NEW YORK STATE OF OPPORTUNITY. Department of Health

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Dear Laboratory Director,

Attached you will find a summary and critique of the Proficiency Testing mail-out from May 2015, 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 which also includes 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.

Yours sincerely,

Gerald J. Mizejewski

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GJM:tlg
Attachments

Fetal Defect Marker Proficiency Test Mailout[1]
May 5, 2015

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

Samples *N = 25	Sample #	MS 326	MS 327	MS 328	MS 329	MS 330
	Gestational Age (weeks)	15.0	17.0	21.0	19.0	20.0
Maternal Race	Ethnic Group	White	Hispanic	White	Asian	White
Maternal Weight	Pounds (lbs)	150	200	145	120	155
Maternal Age	Years	29	30	28	21	23
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	19.9 ± 4.2	23.4 ± 3.8	381.9 ± 30.3	35.7 ± 6.2	114.7 ± 15.0
	MOM ± Std. Dev.	0.70 ± 0.15	0.76 ± 0.13	5.62 ± 0.66	0.62 ± 0.12	2.03 ± 0.30
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	0.34 ± 0.04	0.99 ± 0.09	1.53 ± 0.11	1.46 ± 0.16	1.56 ± 0.11
	MOM ± Std. Dev.	0.54 ± 0.11	1.06 ± 0.25	0.65 ± 0.09	0.86 ± 0.20	0.81 ± 0.09
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	97.9 ± 6.9	29.7 ± 3.1	20.7 ± 2.1	22.3 ± 2.7	22.2 ± 1.9
	MOM ± Std. Dev.	2.25 ± 0.44	1.23 ± 0.23	1.04 ± 0.20	0.87 ± 0.22	1.10 ± 0.20
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	342.8 ± 19.0	136.3 ± 6.5	215.4 ± 10.5	188.8 ± 9.6	217.2 ± 12.7
	MOM ± Std. Dev.	1.83 ± 0.15	0.94 ± 0.08	1.00 ± 0.10	0.99 ± 0.10	1.16 ± 0.10
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (–)	(–) (100%)	(–) (100%)	(+) (100%)	(–) (100%)	(–) (72%)
	Recommended Action**	NFA	NFA	G = 88% U = 100% A = 84%	NFA	NFA
	NTD Risk (median) 1 in	7,320	12,050	13	10,500	403
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (–)	(+) (100%)	(–) (83%)	(–) (92%)	(–) (92%)	(–) (92%)
	Recommended Action**	G = 92% U = 50% A = 83% N = 17%	NFA	NFA	NFA	NFA
	Risk Est. (median) 1 in	40	1,264	6,450	3,285	5,300
2. Quad Test	Pos. (+) or Neg. (–)	(+) (100%)	(–) (92%)	(–) (96%)	(–) (92%)	(–) (96%)
	Recommended Action **	G = 92% U = 56% A = 84% NIPT= 12%	NFA	NFA	NFA	NFA
	Risk Est. (median) 1 in	51	2,860	20,000	3,300	15,250
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (–)	(–) (100%)	(–) (100%)	(–) (100%)	(–) (100%)	(–) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. (median) 1 in	5,000	10,000	10,000	10,000	10,000

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

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

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results (Figs. 4-6):

N = 25 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 326 Wk 15.0	This specimen was obtained from a 29 year old White woman (Gravida = 3, Parity = 1) in her 15th week of gestation with a body weight of 150 lbs. She had a family (siblings) history of pregnancy complications. Her specimen screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100% Triple, 100% Quad). Recommendations for further action from labs reporting a positive T21 quad screen were: genetic counseling, 92%; ultrasound, 56%; amniocentesis, 84%, and noninvasive prenatal testing, 12%; while labs reporting a positive triple test recommended genetic counseling, 92%; ultrasound 50%; and amniocentesis, 83%, and noninvasive prenatal testing, 17%. Specimen MS326 resulted in a negative T18 screen in 100% of the participating labs. This sample was paired to amniotic fluid specimen AF326 which also had a low AFAFP level (MOM = 0.53).
MS 327 Wk 17.0	This specimen was obtained from a 30 year old Hispanic woman (Gravida = 2, Parity = 1) in her 17th week of gestation with a body weight of 200 lbs. She had no personal history of pregnancy complications and her specimen resulted in a negative screen for NTD with a body weight and ethnic correction indicated. There was consensus that both Trisomy screens were negative. Specimen MS327 was not paired with an amniotic fluid specimen.
MS 328 Wk 21.0	This specimen was obtained from a 28 year old White woman (Gravida = 1; Parity = 0) in her 21st week of gestation with a body weight of 145 lbs. She had no pre-existing personal history of pregnancy loss (see critique). Her sample was a positive screen for NTD (100% consensus; MOM = 5.62). Her screen was negative for both Trisomies with all labs in agreement. Recommendations for further action from labs reporting a positive NTD screen were: genetic counseling, 88%; ultrasound, 100%; and amniocentesis, 84%. The MS328 specimen had no amniotic fluid counterpart.
MS 329 Wk 19.0	This specimen was obtained from a 21 year old Asian woman (Gravida = 3, Parity = 2) in her 19th week of gestation with a body weight of 120 lbs. She had no family history of reproductive complications. Her sample screened negative for NTD, and her aneuploidy screens were negative for both Trisomy-18 and Trisomy-21. The MS329 sample was not paired to an amniotic fluid specimen.
MS 330 Wk 20.0	This specimen was obtained from a 23 year old White woman (Gravida = 2; Parity = 0) in her 20th week of gestation with a body weight of 155 lbs. Her sample screened borderline consensus negative for NTD, and her aneuploidy screen was negative for Down syndrome. No further actions were recommended. This sample was paired to amniotic fluid specimen AF330 (MOM = 3.33), which was highly elevated.

Notice of Gravida/Parity Clarification for Present and Future Mail Outs:

For the sake of this program, it will be understood that gravida indicates the pregnant status of a woman and parity is the state of having given birth to a completed term infant or infants. Thus, a gravida = n, indicates number (n) of pregnancies including the present one and parity = m indicates the patient already has m children; however, multiple birth is also considered as a single parity.

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

2) AMNIOTIC FLUID AFP (NTD-analysis) (Fig. 3):

N=19; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 326 Wk 15.0	AFP = 8.5 ± 0.8 µg/ml MOM = 0.53 ± 0.06	The AF326 sample was targeted for a lower AFAFP value in the routine gestational age range and all labs agreed. However, all labs called AF326 screen negative for AFAFP. This sample was matched to maternal serum specimen MS326 whose AFP level was low (MOM = 0.70).
AF 327 Wk 19.0	AFP = 11.7 ± 1.0 µg/ml MOM = 1.52 ± 0.15	The AF327 sample was targeted for a screen negative AFAFP value in the upper gestational age window. All labs reported this specimen as screen negative for AFAFP. The AF327 specimen was not paired with a maternal serum sample.
AF 328 Wk 18.5	AFP = 8.7 ± 0.9 µg/ml MOM = 1.0 ± 0.12	The AF328 sample was targeted as an NTD negative specimen in the upper gestational age screening range. All labs categorized AF328 as a negative NTD screen. This specimen had no maternal serum counterpart.
AF 329 Wk 17.0	AFP = 14.8 ± 1.8 µg/ml MOM = 1.31 ± 0.15	The AF329 sample was targeted for a normal AFAFP value in the upper gestational age range. All labs called AF329 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 330 Wk 20.0	AFP = 21.1 ± 2.4 µg/ml MOM = 3.33 ± 0.42	The AF330 sample was targeted for an elevated AFAFP value in the upper gestational age range. All labs reported this specimen as screen positive for AFAFP. The AF330 specimen was paired with a slightly elevated maternal serum sample (MOM = 2.03).

II. Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 15	**Sample #**	**FT 326**	**FT 327**	**FT 328**	**FT 329**	**FT 330**
	Gestational Age (weeks)	12.4	11.9	13.0	11.1	11.5
Maternal Race	Ethnic Group	Asian	White	Hispanic	Black	Hispanic
Maternal Weight	Pounds (lbs)	130	150	160	155	145
Maternal Age	Years	35	25	21	32	26
Fetal Physical Measurements	Crown Rump Length (mm)	59	53	67	43	48
	NT Thickness (mm)	1.40	2.90	1.60	1.10	1.10
	NT – MOM ± Std. Dev.	0.95 ± 0.06	2.20 ± 0.14	0.96 ± 0.06	0.99 ± 0.07	0.91 ± 0.05
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	52.9 ± 9.8	138.9 ± 16.4	50.9 ± 10.3	85.4 ± 6.7	66.6 ± 6.6
	MOM ± Std. Dev.	0.51 ± 0.05	1.41 ± 0.15	0.61 ± 0.06	0.77 ± 0.10	0.65 ± 0.07
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	3493.2 ± 875.4	2760.5 ± 778.1	6488.7 ± 2323.7	3185.0 ± 1584.2	1796.2 ± 874.2
	MOM ± Std. Dev.	3.50 ± 1.14	3.82 ± 1.26	6.17 ± 2.22	5.14 ± 2.76	2.63 ± 1.24
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(–) (93%)	(–) (80%)	(–) (100%)	(–) (100%)	(–) (100%)
	Recommended Action **	NFA	G = 19% U = 13% A = 6% C = 6% N = 13%	NFA	NFA	NFA
	Risk Estimate 1 in	10,000	485	13,000	16,000	10,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(–) (100%)	(–) (100%)	(–) (100%)	(–) (100%)	(–) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	NFA
	Risk Estimate 1 in	10,000	5,000	10,000	10,000	10,000

*N = total numbers may vary since some labs do not test all analytes. (B) = borderline negative or positive; NFA = no further action; G = genetic counseling; U = ultrasound; 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 below.

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results (Figs. 13, 14):
N = 16 all-lab Consensus Values.

Sample#	Summary Comments:
FT 326 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 blood draw was 12.4 weeks. She had no prior history of pregnancy complications or difficulties. This FT specimen was screen negative and all but one testing lab were in agreement. The FT326 risk estimate for Trisomy-21 was 1 in 10,000 and the Trisomy-18 risk was 1 in 10,000.
FT 327 Wk 11.9	This specimen was obtained from a 25 year old White woman of average body weight (150 lbs). Her gestational age at the time of blood draw was 11.9 weeks. She had no prior history of any pregnancy complications. This FT specimen was borderline negative for Trisomy-21 with 80% of testing labs in agreement. The median FT327 risk estimate for Trisomy-21 was 1 in 485, and for Trisomy-18 was 1 in 5,000.
FT 328 Wk 13.0	This specimen was obtained from a 21 year old Hispanic woman of average body weight (160 lbs.). Her gestational age at the time of blood draw was 13.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative with an all-lab consensus of 100%. The FT328 risk estimate for Trisomy-21 was 1 in 13,000, and the Trisomy-18 risk was 1 in 10,000.
FT 329 Wk 11.1	This specimen came from a 32 year old Black woman with a body weight of 155 lbs. Her gestational age at the time of blood draw was 11.1 weeks. She reported no prior family history of pregnancy problems. This FT specimen was screen negative for both Trisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT329 was 1 in 16,000, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.
FT 330 Wk 11.5	This specimen was procured from a 26 year old Hispanic woman of average body weight (145 lbs). Her gestational age at the time of blood draw was 11.5 weeks. She had no prior family history of pregnancy complications or adverse outcomes. This FT specimen was screen negative for Trisomy-21 with all labs in agreement. The FT330 risk estimate for Trisomy-21 was 1 in 10,000, while the Trisomy-18 risk was 1 in 10,000.

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 **MS330** resulted in a borderline negative NTD screen (Figs. 2a and 3), in contrast with the elevated AFAFP in the matched **AF330** sample (Fig. 2b); all labs were in agreement that all Trisomy screens were negative (Figs. 4-6). The median risk assessment for NTD in MS330 was 1 in 403. To confirm an NTD, a follow-up a polyacrylamide gel electrophoresis would have to be performed to demonstrate the presence of a diagnostic Ache band. If negative, subsequent tests for fetal hemoglobin are indicated to determine whether a fetal-bleed may have caused the elevated amniotic fluid AFP. Taken together, these results would then indicate that **MS330** was a false positive NTD in the initial screen. The maternal serum MOM levels for MS330 were: MSAFP MOM = 2.03; MSuE3 MOM = 0.81; MShCG MOM = 1.10; MSDIA MOM = 1.16.

Sample **MS326** was obtained from a white woman with a prior family history of pregnancy complications. The fetal defect marker MOM values for this specimen (MSAFP MOM = 0.70, MSuE3 MOM = 0.54, MShCG MOM = 2.25, DIA-MOM = 1.83) presented the canonical profile for elevated risk for T21 of low MSAFP and low MSuE3, together with elevated MShCG and raised MSDIA (Fig. 1) resulting in a positive Down Syndrome screen with which all labs agreed (100% by triple and 100% by quad test). In addition, the matched **AF326** specimen showed reduced MSAFP levels (MOM value = 0.53). The median T21 risk was 1 in 40 by triple test and 1 in 51 by quad test (Figs. 4, 5). It is interesting that the triple risk was slightly higher than the risk from the quad tests, possibly due to the high normal MSDIA value. The recommended further actions for sample **MS326** were genetic counseling, 92%; ultrasound, 56%; amniocentesis, 84%, and

noninvasive prenatal testing, 12%, from labs performing the quad screen; and genetic counseling, 92%; ultrasound, 50% amniocentesis 83%, and noninvasive prenatal testing 17% from labs performing the triple screen.

Two other specimens, **MS327** and **MS329**, produced negative screens for NTD, T21, and T18, but a correction for body weight and ethnicity was indicated for **MS327**.

