 IU/ml (MOM = 1.03 $\pm$ 0.12); the all-lab PAPP-A mass measurement was 10,452.6 $\pm$ 7563.7 ng/ml (MOM = 8.38 $\pm$ 4.90). The all-lab T21 screen consensus for **FT292** was negative with a risk assessment of 1 in 20,000 (Fig. 13). No further actions were recommended by the labs. Finally, the **FT292** specimen also screened negative for T18 (1 in 10,000 Fig. 14).

The all lab measurement of the 10.9 week specimen **FT293** was obtained from a 32 year old (155 lbs.) Black woman. Total hCG measurement resulted in a mass mean of 84.5 IU/ml $\pm$ 14.4, with a MOM of 0.97 $\pm$ 0.15. In addition, the all-lab mass mean for PAPP-A was 5900.4 $\pm$ 4066.0 ng/ml with a MOM of 9.27 $\pm$ 5.38. This resulted in an all-lab T21 risk assessment of 1 in 10,000 for the **FT293** specimen and a negative screen (Fig. 13) assessment together with a negative T18 risk assessment of 1 in 10,000 (Fig. 14).

In the **FT294 (**25 year old) White specimen, the gestational age all-lab mean was reported as 11.9 weeks. Assay measurements for **FT294** resulted in an all-lab total hCG mass measurement of 145.7 $\pm$ 32.0 IU/ml (MOM = 1.91 $\pm$ 0.27), while the all-lab PAPP-A mass assessment was 3239.1 $\pm$ 2221.5 ng/ml (MOM = 3.78 $\pm$ 2.17). All labs agreed that the **FT294** sample was screen positive for T21 with a risk of 1 in 177 (Fig. 13) and negative screen for T18 with a risk assessment of 1 in 5,250 (Fig. 14). However, only 75% of labs reported a positive screen for T21, which was just below the 80% required for a consensus. Further action was reported as: genetic counseling, 80%; ultrasound, 33%; amniocentesis, 47%; chorionic villi sampling, 47%.

For the 26 year old (145 lbs) Hispanic **FT295** specimen, the gestational age all-lab mean was reported as 11.5 weeks. Assay measurements resulted in an all-lab total hCG concentration of 65.2 $\pm$ 12.4 IU/ml (MOM = 0.81 $\pm$ 0.10), while the all-lab PAPP-A concentration was 3691.7 $\pm$ 2588.6 ng/ml (MOM = 4.74 $\pm$ 2.68). The all-lab FT T21 risk assessment was 1 in 20,000 and all labs agreed that the **FT295** sample was negative for T21 (Fig. 13). Similarly, the **FT295** specimen was also screen negative for T18 with an all-lab risk assessment of 1 in 10,000 (Fig.14).

D. 1.) First Trimester Assay Kit Performance:

In order to compare the 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 0.9886, a slope of 0.4228 and a Y intercept of -0.0556 (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 measurements by Beckman Access/2 were ~10-15% higher than those by Beckman UNICEL, while the Siemens Immulite DPC instruments measured approximately 20% below the Beckman Access 2/UNICEL instruments. Overall, the hCG MoM values reflected the mass values but the differences were somewhat diminished (Fig. 11B). The results from the three PAPP-A kits, when converted to the same mass units, were not consistent among each other (Fig. 12A). The Beckman UNICEL PAPP-A was less than 30% that of Siemens Immulite 2000, while Anshlite was 25% lower than Beckman UNICEL. In comparison, when the PAPP-A kit MOMs were compared, Siemens Immulite 2000 were more than double those from Anshlite and Beckman (Fig. 12B).

E) First Trimester Screening Software Utilized:

The alpha and Benetech software packages were each used by 31% and 19% of the labs, respectively; Robert Maciel (RMA) software was employed by 31%; and in-house software comprised 19%. None of the labs used programs classified as "other" which are proprietary software packages.

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Teachings on Alpha-fetoprotein

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Can Prenatal Screening for Fetal Alcohol Spectrum Disorder Be Justified?
A Commentary

Abstract

Fetal alcohol spectrum disorder (FASD) is the leading cause of non-genetic mental retardation in the USA possibly exceeding even Down Syndrome, which is currently approaching 1 in 500 livebirths. Alcohol consumption during pregnancy results in brain, cranio-facial and heart defects, neurotoxicity, and immune dysfunction. The preferred action taken to prevent alcohol consumption during pregnancy is abstinence. However, the detection, diagnosis, and treatment of FASD remains a major public health need in this country and throughout the world. The biochemical molecules involved in the developmental anomalities encompass a vast array of signal transduction and synaptic pathways which involve neurotransmittors and neurotrophic peptides. Recent advances in medicine-based therapies for FASD have been reported, and include the use of small molecule agonists, antagonists, and competitive inhibitors. Since biomarkers for FASD have previously been identified in clinical research reports, multi-center screening feasibility studies now seem warranted and could be initiated following adequate funding, protocols, procedures, and institutional review board approvals.

Commentary

One of the leading preventable causes of acquired mental retardation in the western world is Fetal Alcohol Spectrum Disorder (FASD), a cluster disorder of alcohol-related birth defects and learning deficits (1). This non-inherited (acquired) form of developmental brain delays and mental deficiencies can range in incidence from 2 to 3 affected per 1000 livebirths in some geographic areas of the United States (2). In Canada and other parts of the world(France) , incidence has been estimated at 9.1 per 1000 livebirths (3). Alcohol is a teratogen that targets the brain, heart, skeletal bones, and lymporeticular-hematopoietic systems. The brain impairments result in permanent lifelong developmental problems such as intelligence, learning/cognition, memory, speech, vision, and attention-span (4). The FASD-related mental deficiencies can be attenuated if detected early in pregnancy with mothers undergoing counseling and voluntary abstinence (5). Unfortunately, FASD and its less severe effects are typically diagnosed later in childhood (3 – 5 years of age) even though earlier identification would allow interventions and

mitigation of further mental retardation. Thus, the diagnosis and detection of FASD constitute a major public health need in this country and throughout the world. Nonetheless, the most preferred action taken to prevent FASD would be alcohol abstinence by pregnant women via motivational intervention.

The anatomical birth defects in the affected fetus can encompass a wide range of neural and anatomical (structural) anomalies. These can include: cerebellar hypoplasia, microencephaly, cortical thinning, reduced opening at the brain roof/floor palate of the mouth, post-axial septum derangement, altered cerebral cortex and cerebellum, decreased neuron outgrowth and branching, and defective synaptic circuitry (6,7).

The biomolecules affected during pregnancy can encompass the following: gamma amino butryic acid (GABA), receptor alpha-5 subunit, serotonin 5HT1A receptor, glutamate transporter, GSK-3 beta; the homeoproteins PAX-6, SOX-3, and PAX-5, cFOS/cJUNB transcription factors, glial-derived neurotrophic factor (GDNF), sonic hedgehog, vasointestinal peptide (VIP), protein kinase-C (PKC) Family, nerve growth factor (NGF), glutamate receptor, and neurotrophic/protective peptide growth factors (8,9).

In lieu of the potential treatments of FASD looming at the threshold of therapeutic utility discussed below , a rationale for future implementation of FASD prenatal population screening can be presented. Maternal screening biomarkers(analtyes) for the identification and prediction of FASD pregnancies have long been individually investigated, but have not been presently utilized in tri- or quad- combinations. Various biomarkers for FASD have been reported in the biomedical literature, some of which are similar to current neural tube defect (NTD) and Down Sydrome (DS) screening programs. Biomarkers shown to be useful in predicting FASD pregnancies have included alpha-fetoprotein (AFP) and various estrogens. Low maternal levels of both unconjugated estriol (uE3) and estradiol (E2) have been shown to have some FASD predictive value. Even though uE3 was useful for FAS screening, E2 was found to be superior and should be utilized in place of uE3. Unlike DS, human chorionic gonadotrophin (hCG) had no value in identifying FAS pregnancies. Reduced maternal serum concentrations of both pregnancy-specific beta-1-glycoprotein (SP-1) and sex hormone binding globulin (SHBG) have also showed predictive utility in human clinical research studies. The biomarker AFP (low levels) showed a 59% predictive value and 2.46 relative risk, while SP-1 had a predictive value of 56% and 3.29 relative risk. AFP has further been reported to be down-regulated by proteonomic analysis of amniotic fluid proteins in FAS animal models (10). SP-1, a member of the carcinoembryonic (CEA) gene family involved in cell adhesion, was found to exhibit low maternal serum levels in FAS pregnancies in contra-distinction to its elevated levels in DS. Using DS as a sreening model, suggested candidates for a quad screening feasibility study of FAS might consist of low maternal serum profiles of AFP, SP-1,

E2 , and SHBG in second trimester pregnancies (11,12). Such a screening profile would have no overlap with the present quad test employed for either Down syndrome or Trisomy-18. Additional biomarkers worthy of further investigation might also include pro-opiomelanocortin peptides, 5HT1A, brain-derived neurotrophic factor, neurotrophic growth Factor, neuroprotective factor, and S100 beta protein (13).

Even though first trimester FASD screening would be more favorable, maternal screening would have to be enacted in the second trimester due to non-detectable levels of the above analytes during the first trimester (11). Moreover, the quad biomarker screen would not specifically identify FASD from other syndromes; rather , prenatal screening would be able to identify a population of pregnant women at risk for giving birth to an infant with FASD.A screening algorthym for mothers at risk for Down syndrome was previously developed and implemented with noteworthy success; a similar equation taking into account race, maternal age and weight, diabetic status,and gestational age(possibly alcohol weekly intake) could be designed for a FASD screening program. Since prenatal screening of FASD has not progressed beyond the clinical research stages, detection and false- positive rates are not known other than the predictive values and relative risks stated above. Finally, confirmed alcohol consumption during pregnancy would require interview/questionaire documentation by the attending obstetrician , nurse or social worker.To this end, brief questionaires (CAGE, AUDIT, T-ACE., Tweak) have been developed and are currently in place in some clinical locations.

The initiation of maternal serum screening programs for FASD has not yet appeared in the medical community possibly due to a lack of effective postnatal clinical treatments and medicine-based therapies. However, recent advancements at the threshold of development, encompassing three groups of potential FASD-therapeutic agents, have been reported to aid in attenuating FAS and its related mental disorders (14,15). Such agents include 1) small molecule drugs; 2) brain-derived neuropeptides; and 3) nutritional dietary supplements. Potential small molecule class of drugs include: Neurokinin-1 Receptor (NK1R) antagonist LY686017; Sonic Hedgehog pathway agonist (SAG1.1;8, a diaminocyclo-hexane); Serotonin 1A Agonist (ipsapirone, buspirone); superoxide dismutase/catalase mimetic (EUK-134); N-Methyl D-Aspartate (NMDA) receptor modifiers (MK-801, eliprodil); and a phosphodiesterase type-1 inhibitor (vinpocetine). The goal would be to administer pregnant women affected with an FASD fetus, small molecule drugs or peptides (see below),which might mitigate alcohol-induced learning deficits and to increase neurotransmitter receptor expression (14,15). Presently, few if any of the therapeutic small molecule drugs can be recommended for use and the idea may seem premature; however, a time may soon come to consider their potential use.

The second group of therapeutic agents are represented by the glial-derived activity-dependent and neuroprotective (neuotrophic) peptides induced by compounds such as VIP, 5-HT, basic fibroblast growth factor (bFGF), and neurotrophic growth factor (NTGF). Some of these peptide fragments (8-9 amino acids) are capable of preventing further alcohol-induced mental decline, developmental delays, and to enhance learning (16). In the future, it may be possible to administer to FASD pregnant women and/or newborns, small molecule drugs or short neuropeptides to protect, attenuate, and mitigate alcohol-induced mental decline, learning deficits, and neuronal cell death. The neuropeptides have been orally administered in pregnant animal models, were active in picomolar concentrations, and were non-chiral interacting sterochemical isomers (17). Finally, the third group of therapies include the nutritional (diet) supplements such as zinc, copper, fish oils, folic acid,thiamine, and antioxidants (vitamin E) to reduce oxidative stress due to formation of reactive oxygen species (ROS), and to reduce neuronal damage and cell death (18).

In summary, the above discourse suggests the need for screening feasibility studies to investigate specific biomarkers to detect alcohol abuse during early pregnancy. Early detection/identification of FAS pregnancy through prenatal screening could allow for societal interventions and clinical therapies that might diminish or ameliorate brain, bone, and structural damage due to intra-uterine alcohol exposure. Prenatal FASD screening could result in physicians providing follow-up questionaires and perinatal consulting referrals for the afflicted mother; these would inform her of the consequences of further alcohol consumption together with the offer of informational/educational aids. Since FASD continues into adult life costing 1.0 million dollars per incident case (19), the lifetime cost-benefit effect could be enormous if approximately 3000 FASD annual livebirths do indeed occur in the USA (20). Until pilot screening studies are conducted, FASD will remain one of the most preventable and under-diagnosed, pregnancy disorders in the obstetrical community.

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ABSTRACTS

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

(1) Title: Elevated maternal serum alpha-fetoprotein level in a fetus with Beckwith-Wiedemann syndrome in the second trimester of pregnancy.

Source: J Prenat Med 6:7-9, 2012.

Authors: Guanciali-Franchi P, Di Luzio L, Iezzi I, Celentano C, Matarrelli B, Liberati M, Palka G.

Abstract: BACKGROUND: Beckwith-Wiedemann syndrome (BWS) is a rare disorder characterized by macrosomia, macroglossia, visceromegaly, and omphalocele and an increased risk of growing tumors. Prenatal and postnatal high levels of serum alpha-fetoprotein are associated with several diseases and neoplasms including hepatoblastomas and other hepatic tumors. The diagnosis of BWS is usually made in the postnatal period on the basis of physical exam features and hypermethylation of the H19 gene. CASE: A 30-year-old woman gravida 3, para 2, underwent maternal serum screening at 15 weeks' gestation. The screening was negative for Down's syn drome (risk 1/6085), but positive for NTDs. Further ultrasound examination at 20 and 30 weeks' evidenced a fetal overgrowth and a 3-D scan at 33 weeks' gestation presented a protruding tongue, and a fixed opened mouth caused by macroglossia. CONCLUSIONS: BWS was suspected on the basis of clinical features, and molecular analysis of critical region 11p15.5 revealing the hypermethylation of H19 gene supported the diagnosis.

(2) Title: Management strategy in pregnancies with elevated second-trimester maternal serum alpha-fetoprotein based on a second assay.

Source: Am J Obstet Gynecol, 2013.

Authors: Spaggiari E, Ruas M, Dreux S, Valat AS, Czerkiewicz I, Guimiot F, Schmitz T, Delezoide AL, Muller F.

Abstract: OBJECTIVE: To assess maternal-fetal outcomes in pregnancies associated with persistently elevated second-trimester maternal serum alpha-fetoprotein. STUDY DESIGN: A retrospective cohort study in 658 patients with maternal serum alpha-fetoprotein >/=2.5 multiple of median, performed at routine Down syndrome screening. Maternal serum alpha-fetoprotein was assayed a second time in 341 of them. Outcomes were recorded in all cases. RESULTS: The group with unexplained maternal serum alpha-fetoprotein persistently >/=2.5 multiple of median was associated with more pregnancy complications 37 of 92 (40.2%) as fetal death, preeclampsia, intrauterine growth restriction, and congenital nephrotic syndrome, compared with the group with maternal serum alpha-fetoprotein that returned to a normal level 37 of 226 (16.4%) ($P < .001$). CONCLUSION: When maternal serum alpha-fetoprotein returns to a normal level on a second assay, the risk of adverse outcome significantly decreases, but these pregnancies are still at risk of complications and therefore need close surveillance. Repeat maternal serum alpha-fetoprotein assay allows identification of patients who should be offered amniocentesis to evaluate the risk of nephrotic syndrome and epidermolysis bullosa. Alpha-fetoprotein should be monitored in pregnancies associated with unexplained high maternal serum alpha-fetoprotein. A management strategy based on ultrasound examination, second maternal serum alpha-fetoprotein assay and amniocentesis is proposed to improve prenatal counseling and management of such pregnancies. However, a prospective study remains necessary to evaluate it.