The **MS328** specimen at 19 weeks presented an interesting case involving extremely high levels of MSAFP (MOM = 5.6) with normal MShCG, MSuE3, and MSDIA. This biomarker profile resulted in a positive screen for NTD (risk = 1 in 13) (Fig. 3). Sample **MS328** was modeled after several case studies of pregnant women with Epidermolysis Bullosa (EB) which manifested highly elevated levels of MSAFP and AF-AFP and an aberrant presence of acetylcholinesterase (Ache) in amniotic fluid (1, 2). EB was first reported in 1968 and only 144 pregnancy cases have been described thus far in the literature. EB is an inherited malformation of the skin collectively called genodermatosis; however, EB can occur in fetuses without a previous known risk. In some cases, mothers had given birth to a previous child afflicted with EB or a related sporadic skin disorder termed aplasia cutis congenita (ACC) (3). Although the women had been informed of the possibility of a second EB pregnancy, some women chose to continue gestation and underwent further testing which included amniocentesis, ultrasound, fetal skin biopsy, MSAFP and AFAFP measurements, and a possible presence of Ache in the amniotic fluid. Most of the women in the case studies had raised levels of MSAFP determined after their first obstetrician visit. Their ultrasound results did not show any gross anatomical malformations; however, a few images did reveal occurrences of polyhydramnios, gastric dilations, and gastrointestinal blockages (i.e., atresia). Most of the women with EB did not deliver normal term infants, but rather experienced spontaneous second and/or third trimester abortion or pre-term birth with adverse outcomes involving massive fetal skin lesions. In a few instances, however, the skin lesions appeared later in the newborn and neonatal period. In conclusion, **MS328** produced a false positive screen for NTD due to the extremely elevated levels of MSAFP and AFAFP.

Elevated AFP in pregnancy is associated with various fetal malformations such as anencephaly, spina bifida, omphalocoele, gastroschisis, nephrosis, renal agenesis, esophageal/phyoric atresia, aplasia cutis congenita, and epidermolysis bullosa. Such increased serum levels of AFP have been ascribed to five possible mechanisms (4). First, NTD disorders result from AFP leakage from the cerebrospinal fluid into the amniotic sac. Second, AFP transudates from blood vessels of the viscera in the event of defects such as omphalocoele and gastroschisis. Third, congenital nephrosis produces a state of fetal proteinuria in amniotic fluid. Fourth, blockages in the gastrointestinal tract (duodenal/esophageal atresia) cause impairment of fetal swallowing/digestion and the accumulation of fetal proteins in the amniotic sac. Lastly, elevated maternal serum and amniotic fluid AFP levels may arise due to pore formation and leakage from skin lesions in aplasia cutis congenita and epidermolysis bullosa (5).

Epidermolysis bullosa (EB) is an inherited skin connective tissue disorder causing blisters, scarring, erosions, and eruptions within and about the skin and epithelial mucous membranes. This skin disorder can be highly lethal in the fetal, perinatal, and neonatal periods; however, milder forms do exist that allow patients to live into adulthood (6). The major defect in EB lies in a separation of the epidermis from the dermis layer due to disruption of anchoring filaments between the two layers. Gene mutations occur in several connective tissue proteins including collagen, keratin, integrins, laminins, integrin alpha-6 and beta-4, and in collagenase enzymes (7). These mutated, altered proteins result in the formation of defective anchors and filaments that attach the basement membrane of the epidermis to the upper layer of the dermal layer. There is no cure for this blistering and denudation skin disorder and it renders the surviving patients susceptible to skin cancers later in life (8).

The pathophysiology of the EB disorder involves the rivet-like attachments of the outermost epidermis to the innermost dermis of the skin layers. Normal skin has protein anchors that serve as rivets and studs between the two layers with a multi-layered basement membrane between them lined with basal cells. EB patients lack the proper connective tissue-derived protein anchor filaments that hold the two skin layers together. These anchors are comprised of complexes of keratin, collagen, laminin and integrins alpha-6 and beta-4. Blister formation occurs in the lamina lucida and lamina densa of the basement membrane; the damage occurs at the level of the hemi-desmosomes (9). While desmosomes provide cell-to-cell attachments, hemi-desmosomes function by attaching cells to the extracellular matrix proteins. These anchors prevent the two skin layers from moving independently; if defective, a result occurs that causes a shearing effect. Without the proper anchors, the two skin layers slide over each other; and causes friction. The shearing effect results in blisters, scarring, and painful sores comparable to third degree burns after even minor mechanical friction such as rubbing and pressure.

EB occurs worldwide affecting every racial and ethnic group, but especially Lebanese, Turkish, Arab, Bedouin, and American Indian populations due largely to consanguineous marriages (10, 1). While some subtypes of EB are present in less than 9 per million people, the estimated overall livebirths of EB are 50 in 1 million people due to the inherited nature of the disorder. EB is classified into four subtypes, namely, 1) EB simplex, (EBS) and; 2) Dystrophic EB (DEB), both of which are autosomal dominant; 3) Junctional EB (JEB); and 4) EB Letalis (EBL), both being autosomal recessive (11). The percent incidence among the various EB subtypes are as follows: a) EBS at 92%; b) DEB at 5%; c) EBL at 2%; and d) JEB at 1%. Over 300 gene mutations have been identified in connective tissue proteins in EB and the carrier frequency has been determined at 1 in 333 for JEB, 1 in 450 for DEB, and somewhat higher frequencies for EBS and EBL. Newborns rarely survive to adulthood, but those that do require constant treatments such as skin grafting, wearing of skull caps and body tubes (stockings, waist), continuous therapy for blistering and scarring, surgical opening of trachea and esophagus, and clearing of respiratory airways (6).

1) Epidermolysis Bullosa Simplex:

It is important to distinguish among the classification of the four EB subtypes due to their differing anatomical and biochemical etiologies. First in the EB subtype categories is EB-simplex (EBS), which is a consequence of mutations in the keratin protein genes KRT5 and KRT14. Keratins are fibrous structural proteins that assemble into strong bundles of intermediate filaments that comprise the outermost layer of the human epidermis. EBS usually has an onset of the disorder at birth or in early infancy. Skin lesions appear as blisters or vesicles at sites of rubbing of the skin (friction) following minor trauma. The mucus membranes of the epithelial lining of ducts and passageways in various organs are also affected. These lesions appear most frequently in passageways of the esophagus, trachea, mouth, and nose. Skin blistering can also extend to the limbs, hands and feet, with the fingernails and toenails being spared. The friction between the epidermis and dermis causes the skin to become extremely fragile. Following a series of complicated treatments in the neonatal period, some EBS patients may have a good outcome (12).

One case history of EB simplex reported a female infant delivered at full-term, but that died at 43 hours postpartum. The newborn presented with EBS, but diagnosis was complicated by the presence of Aplasia cutis congenital (absence of skin in only certain areas) (13). However, diagnosis of EBS was confirmed by a skin biopsy followed by electron microscopic examination.

2) Junctional Epidermolysis Bullosa:

The second in the EB subtype classification is Junctional EB (Herlitz variant) caused by mutations in the laminin and collagen genes (12, 14). Less than one in a million people have this variant, which can present in both children and adults. Blister formation occurs in the lamina lucida of the skin basement membrane with damage occurring at the level of the hemi-desmosome anchors. Gene mutations can result in nonsense mutations in the integrin-alpha-1 protein in fetuses without a prior risk, but often occurs in consanguineous marriages. EBS is highly lethal with large areas of skin denudation (25%), scarring, thinning skin, kidney malformations, malposition of toes, fisted hands, abnormal ears, and the presence of narrow nostrils.

Diagnostic tests for JEB displayed highly elevated levels of MSAFP and AFAFP together with a presence of Ache in amniotic fluid. Ultrasound imaging revealed the presence of polyhydramnios, dilated stomach, pyloric atresia, and anatomical defects in the brain ventricles of some fetuses (13, 15). Pregnancies are often spontaneously or therapeutically aborted in the third trimester (25-33 weeks) with the delivery of dead fetuses or fetuses that die within several days following birth. Fetal skin biopsies, when recommended and approved by the pregnant patient, require electron microscopy for confirmation of defective anchor lesions in the skin.

3) Dystrophic Epidermolysis Bullosa:

The third in the EB subtype classification is Dystrophic-EB (DEB) caused by mutations in collagen and collagenous enzymes (16). The DEB condition affects both the skin and the linings of visceral organs. The term Dystrophic refers to scar formation resulting from the separation of the epidermis from its underlying basement membrane. DEB often occurs in conjunction with pyloric atresia and aplasia cutis congenital (10, 17). Skin defects can occur in regions of the thorax, flanks, upper arms, eyes, digits, ears, and abdomen, with skin becoming translucent, delicate, and fragile. In extreme cases, almost 95% of the total body epidermis can be missing. Most EB births are pre-term with premature infants sometimes delivered at 23-27 weeks gestation; however, these newborns only survive for a few hours.

Second trimester prenatal biomarkers for DEB include extremely elevated MSAFP levels (24 MOMs) that are mirrored by AFAFP MOMs ranging up to 21 MOMs (18). Ache is usually present and detected in the amniotic fluid, which prompts fetal skin sampling at 20-22 weeks. The presence of Ache is thought to originate from open nerve endings in the skin. However, all EB fetuses (including DEB) display normal karyotypes of both sexes.

4) Epidermolysis Bullosa Letalis:

The fourth and final EB subtype classification is EB Letalis (EBL) in which onset can occur during pregnancy or in the neonatal period in newborns with no known risk (19). EBL is caused by the disconnection of basal cells from the basement membrane. This skin disorder affects less than 1 in 20,000 in the US population. EBL manifests in generalized skin blister formation accompanied by extensive sloughing and denudation of large areas of the skin. The skin separation occurs along the epidermal-dermal junction due to hypoplastic or absent hemi-desmosomes resulting in the formation of attachment lesion plaques.

During pregnancy, EBL often occurs concurrently with gastric dilation, chorioamnionitis, and pyloric atresia but otherwise normal ultrasonography. EBL pregnancies exhibit elevated MSAFP together with elevated AFAFP levels at values averaging 21 MOMs (19). In this subtype of EB, elevated AFP levels can also occur during the neonatal period.

B) Assay Kit Performance:

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in bar-graph format (Figs. 7-10). All participating labs used either a Beckman UNICEL/Access/2 or Siemens Immulite instrument. As shown in Figs. 7A,B, MS-AFP mass measurements showed a substantial difference between the Beckman and Siemens Immulite instruments, with the latter returning values that were 40% lower than those from the Beckman methods. Interestingly, this difference was not seen in the amniotic fluid samples. When the kit specific uE3 MOMs were compared, values from the Siemens Immulite instruments ranged nearly 20% higher than those from the Beckman kits, although there was little difference in the actual mass values (Fig. 8A and 8B). The instrument comparison for Inhibin-A displayed in Figs. 9A,B shows that there was no difference between the results from the Beckman Access/2 and UNICEL instruments. Finally, results for hCG from the Beckman 5th generation kits (BCU/BC2; BCX BC2) were about the same as those from the original Beckman kits (BCU/BC1;BCX/BC1), but both were 20% lower than those from the Siemens Immulite 2000 instrument (Fig. 10A). This difference was increased rather than eliminated by the conversion to MOM values (Fig. 10B).

C) Second Trimester Screening Software Utilized:

The alpha, Benetech PRA and Robert Maciel (RMA) software packages were each used by 28% of the participants whereas in-house and "other" software comprised 16%. Programs classified as "other" are presumably proprietary software packages.

D) First Trimester Screen:

In order to compare the Beckman UNICEL assays (67% users) for PAPP-A with those of the older Siemens Immulite and the AnshLabs assay platforms, a conversion factor given in the AnshLabs/Anshlite package insert of 0.00256 mIU/ml = 1ng/ml was used.

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in Figs. 11 and 12 for the five FT samples. As shown in Fig 11A, FT hCG mass measurements by the Beckman UNICEL or Access/2 original and 5th generation IS hCG kits were 10-20% lower than those by the Siemens Immulite instruments. Overall, the hCG MoM values reflected the mass values but the differences between the kits were exacerbated (Fig. 11B), similar to what was seen with the second trimester MS samples. The results from the three PAPP-A kits, even when converted to the same mass units (ng/ml), were not consistent among one other (Fig. 12A) with Siemens Immulite nearly 2.0 times greater than Beckman, and Anshlite less than half of Beckman. Corresponding MOM values also reflected these differences.

E) First Trimester Screening Software Utilized:

The alpha and Benetech software packages were each used by 20%, while RMA was 40% and in-house software comprised 20%. None of the labs used programs classified as "other".

G.J. Mizejewski, Ph.D.

Critique-Related References:

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1. Tancrede, S., E. Bujold, Y. Giguere, M. H. Renald, J. Girouard and J. C. Forest (2015) Mid-trimester maternal serum AFP and hCG as markers of preterm and term adverse pregnancy outcomes. J Obstet Gynaecol Can 37: 111-6.

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13. Liang, L., S. Ge, L. Li, F. Liu and J. Yu (2015) Microfluidic paper-based multiplex colorimetric immunodevice based on the catalytic effect of Pd/Fe(3)O(4)@C peroxidase mimetics on multiple chromogenic reactions. Anal Chim Acta 862: 70-6.

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VI. Potentially helpful website connections/locations:

1) www.allrefer.com

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

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

4) http://pregnancy.about.com/od/afp

5) http://www.americanpregnancy.org/prenatal-testing

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

(1) Source: J Obstet Gynaecol Can. 2015 Feb;37(2):111-6

Title: Mid-trimester maternal serum AFP and hCG as markers of preterm and term adverse pregnancy outcomes

Authors: Tancrède S, Bujold E, Giguère Y, Renald MH, Girouard J, Forest JC

Abstract: OBJECTIVE: To evaluate the predictive values of mid-trimester serum alpha-fetoprotein (AFP) and human chorionic gonadotropin (hCG) for preterm and term placenta-mediated adverse pregnancy outcomes (PMAPOs). METHODS: We extracted data for nulliparous women with a singleton pregnancy without aneuploidy or lethal fetal anomalies from a prospective cohort study. Maternal serum AFP and hCG measured between 13 and 17 weeks of gestation and expressed as multiples of the median (MoM) for gestational age were compared between women who developed a PMAPO (preeclampsia, intrauterine growth restriction, fetal death) before term or at term and women who did not develop any of these complications. RESULTS: Among 3466 nulliparous women, maternal serum AFP and hCG levels were available in 2110 and 2125 cases, respectively. Women who developed a PMAPO before term had a higher median level of serum AFP (1.4 vs. 1.1 MoM; $P < 0.01$) and hCG (1.3 vs. 1.1 MoM; $P < 0.01$) than controls. A serum hCG > 2.0 MoM was associated with a higher risk of PMAPO before term (RR 4.6; CI 95% 2.3 to 9.1) but had no impact on the risk of PMAPO at term (RR 1.1; CI 95% 0.7 to 1.7). Maternal serum AFP > 2.0 MoM was also associated with a significant increase in the risk of preterm PMAPO (RR 3.9; CI 95% 1.6 to 9.8) but not term PMAPO (RR 1.2; CI 95% 0.6 to 2.3). CONCLUSION: Maternal serum AFP or hCG > 2.0 MoM increases the risk of preterm PMAPO but not term PMAPO in our population. We suggest that women with elevated serum AFP or hCG should receive standard pregnancy care once they have reached 37 weeks of gestation if fetal growth is in the normal range.

(2) Source: Clin Lab. 2014;60(11):1795-800

Title: Rheumatoid factor interference of α-fetoprotein evaluations in human serum by ELISA

Authors: Zhu X, Yuyingguo, Huang Z, Yang S

Abstract: BACKGROUND: Serum a-fetoprotein (AFP) is one of the most common diagnostic markers for hepatocellular cancer (HCC) in the laboratory. As other immunoassays, AFP determination is not free from interferences. Natural antibodies like rheumatoid factors (RFs) may induce falsely elevated results, leading to misdiagnosis and expensive unnecessary explorations. METHODS: Serum samples containing AFP were spiked with moderate- or high-concentration RFs, and samples with high RF levels were spiked with AFP. The original sera, as well as the mixtures were tested for AFP by one-step and two-step sandwich ELISA. RESULTS: In the spiked specimens, addition of RFs caused significant bias in the measurement of AFP. The threshold for triggering significant interference was 260 IU/mL for RFs and 74 ng/mL for AFP. CONCLUSIONS: In studies on the prevalence and clinical significance of serum AFP, RF interference should be considered and RF testing should be performed.

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

(3) Source: Ann Clin Lab Sci. 2015 Winter;45(1):106-9

Title: A case report of a fetus with mosaic autosomal variegated aneuploidies and literature review

Authors: Cho CH, Oh MJ, Lim CS, Lee CK, Cho Y, Yoon SY

Abstract: Mosaic variegated aneuploidy (MVA) is a recessive condition characterized by mosaic aneuploidies, predominantly trisomies and monosomies, involving multiple chromosomes and tissues. The phenotype

of MVA syndrome includes severe microcephaly and growth deficiency, central nervous system anomalies, mental retardation, mild physical anomalies, and predisposition to cancer. We report a case of true fetal mosaicism for variegated aneuploidies detected in amniotic fluid cells. A 33-year-old primigravida woman at 5 weeks 1 day of gestation was referred to our tertiary hospital because of a high-risk pregnancy associated with IgA nephropathy. In a quadruple screening test performed at the 15(th) week of gestation, alpha fetoprotein was 73.4 IU/mL (2.792 MoM), suggesting that she was at high risk of neural tube defect. Following amniocentesis performed at the 17 weeks' gestation, chromosome examination of amniocyte culture showed premature chromatic separation in 63% of the metaphases (58/92) and a high frequency of gain and loss of chromosomes. Repeat amniocentesis at 21 weeks' gestation consistently showed the presence of multiple mosaic autosomal variegated aneuploidies. Ultrasonography at 21 weeks' gestation revealed relatively small head circumference for gestational age (<3%) and vermis defect, suggesting that the fetus would have microcephaly and Dandy-Walker malformation. Cytogenetic analysis with peripheral blood of the parents showed normal karyotype. In summary, we hereby report the cytogenetic analysis and prenatal findings of MVA.

(4) Source: BMJ Case Rep. 2015 Mar 27;2015. pii: bcr2015209695. doi: 10.1136/bcr-2015-209695

Title: The crying sign: the winking umbilical cord

Authors: Smith AM, Healy DB, Ryan CA, Dempsey EM

Abstract: A preterm baby girl, born at 34 weeks gestation, with features of Beckwith-Wiedemann syndrome was noted to have a relatively large umbilical stump. No fetal abnormalities had been detected on anatomy scan at 28 weeks and only mild polyhydramnios and macrosomia were noted on a 32-week ultrasound scan. Although there was no obvious omphalocoele, clinical assessment of the umbilical cord revealed an abdominal wall defect through which bowel would protrude into the umbilicus when the infant was crying. In keeping with an abdominal wall defect α-fetoprotein was found to be elevated. Surgical consultation advised conservative management. Subsequently, detachment of the umbilical cord occurred 1 week postdischarge and a large umbilical hernia persists. Genetic analysis confirmed a diagnosis of Beckwith-Wiedemann syndrome.

(5) Source: Ann Thorac Surg. 2015 Mar;99(3):1073-5. doi: 10.1016/j.athoracsur.2014.05.054

Title: Benign metachronous bilateral ovarian and mediastinal teratomas with an elevated alpha-fetoprotein

Authors: Caposole MZ, Aruca-Bustillo V, Mitchell M, Nam B

Abstract: Teratomas are a common form of non-seminomatous germ cell tumor histologically composed of tissues derived from multiple cell lines of the primary embryonic germ cell layers. There are few cases reported in the literature that describe multiple locations with recurrence of benign teratomas, none of which describe an elevated AFP. We describe a case of metachronous bilateral recurrent ovarian and mediastinal teratomas with a curiously elevated α-fetoprotein. We may be describing a novel syndrome of recurrent metachronous teratomas. Because of the uncertainty of this case, the patient will require close follow-up over the next several years.

C) News of Note: Abstracts of New Markers:

(6) Source: Ultrasound Obstet Gynecol. 2015 Feb 4. doi: 10.1002/uog.14809. [Epub ahead of print]

Title: Serum alpha-fetoprotein in the three trimesters of pregnancy: effects of maternal characteristics and medical history

Authors: Bredaki FE, Sciorio C, Wright A, Wright D, Nicolaides KH

Abstract: OBJECTIVE: To define the contribution of maternal variables which influence the measured maternal serum alpha-fetoprotein (AFP) in screening for pregnancy complications. METHODS: Maternal characteristics and medical history were recorded and serum AFP was measured in women with singleton pregnancies attending for three routine hospital visits at 11^{+0} -13^{+6} , 19^{+0} -24^{+6} and 30^{+0} -34^{+6} weeks' gestation. For pregnancies delivering phenotypically normal live births or stillbirths at ≥24 weeks' gestation, variables from maternal demographic characteristics and medical history important in the prediction of AFP were determined from a linear mixed effects multiple regression. RESULTS: Serum AFP was measured in 17,071 cases in the first trimester, 8,583 in the second trimester and 8,607 in the third trimester. Significant independent contributions to serum AFP were provided by gestational age, maternal weight, racial origin, and gestational age at delivery, birth weight Z-score of the neonate and interval from the previous pregnancy. Cigarette smoking was found to significantly affect serum AFP in the first trimester only. Machine used to measure serum AFP was also found to have a significant effect. Random effects multiple regression analysis was used to define the contribution of maternal variables that influence the measured serum AFP and express the values as multiples of the median (MoMs). The model was shown to provide an adequate fit of MoM values for all covariates both in pregnancies that developed preeclampsia and in those without this pregnancy complication. CONCLUSIONS: A model was fitted to express the measured serum AFP across the three trimesters of pregnancy into MoMs after adjustment for variables from maternal characteristics and medical history that affect this measurement.

(7) Source: Arch Gynecol Obstet. 2014 Dec 30. [Epub ahead of print]

Title: Associations between pregnancy outcomes and unexplained high and low maternal serum alpha-fetoprotein levels

Authors: Puntachai P, Wanapirak C, Sirichotiyakul S, Tongprasert F, Srisupundit K, Luewan S, Traisrisilp K, Tongsong T

Abstract: OBJECTIVE: To determine the relationship between adverse pregnancy outcomes and maternal serum alpha-fetoprotein (MSAFP) levels. MATERIALS AND METHODS: A retrospective cohort study was conducted on consecutive singleton pregnancies, screened for fetal Down syndrome, in the northern part of Thailand. The prospective database of our fetal Down screening program was assessed to recruit all consecutive records. Pregnancies with medical complication and fetal abnormality were excluded. The recruited women were categorized into three groups: normal (≥0.76 to ≤2.0 MoM), low (<0.76 MoM) and high (>2.0 MoM) MSAFP levels. RESULTS: Of 7,110 screened women, 5,486 met inclusion criteria, including 240; 5,016 and 230 in the group of high, normal and low MSAFP levels, respectively. The rates of preterm birth, pregnancy-induced hypertension (PIH), fetal growth restriction (FGR), fetal death, low birth weight (LBW) and low APGAR scores were significantly higher in women with high MSAFP levels (11.7 vs. 6.6 %, 7.5 vs. 3.3 %, 7.5 vs. 3.3 %, 2.1 vs. 0.3 %, 15.8 vs. 6.7 %, and 2.9 vs. 0.5 % respectively), with relative risk of 1.76, 2.28, 2.27, 7.46, 2.35 and 6.09, respectively. The rates of preterm birth, FGR and LBW were significantly lower in low MSAFP levels with relative risk of 0.39, 0.26 and 0.26, respectively, whereas the rates of PIH and fetal death and low Apgar scores were not significantly different. CONCLUSIONS: Pregnant women with high MSAFP levels had an increased risk of poor pregnancy outcomes, while those with low MSAFP levels had a significantly lower risk of such outcomes.

(8) Source: Ultrasound Obstet Gynecol. 2015 Mar 31. doi: 10.1002/uog.14862. [Epub ahead of print]

Title: Prediction of small-for-gestational-age neonates: screening by placental growth factor and soluble fms-like tyrosine kinase-1 at 35-37 weeks

Authors: Fadigas C, Peeva G, Mendez O, Poon LC, Nicolaides KH

Abstract: OBJECTIVE: To investigate the potential value of maternal serum placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) at 35-37 weeks' gestation in the prediction of delivery of small for gestational age (SGA) neonates, in the absence of preeclampsia (PE). METHODS: Screening study in singleton pregnancies at 35-37 weeks, including 158 that delivered SGA neonates with birth weight <5th percentile and 3,701 cases unaffected by SGA, PE or gestational hypertension. Multivariable logistic regression analysis was used to determine if serum PlGF and sFlt-1 improved the prediction of delivery of SGA neonates provided by screening with maternal characteristics and medical history (maternal factors), and estimated fetal weight (EFW) from fetal head circumference, abdominal circumference and femur length. RESULTS: In the SGA group, compared to the normal group, the median PlGF multiple of the median (MoM) was significantly lower and the median sFlt-1 MoM was significantly higher. Combined screening by maternal factors and EFW at 35-37 weeks, predicted 90%, 92% and 94% of SGA neonates with birth weight <10th , <5th and <3rd percentiles delivering at <2 weeks of assessment, at 10% false positive rate; the respective values for SGA delivering at ≥37 weeks were 66%, 73% and 80%. When PlGF and sFlt-1 were added to a model that combines maternal factors and EFW, sFlt-1 did not remain as a significant independent predictor of SGA <5th. Combined screening by maternal factors, and serum PlGF, predicted 88%, 96% and 94% of SGA neonates with birth weight <10th, <5th and <3rd percentiles delivering at <2 weeks of assessment and the respective values for SGA delivering at ≥37 weeks were 64%, 75% and 80%. CONCLUSION: Addition of serum PlGF only marginally improves the screening performance for the delivery of SGA neonates, in the absence of PE, achieved by maternal factors and fetal biometry at 35-37 weeks.

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

(9) Source: Rinsho Byori. 2014 Aug;62(8):795-801

Title: [Laboratory test values in children significantly depend on age: focus on alkaline phosphatase (ALP), alpha-fetoprotein (AFP), and immunoglobulins] *[Article in Japanese]*

Authors: Yamada Y

Abstract: Laboratory test reference values are collected from a group of individuals presumed to be healthy to serve as controls in diagnostic comparisons. Pediatric test reference values sometimes vary from those of adults and/or among children of different ages based on their development and growth. Therefore, it is crucial to obtain age-matched laboratory test reference values. As the acquisition of samples from healthy children has become extremely difficult in recent years, clinical reference ranges calculated from routine laboratory data of patients using a statistical method have been used in some institutions, including our own. In addition, clinical judgment has become increasingly important for interpreting laboratory tests that are relatively less common and/or show marked variation. This article reviews pediatric laboratory test values of markers that are variable at different ages, such as alkaline phosphatase (ALP), alpha-fetoprotein (AFP), and immunoglobulins. In general, the serum level of ALP in a pediatric population is two to three times higher than that of an adult population. Circulating AFP levels are markedly higher in neonates just after birth and then decrease exponentially, reaching close to the average adult value by late infancy. Immunoglobulins, except for IgG, increase after birth in infancy. In contrast, IgG levels drop in early infancy due to the reduction of maternal IgG, and then rise as a result of increased IgG production. As these laboratory test values significantly depend on the age,

they should be used with caution. Furthermore, it is deemed desirable to apply newly developed laboratory tests that have been shown to be beneficial in adults to a pediatric clinical setting.

(10) Source: Ultrasound Obstet Gynecol. 2015 Mar 31. doi: 10.1002/uog.14861. [Epub ahead of print]

Title: Prediction of small-for-gestational-age neonates: maternal biochemical markers at 30-34 weeks

Authors: Bakalis S, Gallo DM, Mendez O, Poon LC, Nicolaides KH

Abstract: OBJECTIVE: To investigate the potential value of serum placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), pregnancy associated plasma protein-A (PAPP-A), free β-human chorionic gonadotropin (β-hCG) and α-fetoprotein (AFP) at 30-34 weeks' gestation in the prediction of small for gestational age (SGA) neonates, in the absence of preeclampsia (PE). METHODS: Screening study in singleton pregnancies at 30-34 weeks including 490 that delivered SGA neonates and 9,850 cases that were unaffected by SGA, PE or gestational hypertension (normal). Multivariable logistic regression analysis was used to determine if serum PlGF, sFlt-1, PAPP-A, free β-hCG and AFP, individually or in combination, improved the prediction of SGA neonates provided by screening with maternal characteristics and medical history (maternal factors), and estimated fetal weight (EFW) from fetal head circumference, abdominal circumference and femur length. RESULTS: In the SGA group with birth weight <5th percentile (SGA <5th) delivering at <5 weeks of assessment, compared to the normal group, the mean log10 multiple of the median (MoM) values of PlGF and AFP were significantly lower and the mean log10 MoM values of sFlt-1 and free β-hCG were significantly higher. The best model for prediction of SGA was provided by a combination of maternal factors, EFW and serum PlGF. Such combined screening, predicted, at 10% false positive rate, 84%, 93% and 92% of SGA neonates delivering at <5 weeks of assessment with birth weight <10th, <5th and <3rd percentiles, respectively; the respective detection rates of combined screening for SGA neonates delivering at ≥5 weeks of assessment were 57%, 64% and 71%. CONCLUSION: Combined screening by maternal factors, EFW and serum PLGF at 30-34 weeks' gestation can identify a high proportion of pregnancies that subsequently deliver SGA neonates.