(3) Title: The effect of hereditary versus acquired thrombophilia on triple test Down's syndrome screening.

Source: Prenat Diagn:1-5, 2013.

Authors: Frank M, Maymon R, Wiener Y, Neeman O, Kurzweil Y, Bar J.

Abstract: OBJECTIVE: To compare the profile of mid gestation triple test serum markers between a cohort of women with history of pregnancy complications with hereditary versus acquired thrombophilia. All were treated with low molecular weight heparin (LMWH) prior to 12 weeks' gestation. METHODS: A retrospective analysis of second trimester maternal serum screening results for Down syndrome was performed comparing women with inherited versus acquired thrombophilia, all treated with LMWH. The test results were calculated from the combination of triple serum markers and maternal age, and expressed as a multiple of the gestation normal medians (MoM). Results in the study groups were compared with MoM values calculated from our local population (controls). RESULTS: The median human chorionic gonadotropin (hCG) level was higher only in the acquired thrombophilia group (N = 47) as compared with the control group (1.3 vs. 0.99 MoM, P = 0.005), and not different between the hereditary thrombophilia group (N = 60) (1.1 MoM) and the control group. Alpha-fetoprotein and unconjugated estriol MoMs did not differ between women with inherited (0.95, 0.97), acquired thrombophilia (0.99, 0.90), and controls (1.01, 0.98), respectively. CONCLUSION: In the interpretation of second trimester maternal serum screening, consideration should be given to the higher hCG maternal serum levels that may occur in women with acquired thrombophilia, even those treated early in pregnancy with LMWH. The higher hCG serum levels may signal the possibility of placental dysfunction, rather than fetal aneuploidy (c) 2013 John Wiley & Sons, Ltd.

(4) Title: Maternal serum second trimester screening for chromosomal disorders and neural tube defects in a government hospital of North India.

Source: Prenat Diagn 32:1192-1196, 2012.

Authors: Kaur G, Srivastav J, Kaur A, Huria A, Goel P, Kaur R, Kataria S, Chavan BS, Kochhar S, Aggarwal P, Kaur N, Panigrahi I, Chawla P.

Abstract: OBJECTIVE: Down syndrome (DS) has major resource implications especially in developing countries being third most important cause of mental handicap. Maternal serum screening for chromosomal aneuploidies and neural tube defects (NTDs) is practiced worldwide in many countries and has been integrated into mainstream health care, while it is gradually gaining momentum in Asian countries. METHODS: This prospective cohort study was carried out in pregnant women undergoing triple screening test between January 2007 and December 2010 after informed consent. Biomarkers alpha-fetoprotein, human-chorionic-gonadotropin and unconjugated-estriol were tested, and risk of pregnancy being affected with DS, Edward's syndrome or NTDs were calculated. Screen-positive patients were referred for detailed ultrasonography and confirmatory amniocentesis. Follow-up record was maintained until delivery. RESULTS: Of 7400 pregnant women enrolled, 419(5.7%) were screen-positive, including 339 positive for DS, two for trisomy 18, and 62 for NTDs. Total eight cases of DS were eventually diagnosed in the population (prevalence of DS = 1 : 925), seven of which were detected in utero following diagnostic evaluation for positive serum screen (DR of DS screen = 87.5%). Total five cases of NTD were observed, yielding NTD prevalence of 0.67/1000. CONCLUSIONS: Triple screening in the second trimester is reasonably effective for the detection of major chromosomal defects and NTDs, and can be implemented successfully also in India.

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

(1) Title: Elevated alpha-fetoprotein levels in Van Wyk-Grumbach syndrome: a case report and review of literature.

Source: J Pediatr Endocrinol Metab 25:761-767, 2012.

Authors: Patni N, Cervantes LF, Diaz A.

Abstract: The association between primary hypothyroidism and precocious puberty secondary to ovarian hyperstimulation has been recognized for over a century. Here, we report the case of a 9-year-old girl with severe primary hypothyroidism, who presented with premature menarche, enlarged pituitary gland, enlarged ovaries with multiple cysts, and elevated prolactin and alpha-feto protein levels. Pituitary and ovarian radiology findings, and alpha-feto protein levels normalized a few weeks after hypothyroidism treatment was started. Reviewing the literature we found several reports of increased levels of tumor markers in girls with this association. Thyroid function tests should be always part of the evaluation of patients with precocious puberty especially if the bone age is delayed. Tumor markers and liver function tests may be abnormal in patients with severe hypothyroidism and improve soon after thyroid hormone replacement is started.

(2) Title: Second trimester maternal serum markers and a predictive model for predicting fetal hemoglobin Bart's disease.

Source: J Matern Fetal Neonatal Med 26:146-149, 2013.

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

Abstract: Objective: To compare the levels of maternal serum alpha-fetoprotein (AFP), unconjugated estriol (uE3) and free beta-human chorionic gonadotropin (free beta-hCG) between pregnancies with fetal Hb Bart's disease and unaffected pregnancies. Methods: 148 pregnancies at risk of fetal Hb Bart's disease scheduled for cordocentesis at 18 to 22 weeks were prospectively recruited into the study. AFP, uE3 and free beta-hCG concentrations were measured before cordocentesis and the final fetal diagnosis of Hb Bart's disease was based on fetal Hb typing using high-performance liquid chromatography. Results: AFP and free beta-hCG were significantly higher whereas uE3 was lower in women with fetal Hb Bart's disease than those with unaffected fetuses (1.94 MoM, 1.38 MoM and 0.81 MoM respectively). Hb Bart's predictive model; probability = 1/1+e(-[2.876 + 1.333(AFP) - 6.310(uE3)]), effectively predicted fetal Hb Bart's disease (AUC ROC 0.91, 95% CI 0.84-0.97) with 61.5% sensitivity and 98.1% specificity using a cut-off probability at greater than 0.5. Conclusions: In triple test, serum AFP and hCG levels are significantly higher while serum uE3 is significantly lower in pregnancies with fetal Hb Bart's disease. Hb Bart's predictive model included AFP and uE3 is relatively effective and may be helpful in Hb Bart's prenatal screening.

(3) Title: Maternal and fetal tyrosinemia type I.

Source: J Inherit Metab Dis, 2012.

Authors: Garcia Segarra N, Roche S, Imbard A, Benoist JF, Greneche MO, Davit-Spraul A, Ogier de Baulny H.

Abstract: A 22 year-old woman with tyrosinemia type I (HT1) married her first cousin who is heterozygous for the same FAH mutation for which the patient is homozygous. During her pregnancy she was treated with diet (prescribed tyrosine intake 300 mg/day), and nitisinone (60 mg/day). Median plasma tyrosine levels were 560 mumol/L (range: 375-838, n = 21) and nitisinone 51 mumol/L (range: 41-57, n = 3) during pregnancy. She gave birth to a clinically healthy girl affected with tyrosinemia type 1. Birth was normal (birth weight 2615 g) and the baby had normal liver

function, normal plasma alpha-fetoprotein concentrations, low urinary excretion of phenolic acids and no detectable succinylacetone. At birth, the baby had hypertyrosinemia (860 mumol/L in blood cord) and nitisinone levels of 14 mumol/L. Following molecular confirmation of the diagnosis of HT1 specific treatment began on day 15 by which time she had detectable urinary succinylacetone.

(4) Title: Hepatoblastoma in the neonatal period: An unusual presentation.

Source: J Cytol 29:252-254, 2012.

Authors: Chattopadhyay S, Mukherjee S, Boler A, Sharma A, Biswas SK.

Abstract: Hepatoblastoma (HBL) is a rare primary malignant liver tumor affecting mainly pediatric patients in the age group 6 months to 3 years. Presentation of HBL in the neonatal period is rare. HBL can be diagnosed on cytology along with subtyping. Estimation of serum alpha-fetoprotein (AFP) is essential as a tumor marker. Fetal type HBL usually shows high AFP level. In this report, diagnosis of HBL in a 10-day-old baby with low serum AFP is being described for its unusual presentation.

(5) Title: Non-Hodgkin B-cell Lymphoma of the Ovary in a Child with Ataxia-Telangiectasia.

Source: J Pediatr Adolesc Gynecol, 2013.

Authors: Danby CS, Allen L, Moharir MD, Weitzman S, Dumont T.

Abstract: Ataxia-telangiectasia (AT) is a multisystem, life-limiting, recessively inherited genetic disorder caused by mutations in the AT mutated gene. It is characterized by the onset of changes in neurological and immunological development, organ maturation in childhood, as well as a high incidence of malignancies. We describe a case of an 11-year-old girl with a history of progressive ataxia and new finding of bilateral pelvic masses. Given an elevated alpha-fetoprotein, the pre-operative working diagnosis was a malignant germ cell tumor. Final ovarian pathology revealed a non-Hodgkin B-cell lymphoma with Burkitt-like morphology. We present the first case of a primary ovarian non-Hodgkin B-cell lymphoma in a child with AT.

C) News of Note: Abstracts of New Markers:

(1) Title: Midtrimester Fetal Herpes Simplex-2 Diagnosis by Serology, Culture and Quantitative Polymerase Chain Reaction.

Source: Fetal Diagn Ther, 2012.

Authors: Curtin WM, Menegus MA, Patru MM, Peterson CJ, Metlay LA, Mooney RA, Stanwood NL, Scheible AL, Dorgan A.

Abstract: The acquisition of herpes simplex virus (HSV) in utero comprises a minority of neonatal herpes infections. Prenatal diagnosis is rare. We describe a midtrimester diagnosis of fetal HSV-2 infection. Ultrasound at 20 weeks for elevated maternal serum alpha-fetoprotein (MSAFP) showed lagging fetal growth, echogenic bowel, echogenic myocardium, and liver with a mottled pattern of echogenicity. Amniocentesis demonstrated normal karyotype, elevated AFP and positive acetylcholinesterase. Culture isolated HSV-2 with an aberrant growth pattern. Maternal serology was positive for HSV-2. Quantitative DNA polymerase chain reaction (PCR) showed 59 million copies/ml. Fetal autopsy demonstrated widespread tissue necrosis but only sparse HSV-2 inclusions. Fetal HSV-2 infection can be suspected when an elevated MSAFP accompanies ultrasound findings suggesting perinatal infection. Maternal HSV serology, amniotic fluid culture and quantitative PCR are recommended for diagnostic certainty and counseling.

(2) Title: Assessment of fetomaternal hemorrhage by kleihauer-betke test, flow cytometry and alpha-fetoprotein after invasive obstetric procedures.

Source: Clin Exp Obstet Gynecol 39:303-306, 2012.

Authors: Meleti D, Caetano AC, Boute T, de Oliveira LG, Araujo E, Jr., Nardozza LM, Moron AF.

Abstract: PURPOSE: The aim of this study was to evaluate the passage of fetal red blood cells to the maternal circulation, after invasive obstetric procedures, through the Kleihauer-Betke test, flow cytometry and by measurement of maternal serum alpha-fetoprotein level. METHODS: This prospective descriptive study with patients submitted to amniocentesis, cordocentesis, chorionic villus sampling (CVS), amnioreduction and ventriculoamniotic shunt was performed for karyotype analysis, treatment of hydrocephalus and polyhydramnios and to assess fetal lung maturity. Maternal blood samples were collected before and 60 minutes after the invasive obstetric procedure to search for fetal erythrocytes using the Kleihauer-Betke test, flow cytometry and serum alpha-fetoprotein measurement. RESULTS: Ten invasive obstetric procedures were performed. The mean age of the patients was 29.2 years and the mean gestational age was 29.6 weeks. The procedures were: five amniocenteses, two cordocenteses, one CVS, one ventriculo-amniotic shunt and one amnioreduction with cephalocentesis. The indications for the procedures were: karyotype analysis in five patients, fetal lung maturity assessment in two patients, amnioreduction in one patient, fetal hydrocephalus shunt in one patient and polyhydramnios related to hydranencephaly in one patient. Regarding the path of puncture, three procedures were accomplished through the placenta and seven apart from it. All punctures were successful at the first attempt. There was no significant increase of fetal erythrocyte quantity in maternal blood samples using the Kleihauer-Betke test. After cordocentesis, a significant increase of fetal erythrocytes was detected by flow cytometry and serum alpha-fetoprotein measurement. CONCLUSION: Invasive obstetric procedures during prenatal care are safe when performed by experienced professionals using adequate techniques, with minimal chance of passage of fetal erythrocytes from the fetal compartment.

(3) Title: [Establishment and application of median serum markers for second trimester screening in Qingdao region].

Source: Zhonghua Yi Xue Yi Chuan Xue Za Zhi 29:587-591, 2012.

Authors: Yu DY, Wang F, Liu Q, Jiang N, Zhao W, Ren HY, Han MY, Zhang K, Li S, Ouyang QQ, Nie Q.

Abstract: OBJECTIVE: To establish the median of serum markers for second trimester screening in Qingdao region and to assess the influence of median correction on the performance of screening. METHODS: Maternal serum alpha-fetoproteins (AFP), human chorionic gonadotrophin, free beta subunit (beta -HCG) and unconjugated oestriol (uE3) were assayed for prenatal screening of 18 188 singleton pregnancies at 15-20(+ 6) weeks gestation from January 2009 to July 2010. The median of serum markers was calculated based on above results and applied for risk estimation in screening for fetal aneuploidy from August 2010 to March 2011. The screening performance, specified in terms of detection rates (DRs), false positive rates (FPRs) and odds of being affected given a positive result (OAPR) were compared between the two groups. The risks of 45 affected pregnancies detected during the study were estimated with both Caucasian and corrected medians. RESULTS: The average level of AFP in local pregnancies was similar to that of the Caucasian population, whilst beta -HCG and uE3 were respectively 11% and 33% higher than those of Caucasians. The multiple of median (MoM) value was between 0.94 and 1.02 for the dataset based on the corrected median. At a cut-off of l in 270, FPR has decreased from 5.2% to 4.9%, and DR of Down syndrome has increased from 60% to 69.2%, and OAPR has increased from 1:79 to 1:59 when evaluating risk based on the corrected median. For the 45 affected pregnancies, three Down syndrome pregnancies could be missed because their risk estimates were lower than the cut-off level based on Caucasian median. CONCLUSION: It is useful to establish and apply population and laboratory-specific medians in order to improve the performance of prenatal screening and diagnosis.

(4) Title: The analysis of second-trimester triple screening for Down syndrome in Chinese normal singleton pregnancies.

Source: Scand J Clin Lab Invest 72:642-647, 2012.

Authors: Wan X, Wen J, Song X, Guo Y, Liu X, Yang B, Lu X.