E) Abstracts of New Assay Methodologies:

(11) Source: Biosens Bioelectron. 2015 Jun 15;68:468-74. doi: 10.1016/j.bios.2015.01.033. Epub 2015 Jan 15

Title: Antibody modified gold nano-mushroom arrays for rapid detection of alpha-fetoprotein

Authors: Li W, Jiang X, Xue J, Zhou Z, Zhou J

Abstract: Localized surface plasmon resonance (LSPR) combined with immunoassay shows greatly potential in fast detection of tumor markers. In this paper, a highly sensitive LSPR substrate has been fabricated and modified for direct detection of alpha-fetoprotein (AFP). The biosensor was prepared by interference lithography, and modified by covalently immobilizing anti-AFP on the surface of gold nano-mushroom arrays (GNMA). The modification process was investigated by Vis-NIR reflectance spectra and cyclic voltammogram measurements. We revealed the optical properties of the modified GNMA by measuring the Vis-NIR reflectance spectra and simulating its electric intensity field distribution under light illumination. The GNMA substrate was highly sensitive, with a refractive index sensitivity of ~465nm/RIU. The substrate can be applied to label-free detection of AFP, with the linear range and the limit of detection determined to be 20-200ng/mL and 24ng/mL (S/N=3), respectively. We also demonstrated its clinical application by directly detecting AFP in human serum samples. It is expected that our biosensor could be integrated on microfluidic chips for high-throughput detection in portable early diagnosis, post-operative and point-of-care (POC) in clinical applications.

(12) Source: Biosens Bioelectron. 2015 Jun 15;68:757-62. doi: 10.1016/j.bios.2015.02.010. Epub 2015 Feb 7

Title: Ultrasensitive sandwich-type electrochemical immunosensor based on a novel signal amplification strategy using highly loaded palladium nanoparticles/carbon decorated magnetic microspheres as signal labels

Authors: Ji L, Guo Z, Yan T, Ma H, Du B, Li Y, Wei Q

Abstract: An ultrasensitive sandwich-type electrochemical immunosensor for quantitative detection of alpha fetoprotein (AFP) was proposed based on a novel signal amplification strategy in this work. Carbon decorated Fe3O4 magnetic microspheres (Fe3O4@C) with large specific surface area and good adsorption property were used as labels to anchor palladium nanoparticles (Pd NPs) and the secondary antibodies (Ab2). Pd NPs were loaded on Fe3O4@C to obtain Fe3O4@C@Pd with core-shell structure by electrostatic attraction, which were further used to immobilize Ab2 due to the bonding of Pd-NH2. A signal amplification strategy was the noble metal nanoparticles, such as Pd NPs, exhibiting high electrocatalytic activities toward hydrogen peroxide (H2O2) reduction. This signal amplification was novel not only because of the great capacity, but also the ease of magnetic separation from the sample solution based on their magnetic property. Moreover, carboxyl-functionalized multi-walled carbon nanotubes (MWCNTs-COOH) were used for the immobilization of primary antibodies (Ab1). Therefore, high sensitivity could be realized by the designed immunosensor based on this novel signal amplification strategy. Under optimal conditions, the immunosensor exhibited a wide linear range of 0.5pg/mL to 10ng/mL toward AFP with a detection limit of 0.16pg/mL (S/N=3). Moreover, it revealed good selectivity, acceptable reproducibility and stability, indicating a potential application in clinical monitoring of tumor biomarkers.

(13) Source: Anal Chim Acta. 2015 Mar 3;862:70-6. doi: 10.1016/j.aca.2014.12.050. Epub 2015 Jan 2

Title: Microfluidic paper-based multiplex colorimetric immunodevice based on the catalytic effect of Pd/Fe_3O_4@C peroxidase mimetics on multiple chromogenic reactions

Authors: Liang L, Ge S, Li L, Liu F, Yu J

Abstract: In this report, a non-toxic method was proposed for the simple synthesis of palladium nanoparticles (Pd)/Fe_3O_4@C peroxidase mimetics by virtue of in situ growth of Pd nanoparticles on Fe_3O_4@C magnetic nanoparticles. And a microfluidic paper-based multiplex colorimetric immunodevice (named α-sheet) was developed by site-selectively immobilizing multiple antigens owing to its intrinsic high-efficiency catalytic activity of peroxidase mimetics to multiple chromogenic reactions. The immunosensor platform was prepared by growing a layer of flower-like gold nanoparticles which could entrap the primary antibodies onto paper sensing zones, and the as-prepared Pd/ Fe_3O_4@C peroxidase mimetics was used to label secondary antibodies. In the presence of 3,3',5,5'-tetramethylbenzidine and o-phenylenediamine chromogenic substrates, Pd/ Fe_3O_4@C peroxidase mimetics catalyzed chromogenic reactions and showed different colors with respective intensity. To precisely identify the intensity, a piece of black wax printed chromatographic paper with three observing windows (named β-sheet) was flatted on α-sheet. Under the optimal condition, the proposed multiplex colorimetric immunodevice displayed wide linear ranges from 0.005 to 30 ng mL(-1) with low detection limits of 1.7 pg mL(-1) for carcinoembryonic antigen (CEA) and α-fetoprotein (α-AFP). Meanwhile, the proposed method provided a non-toxic, low-cost and promising tool for point-of-care diagnosis.

(14) Source: Analyst. 2015 Apr 21;140(8):2603-7. doi: 10.1039/c4an02129k. Epub 2015 Feb 24

Title: Label-free and highly sensitive electrochemiluminescence biosensing using quantum dots/carbon nanotubes in ionic liquid

Authors: Tu W, Fang X, Lou J, Dai Z

Abstract: Combining with the synergic effect of carbon nanotubes and ionic liquids for enhancing electrochemiluminescence (ECL) response of CdSe QDs, a universal strategy for highly sensitive biosensing was designed. Using alpha-fetoprotein as a model and monitoring the variation of ECL intensity before and after immunoreaction, a label-free ECL biosensor was developed.

F) Special Abstract Selection:

(15) Source: Indian J Med Res. 2013;138:83-8

Title: Maternal serum median levels of alpha-foetoprotein, human chorionic gonadotropin & unconjugated estriol in second trimester in pregnant women from north-west India

Authors: Kaur G1, Srivastav J, Sharma S, Huria A, Goel P, Chavan BS

Abstract: BACKGROUND & OBJECTIVES: Triple test as prenatal screening procedure does not form a part of routine health care of pregnant women in India. Hence, median values of triple test biomarkers are lacking for Indian population. This study was undertaken to establish population-specific medians for biomarkers viz. alpha-foetoprotien (AFP), human chorionic gonadotropin (hCGβ), and unconjugated estriol (uE3) for detection of Down's syndrome, Edward's syndrome and neural tube defects (NTDs) in pregnant women in north-west India. METHODS: Serum biomarker values were derived from 5420 pregnant women between 15-20 wk of gestation who were enrolled for triple test investigations at Department of Gynecology and Obstetrics, Government Medical College and Hospital, Chandigarh, India, between January, 2007 to December, 2009. Median values were calculated for rounded weeks using database comprising pregnancies with normal outcomes only. Simple statistical analysis and log-linear regression were used for median estimation of the biomarker values. RESULTS: The levels of the three biomarkers were found to be ranging from 1.38 to 187.00 IU/ml for AFP, 1.06 to 315 ng/ml for hCGβ, and 0.25 to 28.5 nmol/l for uE3. The age of women ranged from 18 to 47 yr and mean weight was 57.9 ± 9.8 kg. Data revealed that AFP, hCGβ and uE3 medians in our study population were not significantly different from those reported from other countries or when compared ethnically. INTERPRETATION & CONCLUSION: The population-specific median values for the three biomarkers (AFP, hCGβ, uE3) may be used as reference values during prenatal screening in Indian pregnant women.

(16) Source: Eur J Obstet Gynecol Reprod Biol. 2015 Mar 23;189:13-18. doi: 10.1016/j.ejogrb.2015.03.016. [Epub ahead of print]

Title: Comparing outcomes and costs between contingent and combined first-trimester screening strategies for Down's syndrome

Authors: Martín I, Gibert MJ, Aulesa C, Alsina M, Casals E, Bauça JM

Abstract: OBJECTIVE: To compare a contingent strategy with a combined strategy for prenatal detection of Down's syndrome (DS) in terms of cost, outcomes and safety. STUDY DESIGN: The contingent strategy was based on a simulation, removing measurement of the free beta subunit of human chorionic gonadotropin (free βhCG) and calculating the DS risk retrospectively in 32,371 pregnant women who had been screened with the combined strategy in the first trimester. In the contingent strategy, a risk between 1:31 and 1:1000 in the first trimester indicated further testing in the second trimester (alpha-fetoprotein, inhibin A, unconjugated oestriol and free βhCG). The cut-off risk values for the contingent and combined strategies in the first trimester were 1:30 and 1:250, respectively, and the cut-off risk value for integrated screening in the second trimester was 1:250. Costs were compared in terms of avoided DS births, and the ratio of loss of healthy fetuses following invasive procedures per avoided DS birth was calculated. RESULTS: The combined strategy had sensitivity of 40/44 (90.9%) and a false-positive rate of 2.8%. Corresponding values for the contingent strategy were 39/44 (88.6%) and 1.3%, respectively. Only 11% of pregnant women required tests in the second trimester, and the approximate cost reduction for each avoided DS birth was 5000€. The ratio of lost healthy fetuses following invasive

procedures per avoided DS birth improved by up to 0.65. CONCLUSION: The contingent strategy has similar effectiveness to the combined strategy, but has lower costs and fewer invasive procedures.

(17) Source: J Perinatol. 2015 Feb 26. doi: 10.1038/jp.2015.6. [Epub ahead of print]

Title: Evaluation of the performance of the insulin-like growth factor-binding protein-1/alpha-fetoprotein test in diagnosing ruptured fetal membranes in pregnant women

Authors: Ruanphoo P, Phupong V

Abstract: OBJECTIVE: The objective of this study was to evaluate the efficacy of IGFBP-1/AFP (insulin-like growth factor-binding protein-1/alpha-fetoprotein) immunoassay (Amnioquick Duo+) in diagnosing rupture of membranes (ROM). STUDY DESIGN: A prospective, observational study was performed in pregnant women with a history of fluid leakage from the vagina. The IGFBP-1/AFP immunoassay and conventional methods were used to diagnose ROM. The obstetricians were blinded to the results of the IGFBP-1/AFP immunoassay. The diagnosis of ROM was finally confirmed by reviewing the medical records after delivery. RESULT: One hundred patients were recruited into this study. The mean gestational age was 37.6 weeks (range 25 to 41 weeks). Twenty-six percent were preterm and 74% were at term. IGFBP-1/AFP immunoassay had a sensitivity of 94.1%, specificity of 87.5%, positive predictive value of 97.5%, negative predictive value of 73.7% and accuracy of 93% in diagnosing ROM.Conclusion:IGFBP-1/AFP immunoassay is a rapid immunoassay test for diagnosing ROM with a high sensitivity and specificity. This test can be used as an alternative method for diagnosis of ROM.

(18) Source: Am J Hum Genet. 2015 Jan 8;96(1):162-9. doi: 10.1016/j.ajhg.2014.11.013. Epub 2014 Dec 31

Title: CRB2 mutations produce a phenotype resembling congenital nephrosis, Finnish type, with cerebral ventriculomegaly and raised alpha-fetoprotein

Authors: Slavotinek A, Kaylor J, Pierce H, Cahr M, DeWard SJ, Schneidman-Duhovny D, Alsadah A, Salem F, Schmajuk G, Mehta L

Abstract: We report five fetuses and a child from three families who shared a phenotype comprising cerebral ventriculomegaly and echogenic kidneys with histopathological findings of congenital nephrosis. The presenting features were greatly elevated maternal serum alpha-fetoprotein (MSAFP) or amniotic fluid alpha-fetoprotein (AFAFP) levels or abnormalities visualized on ultrasound scan during the second trimester of pregnancy. Exome sequencing revealed deleterious sequence variants in Crumbs, Drosophila, Homolog of, 2 (CRB2) consistent with autosomal-recessive inheritance. Two fetuses with cerebral ventriculomegaly and renal microcysts were compound heterozygotes for p.Asn800Lys and p.Trp759Ter, one fetus with renal microcysts was a compound heterozygote for p.Glu643Ala and p.Asn800Lys, and one child with cerebral ventriculomegaly, periventricular heterotopias, echogenic kidneys, and renal failure was homozygous for p.Arg633Trp in CRB2. Examination of the kidneys in one fetus showed tubular cysts at the corticomedullary junction and diffuse effacement of the epithelial foot processes and microvillous transformation of the renal podocytes, findings that were similar to those reported in congenital nephrotic syndrome, Finnish type, that is caused by mutations in nephrin (NPHS1). Loss of function for crb2b and nphs1 in Danio rerio were previously shown to result in loss of the slit diaphragms of the podocytes, leading to the hypothesis that nephrosis develops from an inability to develop a functional glomerular barrier. We conclude that the phenotype associated with CRB2 mutations is pleiotropic and that the condition is an important consideration in the evaluation of high MSAFP/AFAFP where a renal cause is suspected.

May 2015

Teachings on Alpha-fetoprotein

Vol. 7, Part 1

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

Title: 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 live births, alcohol consumption during pregnancy results in brain, craniofacial 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 remain 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 neurotransmitters 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, multicenter 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 1,000 live births in some geographic areas of the USA [2]. In Canada and other parts of the world, e.g. France, the incidence has been estimated at 9.1 per 1,000 live births [3]. Alcohol is a teratogen that targets the brain, heart, skeletal bones, and lymphoreticular-hematopoietic systems. The brain impairments result in permanent lifelong developmental problems in areas 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: γ-aminobutryic acid (GABA), receptor α_5-subunit, serotonin 5-HT_{1A} receptor, glutamate transporter, GSKβ; the homeoproteins PAX-6, SOX-3, and PAX-5, cFOS/cJUN-B transcription factors, glial-derived neurotrophic factor, sonic hedgehog, vasointestinal peptide, protein kinase-C family, nerve growth factor, 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 (analytes) 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 and Down syndrome (DS) screening programs. Biomarkers shown to be useful in predicting FASD pregnancies have included α-fetoprotein (AFP) and various estrogens. Low maternal levels of both unconjugated estriol (uE_3) and estradiol (E_2) have been shown to have some FASD predictive value. Even though uE_3 was useful for FASD screening, E_2 was found to be superior and should be utilized in place of uE_3. Unlike DS, human chorionic gonadotrophin had no value in identifying FASD pregnancies. Reduced maternal serum concentrations of both pregnancy-specific β_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 downregulated by proteonomic analysis of amniotic fluid proteins in FASD animal models [10]. SP-1, a member of the carcinoembryonic gene family involved in cell adhesion, was found to exhibit low maternal serum levels in FASD pregnancies in contradistinction to its elevated levels in DS. Using DS as a screening model, suggested candidates for a quad screening feasibility study of FASD might consist of low maternal serum profiles of AFP, SP-1, E_2, and SHBG in second-trimester pregnancies [11, 12]. Such a screening profile would have no overlap with the present quad test employed for either DS or trisomy-18. Additional biomarkers worthy of further investigation might also include pro-opiomelanocortin peptides, 5-HT_{1A}, brain-derived neurotrophic factor, neurotrophic growth factor, neuroprotective factor, and Sl00 β 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 algorithm for mothers at risk for DS 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/questionnaire documentation by the attending obstetrician, nurse or social worker. To this end, brief questionnaires (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 FASD 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 drugs include: neurokinin-1 receptor (NKlR) antagonist LY686017; sonic hedgehog pathway agonist (SAG1.1;8, a diaminocyclohexane); serotonin 1A agonist (ipsapirone, buspirone); superoxide dismutase/catalase mimetic (EUK-134); N-methyl-D-aspartate 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 (neurotrophic) peptides induced by compounds such as vasointestinal peptide, 5-HT, basic fibroblast growth factor, and neurotrophic growth factor. Some of these peptide fragments (8-9 amino acids) are capable of preventing further alcohol-induced mental decline, developmental delays, and enhancing 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 that 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 includes 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, 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 FASD pregnancy through prenatal screening could allow for societal interventions and clinical therapies that might diminish or ameliorate brain, bone, and structural damage due to intrauterine alcohol exposure. Prenatal FASD screening could result in physicians providing follow-up questionnaires 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 3,000 FASD annual live births do indeed occur in the USA [20]. Until pilot screening studies are conducted, FASD will remain one of the most preventable and underdiagnosed pregnancy disorders in the obstetrical community.

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New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of Second Trimester Results

	MS 326	MS 327	MS 328	MS 329	MS 330
Gestational Age All Lab Mean:					
Mean	15.0	17.0	21.0	19.0	20.0
SD	0.00	0.00	0.02	0.00	0.00
%CV	0.0%	0.0%	0.1%	0.0%	0.0%
mean+3*SD	15.0	17.0	21.1	19.0	20.0
mean-3*SD	15.0	17.0	20.9	19.0	20.0
N	25	25	25	25	25

	MS 326	MS 327	MS 328	MS 329	MS 330
MS AFP All Lab Mean:					
mean	19.9	23.4	381.9	35.7	114.7
SD	4.2	3.8	30.3	6.2	15.0
%CV	21.2%	16.2%	7.9%	17.4%	13.1%
mean+3SD	32.5	34.8	472.9	54.4	159.7
mean-3SD	7.2	12.0	290.8	17.1	69.7
N	25	25	25	25	25
median	20.6	24.2	388.8	36.9	117.7
mean/all kit median	0.98	0.99	0.99	0.99	1.00
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	21.3	24.7	386.9	37.9	120.2
SD	1.2	1.3	27.0	2.2	6.7
%CV	5.8%	5.3%	7.0%	5.8%	5.5%
mean + 3SD	25.0	28.6	467.8	44.6	140.2
mean - 3SD	17.5	20.8	305.9	31.3	100.2
N	16	16	16	16	16
Median	21.2	24.4	394.4	37.6	120.1
mean/All kit median	1.05	1.05	1.00	1.06	1.05
MS AFP Beckman Access/2 (BCX/BC1) mean:					
mean	20.2	23.6	388.5	35.9	114.3
SD	0.7	0.8	12.0	1.6	3.7
%CV	3.7%	3.3%	3.1%	4.6%	3.2%
mean+3SD	22.4	25.9	424.6	40.8	125.2
mean-3SD	18.0	21.3	352.5	31.0	103.3
N	6	6	6	6	6
median	20.5	23.6	390.0	36.7	114.9
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS AFP Siemens Immulite 2000 (DPD/DP5) mean:					
mean	6.4	11.5	319.5	16.3	69.4
N	2	2	2	2	2
mean/all kit median	0.32	0.49	0.83	0.45	0.61
MS AFP kit average:					
mean	16.0	19.9	365.0	30.0	101.3
SD	8.3	7.3	39.4	12.0	27.8
all kit median	20.2	23.6	386.9	35.9	114.3

	MS 326	MS 327	MS 328	MS 329	MS 330
MS AFP MoM All Lab Mean:					
mean	0.70	0.76	5.62	0.62	2.03
SD	0.15	0.13	0.66	0.12	0.30
%CV	21.5%	17.3%	11.8%	19.2%	15.0%
mean+3SD	1.15	1.15	7.61	0.98	2.95
mean-3SD	0.25	0.36	3.63	0.27	1.12
N	25	25	25	25	25
All Median	0.73	0.79	5.81	0.65	2.05
mean/all kit median	0.98	1.01	0.98	0.97	1.00
MS AFP MoM Beckman Unicel (BCU/BC1) mean:					
Mean	0.75	0.81	5.75	0.66	2.15
SD	0.04	0.05	0.44	0.05	0.13
%CV	5.2%	5.9%	7.7%	7.2%	6.3%
mean + 3SD	0.86	0.95	7.07	0.80	2.55
mean - 3SD	0.63	0.66	4.42	0.52	1.74
N	16	16	16	16	16
Median	0.75	0.82	5.90	0.67	2.14
mean/all kit median	1.05	1.07	1.00	1.03	1.05
MS AFP MoM Beckman Access/2 (BCX/BC1) mean:					
Mean	0.71	0.75	5.83	0.64	2.04
SD	0.07	0.06	0.56	0.07	0.13
%CV	9.8%	8.4%	9.6%	11.5%	6.6%
mean + 3SD	0.92	0.94	7.52	0.86	2.44
mean - 3SD	0.50	0.56	4.14	0.42	1.64
N	6	6	6	6	6
Median	0.71	0.76	5.66	0.63	2.04
mean/all kit median	1.00	1.00	1.01	1.00	1.00
MS AFP MoM Siemens Immulite 2000 (DPD/DP5) mean:					
Mean	0.23	0.36	4.02	0.27	1.13
N	2	2	2	2	2
mean/all kit median	0.32	0.48	0.70	0.41	0.55
MS AFP MoM kit average:					
mean	0.56	0.64	5.20	0.52	1.77
SD	0.29	0.24	1.03	0.22	0.56
all kit median	0.71	0.75	5.75	0.64	2.04

New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of Second Trimester Results

	MS 326	MS 327	MS 328	MS 329	MS 330		MS 326	MS 327	MS 328	MS 329	MS 330
MS uE3 All Lab Mean:						**MS uE3 MoM All Lab Mean:**					
mean	0.34	0.99	1.53	1.46	1.56	Mean	0.54	1.06	0.65	0.86	0.81
SD	0.04	0.09	0.11	0.16	0.11	SD	0.11	0.25	0.09	0.20	0.09
%CV	11.5%	8.9%	7.4%	11.0%	7.2%	%CV	19.9%	23.9%	14.0%	23.3%	11.5%
mean+3SD	0.46	1.26	1.87	1.95	1.90	mean+3SD	0.87	1.82	0.92	1.46	1.09
mean-3SD	0.22	0.73	1.19	0.98	1.23	mean-3SD	0.22	0.30	0.37	0.26	0.53
N	24	24	24	24	24	N	24	24	24	24	24
mean/all kit median	1.03	0.94	0.96	0.98	1.02	mean/all kit Median	0.98	0.99	1.02	1.01	1.01
MS uE3 Beckman Unicel (BCU/BC1) mean:						**MS uE3 MoM Beckman Unicel (BCU/BC1) Mean:**					
Mean	0.33	0.96	1.50	1.42	1.54	Mean	0.52	0.99	0.63	0.81	0.79
SD	0.03	0.08	0.12	0.13	0.12	SD	0.06	0.06	0.07	0.08	0.07
%CV	10.1%	8.0%	8.1%	9.2%	7.9%	%CV	12.3%	6.2%	10.9%	9.4%	8.9%
mean+3SD	0.43	1.19	1.86	1.82	1.91	mean+3SD	0.70	1.17	0.84	1.04	1.00
mean-3SD	0.23	0.73	1.13	1.03	1.17	mean-3SD	0.33	0.80	0.43	0.58	0.58
N	16	16	16	16	16	N	16	16	16	16	16
mean/all kit median	1.00	0.91	0.94	0.96	1.00	mean/all kit Median	0.93	0.92	1.00	0.95	0.98
MS uE3 Beckman Access/2 (BCX/BC1) mean:						**MS uE3 MoM Beckman Access/2 (BCX/BC1) Mean:**					
mean	0.38	1.05	1.59	1.49	1.64	Mean	0.56	1.07	0.62	0.85	0.80
SD	0.04	0.04	0.06	0.07	0.04	SD	0.05	0.07	0.09	0.08	0.09
%CV	9.6%	4.3%	4.0%	4.6%	2.7%	%CV	9.9%	6.5%	14.0%	9.9%	10.9%
mean+3SD	0.49	1.19	1.78	1.69	1.77	mean+3SD	0.72	1.28	0.88	1.11	1.06
mean-3SD	0.27	0.92	1.40	1.28	1.50	mean-3SD	0.39	0.86	0.36	0.60	0.54
N	6	6	6	6	6	N	6	6	6	6	6
mean/all kit median	1.15	1.00	1.00	1.00	1.06	mean/all kit Median	1.00	1.00	0.98	1.00	1.00
MS uE3 Siemens Immulite/2000 (DPD/DP5 or 6) mean:						**MS uE3 MoM Siemens Immulite/2000 (DPD/DP5 or 6) Mean:**					
Mean	0.32	1.11	1.62	1.72	1.53	Mean	0.72	1.61	0.84	1.33	1.01
N	2	2	2	2	2	N	2	2	2	2	2
mean/all Kit Median	0.96	1.05	1.01	1.15	0.99	mean/all kit Median	1.29	1.50	1.32	1.56	1.25
MS uE3 kit average:						**MS uE3 MoM kit average:**					
mean	0.34	1.04	1.57	1.54	1.57	mean	0.60	1.22	0.70	0.99	0.87
SD	0.03	0.08	0.06	0.15	0.06	SD	0.11	0.34	0.12	0.29	0.12
all kit median	0.33	1.05	1.59	1.49	1.54	all kit median	0.56	1.07	0.63	0.85	0.80

New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of Second Trimester Results

	MS 326	MS 327	MS 328	MS 329	MS 330
MS hCG All Lab mean:					
mean	97.9	29.7	20.7	22.3	22.2
SD	6.9	3.1	2.1	2.7	1.9
%CV	7.0%	10.5%	10.0%	12.1%	8.4%
mean+3SD	118.4	39.0	26.9	30.4	27.7
mean-3SD	77.3	20.3	14.4	14.2	16.6
N	24	24	24	24	24
mean/all kit median	0.98	0.97	1.00	0.96	0.99
MS hCG Beckman Unicel (BCU/BC1 or 2) mean:					
mean	96.6	28.6	20.1	21.5	21.7
SD	7.3	2.1	1.6	2.1	1.4
%CV	7.6%	7.3%	7.8%	9.8%	6.6%
mean+3SD	118.6	34.8	24.8	27.8	26.0
mean-3SD	74.6	22.3	15.4	15.1	17.4
N	17	17	17	17	17
median	96.1	28.5	20.1	21.9	21.2
mean/All kit median	0.97	0.94	0.97	0.93	0.97
MS hCG Beckman Access/2 (BCX/BC1 or 2) mean:					
mean	99.4	30.5	20.7	23.1	22.3
SD	2.6	1.8	1.9	2.0	1.8
%CV	2.6%	5.8%	9.1%	8.7%	8.0%
X+3SD	107.2	35.8	26.4	29.1	27.7
X-3SD	91.7	25.2	15.0	17.1	17.0
N	5	5	5	5	5
median	98.7	31.3	21.5	23.2	21.8
mean/All kit median	1.01	1.03	1.01	1.04	1.01
MS hCG Siemens Immulite 2000 (DPD/DP5) mean:					
mean	104.6	36.8	25.1	27.3	25.7
N	2	2	2	2	2
mean/all kit median	1.11	1.30	1.24	1.30	1.22
MS hCG kit average:					
mean	100.2	31.9	22.0	24.0	23.2
SD	4.1	4.3	2.7	3.0	2.2
all kit median	99.4	30.5	20.7	23.1	22.3

	MS 326	MS 327	MS 328	MS 329	MS 330
MS hCG MoM All Lab Mean:					
mean	2.25	1.23	1.04	0.87	1.10
SD	0.44	0.23	0.20	0.22	0.20
%CV	19.4%	18.8%	18.8%	25.5%	18.2%
mean+3SD	3.56	1.93	1.62	1.54	1.71
mean-3SD	0.94	0.54	0.45	0.20	0.50
N	24	24	24	24	24
mean/All Kit Median	1.03	1.04	1.01	1.05	1.01
MS hCG MoM Beckman Unicel (BCU/BC1 or 2) mean:					
mean	2.16	1.18	1.03	0.83	1.10
SD	0.25	0.11	0.12	0.18	0.14
%CV	11.6%	9.1%	11.9%	21.5%	12.3%
X+3SD	2.91	1.50	1.39	1.36	1.50
X-3SD	1.41	0.86	0.66	0.29	0.69
N	17	17	17	17	17
median	2.13	1.17	1.00	0.83	1.07
mean/All kit median	0.99	1.00	1.00	1.00	1.00
MS hCG MoM Beckman Access/2 (BCX/BC1 or 2) mean:					
mean	2.19	1.16	0.90	0.82	0.97
SD	0.51	0.20	0.12	0.12	0.16
%CV	23.4%	17.0%	13.5%	14.4%	16.4%
X+3SD	2.91	1.50	1.39	1.36	1.50
X-3SD	1.41	0.86	0.66	0.29	0.69
N	5	5	5	5	5
median	2.35	1.12	0.93	0.76	0.98
mean/All kit median	1.00	0.98	0.88	0.99	0.89
MS hCG MoM Siemens Immulite 2000 (DPD/DP5) mean:					
mean	3.23	1.85	1.49	1.34	1.51
N	2	2	2	2	2
mean/All kit median	1.63	1.71	1.65	1.87	1.60
MS hCG MoM kit average:					
mean	2.53	1.40	1.14	1.00	1.19
SD	1.36	0.77	0.62	0.55	0.64
all kit median	2.19	1.18	1.03	0.83	1.10