Abstract: BACKGROUND AND OBJECTIVES: The study aimed to compare the alpha fetoprotein (AFP), total beta-human chorionic gonadotropin (hCG), and unconjugated estriol (uE3) levels in the second-trimester triple screening for Down syndrome with different regions, and to analyse the related factors that influenced the screening performance. MATERIALS AND METHODS: The study was conducted between February 2007 and November 2010 in Beijing Tongren Hospital, P. R. China. Data derived from 7,647 normal singleton pregnancies between 14 and 21 weeks of gestation were examined. The regressed median values in different gestational ages were compared with earlier published data from other regions. The distribution of median values and multiples of median (MoM) of AFP, hCG and uE3 according to maternal age and weight in normal pregnancies were described. Statistic parameters for AFP, hCG, and uE3 (based on log(10) MoM values) were compared with earlier published data from other studies. RESULTS: There were significantly increasing trends for AFP ($p < 0.001$) and uE3 ($p < 0.001$), and a significantly decreasing trend for hCG ($p < 0.001$) in the second trimester. There were significantly decreasing trends with increasing maternal weight for all the markers and their MoMs ($p < 0.001$). The distribution of the log(10) MoM marker values were Gaussian for the three parameters. CONCLUSIONS: Ethnic and laboratory variance should be taken into account in the second trimester triple screening for Down syndrome. The parameters of maternal serum markers should

be calculated using local data, and the algorithm modified to match the screening achievable for the local population.

(5) Title: Quantitative morphometric analysis of hepatocellular carcinoma: development of a programmed algorithm and preliminary application.

Source: Diagn Interv Radiol, 2012.

Authors: Yap FY, Bui JT, Knuttinen MG, Walzer NM, Cotler SJ, Owens CA, Berkes JL, Gaba RC.

Abstract: PURPOSE: The quantitative relationship between tumor morphology and malignant potential has not been explored in liver tumors. We designed a computer algorithm to analyze shape features of hepatocellular carcinoma (HCC) and tested feasibility of morphologic analysis. MATERIALS AND METHODS: Cross-sectional images from 118 patients diagnosed with HCC between 2007 and 2010 were extracted at the widest index tumor diameter. The tumor margins were outlined, and point coordinates were input into a MATLAB (Math- Works Inc., Natick, Massachusetts, USA) algorithm. Twelve shape descriptors were calculated per tumor: the compactness, the mean radial distance (MRD), the RD standard deviation (RDSD), the RD area ratio (RDAR), the zero crossings, entropy, the mean Feret diameter (MFD), the Feret ratio, the convex hull area (CHA) and perimeter (CHP) ratios, the elliptic compactness (EC), and the elliptic irregularity (EI). The parameters were correlated with the levels of alpha-fetoprotein (AFP) as an indicator of tumor aggressiveness. RESULTS: The quantitative morphometric analysis was technically successful in all cases. The mean parameters were as follows: compactness 0.88+/-0.086, MRD 0.83+/-0.056, RDSD 0.087+/-0.037, RDAR 0.045+/-0.023, zero crossings 6+/-2.2, entropy 1.43+/-0.16, MFD 4.40+/-3.14 cm, Feret ratio 0.78+/-0.089, CHA 0.98+/-0.027, CHP 0.98+/-0.030, EC 0.95+/-0.043, and EI 0.95+/-0.023. MFD and RDAR provided the widest value range for the best shape discrimination. The larger tumors were less compact, more concave, and less ellipsoid than the smaller tumors ($P < 0.0001$). AFP-producing tumors displayed greater morphologic irregularity based on several parameters, including compactness, MRD, RDSD, RDAR, entropy, and EI ($P < 0.05$ for all). CONCLUSION: Computerized HCC image analysis using shape descriptors is technically feasible. Aggressively growing tumors have wider diameters and more irregular margins. Future studies will determine further clinical applications for this morphologic analysis.

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

(1) Title: A novel amperometric immunosensor constructed with gold-platinum nanoparticles and horseradish peroxidase nanoparticles as well as nickel hexacyanoferrates nanoparticles.

Source: Analyst 138:620-626, 2012.

Authors: Zhu Q, Yuan R, Chai Y, Han J, Li Y, Liao N.

Abstract: In this study, three nano-materials comprising gold-platinum nanoparticles (Au-PtNPs), horseradish peroxidase nanoparticles (HRPNPs) and nickel hexacyanoferrate nanoparticles (NiHCFNPs) were used to construct a signal-off immunosensor. Au-PtNPs and NiHCFNPs were assembled on a glass carbon electrode (GCE) by electrodeposition and gold-cyanide bond formation, respectively; anti-fetoproteins (anti-AFP) were immobilized on the Au-PtNPs. HRPNPs were employed to block the possible remaining active sites and avoid nonspecific adsorption. Here, NiHCFNPs served as redox probes, while Au-PtNPs and HRPNPs were used for the synergistic catalysis of H(2)O(2) to amplify the signal. With more and more immunocomplex produced by the antibody-antigen reaction covering the biosensing surface, thus hindering electron transfer, the catalytic peak current will decrease quantitatively in relation to the concentration of

target antigen. The resulting immunosensors exhibited a fast response and excellent sensitivity to alpha-fetoprotein (AFP), and showed two linear ranges in the concentration ranges of 0.06-13 ng mL(-1) and 13-200 ng mL(-1) with a detection limit of 0.017 ng mL(-1).

(2) Title: Microfluidic beads-based immunosensor for sensitive detection of cancer biomarker proteins using multienzyme-nanoparticle amplification and quantum dots labels.

Source: Biosens Bioelectron 42C:23-30, 2012.

Authors: Zhang H, Liu L, Fu X, Zhu Z.

Abstract: This study reports the development of a microfluidic beads-based immunosensor for sensitive detection of cancer biomarker alpha-fetoprotein (AFP) that uses multienzyme-nanoparticle amplification and quantum dots labels. This method utilizes microbeads functionalized with the capture antibodies (Ab(1)) and modified electron rich proteins as sensing platform that was fabricated within a microfluidic channel, and uses gold nanoparticles (AuNPs) functionalized with the horseradish peroxidase (HRP) and the detection antibodies (Ab(2)) as label. Greatly enhanced sensitivity for the cancer biomarker is based on a dual signal amplification strategy: first, the large surface area of Au nanoparticle carrier allows several binding events of HRP on each nanosphere. Enhanced sensitivity was achieved by introducing the multi-HRP-antibody functionalized AuNPs onto the surface of microbeads through "sandwich" immunoreactions and subsequently multiple biotin moieties could be deposited onto the surface of beads resulted from the oxidation of biotin-tyramine by hydrogen peroxide. Streptavidin-labeled quantum dots were then allowed to bind to the deposited biotin moieties and displayed the signal. Secondly, enhanced mass transport capability inherent from microfluidics leads to higher capture efficiency of targets because continuous flow within micro-channel delivers fresh analyte solution to the reaction site which maintains a high concentration gradient differential to enhance mass transport. Based on the dual signal amplification strategy, the developed microfluidic bead-based immunosensor could discriminate as low as 0.2pgmL(-1) AFP in 10muL of undiluted calf serum (0.2fg/chip), and showed a 500-fold increase in detection limit compared to the off-chip test and 50-fold increase in detection limit compared to microfluidic beads-based immunoassay using single label HRP-Ab(2). The immunosensor showed acceptable repeatability and reproducibility. This microfluidic beads-based immunosensor is a promising platform for disease-related biomolecules at the lowest level at their earliest incidence.

(3) Title: Amperometric immunobiosensor for alpha-fetoprotein using Au nanoparticles/chitosan/TiO(2)-graphene composite based platform.

Source: Bioelectrochemistry 90:18-23, 2013.

Authors: Huang KJ, Li J, Wu YY, Liu YM.

Abstract: A simple label-free amperometric immunosensor for protein detection is developed based on TiO(2)-graphene (TiO(2)-Gr), chitosan and gold nanoparticles (AuNPs) composite film modified glassy carbon electrode (GCE). The negatively charged AuNPs can be adsorbed on the positively charged chitosan/TiO(2)-Gr composite film by electrostatic adsorption, and then is used to immobilize alpha-fetoprotein antibody for the assay of alpha-fetoprotein (AFP). The interaction of antigen and antibody on the electrode interface makes a barrier for electrons and inhibits the electro-transfer, resulting in the decreased DPV signals of probe Fe(CN)(6)(3-/4-). Using this strategy, a wide detection range (0.1-300 ng mL(-1)) with the correlation coefficients of 0.992-0.994 for model target AFP is obtained. The limit of detection is 0.03 ng mL(-1) at a signal-to-noise ratio of 3. The results prove that the sensing strategy possesses sensitivity, selectivity, stability and generality, and it may be used to immobilize other biomoleculars to develop biosensor for the detection of other antigens or biocompounds.

(4) Title: Novel nanoimaging approach: Antibodious polymeric nanolabel for intracellular alpha-fetoprotein targeted monitoring.

Source: Biotechnol Prog, 2012.

Authors: Unluer OB, Ersoz A, Say R, Tomsuk O, Sivas H.

Abstract: This study describes preparation and use of novel labeled and antibodious polymeric nanolabels (anti-alpha fetoprotein cross-linked nanolabels) as an immunogenic and semisynthetic nanolabel with potential prognostic and therapeutic roles for hepatoma cancer. Specificity, uptake, and binding efficiencies of the nanolabel have been examined in a human hepatosarcoma cell line HepG2, a human colorectal cell line DLD-1, and a mouse myoblast cell line C2. Labeling of the cells has been performed by treating live and fixed cells with varying concentrations of the nanolabels and then, the cells have been examined under a fluorescence microscope. In addition, all cell lines have also been labeled using FITC-conjugated nanotrastuzumab to compare the results obtained with those of the binding of the FITC-nanoanti-alpha fetoprotein nanolabels. Results show that FITC-conjugated anti-alpha fetoprotein cross-linked nanolabels have been taken up by both live and fixed cells and have efficiently and specifically labeled HepG2 cells at a quite low concentration. Taken all together, the results indicate that the novel targeted nanoimaging tools and technique demonstrated their ability to detect the distribution of the nanolabels as probes in hepatoma cells. (c) 2012 American Institute of Chemical Engineers Biotechnol. Prog., 2012.

E) Abstracts of New Assay Methodologies:

(1) Title: Ultrasensitive Immunoassay Based on Anodic Near-Infrared Electrochemiluminescence from Dual-Stabilizer-Capped CdTe Nanocrystals.

Source: Anal Chem 84:10645-10649, 2012.

Authors: Liang G, Liu S, Zou G, Zhang X.

Abstract: A sandwich-typed near-infrared (NIR) electrochemiluminescence (ECL) immunoassay was developed with dual-stabilizer-capped CdTe nanocrystals (NCs) as ECL labels and alpha fetoprotein antigen (AFP) as model protein. The dual-stabilizer-capped NIR CdTe NCs were promising ECL labels because of their NIR ECL emission of 800 nm, low anodic ECL potential of +0.85 V, and high biocompatibity, which can facilitate interference-free and highly sensitive ECL bioassays. Upon the immunorecognition of the immobilized AFP to its antibody labeled with dual-stabilizer-capped CdTe NCs, the proposed immunoassay displayed increasing ECL intensity, leading to a wide calibration range of 10.0 pg/mL to 80.0 ng/mL with a detection limit of 5.0 pg/mL [signal-to-noise ratio (S/N) = 3] without coupling any signal amplification procedures. The NIR ECL immunoassay for real samples displayed very similar results with those of Ru(bpy)(3)(2+) reagent kit based commercial ECL immunoassay, which not only proved for the efficiency of NIR ECL from dual-stabilizer-capped CdTe NCs but also paved the road for development of novel ECL emitters and corresponding reagent kits.

(2) Title: A bead-based multiplexed immunoassay to evaluate breast cancer biomarkers for early detection in pre-diagnostic serum.

Source: Int J Mol Sci 13:13587-13604, 2012.

Authors: Opstal-van Winden AW, Rodenburg W, Pennings JL, van Oostrom CT, Beijnen JH, Peeters PH, van Gils CH, de Vries A.

Abstract: This study investigates whether a set of ten potential breast cancer serum biomarkers and cancer antigens (osteopontin (OPN), haptoglobin, cancer antigen 15-3 (CA15-3), carcinoembryonic antigen (CEA), cancer antigen 125 (CA-125), prolactin, cancer antigen 19-9 (CA19-9), alpha-fetoprotein (AFP), leptin and migration inhibitory factor (MIF)) can predict early stage breast cancer in samples collected before clinical diagnosis (phase III samples). We performed a nested case-control study within the Prospect-EPIC (European Prospective Investigation into Cancer and nutrition) cohort. We examined to what extent the biomarker panel could discriminate between 68 women diagnosed with breast cancer up to three years after enrollment and 68 matched healthy controls (all 56-64 years at baseline). Using a quantitative bead-based multiplexed assay, we determined protein concentrations in serum samples collected at enrollment. Principal Component Analysis (PCA) and Random Forest (RF) analysis revealed that on the basis of all ten proteins, early cases could not be separated from controls. When we combined serum protein concentrations and subject characteristics related to breast cancer risk in the RF analysis, this did not result in classification accuracy scores that could correctly classify the samples (sensitivity: 50%, specificity: 50%). Our findings indicate that this panel of selected tumor markers cannot be used for diagnosis of early breast cancer.

F) Special Abstract Selection:

(1) Title: [Relationship of adverse pregnancy outcomes and a high risk serum screen for Down syndrome in the second trimester].

Source: Zhonghua Fu Chan Ke Za Zhi 47:427-430, 2012.

Authors: Hu XY, Bian XM, Jiang YL, Liu SY.

Abstract: OBJECTIVE: To investigate the the relationship of a high risk serum screen for Down syndrome in second trimester and adverse pregnancy outcomes, and to evaluate the predictive value for adverse pregnancy outcomes. METHODS: The tri-marker second trimester maternal serum screening for Down syndrome (alpha-fetoprotein, free beta-hCG and unconjugated estriol) was performed on the pregnant women at Peking Union Medical Hospital from January 2009 to January 2011. The cutoff valvue was 1/270. Pregnancy outcomes were followed up. The general condition and pregnancy complications of the pregnant women with high risk (high-risk group) were compared to that of the pregnant women with low risk (low-risk group); and with 35 years old as a demarcation, the incidences of adverse pregnancy outcomes were calculated in the two groups. RESULTS: (1) A total of 1935 cases were collected. And 1784 cases were with low risk, and 151 cases were with high risk. The difference of weight and gestational age between the two groups was not statistically significant ($P > 0.05$); the difference of age between the two groups was statistically significant ($P < 0.01$). (2) Pregnancy complications were found in 791 cases. In high-risk group, the incidences of gestational diaetes mellitus (GDM, 13.9%), neonatal asphyxia (4.0%) and small for gestational age infant (SGA, 4.6%) were higher than that in low-risk group (8.4%, 1.0%, 1.6%), the difference was statistically significant ($P < 0.05$). The incidences of gestational hypertension disease, premature labor, oligohydramnios, placenta previa, placenta abruption, fetal macrosomia in the two groups was not statistically different ($P > 0.05$). (3) In 1705 cases aged less than 35 years, 129 cases (7.6%) were GDM, 43 cases (2.5%) were gestational hypertension disease, 61 cases (3.9%) were premature labor; in 230 cases aged 35 years or more, 41 cases (17.8%) were GDM, 12 cases (5.2%) were gestational hypertension disease, 15 cases (6.5%) were premature labor, and the difference between the two groups was statistically significant ($P < 0.05$). In < 35 years old group, the incidences of GDM, neonatal asphyxia and SGA (12.3%, 4.4%, 5.3%) were higher in the high-risk group than that (7.2%, 0.9%, 1.6%) in the low-risk group, and the difference was statistically significant ($P < 0.05$). In >/= 35 years old group, the incidences of GDM, neonatal asphyxia and SGA (18.9%, 2.7%, 2.7%) were slightly higher in the high-risk group than that (17.6%, 1.6%, 1.6%) in the low-risk group, the difference between the two groups was not statistically significant ($P > 0.05$). CONCLUSIONS: The present study revealed apparent increase in the adverse pregnancy outcomes in women with a high risk of

Down syndrome screening test. Advanced age is the most important risk factor for a high risk of Down syndrome screening test and adverse pregnancy outcomes. More attention should be attached to the patients whose age were < 35 years old and with a high risk of Down syndrome screening test.