New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of Second Trimester Results

	MS 326	MS 327	MS 328	MS 329	MS 330
MS Inhibin A all lab mean:					
Mean	342.8	136.3	215.4	188.8	217.2
SD	19.0	6.5	10.5	9.6	12.7
%CV	5.6%	4.8%	4.9%	5.1%	5.8%
mean + 3SD	399.9	155.9	246.8	217.6	255.2
mean- 3SD	285.7	116.8	184.0	160.0	179.2
N	24	24	24	24	24
All Lab Median	349.6	137.6	215.6	190.9	217.0
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	341.1	136.5	213.8	187.5	216.1
SD	19.1	5.6	11.4	7.9	11.8
%CV	5.6%	4.1%	5.3%	4.2%	5.5%
mean + 3SD	398.3	153.2	248.1	211.3	251.4
mean- 3SD	283.8	119.8	179.6	163.7	180.7
N	16	16	16	16	16
Kit median	342.7	136.9	214.7	190.2	216.3
mean/all kit median	0.99	1.00	0.99	0.99	0.99
MS Inhibin A Beckman Access/2 (BCX/BC1) mean:					
Mean	346.4	136.0	218.4	191.5	219.4
SD	19.7	8.6	8.0	12.5	14.9
%CV	5.7%	6.3%	3.7%	6.5%	6.8%
mean + 3SD	405.5	161.7	242.5	228.8	264.0
mean- 3SD	287.4	110.3	194.3	154.1	174.8
N	8	8	8	8	8
Kit median	351.6	139.2	216.9	196.2	219.1
mean/All kit median	1.01	1.00	1.01	1.01	1.01
MS Inhibin A kit average:					
mean	343.7	136.3	216.1	189.5	217.7
SD	3.8	0.3	3.2	2.8	2.3
all kit median	343.7	136.3	216.1	189.5	217.7

	MS 326	MS 327	MS 328	MS 329	MS 330
MS Inhibin A MoM All Lab mean:					
mean	1.83	0.94	1.00	0.99	1.16
SD	0.15	0.08	0.10	0.10	0.10
%CV	8.2%	8.1%	9.6%	10.2%	8.7%
mean+3SD	2.28	1.17	1.29	1.30	1.47
mean-3SD	1.38	0.71	0.71	0.69	0.86
N	24	24	24	24	24
mean/all kit median	1.00	1.01	1.01	0.99	1.00
MS Inhibin A MoM Beckman Unicel (BCU/BC1) mean:					
Mean	1.84	0.96	1.02	0.98	1.18
SD	0.16	0.07	0.10	0.10	0.11
%CV	8.6%	7.7%	10.2%	10.0%	9.4%
mean + 3SD	2.31	1.18	1.33	1.28	1.51
mean- 3SD	1.37	0.74	0.70	0.69	0.85
N	16	16	16	16	16
Kit Median	1.79	0.94	1.00	0.98	1.17
mean/all kit median	1.00	1.03	1.02	0.98	1.01
MS Inhibin A MoM Beckman Access (BCX/BC1) mean:					
Mean	1.82	0.91	0.97	1.02	1.14
SD	0.14	0.08	0.07	0.11	0.08
%CV	7.7%	8.4%	7.7%	10.9%	7.2%
mean + 3SD	2.24	1.14	1.19	1.35	1.39
mean- 3SD	1.40	0.68	0.75	0.68	0.90
N	8	8	8	8	8
Kit Median	1.80	0.95	0.98	0.98	1.17
mean/all kit median	1.00	0.97	0.98	1.02	0.99
MS Inhibin A MoM kit average:					
mean	1.83	0.93	0.99	1.00	1.16
SD	0.01	0.03	0.03	0.02	0.02
all kit median	1.83	0.93	0.99	1.00	1.16

New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of Second Trimester Results

	AF326	AF327	AF328	AF329	AF330
AF AFP All Lab mean :					
mean	8.5	11.7	8.7	14.8	21.1
SD	0.8	1.0	0.9	1.8	2.4
%CV	9.1%	8.4%	10.3%	11.9%	11.4%
mean+3SD	10.8	14.7	11.5	20.0	28.4
mean-3SD	6.2	8.8	6.0	9.5	13.9
N	19	19	19	19	19
All kit median	9.0	12.1	8.9	14.9	22.0
mean/all kit mean	0.94	0.97	0.98	0.99	0.96
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	8.2	11.4	8.4	14.3	20.4
SD	0.7	0.9	0.8	1.8	2.3
%CV	8.4%	8.0%	9.9%	12.7%	11.5%
X+3SD	10.3	14.2	10.9	19.8	27.4
X-3SD	6.2	8.7	5.9	8.8	13.4
N	13	13	13	13	13
median	8.1	11.7	8.4	14.6	20.5
mean/all kit median	0.91	0.94	0.95	0.96	0.93
AF AFP Beckman Access/2 (BCX/BC1) mean:					
Mean	9.0	12.1	8.9	14.9	22.0
SD	0.7	0.8	0.5	0.8	2.2
%CV	8.2%	6.7%	5.1%	5.1%	10.0%
X+3SD	11.24	14.56	10.27	17.22	28.58
X-3SD	6.8	9.7	7.5	12.6	15.4
N	3	3	3	3	3
median	9.3	12.6	9.0	15.1	22.9
mean/all kit median	1.00	1.00	1.00	1.00	1.00
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	9.3	12.8	9.5	16.3	23.3
N	2	2	2	2	2
mean/all kit median	1.02	1.05	1.07	1.09	1.06
AF AFP kit average:					
mean	6.6	9.1	6.7	11.4	16.4
SD	0.5	0.7	0.5	1.0	1.4
all kit median	9.0	12.1	8.9	14.9	22.0

	AF326	AF327	AF328	AF329	AF330
AF AFP MoM All Lab Mean:					
mean	0.53	1.52	1.00	1.31	3.33
SD	0.06	0.15	0.12	0.15	0.42
%CV	11.4%	10.0%	11.6%	11.3%	12.7%
mean+3SD	0.71	1.98	1.35	1.75	4.60
mean-3SD	0.35	1.07	0.65	0.86	2.06
N	19	19	19	19	19
All median	0.52	1.49	0.97	1.27	3.39
mean/all kit median	1.01	1.02	1.03	1.03	0.98
AF AFP MoM Beckman Unicel(BCU/BC1) mean:					
Mean	0.53	1.49	0.97	1.30	3.21
SD	0.06	0.13	0.10	0.15	0.34
%CV	12.2%	8.9%	9.8%	11.9%	10.6%
X+3SD	0.72	1.89	1.26	1.76	4.24
X-3SD	0.33	1.09	0.69	0.83	2.19
N	13	13	13	13	13
median	0.52	1.45	0.95	1.26	3.39
mean/all kit median	1.00	0.95	0.98	1.00	0.91
AF AFP MoM Beckman Access (BCX/BC1) mean:					
Mean	0.50	1.57	1.06	1.26	3.54
SD	0.08	0.25	0.13	0.13	0.75
%CV	14.9%	16.2%	12.7%	10.2%	21.2%
X+3SD	0.73	2.33	1.46	1.65	5.79
X-3SD	0.28	0.81	0.66	0.88	1.28
N	3	3	3	3	3
median	0.50	1.46	1.02	1.21	3.18
mean/all kit median	0.96	1.00	1.07	0.98	1.00
AF AFP MoM DPC Immulite 2000 (DPD/DP5) mean:					
Mean	0.55	1.62	0.99	1.41	3.62
N	2	2	2	2	2
mean/all kit median	1.04	1.03	1.00	1.08	1.02
AF AFP MoM kit average:					
mean	0.53	1.56	1.01	1.32	3.45
SD	0.02	0.06	0.05	0.07	0.21
all kit median	0.53	1.57	0.99	1.30	3.54

New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of First Trimester Results

	FT326	FT327	FT328	FT329	FT330
FT Gestational Age All Lab Mean:					
Mean	12.4	11.9	13.0	11.1	11.5
SD	0.05	0.11	0.05	0.10	0.13
%CV	0.4%	0.9%	0.4%	0.9%	1.1%
mean+3*SD	12.5	12.2	13.1	11.4	11.9
mean-3*SD	12.2	11.6	12.8	10.7	11.1
N	16	16	16	16	16

	FT326	FT327	FT328	FT329	FT330
FT NT MoM All Lab Mean:					
Mean	0.95	2.20	0.96	0.99	0.91
SD	0.06	0.14	0.06	0.07	0.05
%CV	6.1%	6.5%	6.0%	7.4%	6.0%
mean+3*SD	1.13	2.62	1.14	1.21	1.07
mean- 3*SD	0.78	1.77	0.79	0.77	0.74
N	15	15	15	15	15
All Median	0.94	2.15	0.97	0.97	0.90

New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of First Trimester Results

	FT326	FT327	FT328	FT329	FT330		FT326	FT327	FT328	FT329	FT330
FT hCG All Lab Mean:						**FT hCG MoM All Lab Mean:**					
mean	52.9	138.9	50.9	85.4	66.6	Mean	0.51	1.41	0.61	0.77	0.65
SD	9.8	16.4	10.3	6.7	6.6	SD	0.05	0.15	0.06	0.10	0.07
%CV	18.6%	11.8%	20.3%	7.9%	9.9%	%CV	10.6%	10.5%	9.4%	12.6%	11.3%
mean+3*SD	82.4	188.1	81.9	105.5	86.3	mean+3*SD	0.67	1.86	0.79	1.06	0.86
mean- 3*SD	23.3	89.6	20.0	65.2	46.8	mean - 3*SD	0.35	0.97	0.44	0.48	0.43
N	15	15	15	15	15	N	12	12	12	12	12
All lab median	50.5	134.1	47.8	86.4	66.1	All lab Median	0.51	1.38	0.60	0.77	0.67
mean/All kit median	0.85	0.91	0.83	0.95	0.95	mean/All kit Median	0.60	0.70	0.63	0.72	0.74
FT hCG Beckman Unicel/Access 2 (BCU or X/BC1 or 2) mean:						**FT hCG MoM Beckman Unicel or Access 2 (BCU or X/BC1 or 2) mean:**					
mean	49.5	133.8	47.2	83.7	65.3	mean	0.51	1.41	0.61	0.77	0.65
SD	3.1	8.4	2.6	5.2	6.2	SD	0.05	0.15	0.06	0.10	0.07
%CV	6.2%	6.3%	5.4%	6.2%	9.5%	%CV	10.6%	10.5%	9.4%	12.6%	11.3%
mean+3SD	58.7	159.0	54.9	99.3	83.9	mean+3SD	0.67	1.86	0.79	1.06	0.86
mean- 3SD	40.2	108.6	39.5	68.1	46.8	mean-3SD	0.35	0.97	0.44	0.48	0.43
N	13	13	13	13	13	N	12	12	12	12	12
median	50.2	133.9	47.3	84.4	65.8	median	0.51	1.38	0.60	0.77	0.67
mean/All kit median	0.80	0.88	0.77	0.93	0.93	mean/All kit median	0.60	0.70	0.63	0.72	0.74
FT hCG DPC Immulite 2000(DPD/DP5) mean:						**FT hCG MoM DPC Immulite2000 (DPD/DP5) mean:**					
mean	75.0	171.8	75.1	95.9	74.5	mean	1.18	2.61	1.35	1.36	1.10
N	2	2	2	2	2	N	2	2	2	2	2
mean/All kit median	1.20	1.12	1.23	1.07	1.07	mean/All kit median	1.40	1.30	1.37	1.28	1.26
FT hCG kit average:						**FT hCG MoM kit average:**					
mean	62.2	152.8	61.2	89.8	69.9	mean	0.85	2.01	0.98	1.07	0.87
SD	18.0	26.9	19.7	8.6	6.5	SD	0.47	0.84	0.52	0.42	0.32
all kit median	62.2	152.8	61.2	89.8	69.9	all kit median	0.85	2.01	0.98	1.07	0.87

New York State Fetal Defect Markers Proficiency Test,
May 2015
Summary of First Trimester Results

	FT326	FT327	FT328	FT329	FT330
FT PAPP-A All Lab Mean:					
Mean	3493.2	2760.5	6488.7	3185.0	1796.2
SD	875.4	778.1	2323.7	1584.2	874.2
%CV	25.1%	28.2%	35.8%	49.7%	48.7%
mean + 3SD	6119.5	5094.7	13459.7	7937.5	4418.7
mean- 3SD	866.9	426.3	-482.2	-1567.5	-826.3
N	15	15	15	15	15
All Lab Median	3867.2	3038.0	6167.3	2816.0	1542.0
mean/All kit median	0.90	0.93	1.04	1.13	1.16
FT PAPP-A Beckman Unicel(BCU/BC1) Mean:					
Mean	3876.5	3135.8	6254.5	2824.5	1549.6
SD	236.5	307.1	599.7	201.0	154.0
%CV	6.1%	9.8%	9.6%	7.1%	9.9%
mean + 3SD	4586.0	4057.0	8053.6	3427.6	2011.6
mean - 3SD	3166.9	2214.6	4455.4	2221.3	1087.7
N	10	10	10	10	10
Kit Median	3900.9	3094.9	6245.7	2829.2	1554.0
mean/All kit median	1.00	1.06	1.00	1.00	1.00
***FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:**					
Mean	3964.8	2968.8	12597.7	7910.2	4453.1
N	2	2	2	2	2
mean/All kit median	1.02	1.00	2.01	2.80	2.87
***Note: The above table contains converted values (mIU/ml->ng/ml) from conversion factor from Anshlabs PAPP-A Elisa Package insert.(see critique)**					
FT PAPP-A AnshLite (SMR, MPR or APM/AN1) Mean:					
Mean	1901.1	1370.6	3196.8	1236.4	846.9
SD	526.1	191.8	338.9	61.6	237.4
%CV	27.7%	14.0%	10.6%	5.0%	28.0%
mean + 3SD	3479.5	1946.0	4213.5	1421.4	1559.0
mean - 3SD	322.8	795.1	2180.0	1051.5	134.8
N	3	3	3	3	3
Kit Median	1969.0	1445.0	3188.0	1221.0	791.7
mean/All kit median	0.49	0.46	0.51	0.44	0.55
FT PAPP-A kit average:					
mean	3247.5	2491.7	7349.7	3990.4	2283.2
SD	1166.8	974.5	4795.2	3486.3	1911.8
all kit median	3876.5	2968.8	6254.5	2824.5	1549.6

	FT326	FT327	FT328	FT329	FT330
FT PAPP-A MoM All Lab Mean:					
Mean	3.50	3.82	6.17	5.14	2.63
SD	1.14	1.26	2.22	2.76	1.24
%CV	32.6%	32.8%	36.0%	53.6%	47.0%
mean + 3SD	6.92	7.59	12.83	13.41	6.34
mean- 3SD	0.08	0.06	-0.50	-3.12	-1.08
N	15	15	15	15	15
All Lab Median	3.57	4.25	6.32	5.24	2.38
mean/ All kit median	1.10	1.15	0.96	1.01	1.07
FT PAPP-A MoM Beckman Unicel(BCU/BC1) Mean:					
Mean	4.11	4.56	6.44	5.07	2.46
SD	0.55	0.39	0.90	0.78	0.22
%CV	13.3%	8.6%	14.0%	15.4%	9.0%
mean + 3SD	5.74	5.74	9.14	7.41	3.12
mean - 3SD	2.47	3.39	3.74	2.73	1.80
N	10	10	10	10	10
Kit Median	4.09	4.48	6.37	5.34	2.40
mean/All kit median	1.28	1.37	1.00	1.00	1.00
FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:					
Mean	3.20	3.33	9.53	10.26	5.34
N	2	2	2	2	2
mean/All kit median	1.00	1.00	1.48	2.02	2.17
FT PAPP-A MoM (SMR or APM/AN1) Mean:					
Mean	2.22	2.14	3.69	2.04	1.48
N	2	2	2	2	2
mean/ All kit median	0.69	0.64	0.57	0.40	0.60
FT PAPP-A MoM kit average:					
mean	3.17	3.34	6.55	5.79	3.09
SD	0.95	1.21	2.92	4.16	2.00
all kit median	3.20	3.33	6.44	5.07	2.46

Figure 1

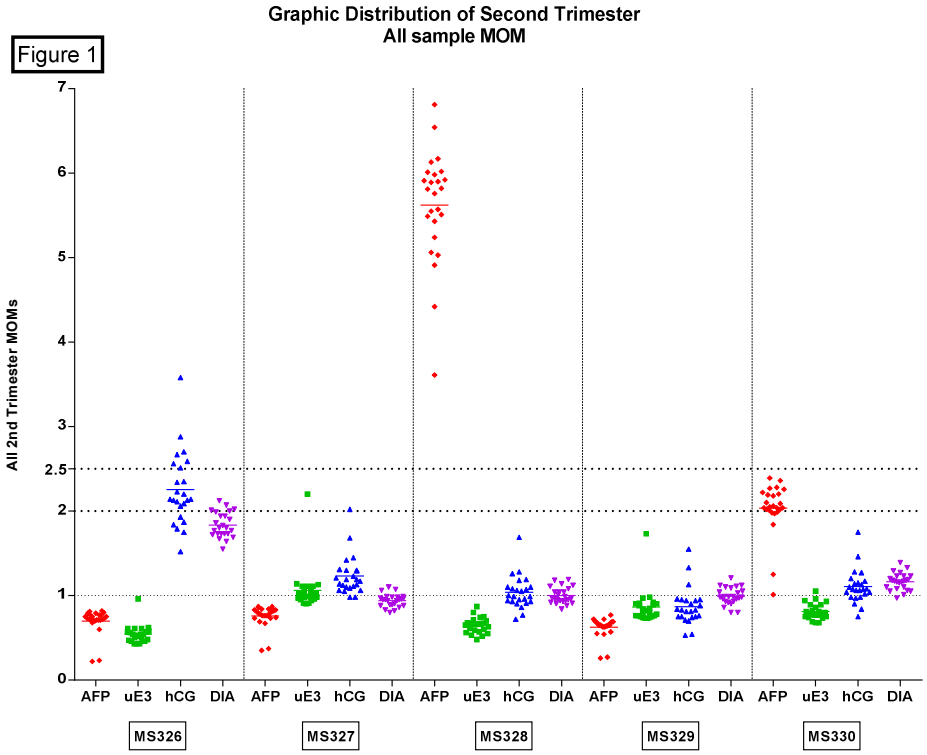


Figure 2a

Maternal Sera AFP MoM

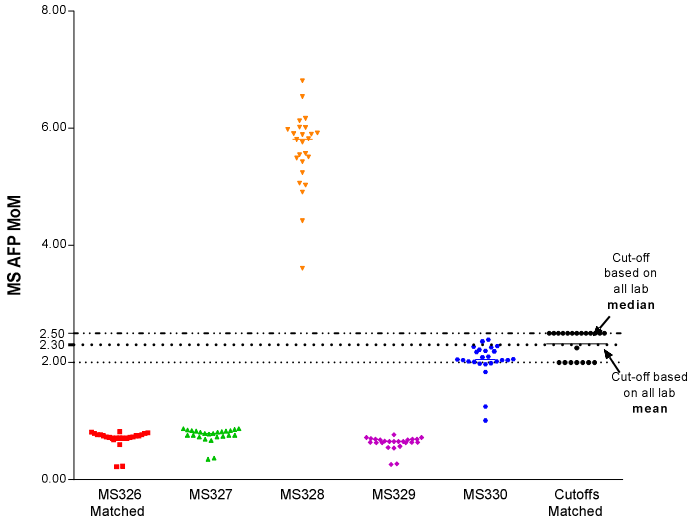


Figure 2b

Amniotic Fluid AFP MoM

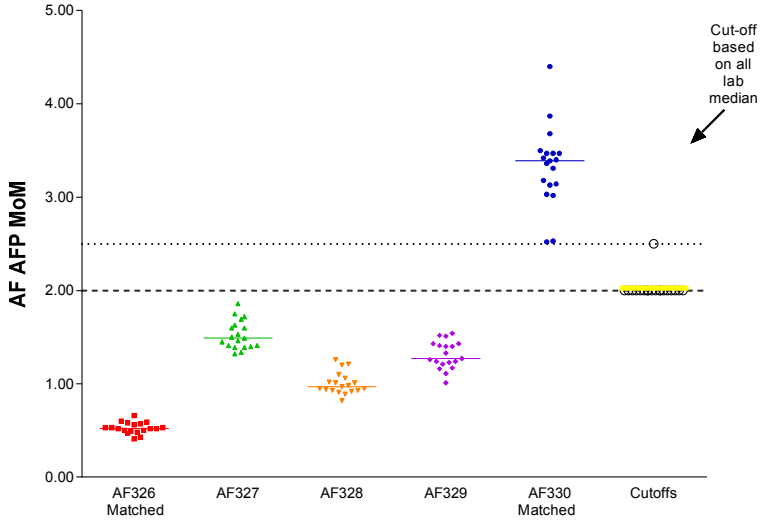


Figure 3

Graphic Distribution of Second Trimester Neural Tube Defect Risk Estimates

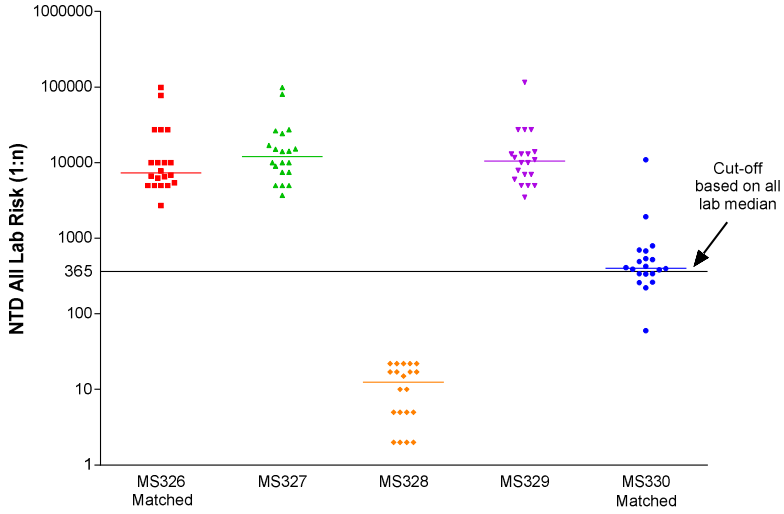


Figure 4

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

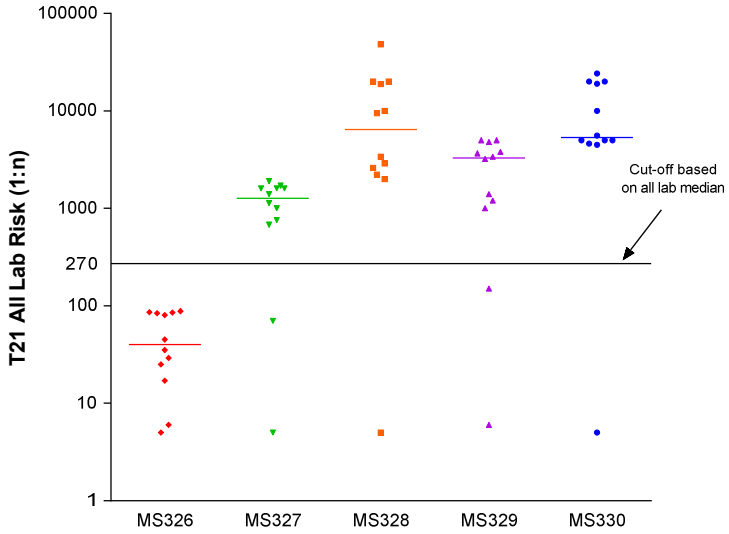


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

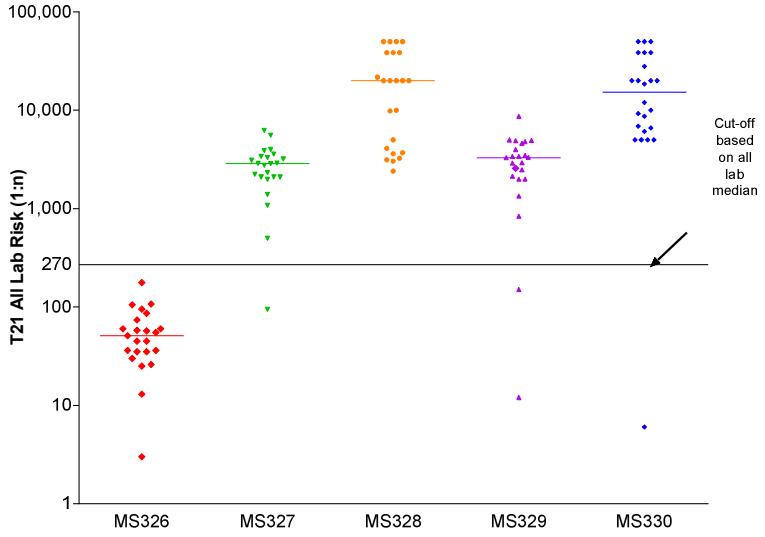
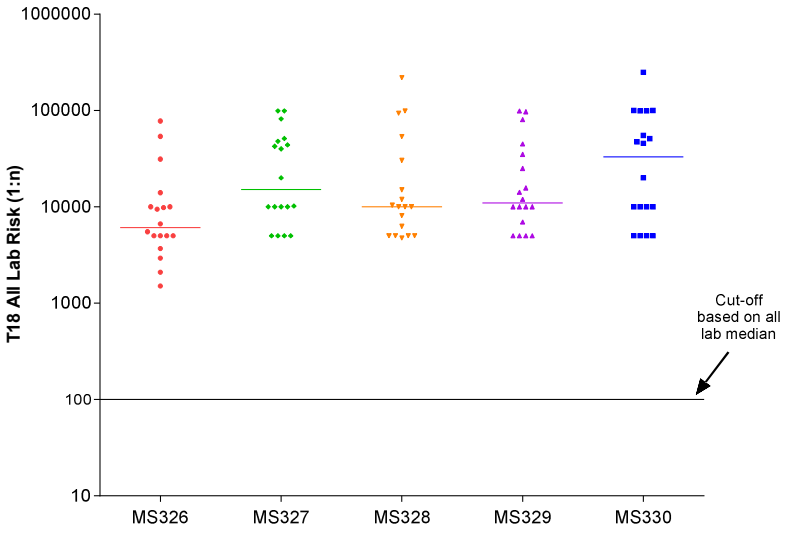


Figure 6

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates



NYS FEDM PT 5/15
Second Trimester

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

Figure 7A

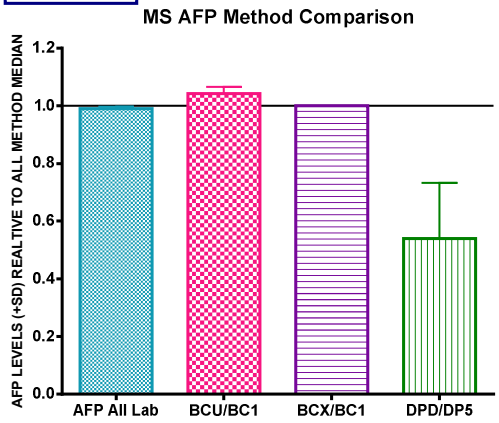


Figure 7B

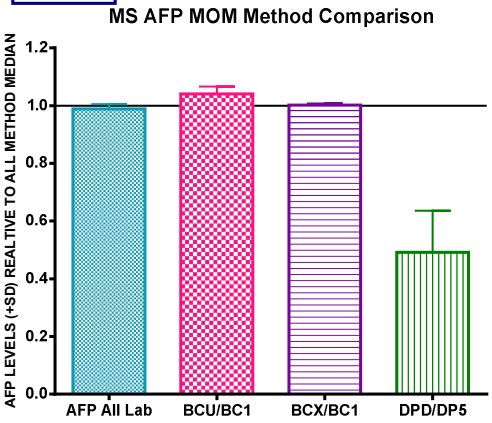


Figure 7C

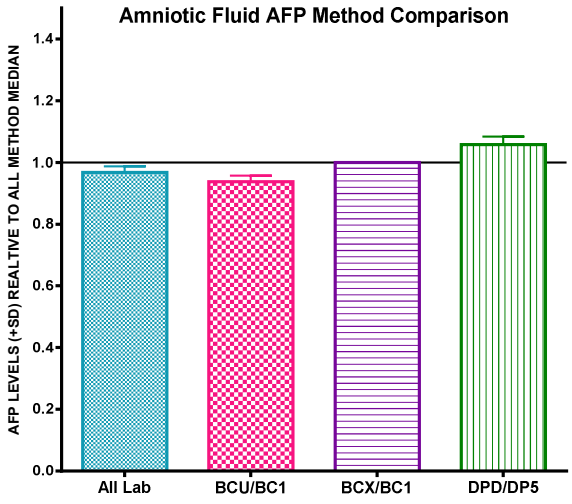
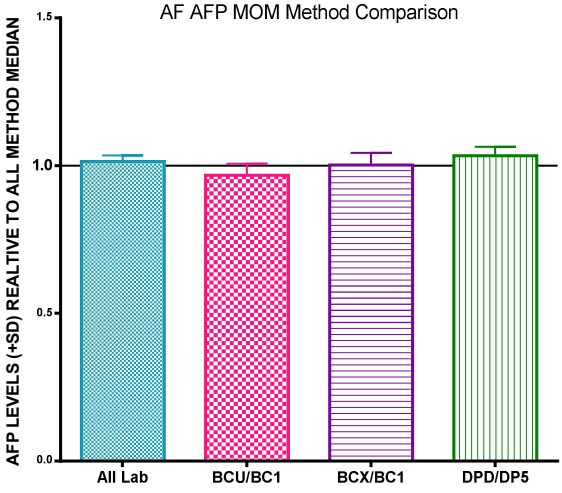