(2) Title: [Establishment of median values for second trimester maternal serum biomarkers in Weihai region].

Source: Zhonghua Yi Xue Yi Chuan Xue Za Zhi 29:693-696, 2012.

Authors: Lan X, Wang S, Deng Y.

Abstract: OBJECTIVE: To establish the median values for second trimester biomarkers in Weihai region, and to assess its value for improving the performance and efficiency of prenatal screening. METHODS: Maternal serum alpha-fetoprotein (AFP) and free beta human chorionic gonadotropin (Free beta-hCG) were determined for 24 400 pregnant women at 105 to 146 gestational days. A regression equation was derived after adjusting for different gestational ages. The median values were further adjusted with body weight. RESULTS: The median values of AFP and Free beta-hCG were respectively 6% and 24% higher than those embedded in a 2T software. After adjusting with gestational age and weight, there is a significant difference in multiple of the median (MoM) of serum biomarkers between local population and that embedded in the 2T model. CONCLUSION: To establish the median values for different gestational ages for local region may help to improve the efficiency of prenatal screening.

(3) Title: Placenta accreta and the risk of adverse maternal and neonatal outcomes.

Source: J Perinat Med:1-9, 2012.

Authors: Balayla J, Bondarenko HD.

Abstract: Abstract Objective: Placenta accreta is an increasingly prevalent and potentially dangerous complication of pregnancy. Although most studies on the subject have addressed the risk factors for the development of this condition, evidence on maternal and neonatal outcomes for these pregnancies is scarce. The objective of the present study is to compile current evidence with regard to risk factors as well as adverse outcomes associated with placenta accreta. Methods: We conducted a complete literature review using PubMed, MEDLINE, Cochrane Database Reviews, UptoDate, DocGuide, as well as Google scholar and textbook literature for all articles on placenta accreta, and any one of the following keywords: "risk factors", "maternal outcomes", "neonatal outcomes", "morbidity", and "mortality". Individual case reports were excluded. Results: We reviewed 34 studies conducted between 1977 and 2012. A total number of 508,617 deliveries were studied, with 865 cases of confirmed placenta accreta (average pooled incidence=1/588). The development of placenta accreta appears to be most strongly predicted by a history of cesarean section, low-lying placenta/previa, in vitro fertilization pregnancy, as well as elevated second-trimester levels of alpha-fetoprotein and beta-human chorionic gonadotropin. The most significant maternal outcomes include the need for postpartum transfusion due to hemorrhage and peripartum hysterectomy. Maternal mortality remains rare but significantly higher than among matched, postpartum controls. Important neonatal outcomes include preterm birth, low birth weight, small for gestational age, and reduced 5-min Apgar scores. Whether the need for neonatal intensive care unit admission and steroid administration is iatrogenic and whether an increased risk of perinatal mortality is a clinically significant and independent outcome remain controversial. Conclusion: Although there is a significant shortage of studies on the subject, it appears that placenta accreta is associated with adverse maternal and neonatal outcomes, some of which may be life threatening.

Prenatal diagnosis and adequate planning, particularly in high-risk populations, may be indicated for the reduction of these adverse outcomes.

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

Maternal Sera AFP MoM

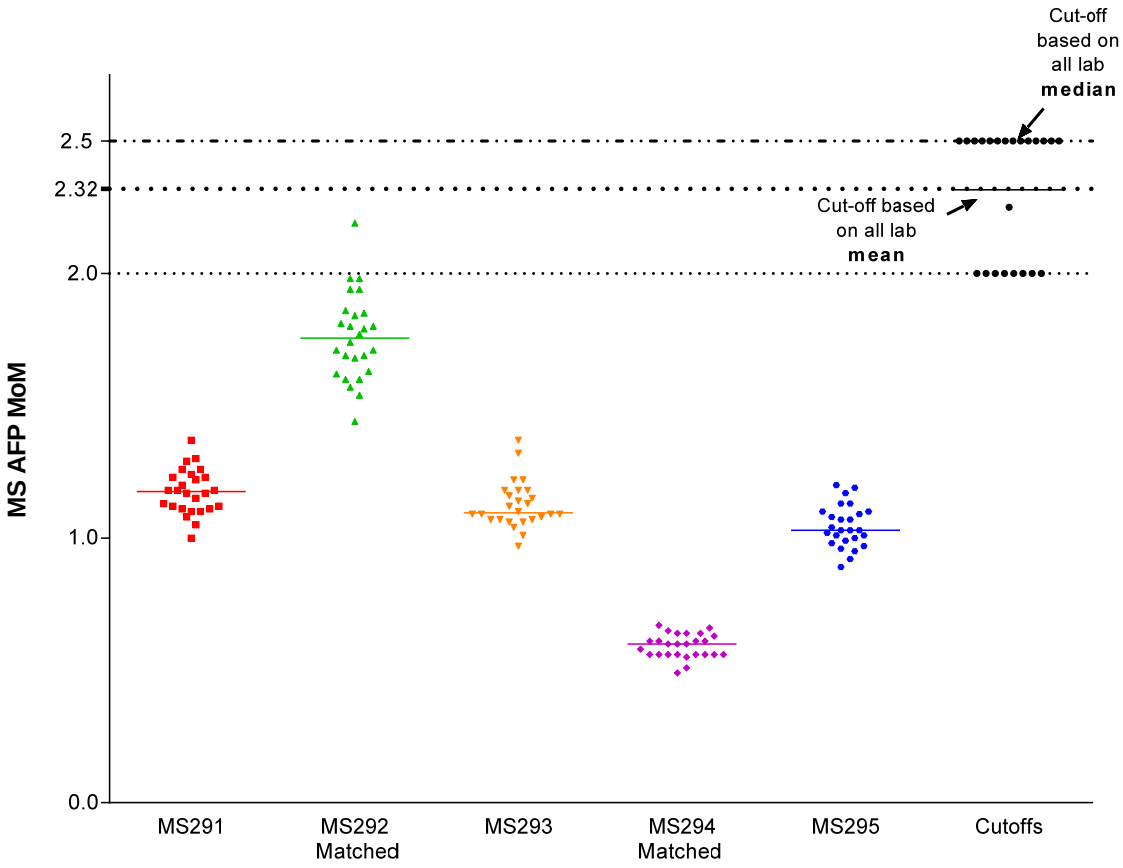


Figure 2

Amniotic Fluid AFP MoM

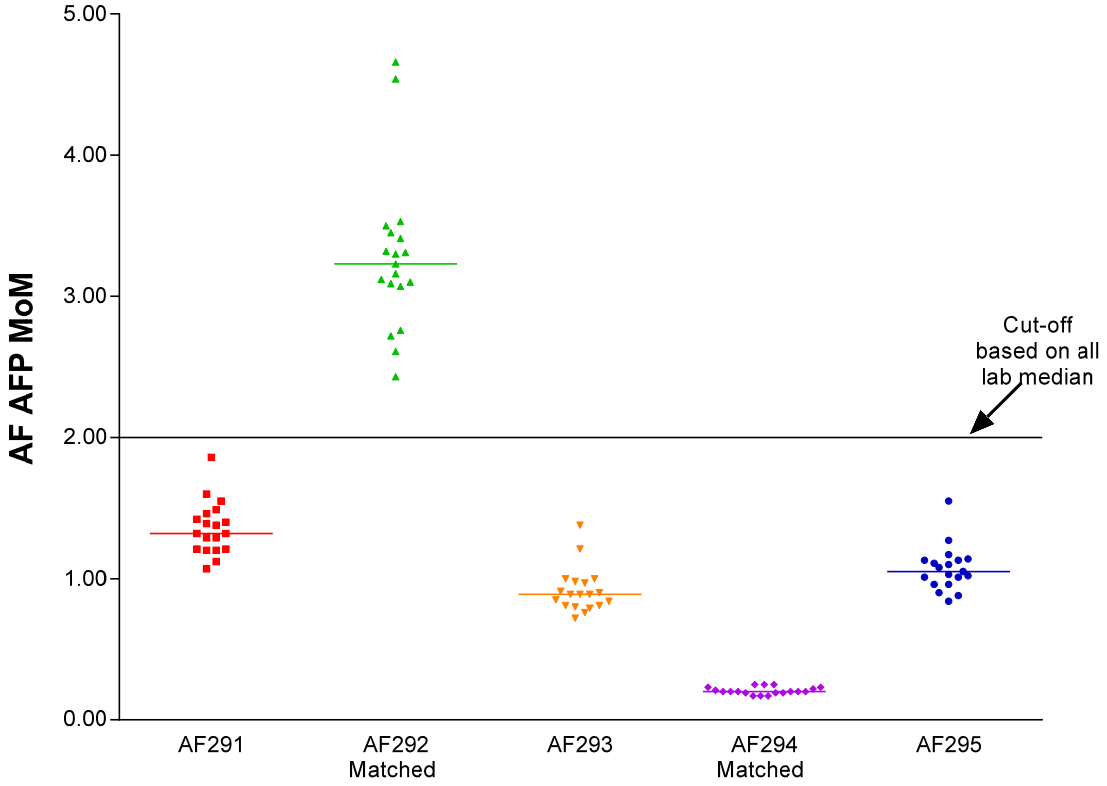


Figure 3

Graphic Distribution of Second Trimester Neural Tube Defect Risk Estimates

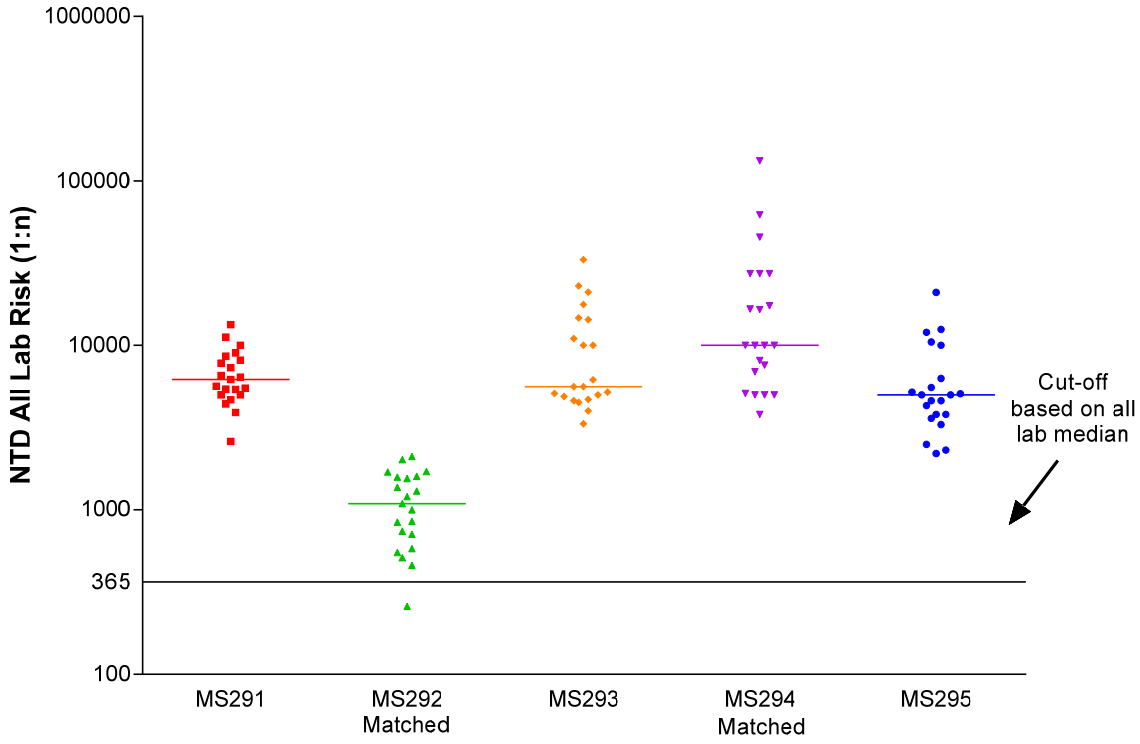


Figure 4

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates

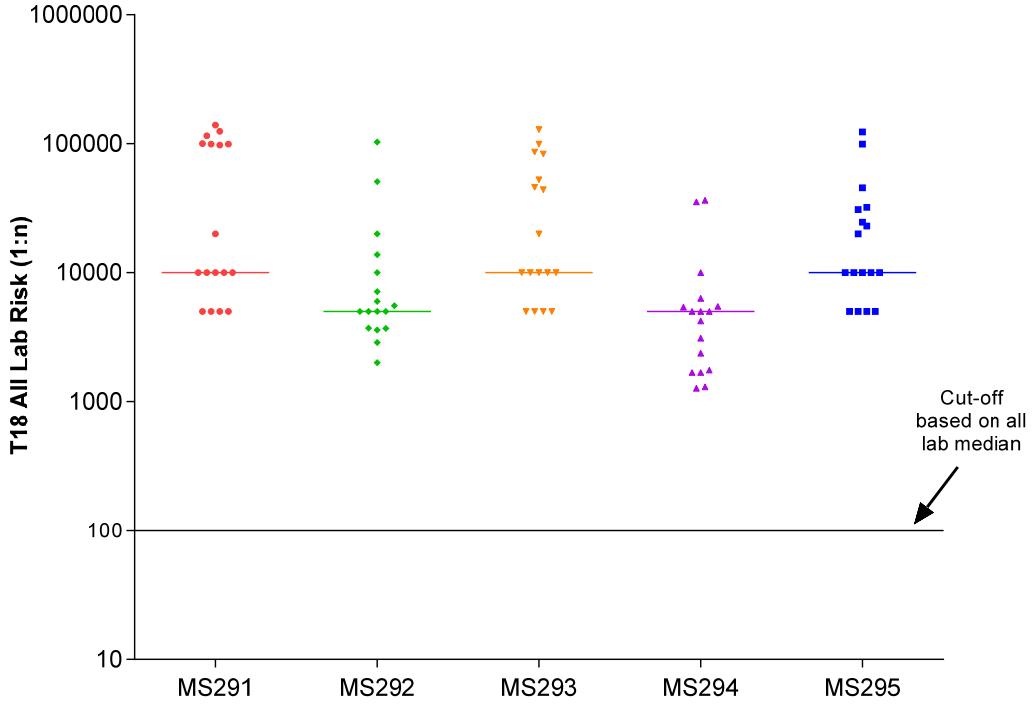


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

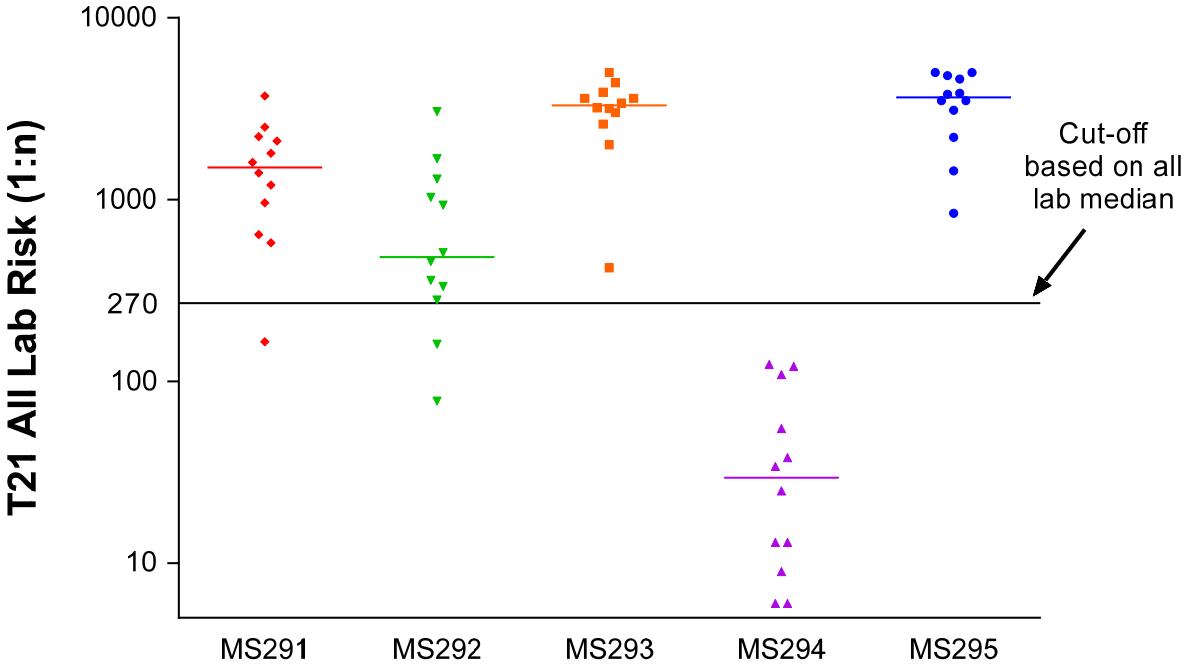
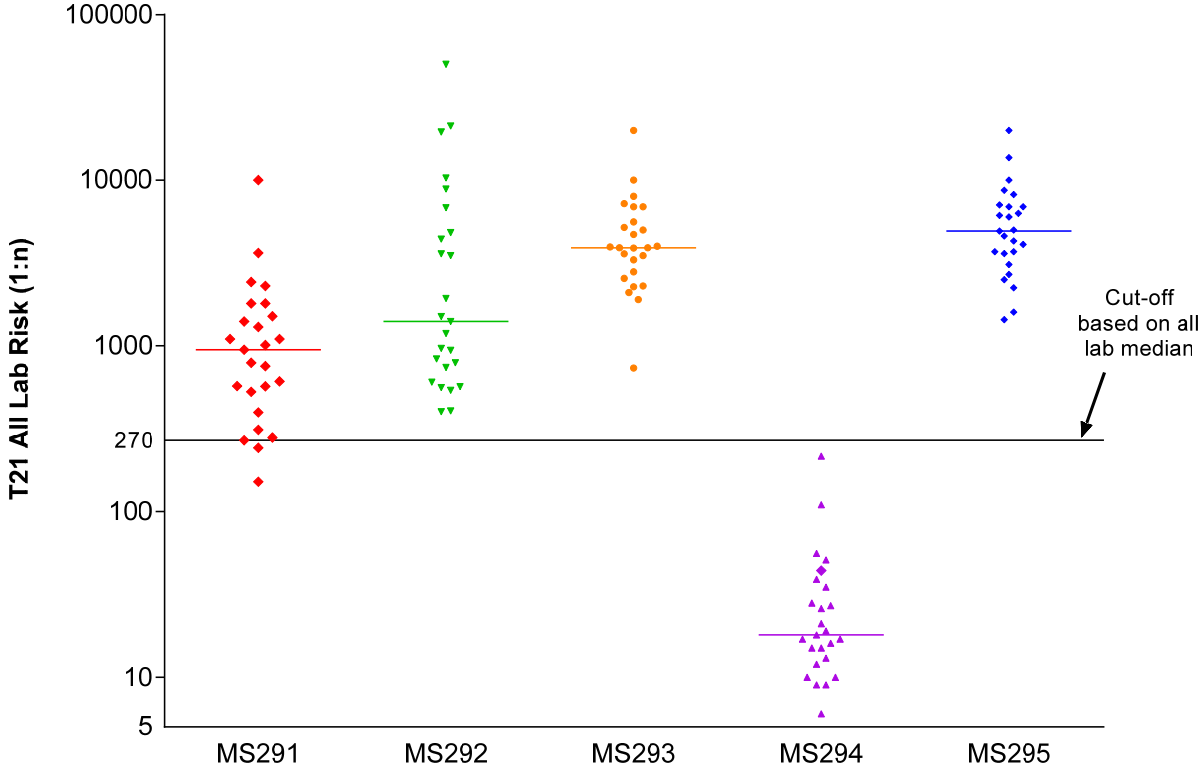


Figure 6

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates



NYS FEDM PT 1/13
Second Trimester

BCX/BC1 = Beckman Access/2
BCU/BC1 = Beckman Unicel
DPD/DP5 = Siemens Immulite 2000
DS1 = Diagnostic Systems Labs

Figure 7A

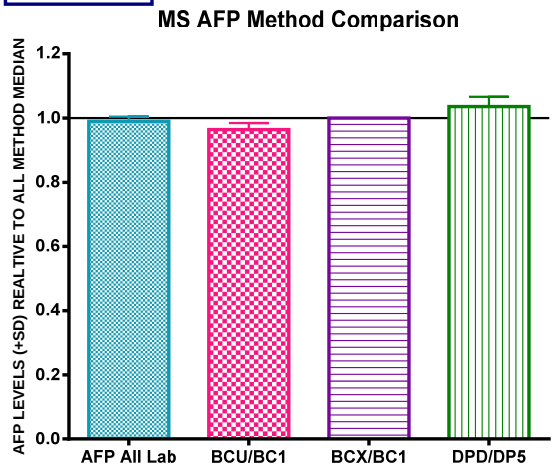


Figure 7B

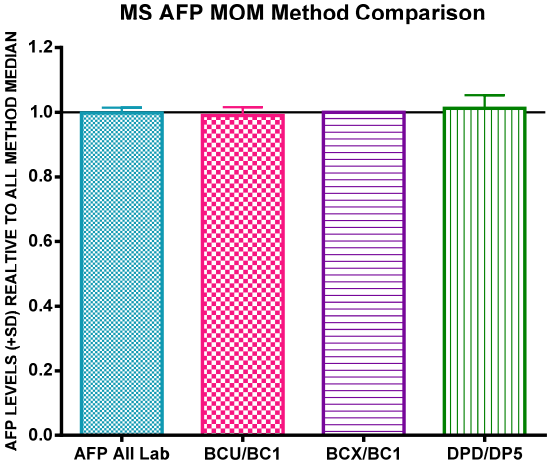


Figure 7C

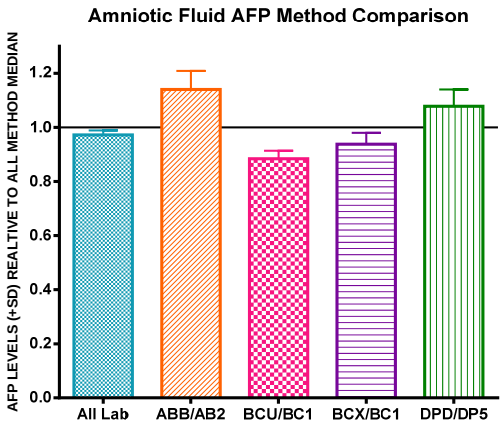


Figure 7D

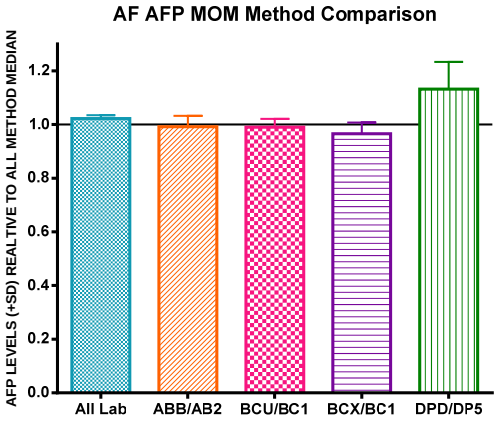


Figure 8A

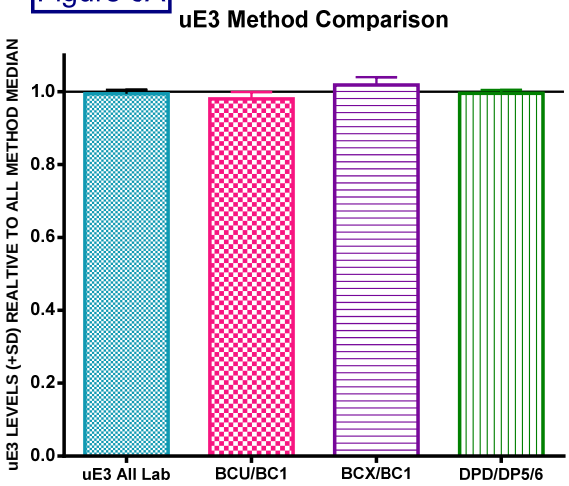
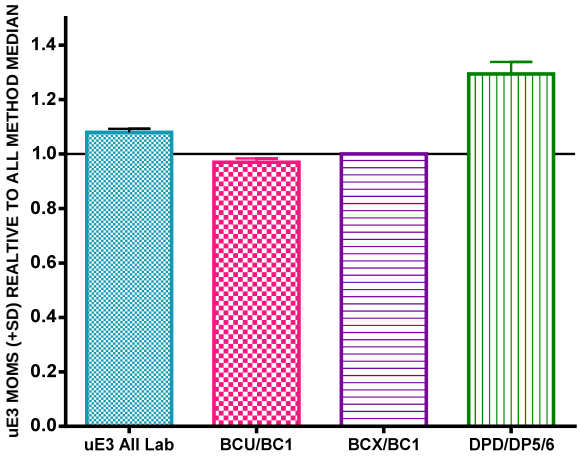


Figure 8B



NYS FEDM PT 1/13
Second Trimester

Figure 9A

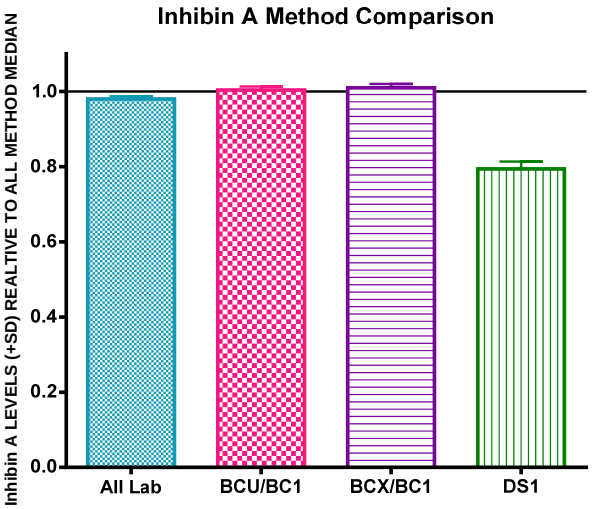


Figure 9B

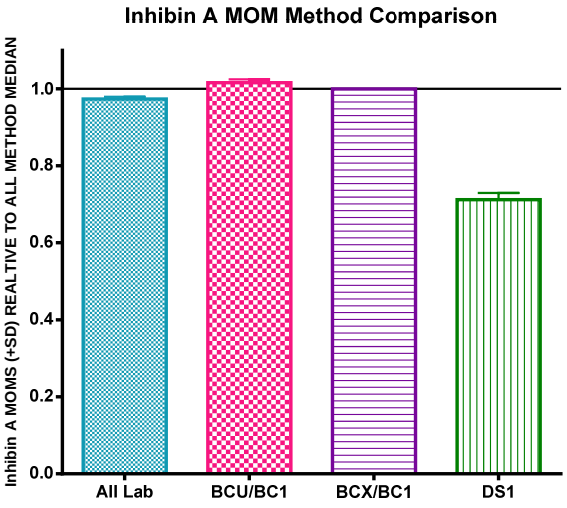


Figure 10A

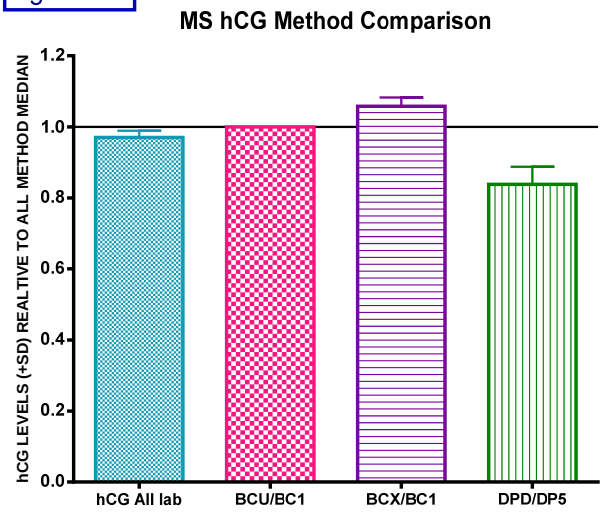
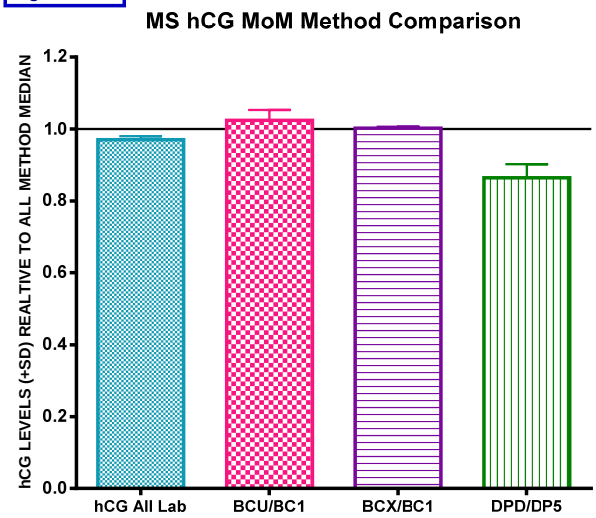


Figure 10B



ABB/AB1 = Abbott Asxym
BCX/BC1 = Beckman Access/2
BCU/BC1 = Beckman Unicel
DPD/DP5 = Siemens Immulite 2000
DS1 = Diagnostic Systems Labs

NYS FEDM PT 1/13
First Trimester

Figure 11A

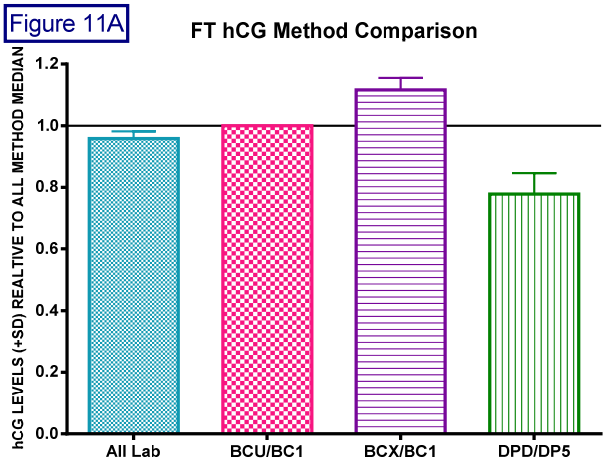


Figure 11B

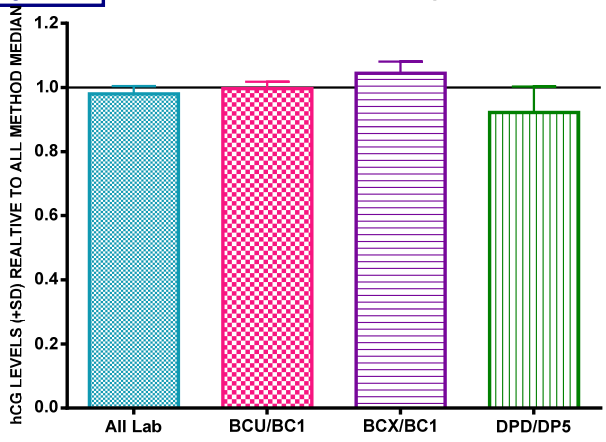


Figure 12A

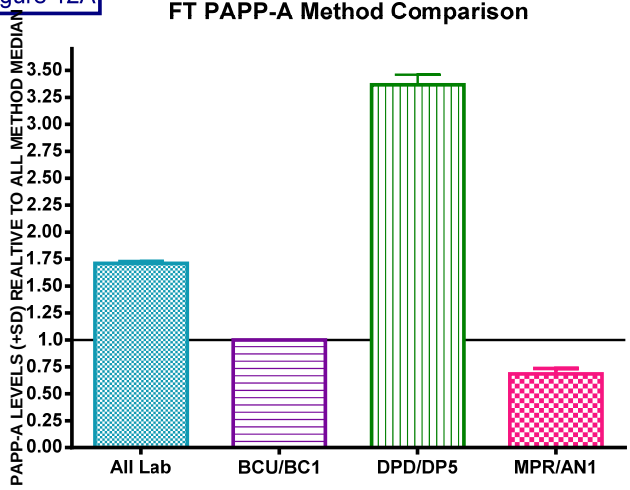
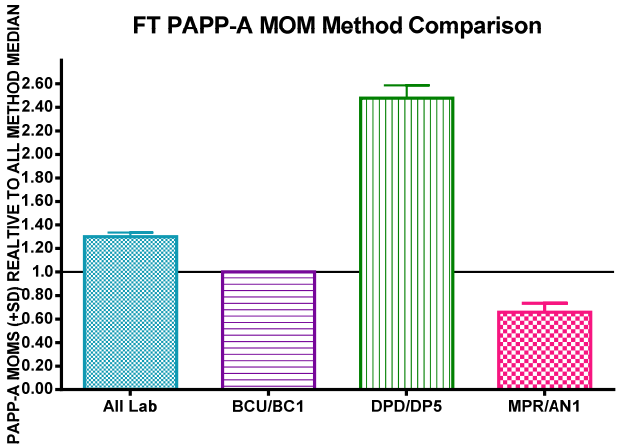


Figure 12B



ABB/AB1 = Abbott Asxym
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

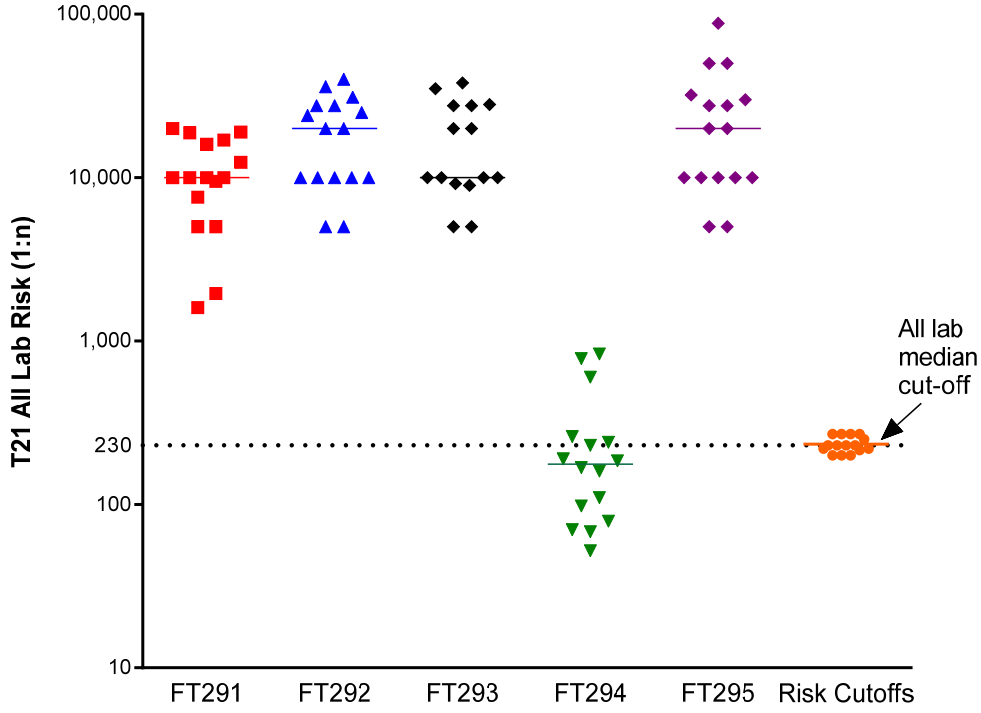


Figure 14

Graphic Distribution of First Trimester Trisomy 18 Risk Estimates

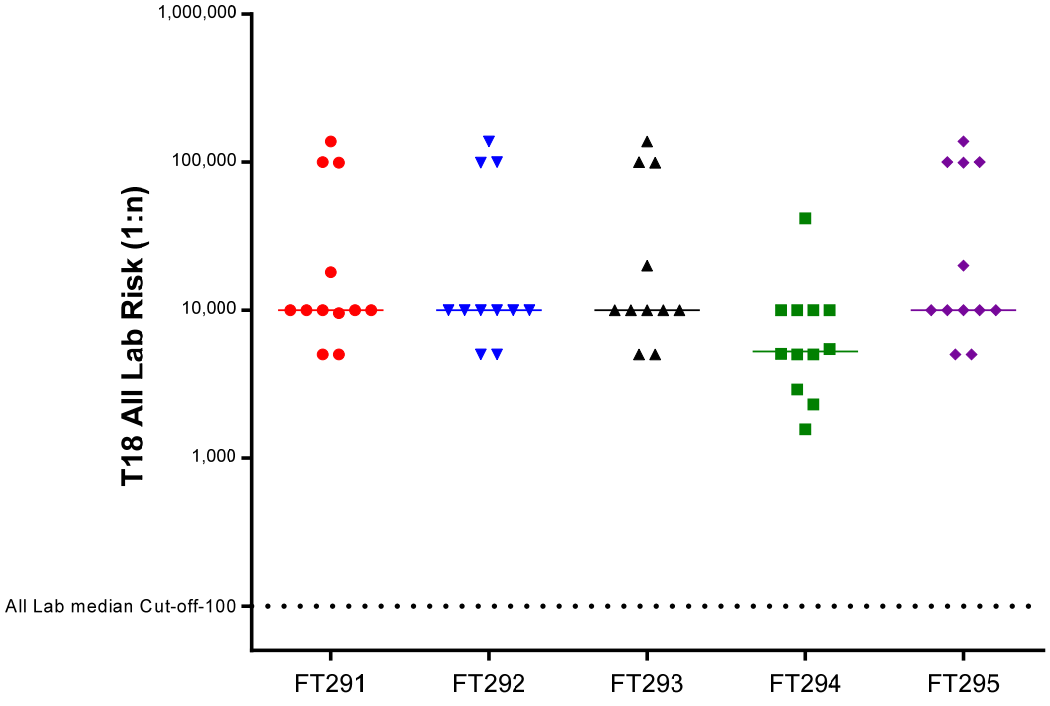
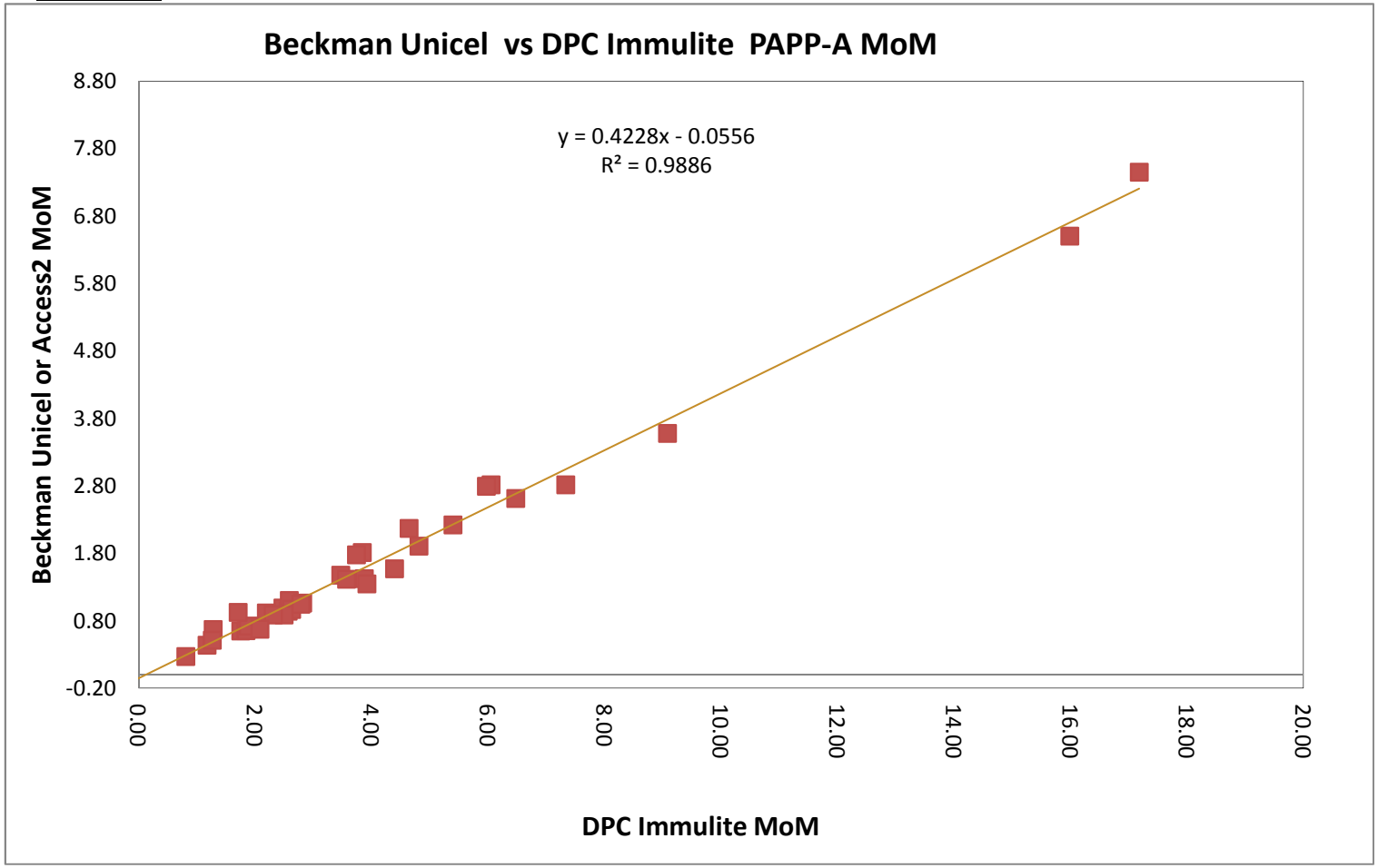


Fig. 15


Beckman Unicel vs DPC Immulite PAPP-A MoM
y = 0.4228x - 0.0556
R² = 0.9886
Beckman Unicel or Access2 MoM
DPC Immulite 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

New York State Fetal Defect Markers Proficiency Test,
January 2013
Summary of Results

	MS 291	MS 292	MS 293	MS 294	MS 295
Gestational Age All Lab Mean:					
Mean	17.0	19.0	19.4	18.0	20.0
SD	0.00	0.00	0.08	0.00	0.00
%CV	0.0%	0.0%	0.4%	0.0%	0.0%
mean+3*SD	17.0	19.0	19.6	18.0	20.0
mean-3*SD	17.0	19.0	19.1	18.0	20.0
N	26	26	26	26	26

	MS 291	MS 292	MS 293	MS 294	MS 295
MS AFP All Lab Mean:					
mean	45.1	93.5	64.9	26.4	70.3
SD	3.5	8.7	4.9	2.1	4.8
%CV	7.7%	9.4%	7.6%	8.1%	6.8%
mean+3SD	55.5	119.7	79.7	32.8	84.7
mean-3SD	34.7	67.3	50.1	20.0	56.0
N	26	26	26	26	26
median	45.05	92.2	63.8	26.8	69.5
mean/all kit median	0.98	0.98	1.01	0.98	1.00
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	43.5	91.6	63.0	25.5	69.6
SD	2.3	5.8	3.4	1.6	3.1
%CV	5.2%	6.3%	5.4%	6.5%	4.5%
mean + 3SD	50.3	109.0	73.2	30.4	78.9
mean - 3SD	36.8	74.3	52.8	20.5	60.3
N	11	11	11	11	11
Median	44.0	89.6	62.9	24.7	68.7
mean/All kit median	0.95	0.96	0.98	0.94	0.99
MS AFP Beckman Access/2 (BCX/BC1) mean:					
mean	46.0	95.2	64.2	27.0	70.6
SD	2.2	8.1	3.9	1.7	5.4
%CV	4.9%	8.5%	6.1%	6.2%	7.6%
mean+3SD	52.7	119.5	76.0	32.0	86.8
mean-3SD	39.3	70.9	52.4	22.0	54.5
N	6	6	6	6	6
median	46.6	96.8	63.0	26.8	68.7
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS AFP Siemens Immulite 2000 (DPD/DP5) mean:					
mean	47.4	97.1	69.7	27.4	72.2
SD	4.6	12.8	5.1	2.6	6.2
%CV	9.7%	13.1%	7.3%	9.6%	8.6%
mean+3SD	61.1	135.3	85.0	35.3	90.9
mean-3SD	33.6	58.8	54.4	19.5	53.5
N	7	7	7	7	7
median	47.0	94.0	70.8	27.8	71.8
mean/all kit median	1.03	1.02	1.09	1.02	1.02
MS AFP kit average:					
mean	45.6	94.6	65.6	26.6	70.8
SD	1.9	2.8	3.6	1.0	1.3
all kit median	46.0	95.2	64.2	27.0	70.6

	MS 291	MS 292	MS 293	MS 294	MS 295
MS AFP MoM All Lab Mean:					
mean	1.18	1.76	1.13	0.59	1.04
SD	0.08	0.16	0.09	0.05	0.08
%CV	7.2%	9.3%	7.9%	7.6%	7.7%
mean+3SD	1.43	2.25	1.39	0.73	1.28
mean-3SD	0.92	1.27	0.86	0.46	0.80
N	26	26	26	26	26
All Median	1.18	1.76	1.10	0.60	1.03
mean/all kit median	0.98	0.99	1.02	0.99	1.01
MS AFP MoM Beckman Unicel (BCU/BC1) mean:					
Mean	1.15	1.74	1.10	0.58	1.07
SD	0.07	0.12	0.05	0.04	0.07
%CV	6.2%	6.7%	4.7%	6.5%	6.3%
mean + 3SD	1.37	2.09	1.26	0.69	1.27
mean - 3SD	0.94	1.39	0.95	0.47	0.87
N	11	11	11	11	11
Median	1.13	1.74	1.09	0.56	1.07
mean/all kit median	0.97	0.98	1.00	0.97	1.03
MS AFP MoM Beckman Access/2 (BCX/BC1) mean:					
Mean	1.20	1.78	1.11	0.60	1.04
SD	0.08	0.20	0.10	0.05	0.09
%CV	7.0%	11.0%	8.8%	7.8%	9.1%
mean + 3SD	1.45	2.37	1.40	0.74	1.32
mean - 3SD	0.94	1.19	0.82	0.46	0.76
N	6	6	6	6	6
Median	1.21	1.81	1.09	0.59	1.03
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS AFP MoM Siemens Immulite 2000 (DPD/DP5) mean:					
Mean	1.20	1.79	1.19	0.60	1.01
SD	0.11	0.23	0.11	0.06	0.10
%CV	9.5%	12.7%	9.3%	10.2%	9.4%
mean + 3SD	1.54	2.47	1.53	0.79	1.30
mean - 3SD	0.86	1.10	0.86	0.42	0.72
N	7	7	7	7	7
Median	1.20	1.79	1.15	0.61	0.98
mean/all kit median	1.00	1.00	1.08	1.01	0.97
MS AFP MoM kit average:					
mean	1.18	1.77	1.14	0.59	1.04
SD	0.02	0.02	0.05	0.01	0.03
all kit median	1.20	1.78	1.11	0.60	1.04

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	MS 291	MS 292	MS 293	MS 294	MS 295
MS uE3 All Lab Mean:					
mean	0.97	0.76	1.38	0.58	1.55
SD	0.11	0.07	0.10	0.06	0.09
%CV	11.0%	9.5%	7.4%	10.3%	5.9%
mean+3SD	1.29	0.98	1.69	0.76	1.83
mean-3SD	0.65	0.55	1.07	0.40	1.28
N	25	25	25	25	25
mean/all kit median	0.98	1.00	1.01	0.99	0.99
MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	0.94	0.75	1.37	0.58	1.53
SD	0.07	0.07	0.08	0.05	0.08
%CV	7.7%	9.4%	5.6%	9.3%	5.0%
mean+3SD	1.16	0.97	1.60	0.74	1.76
mean-3SD	0.72	0.54	1.14	0.41	1.30
N	11	11	11	11	11
mean/all kit median	0.95	0.99	1.00	0.98	0.98
MS uE3 Beckman Access/2 (BCX/BC1) mean:					
mean	0.99	0.78	1.44	0.59	1.58
SD	0.09	0.06	0.07	0.04	0.08
%CV	8.9%	7.9%	5.2%	7.0%	5.0%
mean+3SD	1.26	0.97	1.66	0.71	1.82
mean-3SD	0.72	0.60	1.21	0.46	1.34
N	6	6	6	6	6
mean/all kit median	1.00	1.03	1.05	1.00	1.01
MS uE3 Siemens Immulite/2000 (DPD/DP6) mean:					
Mean	0.99	0.76	1.35	0.59	1.57
SD	0.16	0.09	0.14	0.08	0.12
%CV	15.7%	11.3%	10.3%	14.2%	7.6%
mean+3SD	1.45	1.02	1.76	0.84	1.92
mean-3SD	0.52	0.50	0.93	0.34	1.21
N	8	8	8	8	8
mean/all Kit Median	1.00	1.00	0.98	1.00	1.00
MS uE3 kit average:					
mean	0.97	0.77	1.39	0.58	1.56
SD	0.03	0.02	0.05	0.01	0.03
all kit median	0.99	0.76	1.37	0.59	1.57

	MS 291	MS 292	MS 293	MS 294	MS 295
MS uE3 MoM All Lab Mean:					
Mean	1.05	0.53	0.90	0.49	0.83
SD	0.22	0.09	0.14	0.09	0.15
%CV	21.3%	18.0%	15.8%	18.7%	18.4%
mean+3SD	1.72	0.81	1.33	0.77	1.29
mean-3SD	0.38	0.24	0.47	0.22	0.37
N	25	25	25	25	25
mean/all kit Median	1.10	1.07	1.07	1.08	1.08
MS uE3 MoM Beckman Unicel (BCU/BC1) Mean:					
Mean	0.92	0.47	0.81	0.45	0.75
SD	0.09	0.05	0.05	0.05	0.04
%CV	10.1%	9.9%	6.4%	10.6%	5.8%
mean+3SD	1.20	0.61	0.97	0.60	0.88
mean-3SD	0.64	0.33	0.66	0.31	0.62
N	11	11	11	11	11
mean/all kit Median	0.96	0.96	0.96	0.99	0.98
MS uE3 MoM Beckman Access/2 (BCX/BC1) Mean:					
Mean	0.95	0.49	0.84	0.46	0.77
SD	0.13	0.07	0.09	0.06	0.10
%CV	13.3%	13.6%	10.5%	12.4%	13.2%
mean+3SD	1.33	0.69	1.11	0.63	1.08
mean-3SD	0.57	0.29	0.58	0.29	0.46
N	6	6	6	6	6
mean/all kit Median	1.00	1.00	1.00	1.00	1.00
MS uE3 MoM Siemens Immulite/2000 (DPD/DP6) Mean:					
Mean	1.31	0.63	1.07	0.58	0.99
SD	0.19	0.08	0.12	0.11	0.17
%CV	14.6%	12.8%	11.1%	18.1%	17.0%
mean+3SD	1.88	0.87	1.42	0.90	1.50
mean-3SD	0.73	0.39	0.71	0.27	0.48
N	8	8	8	8	8
mean/all kit Median	1.37	1.28	1.26	1.27	1.29
MS uE3 MoM kit average:					
mean	1.06	0.53	0.91	0.50	0.84
SD	0.21	0.09	0.14	0.07	0.13
all kit median	0.95	0.49	0.84	0.46	0.77

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	MS 291	MS 292	MS 293	MS 294	MS 295
MS hCG All Lab mean:					
mean	58.0	30.2	20.3	45.2	18.2
SD	7.0	3.9	1.9	7.2	2.5
%CV	12.1%	12.8%	9.2%	15.9%	13.8%
mean+3SD	79.0	41.7	26.0	66.8	25.8
mean-3SD	37.1	18.6	14.7	23.6	10.7
N	25	25	25	25	25
mean/all kit median	0.98	0.97	0.99	0.94	0.97
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	59.5	31.0	20.5	48.2	18.8
SD	4.5	2.6	1.5	6.7	2.4
%CV	7.5%	8.4%	7.1%	13.8%	12.9%
mean+3SD	72.87	38.82	24.91	68.13	26.05
mean-3SD	46.04	23.24	16.18	28.20	11.55
N	11	11	11	11	11
median	59.50	30.90	20.80	46.00	18.30
mean/All kit median	1.00	1.00	1.00	1.00	1.00
MS hCG Beckman Access/2 (BCX/BC1) mean:					
mean	64.2	33.4	21.7	49.0	19.8
SD	4.8	2.7	1.4	3.6	2.0
%CV	7.5%	8.0%	6.6%	7.3%	10.3%
mean+3SD	78.6	41.5	26.0	59.8	25.9
mean-3SD	49.8	25.4	17.4	38.2	13.6
N	6	6	6	6	6
median	64.5	33.4	21.5	48.1	19.2
mean/all kit median	1.08	1.08	1.06	1.02	1.05
MS hCG Siemens Immulite 2000 (DPD/DP5) mean:					
mean	49.7	25.8	18.6	37.0	15.8
SD	4.0	2.7	1.7	3.7	1.5
%CV	8.1%	10.6%	9.3%	10.0%	9.2%
mean+3SD	61.8	34.0	23.8	48.2	20.2
mean-3SD	37.7	17.6	13.4	25.9	11.4
N	7	7	7	7	7
median	49.4	25.5	18.7	35.8	16.2
mean/all kit median	0.84	0.83	0.91	0.77	0.84
MS hCG kit average:					
mean	57.8	30.1	20.3	44.7	18.1
SD	7.4	3.9	1.6	6.7	2.1
all kit median	59.5	31.0	20.5	48.2	18.8

	MS 291	MS 292	MS 293	MS 294	MS 295
MS hCG MoMs All Lab Mean:					
mean	2.46	1.61	1.14	2.19	0.94
SD	0.24	0.18	0.10	0.33	0.14
%CV	9.6%	11.2%	9.1%	14.9%	14.5%
mean+3SD	3.16	2.15	1.45	3.17	1.35
mean-3SD	1.75	1.07	0.82	1.21	0.53
N	25	25	25	25	25
mean/All Kit Median	0.96	0.96	0.98	0.97	0.98
MS hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	2.56	1.68	1.18	2.39	1.02
SD	0.11	0.10	0.09	0.27	0.11
%CV	4.3%	5.9%	7.4%	11.4%	10.3%
mean+3SD	2.89	1.98	1.44	3.21	1.34
mean-3SD	2.22	1.38	0.92	1.57	0.71
N	11	11	11	11	11
median	2.53	1.72	1.21	2.33	0.99
mean/All kit median	1.00	1.00	1.01	1.05	1.06
MS hCG MoM Beckman Access/2 (BCX/BC1) mean:					
mean	2.59	1.68	1.16	2.27	0.96
SD	0.24	0.24	0.13	0.23	0.11
%CV	9.3%	14.3%	11.2%	10.2%	11.2%
X+3SD	3.31	2.40	1.55	2.96	1.29
X-3SD	1.87	0.96	0.77	1.57	0.64
N	6	6	6	6	6
median	2.60	1.71	1.14	2.26	0.98
mean/All kit median	1.01	1.00	1.00	1.00	1.00
MS hCG MoM Siemens Immulite 2000 (DPD/DP5) mean:					
mean	2.23	1.46	1.06	1.87	0.81
SD	0.20	0.13	0.07	0.19	0.11
%CV	9.1%	8.7%	6.8%	10.2%	13.8%
X+3SD	2.84	1.84	1.28	2.44	1.15
X-3SD	1.62	1.08	0.85	1.30	0.47
N	7	7	7	7	7
median	2.18	1.40	1.07	1.83	0.80
mean/All kit median	0.87	0.87	0.92	0.82	0.84
MS hCG MoM kit average:					
mean	2.5	1.6	1.1	2.2	0.9
SD	0.2	0.1	0.1	0.3	0.1
all kit median	2.6	1.7	1.2	2.3	1.0

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	MS 291	MS 292	MS 293	MS 294	MS 295
MS Inhibin A all lab mean:					
Mean	359.3	144.1	209.8	298.0	221.5
SD	43.1	17.2	22.6	33.7	25.3
%CV	12.0%	11.9%	10.8%	11.3%	11.4%
mean + 3SD	488.5	195.6	277.7	399.2	297.4
mean- 3SD	230.2	92.7	141.9	196.8	145.6
N	25	25	25	25	25
All Lab Median	363.8	148.2	211.3	301.7	225.5
mean/all kit median	0.97	0.97	0.98	0.98	0.97
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	373.7	149.9	218.3	312.1	230.2
SD	27.5	10.0	14.3	16.5	13.8
%CV	7.4%	6.6%	6.5%	5.3%	6.0%
mean + 3SD	456.2	179.8	261.1	361.5	271.6
mean- 3SD	291.1	120.0	175.4	262.7	188.7
N	14	14	14	14	14
kit median	365.0	149.8	214.0	313.4	225.7
mean/all kit median	1.01	1.01	1.02	1.03	1.01
MS Inhibin A Beckman Access/2 (BCX/BC1) mean:					
Mean	371.2	149.1	214.0	303.1	229.0
SD	14.2	6.1	9.0	15.1	7.3
%CV	3.8%	4.1%	4.2%	5.0%	3.2%
mean + 3SD	413.7	167.2	240.9	348.3	250.8
mean- 3SD	328.6	130.9	187.1	257.8	207.2
N	8	8	8	8	8
kit median	366.3	148.1	211.6	303.5	229.1
mean/All kit median	1.00	1.00	1.00	1.00	1.00
MS Inhibin A Diagnostic System Labs (DS1) mean:					
Mean	260.9	103.9	158.9	218.5	161.1
SD	9.8	1.8	9.5	11.3	6.9
%CV	3.7%	1.7%	6.0%	5.2%	4.3%
mean + 3SD	290.2	109.4	187.5	252.3	182.0
mean- 3SD	231.6	98.5	130.3	184.7	140.3
N	3	3	3	3	3
kit median	257.0	104.2	163.3	216.0	162.0
mean/all kit median	0.70	0.70	0.74	0.72	0.70
MS Inhibin A kit average:					
mean	335.2	134.3	197.1	277.9	206.8
SD	64.4	26.3	33.1	51.6	39.5
all kit median	371.2	149.1	214.0	303.1	229.0

	MS 291	MS 292	MS 293	MS 294	MS 295
MS Inhibin A MoM All Lab mean:					
mean	2.14	0.79	1.20	1.76	1.07
SD	0.25	0.09	0.13	0.20	0.15
%CV	11.9%	11.2%	11.1%	11.4%	13.8%
mean+3SD	2.91	1.06	1.60	2.36	1.51
mean-3SD	1.38	0.53	0.80	1.16	0.63
N	25	25	25	25	25
mean/all kit median	0.98	0.97	1.02	1.00	1.00
MS Inhibin A MoM Beckman Unicel (BCU/BC1) mean:					
Mean	2.20	0.82	1.27	1.84	1.12
SD	0.22	0.07	0.10	0.14	0.14
%CV	9.8%	8.0%	7.5%	7.8%	12.8%
mean + 3SD	2.85	1.02	1.56	2.27	1.55
mean- 3SD	1.55	0.62	0.99	1.41	0.69
N	14	14	14	14	14
Kit Median	2.14	0.80	1.21	1.79	1.08
mean/all kit median	1.00	1.01	1.08	1.05	1.05
MS Inhibin A MoM Beckman Access (BCX/BC1) mean:					
Mean	2.22	0.82	1.18	1.76	1.07
SD	0.15	0.05	0.04	0.11	0.07
%CV	6.7%	6.2%	3.5%	6.4%	6.8%
mean + 3SD	2.67	0.97	1.30	2.10	1.28
mean- 3SD	1.77	0.66	1.05	1.42	0.85
N	8	8	8	8	8
Kit Median	2.18	0.81	1.21	1.82	1.09
mean/all kit median	1.01	1.00	1.00	1.00	1.00
MS Inhibin A MoM Diagnostic System Labs (DS1) mean:					
Mean	1.68	0.62	0.94	1.37	0.83
SD	0.18	0.06	0.07	0.19	0.08
%CV	10.6%	9.9%	7.9%	13.8%	9.1%
mean + 3SD	2.21	0.80	1.16	1.94	1.06
mean- 3SD	1.15	0.43	0.72	0.81	0.60
N	3	3	3	3	3
Kit Median	1.62	0.63	0.91	1.44	0.84
mean/all kit median	0.76	0.76	0.80	0.78	0.78
MS Inhibin A MoM kit average:					
mean	2.03	0.75	1.13	1.66	1.01
SD	0.31	0.12	0.17	0.25	0.15
all kit median	2.20	0.82	1.18	1.76	1.07

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	AF 291	AF 292	AF 293	AF 294	AF 295
AF AFP All Lab mean :					
mean	8.4	24.7	10.4	1.9	6.6
SD	1.1	3.7	1.6	0.2	1.0
%CV	12.8%	15.0%	15.3%	12.3%	14.4%
mean+3SD	11.6	35.8	15.2	2.7	9.5
mean-3SD	5.2	13.6	5.6	1.2	3.8
N	19	19	19	18	19
All kit median	8.7	25.2	10.6	2.0	7.0
mean/all kit mean	0.96	0.98	0.98	0.99	0.95
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	7.6	22.4	9.4	1.8	5.9
SD	0.8	2.3	1.3	0.2	0.6
%CV	10.9%	10.3%	13.4%	12.7%	10.4%
X+3SD	10.0	29.3	13.1	2.5	7.7
X-3SD	5.1	15.5	5.6	1.1	4.1
N	8	8	8	8	8
median	7.6	21.9	9.0	1.8	6.0
mean/all kit median	0.87	0.89	0.88	0.93	0.85
AF AFP Beckman Access/2 (BCX/BC1) mean:					
mean	8.4	23.1	9.7	2.0	6.3
SD	0.2	1.5	0.2	0.2	0.2
%CV	2.6%	6.5%	2.5%	8.9%	2.8%
mean+3SD	9.0	27.6	10.4	2.5	6.8
mean-3SD	7.7	18.5	8.9	1.4	5.7
N	4	4	4	4	4
median	8.4	23.4	9.55	1.95	6.2
mean/all kit median	0.96	0.92	0.91	1.00	0.90
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	9.1	27.3	12.3	2.0	7.7
SD	1.0	2.9	1.2	0.2	0.7
%CV	11.5%	10.5%	9.9%	10.6%	9.3%
mean+3SD	12.2	35.9	16.0	2.6	9.8
mean-3SD	5.9	18.7	8.7	1.3	5.6
N	4	4	4	3	4
median	8.9	26.3	11.9	1.9	7.55
mean/all kit median	1.04	1.08	1.16	1.00	1.11
AF AFP Abbott Axsym (ABB/AB2) mean:					
mean	9.9	31.7	11.6	2.2	7.7
N	2	2	2	2	2
mean/all kit median	1.13	1.26	1.09	1.12	1.10
AF AFP kit average:					
mean	8.7	26.1	10.7	2.0	6.9
SD	1.0	4.3	1.4	0.2	0.9
all kit median	8.7	25.2	10.6	2.0	7.0

	AF 291	AF 292	AF 293	AF 294	AF 295
AF AFP MoM All Lab Mean:					
mean	1.36	3.28	0.92	0.21	1.04
SD	0.19	0.56	0.16	0.03	0.11
%CV	13.8%	17.0%	17.3%	12.5%	10.6%
mean+3SD	1.92	4.95	1.39	0.28	1.37
mean-3SD	0.80	1.61	0.44	0.13	0.71
N	19	19	19	19	18
All median	1.32	3.23	0.89	0.20	1.04
mean/all kit median	1.03	1.02	1.03	1.03	1.00
AF AFP MoM Beckman Unicel(BCU/BC1) mean:					
Mean	1.30	3.22	0.89	0.20	1.02
SD	0.17	0.63	0.14	0.03	0.13
%CV	13.0%	19.6%	15.8%	13.9%	12.9%
X+3SD	1.81	5.12	1.31	0.29	1.42
X-3SD	0.79	1.33	0.47	0.12	0.63
N	8	8	8	8	8
median	1.31	3.10	0.87	0.20	1.02
mean/all kit median	0.97	0.96	1.04	0.99	0.99
AF AFP MoM Beckman Access (BCX/BC1) mean:					
Mean	1.34	3.01	0.83	0.21	1.00
SD	0.18	0.50	0.08	0.03	0.11
%CV	13.8%	16.7%	9.8%	15.9%	11.0%
X+3SD	1.89	4.52	1.07	0.31	1.33
X-3SD	0.78	1.50	0.58	0.11	0.67
N	4	4	4	4	4
median	1.27	3.04	0.85	0.21	0.99
mean/all kit median	0.99	0.90	0.96	1.01	0.97
AF AFP MoM DPC Immulite 2000 (DPD/DP5) mean:					
Mean	1.48	3.58	1.09	0.21	1.24
SD	0.28	0.64	0.20	0.03	0.21
%CV	18.7%	18.0%	18.0%	12.9%	17.0%
X+3SD	2.31	5.51	1.67	0.29	1.87
X-3SD	0.65	1.65	0.50	0.13	0.61
N	4	4	4	4	4
median	1.43	3.31	1.00	0.20	1.15
mean/all kit median	1.10	1.07	1.27	1.02	1.20
AF AFP MoM Abbott Axsym (ABB/AB2) mean					
Mean	1.36	3.48	0.82	0.20	1.05
N	2	2	2	2	2
mean/all kit median	1.01	1.04	0.95	0.95	1.01
AF AFP MoM kit average:					
mean	1.37	3.32	0.91	0.20	1.08
SD	0.08	0.26	0.12	0.01	0.11
all kit median	1.35	3.35	0.86	0.21	1.03

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January 2013
Summary of First Trimester Results

	FT291	FT292	FT293	FT294	FT295
FT Gestational Age All Lab Mean:					
Mean	12.4	13.0	10.9	11.9	11.5
SD	0.06	0.05	0.11	0.11	0.13
%CV	0.5%	0.4%	1.0%	0.9%	1.1%
mean+3*SD	12.5	13.1	11.2	12.2	11.9
mean-3*SD	12.2	12.8	10.6	11.6	11.1
N	17	17	17	17	17

	FT291	FT292	FT293	FT294	FT295
FT NT MoM All Lab Mean:					
Mean	0.96	0.98	1.04	2.21	0.91
SD	0.06	0.06	0.06	0.12	0.05
%CV	6.2%	6.2%	5.8%	5.3%	5.2%
mean+3SD	1.14	1.16	1.22	2.56	1.05
mean- 3SD	0.78	0.80	0.86	1.86	0.77
N	16	16	15	16	16
All Median	0.96	0.98	1.03	2.20	0.91

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	FT291	FT292	FT293	FT294	FT295
FT hCG All Lab Mean:					
mean	62.7	65.5	84.5	145.7	65.2
SD	9.3	10.8	14.4	32.0	12.4
%CV	14.9%	16.5%	17.0%	22.0%	19.0%
mean+3SD	90.7	97.9	127.6	241.7	102.3
mean- 3SD	34.7	33.1	41.4	49.7	28.0
N	16	16	16	16	16
All lab median	65.3	66.8	86.2	158.7	67.4
mean/All kit median	0.97	0.97	0.95	0.92	0.98
FT hCG Beckman Unicel (BCU/BC1) mean:					
mean	65.0	67.3	88.8	158.1	66.2
SD	5.8	5.5	9.9	7.6	9.0
%CV	9.0%	8.1%	11.1%	4.8%	13.6%
mean+3SD	82.7	85.9	110.6	211.4	105.0
mean- 3SD	58.0	67.0	81.3	140.5	49.7
N	7	7	7	7	7
median	65.3	67.9	87.9	161.1	71.4
mean/All kit median	1.00	1.00	1.00	1.00	1.00
FT hCG Beckman Access (BCX/BC1) mean:					
mean	70.4	76.5	96.0	176.0	77.4
SD	4.1	3.2	4.9	11.8	9.2
%CV	5.8%	4.1%	5.1%	6.7%	11.9%
mean+3SD	82.7	85.9	110.6	211.4	105.0
mean- 3SD	58.0	67.0	81.3	140.5	49.7
N	4	4	4	4	4
median	69.3	76.6	95.9	177.3	74.3
mean/All kit median	1.08	1.14	1.08	1.11	1.17
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	53.5	54.2	69.4	104.2	54.0
SD	9.5	10.0	12.6	17.4	9.2
%CV	17.7%	18.4%	18.2%	16.7%	17.0%
mean+3SD	81.9	84.1	107.2	156.4	81.5
mean- 3SD	25.1	24.3	31.5	51.9	26.6
N	5	5	5	5	5
median	48.7	51.5	66.7	100.0	49.4
mean/All kit median	0.82	0.81	0.78	0.66	0.82
FT hCG kit average:					
mean	62.9	66.0	84.7	146.1	65.9
SD	8.6	11.2	13.8	37.4	11.7
all kit median	65.0	67.3	88.8	158.1	66.2

	FT291	FT292	FT293	FT294	FT295
FT hCG MoM All Lab Mean:					
Mean	0.82	1.03	0.97	1.91	0.81
SD	0.10	0.12	0.15	0.27	0.10
%CV	12.3%	11.5%	15.2%	14.3%	11.9%
mean+3*SD	1.13	1.39	1.40	2.73	1.10
mean - 3*SD	0.52	0.68	0.53	1.09	0.52
N	15	15	14	15	15
All lab Median	0.82	1.04	0.98	1.90	0.80
mean/All kit Median	1.00	0.98	0.98	0.94	1.00
MS hCG MoM Beckman Unicel (BCU/BC1) mean:					
mean	0.83	1.05	0.99	2.03	0.78
SD	0.10	0.09	0.12	0.17	0.09
%CV	12.5%	9.0%	12.4%	8.3%	11.4%
mean+3SD	1.14	1.34	1.36	2.54	1.04
mean-3SD	0.52	0.77	0.62	1.53	0.51
N	7	7	6	7	7
median	0.84	1.04	0.92	1.98	0.78
mean/All kit median	1.02	1.00	1.00	1.00	0.96
MS hCG MoM Beckman Access (BCX/BC1) mean:					
mean	0.82	1.09	1.04	2.11	0.89
SD	0.01	0.04	0.04	0.06	0.06
%CV	1.2%	3.8%	3.8%	2.8%	6.8%
mean+3SD	0.85	1.21	1.16	2.28	1.07
mean-3SD	0.79	0.96	0.92	1.93	0.71
N	3	3	3	3	3
median	0.82	1.10	1.04	2.13	0.86
mean/All kit median	1.00	1.03	1.05	1.04	1.10
MS hCG MoM DPC Immulite2000 (DPD/DP5) mean:					
mean	0.81	0.97	0.89	1.62	0.81
SD	0.14	0.17	0.20	0.23	0.11
%CV	17.0%	17.1%	21.9%	14.5%	13.9%
mean+3SD	1.22	1.47	1.48	2.32	1.15
mean-3SD	0.40	0.47	0.31	0.92	0.47
N	5	5	5	5	5
median	0.77	0.98	0.96	1.67	0.80
mean/All kit median	0.99	0.92	0.90	0.80	1.00
FT hCG MoM kit average:					
mean	0.8	1.0	1.0	1.9	0.8
SD	0.0	0.1	0.1	0.3	0.1
all kit median	0.8	1.1	1.0	2.0	0.8

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	FT291	FT292	FT293	FT294	FT295
FT PAPP-A All Lab Mean:					
Mean	4057.9	10452.6	5900.4	3239.1	3691.7
SD	2782.8	7363.7	4066.0	2221.5	2588.6
%CV	68.6%	70.4%	68.9%	68.6%	70.1%
mean + 3SD	12406.4	32543.7	18098.5	9903.6	11457.4
mean- 3SD	-4290.6	-11638.5	-6297.7	-3425.4	-4074.1
N	16	16	16	16	16
All Lab Median	2480.5	6261.5	3550.0	1945.0	2221.9
mean/All kit median	1.70	1.71	1.68	1.72	1.74

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A MoM All Lab Mean:					
Mean	3.38	8.38	9.29	3.78	4.74
SD	1.91	4.90	5.38	2.17	2.68
%CV	56.3%	58.5%	58.0%	57.4%	56.4%
mean + 3SD	9.10	23.08	25.44	10.28	12.77
mean- 3SD	-2.33	-6.32	-6.86	-2.73	-3.28
N	16	16	15	16	16
All Lab Median	2.74	6.31	8.27	2.90	3.57
mean/ All kit median	1.29	1.29	1.25	1.34	1.33

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A Beckman Unicel(BCU/BC1) Mean:					
Mean	2393.8	6115.5	3506.4	1887.2	2122.4
SD	223.0	429.2	185.9	142.5	212.3
%CV	9.3%	7.0%	5.3%	7.6%	10.0%
mean + 3SD	3062.8	7403.0	4064.2	2314.9	2759.2
mean - 3SD	1724.7	4828.0	2948.6	1459.6	1485.6
N	9	9	9	9	9
Kit Median	2411.0	6130.0	3446.0	1824.9	2186.7
mean/All kit median	1.00	1.00	1.00	1.00	1.00

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A MoM Beckman Unicel(BCU/BC1) Mean:					
Mean	2.61	6.50	7.45	2.82	3.58
SD	0.26	1.18	1.67	0.30	0.52
%CV	10.0%	18.1%	22.4%	10.5%	14.6%
mean + 3SD	3.40	10.04	12.45	3.70	5.14
mean - 3SD	1.83	2.96	2.45	1.93	2.02
N	9	9	8	9	9
Kit Median	2.72	6.27	8.15	2.88	3.55
mean/All kit median	1.00	1.00	1.00	1.00	1.00

	FT291	FT292	FT293	FT294	FT295
***FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:**					
Mean	7935.3	20207.1	11539.7	6476.7	7428.0
SD	231.6	1058.6	749.5	277.2	501.5
%CV	2.9%	5.2%	6.5%	4.3%	6.8%
mean + 3SD	8630.1	23382.9	13788.2	7308.4	8932.3
mean - 3SD	7240.6	17031.2	9291.2	5644.9	5923.6
N	4	4	4	4	4
Kit Median	8019.9	20048.5	11381.1	6561.2	7533.7
mean/All kit median	3.31	3.30	3.29	3.43	3.50

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:					
Mean	6.48	15.99	17.18	7.34	9.08
SD	0.24	2.81	2.21	0.37	0.77
%CV	3.8%	17.6%	12.8%	5.0%	8.5%
mean + 3SD	7.21	24.41	23.80	8.44	11.40
mean - 3SD	5.75	7.57	10.56	6.23	6.76
N	4	4	4	4	4
Kit Median	6.375	16.34	17.305	7.4	9.135
mean/All kit median	2.48	2.46	2.31	2.60	2.54

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

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A AnshLite (MPR or APM/AN1) Mean:					
Mean	1628.0	3893.0	2225.5	1395.5	1541.5
N	2	2	2	2	2
Kit Median	1628.0	3893.0	2225.5	1395.5	1541.5
mean/All kit median	0.68	0.64	0.63	0.74	0.73

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A MoM (MPR or APM/AN1) Mean:					
Mean	1.66	4.27	4.03	2.02	2.61
N	2	2	2	2	2
Kit Median	1.66	4.27	4.03	2.02	2.61
mean/ All kit median	0.64	0.66	0.54	0.72	0.73

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A kit average:					
mean	3985.7	10071.9	5757.2	3253.1	3697.3
SD	3441.8	8847.4	5048.6	2802.5	3243.9
all kit median	2393.8	6115.5	3506.4	1887.2	2122.4

	FT291	FT292	FT293	FT294	FT295
FT PAPP-A MoM kit average:					
mean	3.58	8.92	9.55	4.06	5.09
SD	2.55	6.22	6.82	2.87	3.49
all kit median	2.61	6.50	7.45	2.82	3.58