Figure 7D



NYS FEDM PT 5/15
Second Trimester

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

Figure 8A

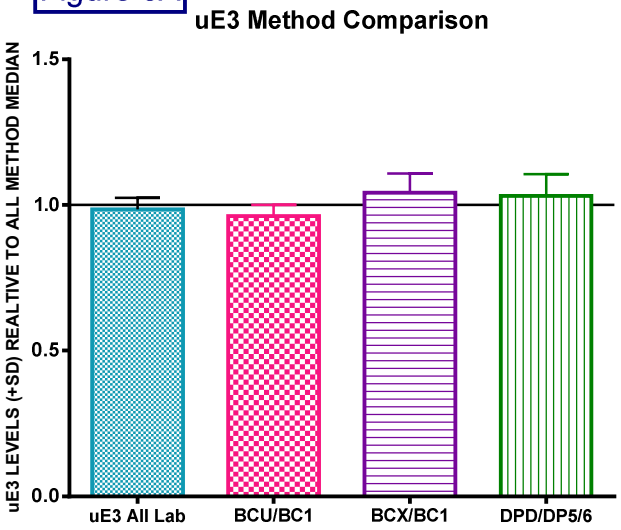


Figure 8B

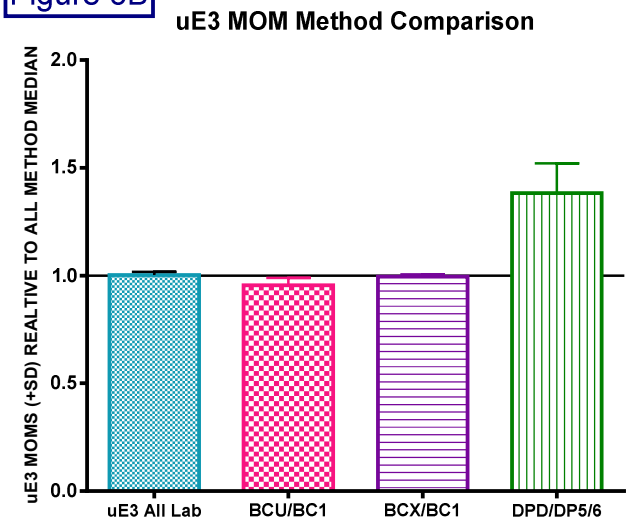


Figure 9A

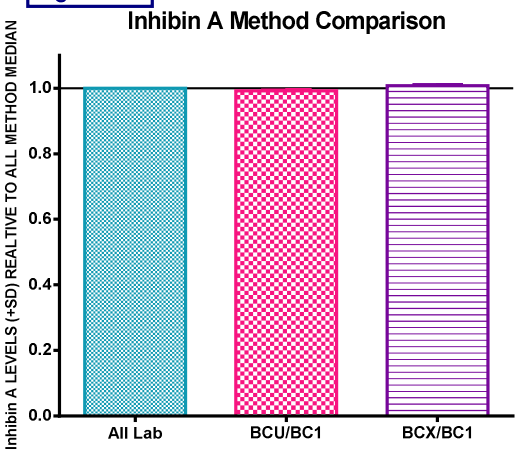


Figure 9B

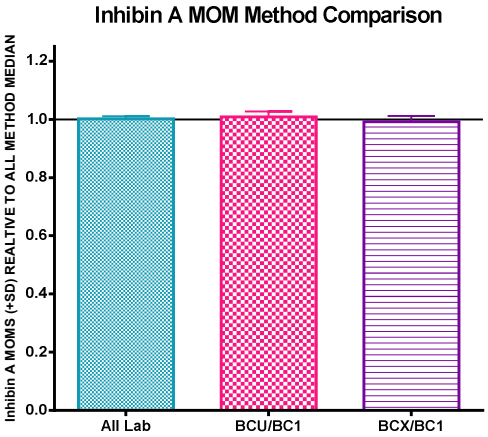


Figure 10A

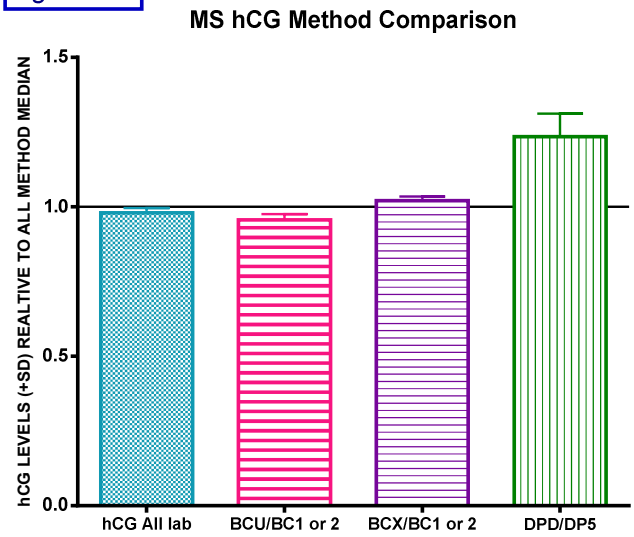
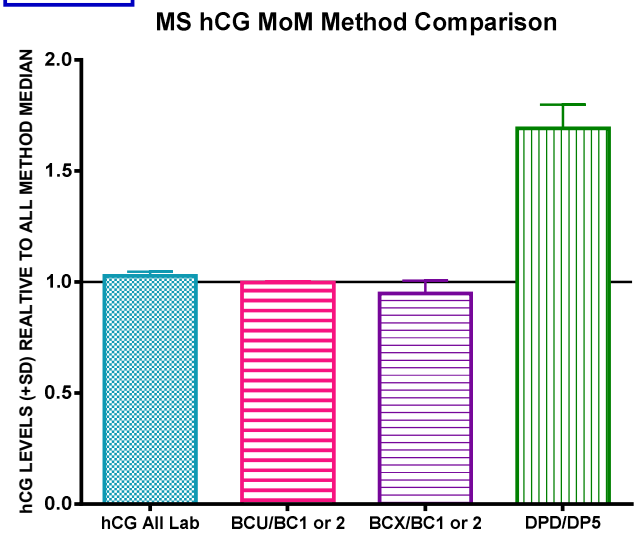


Figure 10B



NYS FEDM PT 5/15
First Trimester

Figure 11A

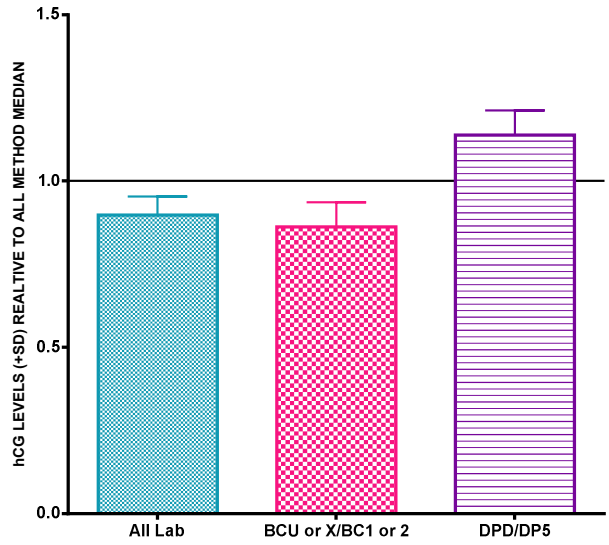


Figure 11B

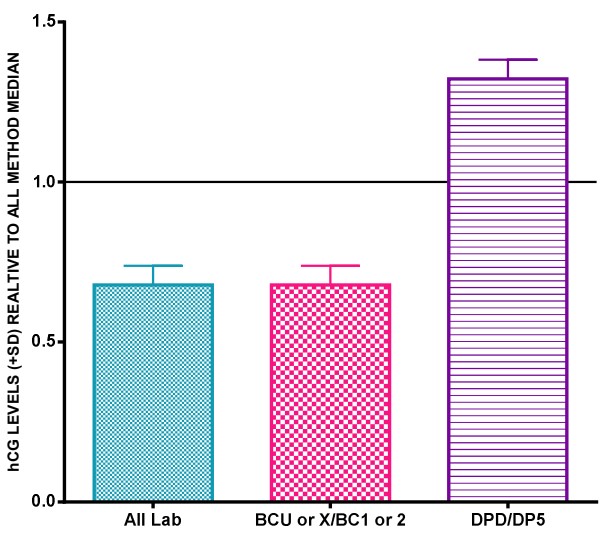


Figure 12A

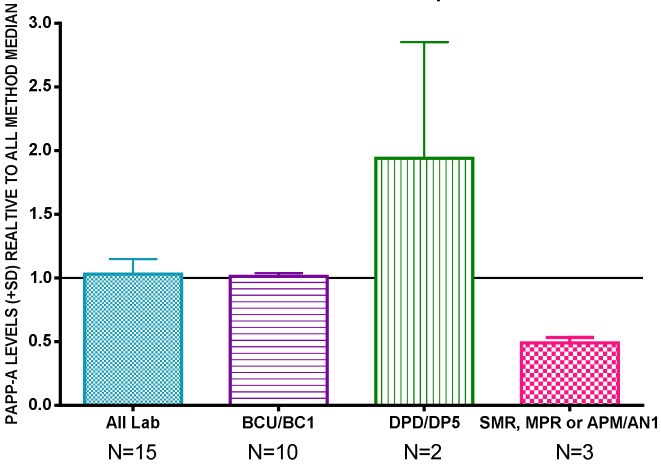
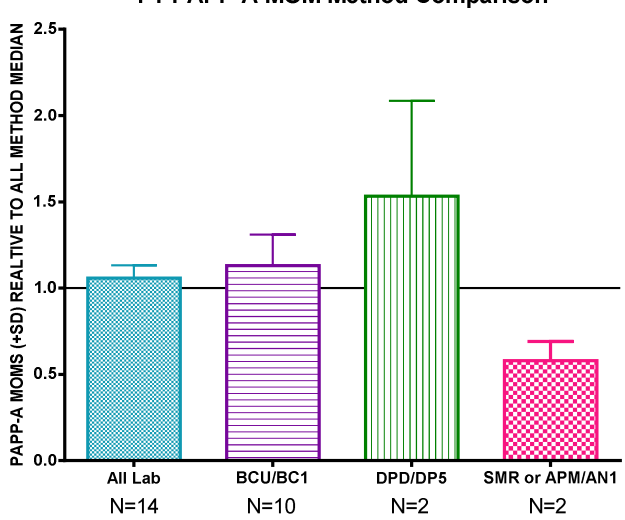


Figure 12B



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

Figure 13

Graphic Distribution of First Trimester
Trisomy 21 Risk Estimates

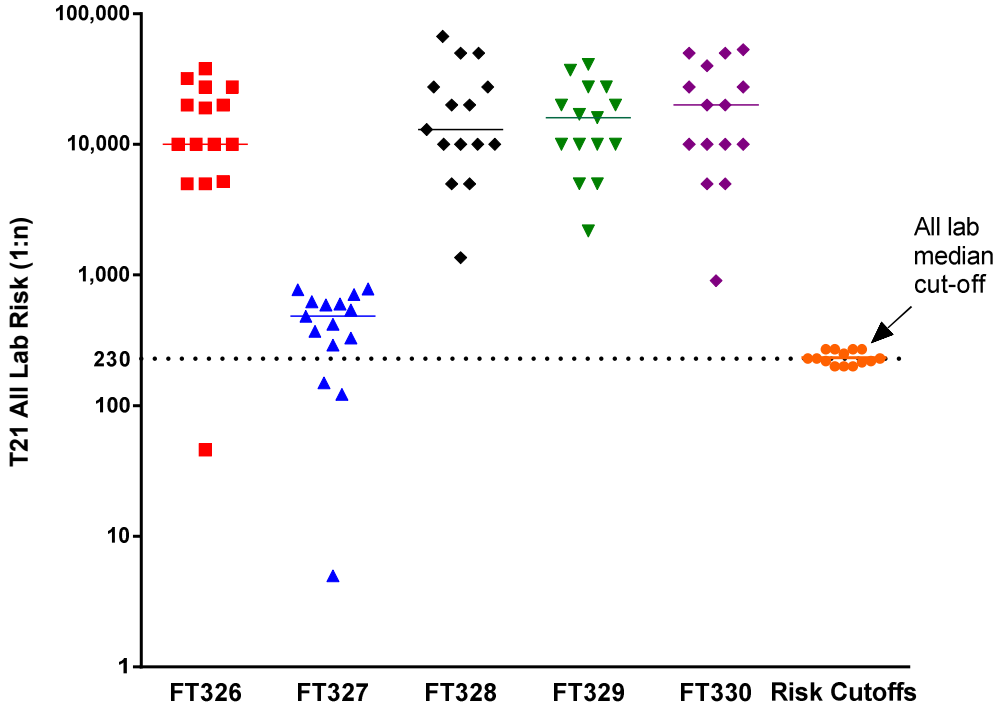


Figure 14

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates