NEW YORK STATE OF OPPORTUNITY. Department of Health

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October 9, 2015

Dear Laboratory Director,

Attached you will find a summary and critique of the Proficiency Testing mail-out from September 1, 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; also included are the grades from the previous two PT events. These will be mailed to you separately. Please review and sign your evaluation. Retain the signed evaluation in your files. You will need it for your next laboratory survey to demonstrate participation in the NYSPT program.

PLEASE NOTE:

The New York State (NYS) Proficiency Testing (PT) program for Fetal Defect Markers will officially be terminated as of December 31, 2015. Starting January 2016, you must secure alternative means to satisfy requirements under the NYS Clinical Laboratory Evaluation Program standard QA S3, which requires laboratories to verify the accuracy of each test at least twice per year. We do not stipulate what labs must do to comply with QA S3. Acceptable mechanisms include exchanging samples with another lab, re-testing blinded samples, or enrolling in another provider's PT that is appropriate for the testing offered by our lab. If you choose to enroll in another provider's PT, the results will be sent to us for review; unsatisfactory or unsuccessful performance may result in citations or the loss of a permit. Documentation of other QA S3 mechanisms should be available during inspection. It has been both an honor and a pleasure to have served you as assistant director of this program in the past years.

Yours sincerely,

Gerald J. Mizejewski

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Assistant Director, Fetal Defect Markers Section
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Fetal Defect Marker Proficiency Test Mailout[1]
September 2015

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

Samples *N = 24	Sample #	MS 331	MS 332	MS 333	MS 334	MS 335
	Gestational Age (weeks)	18.0	17.0	20.0	16.0	20.0
Maternal Race	Ethnic Group	White	Hispanic	White	Black	White
Maternal Weight	Pounds (lbs)	155	140	150	165	160
Maternal Age	Years	32	20	25	28	25
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	23.5 ± 2.0	40.7 ± 3.3	217.3 ± 19.3	34.6 ± 3.1	193.7 ± 18.9
	MOM ± Std. Dev.	0.54 ± 0.04	1.03 ± 0.11	3.86 ± 0.51	0.99 ± 0.12	3.45 ± 0.28
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	0.48 ± 0.06	0.74 ± 0.09	1.87 ± 1.13	0.56 ± 0.06	1.21 ± 0.10
	MOM ± Std. Dev.	0.37 ± 0.06	0.68 ± 0.08	0.96 ± 0.13	0.70 ± 0.09	0.63 ± 0.07
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	49.5 ± 5.4	26.8 ± 3.1	19.2 ± 1.9	32.6 ± 3.7	20.0 ± 2.0
	MOM ± Std. Dev.	1.99 ± 0.16	0.88 ± 0.08	0.92 ± 0.09	0.89 ± 0.14	1.00 ± 0.10
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	409.9 ± 25.5	188.2 ± 11.9	264.7 ± 15.8	165.0 ± 20.0	286.5 ± 19.6
	MOM ± Std. Dev.	2.49 ± 0.21	1.10 ± 0.08	1.40 ± 0.13	1.04 ± 0.09	1.56 ± 0.15
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (–)	(–) (100%)	(–) (100%)	(+) (100%)	(–) (100%)	(+) (100%)
	Recommended Action**	NFA	NFA	G = 96% U = 100% A = 83%	NFA	G = 96% U = 96% A = 88%
	NTD Risk (median) 1 in	15,400	9,085	19	11,500	26
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (–)	(+) (100%)	(–) (100%)	(–) (100%)	(–) (100%)	(–) (100%)
	Recommended Action**	G = 100% U = 50% A = 83% NIPT = 17%	NFA	NFA	NFA	NFA
	Risk Est. (median) 1 in	14	3,150	10,323	2,955	4,900
2. Quad Test	Pos. (+) or Neg. (–)	(+) (100%)	(–) (96%)	(–) (96%)	(–) (96%)	(–) (100%)
	Recommended Action **	G = 100% U = 54% A = 88% NIPT = 13%	NFA	NFA	NFA	NFA
	Risk Est. (median) 1 in	8	5,500	20,000	4,300	19,600
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	725	10,000	10,000	8,910	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 NIPT = Noninvasive prenatal testing.**This percentage is normalized to labs requesting further action. ‡ Insulin Dependent Diabetic pregnancy.

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

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:

N = 24 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 331 Wk 18.0	This specimen was obtained from a 32 year old White woman (Gravida = 4, Parity = 2) in her 18th week of gestation with a body weight of 155 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, 100%; ultrasound, 54%; amniocentesis, 88%, and noninvasive prenatal testing, 13%; while labs reporting a positive triple test recommended genetic counseling, 100%; ultrasound 50%; and amniocentesis, 83%, and noninvasive prenatal testing, 17%. Specimen MS331 resulted in a negative T18 screen in 100% of the participating labs. This sample was paired to an amniotic fluid specimen AF326 which also had a slightly reduced AFAFP level (MOM = 0.87).
MS 332 Wk 17.0	This specimen was obtained from a 20 year old Hispanic woman (Gravida = 1, Parity = 0) in her 17th week of gestation with a body weight of 140 lbs. She had no personal history of pregnancy complications and her specimen resulted in negative screens for NTD and T21 with neither a body weight nor ethnic correction indicated. Specimen MS332 was not paired with an amniotic fluid specimen.
MS 333 Wk 20.0	This specimen was obtained from a 25 year old White woman (Gravida = 3; Parity = 1) in her 20th week of gestation with a body weight of 150 lbs. She had a personal history of pregnancy loss (see critique). Her sample was a positive screen for NTD (100% consensus; MSAFP MOM = 3.86). 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, 96%; ultrasound, 100%; and amniocentesis, 83%. The MS333 specimen had no amniotic fluid counterpart.
MS 334 Wk 16.0	This specimen was obtained from a 28 year old Black woman (Gravida = 1, Parity = 0) in her 16th week of gestation with a body weight of 165 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 MS334 sample was not paired to an amniotic fluid specimen.
MS 335 Wk 20.0	This specimen was obtained from a 25 year old White woman (Gravida = 3; Parity = 1) in her 20th week of gestation with a body weight of 160 lbs and no history of pregnancy complications. Her sample screened positive for NTD (MSAFP MOM = 3.45), and her aneuploidy screen was negative for Down syndrome and T18. Further actions for the NTD were recommended as: genetic counseling, 96%; ultrasound, 96%; amniocentesis, 88%. This sample was paired to an amniotic fluid specimen (MOM = 3.28), which was in the very high range.

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

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

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

2) AMNIOTIC FLUID AFP (NTD-analysis):

N=20; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 331 Wk 18.0	AFP = 8.1 ± 0.7 µg/ml MOM = 0.87 ± 0.09	The AF331 sample was targeted for an AFAFP less than 1.0 median in the routine gestational age range. All labs called AF331 a negative screen for AFAFP. The AFAFP sample was matched to maternal serum specimen MS331 whose AFP level was low (MOM = 0.54).
AF 332 Wk 19.5	AFP = 10.0 ± 0.6 µg/ml MOM = 1.40 ± 0.14	The AF332 sample was targeted for a screen negative AFAFP value in the upper gestational age window. All labs reported this specimen as screen negative AFAFP. The AF332 specimen was not paired with a maternal serum sample.
AF 333 Wk 17.0	AFP = 9.8 ± 0.7 µg/ml MOM = 0.87 ± 0.09	The AF333 sample was targeted as an AFAFP normal value in the routine gestational age screening range. All labs categorized AF333 as a negative screen. This specimen had no maternal serum counterpart.
AF 334 Wk 19.0	AFP = 5.8 ± 0.5 µg/ml MOM = 0.74 ± 0.10	The AF334 sample was targeted for an AFAFP less than 1.0 median value in the upper gestational age range. All labs called AF334 a non-elevated specimen. This AFAFP sample was not matched to a maternal serum specimen.
AF 335 Wk 20.0	AFP = 20.9 ± 1.7 µg/ml MOM = 3.28 ± 0.42	The AF335 sample was targeted for a highly elevated screen AFAFP value in the upper gestational age range. All labs reported this specimen as screen positive for AFAFP. The AF335 specimen was paired with an elevated maternal serum sample (MOM = 3.45).

II. Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 15	**Sample #**	**FT 331**	**FT 332**	**FT 333**	**FT 334**	**FT 335**
	Gestational Age (weeks)	13.0	11.4	11.1	13.0	12.4
Maternal Race	Ethnic Group	Black	White	White	Hispanic	Asian
Maternal Weight	Pounds (lbs)	160	145	132	150	140
Maternal Age	Years	21	25	35	30	28
Fetal Physical Measurements	Crown Rump Length (mm)	67	47	44	67	60
	NT Thickness (mm)	1.50	2.50	1.19	1.50	1.40
	NT – MOM ± Std. Dev.	0.92 ± 0.05	2.11 ± 0.12	1.07 ± 0.06	0.92 ± 0.05	0.95 ± 0.05
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	70.7 ± 8.9	201.9 ± 35.6	94.2 ± 14.7	65.8 ± 9.6	74.1 ± 10.5
	MOM ± Std. Dev.	0.90 ± 0.06	1.95 ± 0.18	0.84 ± 0.11	0.83 ± 0.07	0.82 ± 0.09
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	2072.8 ± 1287.2	2096.7 ± 5282.3	1308.7 ± 916.3	2073.1 ± 1288.2	1780.6 ± 1205.4
	MOM ± Std. Dev.	1.70 ± 0.91	1.18 ± 0.59	1.93 ± 0.96	1.83 ± 0.81	1.80 ± 0.92
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (–)	(–) (100%)	(+) (86%)	(–) (100%)	(–) (100%)	(–) (100%)
	Recommended Action **	NFA	G = 80% U = 47% A = 33% C = 53% NIPT = 33%	NFA	NFA	NFA
	Risk Estimate 1 in	12,800	59	6,400	11,450	14,300
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	2,800	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, NIPT = Noninvasive prenatal testing; 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:

N = 15 all-lab Consensus Values.

Sample#	Summary Comments:
FT 331 Wk 13.0	This specimen was obtained from a 21 year old Black woman with a body weight of 160 lbs. Her gestational age at the time of screening was 13.0 weeks. She had no prior history of pregnancy complications or difficulties. This FT specimen was screen negative and all testing labs were in agreement. The FT331 risk estimate for Trisomy-21 was 1 in 12,900 and the Trisomy-18 risk was 1 in 10,000.
FT 332 Wk 11.4	This specimen was obtained from a 25 year old White woman of average body weight (145 lbs.). Her gestational age at the time of screening was 11.4 weeks. She had no prior history of any pregnancy complications. This FT specimen was screen positive for Trisomy-21 with 86% of labs reporting an elevated risk. Recommendations for further action from labs were: genetic counseling, 80%; ultrasound, 47%; amniocentesis, 33%, CVS, 53% and noninvasive prenatal testing, 33%. The FT332 risk estimate for Trisomy-21 was 1 in 59, and the Trisomy-18 risk was 1 in 2,800.
FT 333 Wk 11.1	This specimen was obtained from a 35 year old White woman of average body weight (132 lbs.). Her gestational age at the time of screening was 11.1 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 FT333 risk estimate for Trisomy-21 was 1 in 6,400, and the Trisomy-18 risk was 1 in 10,000.
FT 334 Wk 13.0	This specimen came from a 30 year old Hispanic woman with a body weight of 150 lbs. Her gestational age at the time of screening was 13.0 weeks. She reported no prior family history of pregnancy problems. This FT specimen was screen negative for both Trisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT334 was 1 in 11,450, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.
FT 335 Wk 12.4	This specimen was procured from a 28 year old Asian woman of average body weight (140 lbs.). Her gestational age at the time of screening was 12.4 weeks. She had no prior family history of pregnancy complications or adverse outcomes. This FT specimen was screen negative for Trisomy-21. The FT335 risk estimate for Trisomy-21 was 1 in 14,300, 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 **MS335** was targeted as a screen positive specimen for NTD (Figs. 2a and 3) and was matched to an elevated **AF335** sample (Fig. 2b). All labs agreed that specimen MS335 was screen positive for NTD and all labs agreed that both Trisomy screens were negative (Figs. 4-6). The risk assessment for NTD in **MS335** was 1 in 26. As a follow-up, a polyacrylamide gel electrophoresis would be indicated to determine the absence or presence of a diagnostic Ache band, which would confirm an NTD. The maternal serum MOM levels for **MS335** were: MSAFP MOM = 3.45; MSuE3 MOM = 0.63; MShCG MOM = 1.00; MSDIA MOM = 1.56.

Sample **MS331** was obtained from a white woman with a prior family history of pregnancy complications. The fetal defect biomarker MOM values for this specimen (MSAFP MOM = 0.54, MSuE3 MOM = 0.37, MShCG MOM = 1.99, DIA-MOM = 2.49) presented the canonical profile for elevated risk for T21 of low MSAFP and low MSuE3, together with elevated MShCG and MSDIA (Fig. 1). This biomarker pattern resulted in a positive Down Syndrome screen in which all labs agreed (100% by both triple and quad test). In addition, the matched **AF331** specimen showed reduced AFP levels (MOM value = 0.87) in amniotic fluid. The median T21 risk was 1 in 14 by triple test and 1 in 8 by quad test (Figs. 4, 5). The recommended further actions for sample **MS331** were genetic counseling, 100%; ultrasound, 54%; amniocentesis, 88%, and noninvasive prenatal testing, 12%, from labs performing the quad screen; and genetic counseling, 100%; ultrasound, 50%; amniocentesis, 83%, and noninvasive prenatal testing, 17% from labs performing the triple screen.

Two other specimens, **MS332** and **MS334**, produced negative screens for NTD, T21, and T18 with no corrections for body weight or race being indicated.

The **MS333** specimen at 20 weeks presented an interesting case involving extremely high MSAFP (MOM = >3.0) together with normal MShCG, MSuE3, and MSDIA levels. This biomarker profile resulted in a positive screen for NTD (risk = 1 in 19) (Fig. 5). Sample **MS333** was mimicked after several case studies of pregnant women bearing fetuses with Ventral Wall Defects (VWD), which manifested with highly elevated levels of MSAFP and AFAFP and an aberrant pattern of acetylcholinesterase (Ache) in amniotic fluid (1). A VWD was first reported in 1977 and many such pregnancies have since been described in the literature. VWD is not inherited in a Mendelian pattern but fits a multifactorial inheritance profile (2, 3). In certain cases, mothers had given birth to a previous child afflicted with a VWD defect, demonstrating a sibling re-occurrence effect. Behavioral and/or environmental factors may be involved since a recent increase in birth prevalence of VWD has been recorded worldwide. MSAFP and AFAFP measurements are elevated and the presence of Ache shows an aberrant pattern in the amniotic fluid. All of the women in the case studies had extremely high levels of MSAFP determined early in the second trimester. Their ultrasound results did not show any gross anatomical malformations but did display gastrointestinal obstructions such as atresias, stenosis, gastroschisis, and omphalocoele (4). Most of the women with VWDs do not deliver normal term infants, but rather experience either pre-term birth, intra-uterine growth retardation, or immature newborns. In some instances, discussed below, chromosomal abnormalities were observed following amniocentesis. In summary, the present specimen **MS333** produced a false positive screen for NTD due to the extremely elevated levels of MSAFP. Following amniocentesis, VWD can only be distinguished from NTD by a unique Ache electrophoretic pattern (see Fig. 9).

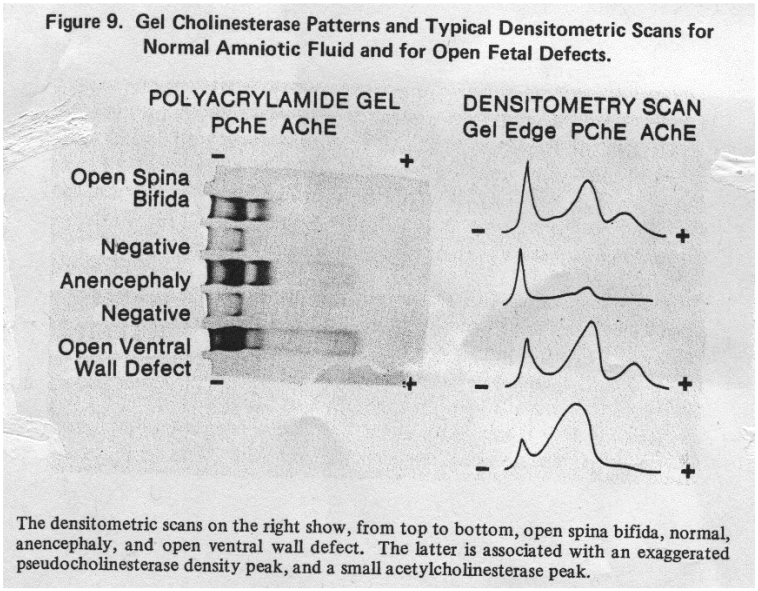

Figure 9. Gel Cholinesterase Patterns and Typical Densitometric Scans for Normal Amniotic Fluid and for Open Fetal Defects.

The densitometric scans on the right show, from top to bottom, open spina bifida, normal, anencephaly, and open ventral wall defect. The latter is associated with an exaggerated pseudocholinesterase density peak, and a small acetylcholinesterase peak.

Haddow, J.E. and Goldfine, C. The Evolving Role of Amniotic Fluid Gel Acetylcholinesterase Analysis for Identifying Open Fetal Defects during Second Trimester. In: Alpha-fetoprotein and Congenital Disorders, Edited by G.J. Mizejewski and I.H. Porter, Academic Press, New York, 1985, pp 215-237.

Maternal serum elevated AFP in pregnancy has been shown to be associated with various fetal malformations such as anencephaly, spina bifida, omphalocoele, gastroschisis, nephrosis, renal agenesis, and esophageal/phyoric atresia (5). Some increased serum levels of AFP in NTD pregnancies have been ascribed to AFP leakage from the cerebrospinal fluid into the amniotic sac. AFP can also transudate from blood vessels of the viscera in the event of defects such as omphalocoele and gastroschisis. Blockages in the gastrointestinal tract (duodenal/esophageal atresia) can also cause impairment of fetal swallowing/digestion resulting in the accumulation of fetal proteins in the amniotic sac.

Ventral wall defects consist of two major types, namely, gastroschisis (GTS) and omphalocele (OPC) (6). GTS is an opening in the abdominal wall lateral to the insertion of the umbilical cord (7). As a result of the wall separation, the viscera protrude out through the opening and float uncovered (lack of membrane) in the amniotic fluid. The bowels are shortened as a consequence and acquire an inflammatory coating over the surface of the viscera. Intestinal function in such newborns is impaired and the overall lesion requires surgical repair at birth (8).

Intrauterine growth retardation is common in GTS pregnancies (9). Chromosomal abnormalities are not usually associated with GTS.

In comparison to GTS, an OPC defect is a persistent and vast enlargement of the body stalk of the umbilical cord protruding out of the abdominal wall. A membranous sac covers the herniated viscera which emerge alongside the umbilical cord and open into the abdominal wall. OPC is associated with a high incidence of fetal malformations compared to GTS and is co-morbid with chromosomal anomalies. Complications such as immature infant birth (<36 weeks), low birthweight (<1500 g), and high mortality rates are also observed with OPC (10). High mortality rates have been attributed to fetal total protein loss (11).

VWDs occur in 0.04-0.05% of live births with the incidence of GST being twice as high as OPC (12, 13). Both GST and OPC display evisceration of the bowels; hence VWD-bearing fetuses/newborns are vulnerable to hypothermia, acute fluid loss, and dehydration. The importance of prenatal screening and diagnostic ultrasound is evident with VWDs (7). Early diagnosis allows planning for optimal perinatal care at delivery, and surgical treatment when necessary. Cesarean section deliveries are often performed in such pregnancies (14, 15).

Other birth defects and anatomical malformations are also associated with VWDs, especially with OPCs. Such defects include intestinal atresias, stenosis, and other blocked passages and orifices including obstructions in the urinary tract (16). Mal-rotation of anatomical structures (organs, etc.) can occur in addition to amyoplasia, a lack of muscular development at the joints (17, 18). More rarely, cryptorchidism (lack of testis descent) and cardiac and limb defects can occur. Both fetal and neonatal mortality rates are increased in VWD pregnancies with rates ranging from 20-30% (19).

OPC, but not GTS, is highly correlated with chromosomal abnormalities in approximately 54% of OPC cases. In a study of 28 cases of detected VWDs, three cases of Trisomy-18 were found in the OPC group in addition to multiple other chromosomal abnormalities (20). Among fetuses with VWD, those with GTS have a better outcome because fewer chromosomal aberrations and anatomical defects are found. For example, OPC can be associated with Beckwith-Wiedemann syndrome (an organ overgrowth condition) and with exomphalos macroglossia gigantism (EMG) syndrome. The EMG syndrome displays defects in the muscles of the body wall and an enlarged tongue that extrudes from the mouth.

MSAFP screening leads to the detection of 95% of fetal GTS cases at 15-20 weeks gestation. In a study of 73,782 pregnancies (Maine and Rhode Island), 20 cases of GTS and 13 cases of OPC were identified (21). The median ranges of MSAFP MOMs were 3.6 to 13.5 for GTS and 0.5 to 29.8 for OPC cases. The combined rate of VWD was 5.4 per 10,000 livebirths in this particular study. In a second study of 35 VWD pregnancies and 200 controls, median values for triple screen markers were determined (22). The MSAFP median MOM for GTS was 9.42 MOMs, while the median for OPC was 4.14 MOMs. In comparison, the median MShCG for GTS was 1.10 MOM while that for OPC was 1.13; the median MSuE3 MOM for GTS was 1.10 MOM and for OPC was 0.9 MOM. Thus, MSAFP detects most cases of VWDs without association with MShCG and MSuE3.

The amniotic fluid biomarkers and ultrasound values for VWDs have also been determined (23, 24). In GTS specimens, 89% of specimens displayed elevated AFAFP levels, while only 29% of OPC specimens showed elevations. Similarly, 73% of GTS specimens were positive for Ache in amniotic fluid, but only 27% of OPC specimens were (25, 26). When ultrasound imaging was performed, 90% of GTS fetuses showed abnormalities, while a comparable 89% of OPC fetuses did likewise (27). Thus, the diagnosis of OPCs becomes more clear when amniotic fluid analysis and ultrasound imaging are applied (28).

VWDs do not show a genetic pre-disposition; however, behavioral and/or environmental factors may be involved since a recent increase in prevalence has been observed in some countries. Past studies have shown that certain risk factors were evident; these included young maternal age and cigarette smoking (29). Data revealed that women less than 20 years of age were 7.3 times more likely to have a child with VWD than those 25 years or older. Further analyses showed that the likelihood for women aged 20 to 24 years was only 1.9 times greater than that for women 25 years or older to have a child with VWD. In regards to cigarette smoking in pregnant women, it was reported that these women were at a 2.1 times greater risk for a VWD pregnancy than non-smoking pregnant women. Infants' month of birth was also investigated but showed no association with VWDs. Thus, the greatest risk for VWD was linked to mothers less than 20 years of age.

The proposed etiology of VWDs, especially GTS, may relate to several characteristics. First, there is a negative correlation with obesity. Second, GTSs occur mostly in young white pregnant women, but not in African-American women. Third, an association with thrombotic events early in pregnancy has been found. Apparently, young white women have higher first trimester estrogen levels, which can induce thrombosis: this event produces thrombic by-products that may interfere with developmental signaling. African-American women have a different thrombic gene background and lower estrogen levels that appear to result in less thrombotic events. Furthermore, in GTS-related pregnancies, women display an excess of omega-6 fatty acids (i.e. linoleic acid) which may pose a dietary risk factor for thrombosis (30). GTS-associated defects are associated with a disruption in intrauterine blood flow and omega-6 fatty acids serve as substrates for inflammatory eicosanoids/cytokines involved in vascular regulation; such fatty acids are also associated with oxidation reactions. Thus, a higher intake of omega-6 polyunsaturated fatty acids might increase the risk of GTS. Finally, mothers with GTS fetuses were found to have

higher serum levels of palmitoleic and linoleic acids and lower levels of oleic and decosohexanic acids during pregnancy and at term (31).

B) Second Trimester 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 method. As shown in Figs. 7A-7D, MSAFP and AFAFP mass measurements among the individual kits mostly agreed. The exception was Siemens Immulite (DPD/DP5) in serum with mass values and MOMs that were 5-20% higher than those from the Beckman methods. When the kit specific uE3 MOMs were compared, values from Siemens DPC Immulite 2000/2500 ranged nearly 40% higher than those from the Beckman kits, although the actual mass values were somewhat lower (Figs. 8A and 8B). The method comparison for Inhibin-A displayed in Fig. 9A shows that there was no difference between the results from the Beckman Access/2 and UNICEL instruments (Fig. 9B). Finally, regarding the hCG kits (Fig. 10A), results from the Beckman Access/2 5th generation kits (BCX/BC1; BCX/BC2) were 20% higher than those from the Beckman UNICEL kits (BCU/BC1; BCU/BC2), whereas those from Siemens Immulite 2000 (DPD/DP5) were 20% lower. However, this difference was largely 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 25%, 29.2%, and 29.2%, respectively, whereas in-house and "other" software comprised 16%. Programs classified as "other" are presumably proprietary software packages.

D) First Trimester Maternal Serum

In general, all lab results were consistent with the targeted values for the Trisomy screens for risks and outcomes. The Caucasian maternal serum sample FT332 was targeted as a screen positive specimen for T21 (Figs. 13, 14). Most labs (86%) agreed that specimen FT332 was positive for T21 and all labs reported that this specimen was T18 negative. The risk assessment was 1 in 59. The maternal serum MOM levels were: hCG MOM = 1.95; and PAPP-A MOM = 1.18. Furthermore, Nuchal Translucency = 2.5 mm; MOM = 2.5

The other specimens, FT331, FT333, FT334, and FT335 produced negative screens for T21 and T18.

E) First Trimester Assay Kit Performance:

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 = 1 ng/ml was used.

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in Figs. 11 and 12 for the five FT samples. As shown in Fig. 11A, FT hCG mass measurements by Beckman UNICEL or Access/2 original and 5th IS hCG kit were 20% higher than those by the Siemens Immulite DPC instruments, but those differences between the kits were eliminated by the conversion to MOMs (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 another (Fig. 12A) with Siemens Immulite more than 6.0 times greater than Beckman, and Anshlite about half that of Beckman. Corresponding MOM values reflected similar, though somewhat diminished differences.

F) First Trimester Screening Software Utilized:

The alpha, Benetech and Maciel (RMA) software packages were each used by 14%, 21%, and 43% respectively; and in-house software comprised of 21%. None of the labs used programs classified as "other".

G.J. Mizejewski, Ph.D.

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October 2015

Teachings on Alpha-fetoprotein

Vol. 8, Part 1

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

Title: a-Fetoprotein and folate deficiency

To the Editors: A previous article by the Medical Research Council Vitamin Study Research Group (Wald N, Hall M, Campbell D, et al. Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. Lancet 1991;338:131-7) on the prevention of neural tube defects with periconceptional folic acid supplementation has aroused my interest concerning an involvement with α-fetoprotein (AFP). Since the relationship of neural tube defects with AFP is now well established, I believe that a consideration of AFP in light of folic acid deficiencies during pregnancy is now in order. The increased demand for folates during pregnancy results from transport to the fetus, placental tissue growth, excess erythrocyte synthesis, and urinary loss replacement.[1] It is generally accepted that a steady depletion of maternal serum folate occurs during pregnancy, which is accompanied by a mild physiologic anemia.[2] The serum folate decline has been attributed to rapid plasma clearance, fetal transfer, hemodilution, and possibly hormonal effects as seen in users of oral contraceptives.[3] There appears to be a higher incidence of the megaloblastic-type anemias in folate-deficient pregnant women. In addition, a number of complications have been associated with folate deficiency in pregnant women, although some are considered controversial. Such complications include: (1) abruptio placentae, (2) toxemia of pregnancy, (3) spontaneous abortion and fetal death (loss), (4) neural tube defects, including spina bifida and anencephaly, (5) hydrocephalus, (6) prematurity-low birth weight, (7) hemolytic anemias, (8) various anatomic congenital malformations, including heart disorders, and (9) twin pregnancies. It is quite remarkable that AFP concentrations tend to be elevated in most if not every pregnancy complication listed. This relationship might be considered a mere coincidence because elevated AFP levels are often associated with anatomic anomalies such as ductal or vessel obstruction, failure of a structure to form or close, membrane rupture, fluid accumulation, compartmental leakage, and circulatory complications. However, AFP is elevated in many instances where folic acid deficiency is implicated, some of which imply a biochemical rather than an anatomic basis.

In light of these associations, one may consider the following reported observations. First, both human AFP (author's data) and albumin can bind folic acid or unconjugated pterins in a low-affinity, high-capacity manner.[4] Second, elevated AFP serum levels have been reported in patients with severe anemia.[5] Third, the normal decline of maternal serum folate appears to parallel the normal fall in human AFP concentrations (Fig. 1) in both amniotic fluid (13 to 40 weeks' gestation) and maternal serum (0 to 10 weeks' gestation). Fourth, in pups burn to nude mice both elevated AFP serum levels and liver hematopoiesis are maintained well into adulthood.[6] Fifth, high serum AFP concentrations have been observed to persist in tumor-bearing mice with verified hemolytic anemia in the hosts.[7] Finally, AFP serum levels are elevated in weanling rats born to mothers with anemia induced by serial bleeding episodes during pregnancy.[8] Thus conditions that modulate or interfere with hematologic maturation processes in the prenatal, perinatal, and postnatal periods appear to influence AFP fluid concentrations. It is tempting to speculate that a relationship exists between elevated AFP and folic acid deficiencies in excess of physiologic anemia during ontogenetic development. Alternatively, studies of AFP interaction with folate-binding proteins and receptors also appear warranted. Only future studies will elucidate whether such relationships exist and, if so, whether the interaction is causal or consequential. To this end it may be expedient to rule out folic acid deficiency in cases of unexplained elevated AFP levels.

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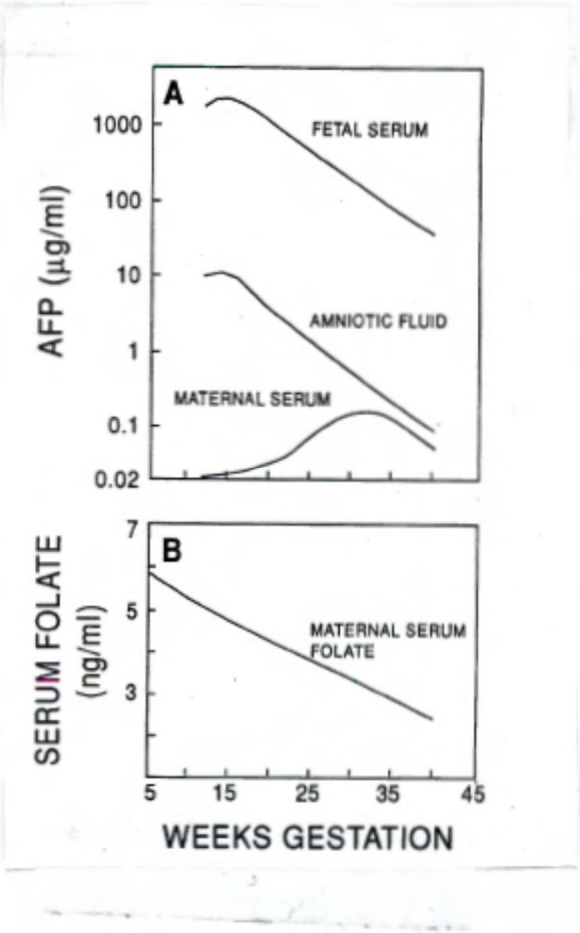
Figure 1



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

(1) Source: Obstet Gynecol. 2015 Jun 29. [Epub ahead of print]

Title: Committee Opinion No. 640: Cell-free DNA Screening for Fetal Aneuploidy

Authors: [No authors listed]

Abstract: Noninvasive prenatal screening that uses cell-free DNA from the plasma of pregnant women offers tremendous potential as a screening method for fetal aneuploidy. A number of laboratories have validated different techniques for the use of cell-free DNA as a screening test for fetal aneuploidy. All tests have a high sensitivity and specificity for trisomy 18 and trisomy 21, regardless of which molecular technique is used. Women whose results are not reported, indeterminate, or uninterpretable (a "no call" test result) from cell-free DNA screening should receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy. Patients should be counseled that cell-free DNA screening does not replace the precision obtained with diagnostic tests, such as chorionic villus sampling or amniocentesis and, therefore, is limited in its ability to identify all chromosome abnormalities. Cell-free DNA screening does not assess risk of fetal anomalies such as neural tube defects or ventral wall defects. Patients who are undergoing cell-free DNA screening should be offered maternal serum alpha-fetoprotein screening or ultrasound evaluation for risk assessment. The cell-free DNA screening test should not be considered in isolation from other clinical findings and test results. Management decisions, including termination of the pregnancy, should not be based on the results of the cell-free DNA screening alone. Patients should be counseled that a negative cell-free DNA test result does not ensure an unaffected pregnancy. Given the performance of conventional screening methods, the limitations of cell-free DNA screening performance, and the limited data on cost-effectiveness in the low-risk obstetric population, conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.

(2) Source: Ultrasound Obstet Gynecol. 2015 May 18. doi: 10.1002/uog.14904. [Epub ahead of print]

Title: Prediction of small for gestational age neonates: screening by biophysical and biochemical markers at 19-24 weeks

Authors: Poon LC, Lesmes C, Gallo DM, Akolekar R, Nicolaides KH

Abstract: OBJECTIVE: To investigate the potential value of combined screening, by maternal characteristics and medical history, fetal biometry and biophysical and biochemical markers at 19-24 weeks' gestation, for prediction of delivery of small for gestational age (SGA) neonates in the absence of preeclampsia (PE) and examine the potential value of such assessment in deciding whether the third-trimester scan should be at 32 and / or 36 weeks' gestation.

METHODS: Screening study in 7,816 singleton pregnancies, including 389 (4.9%) that delivered SGA neonates with birth weight <5th percentile (SGA <5th) in the absence of PE. Multivariable logistic regression analysis was used to determine if screening by a combination of maternal factors, fetal biometry, uterine artery pulsatility index (PI), and maternal serum concentration of placental growth factor (PlGF) and α-fetoprotein (AFP) had significant contribution in predicting SGA neonates. A model was developed in selecting the gestational age for third-trimester assessment, at 32 and / or 36 weeks, based on the results of screening at 19-24 weeks.

RESULTS: Significant independent contributions to the prediction of SGA <5th were provided by maternal factors, fetal biometry, uterine artery PI and serum PlGF and AFP. The detection rate (DR) of such combined screening at 19-24 weeks, was 100%, 78% and 42% for SGA <5th delivering at <32, at 32-36 and at ≥37 weeks' gestation, respectively, at false positive rate (FPR) of 10%. In a hypothetical model, it was estimated that if the desired objective of prenatal screening is

to predict about 80% of the cases of SGA <5th , it would be necessary at the 19-24 weeks assessment to select 11% of the population to be reassessed at 32 weeks, 43% to be reassessed at 36 weeks and 57% that do not require a third-trimester scan.

CONCLUSION: Prenatal prediction of a high proportion of SGA neonates necessitates the undertaking of screening in the third-trimester of pregnancy, in addition to assessment in the second-trimester, and the timing of such screening, at 32 and / or 36 weeks, should be contingent on the results of the assessment at 19-24 weeks.

(3) Source: Prenat Diagn. 2015 Jul;35(7):709-16. doi: 10.1002/pd.4597. Epub 2015 May 19.

Title: First trimester screening for Down syndrome using nuchal translucency, maternal serum pregnancy-associated plasma protein A, free-β human chorionic gonadotrophin, placental growth factor, and α-fetoprotein

Authors: Huang T, Dennis A, Meschino WS, Rashid S, Mak-Tam E, Cuckle H

Abstract: OBJECTIVE: The aim of this study was to assess the screening performance for Down syndrome using first trimester combined screening (FTS) and two additional markers, serum placental growth factor (PlGF) and α-fetoprotein (AFP).

METHODS: This is a retrospective case-control study of 137 pregnancies affected by Down syndrome and 684 individually matched unaffected pregnancies. Stored serum samples were tested for all four markers, and results were expressed as multiples of the gestation-specific median (MoM). Multivariate Gaussian modeling was used to calculate risks for different combinations of markers and to predict the detection rate (DR) and false positive rate (FPR). The predicted performance of enhanced FTS (FTS plus PlGF and AFP) was compared with FTS; the performance without nuchal translucency (first trimester quad) was assessed.

RESULTS: For affected pregnancies, the median PlGF level was 0.622 MoM and median AFP 0.764 MoM. Adding PlGF and AFP improved the screening performance. At 3% FPR, DR increased by 4.4% from 83.8% to 88.2% using enhanced FTS; at 95% DR, FPR decreased by 8.3%, from 19.3% to 11.0%. At 3% FPR, DR using first trimester quad test was 76.4%.

CONCLUSIONS: The performance of FTS can be enhanced by adding PlGF and AFP. Even without nuchal translucency, the test would perform well.

(4) Source: Clin Med Insights Reprod Health. 2015 Jun 11;9:13-20. doi: 10.4137/CMRH.S21865

Title: Combination of PAPPA, fhCGβ, AFP, PlGF, sTNFR1, and Maternal Characteristics in Prediction of Early-onset Preeclampsia

Authors: Yliniemi A, Makikallio K, Korpimaki T, Kouru H, Marttala J, Ryynanen M

Abstract: OBJECTIVE: To evaluate the efficacy of first-trimester markers-pregnancy-associated plasma protein A (PAPPA), free human chorionic gonadotropin β (fhCGβ), alpha-fetoprotein (AFP), placental growth factor (PlGF), and soluble tumor necrosis factor receptor-1 (sTNFR1) together with maternal characteristics (MC) for prediction of early-onset preeclampsia (EOPE).

METHODS: During 2005-2010, the abovementioned biomarkers were analyzed with logistic regression analysis in 64 EOPE and 752 control subjects to determine whether these biomarkers separately and in combination with MC would predict development of EOPE.

RESULTS: PAPPA, fhCGβ, and PlGF levels were lower, whereas AFP and sTNFR1 levels were higher in mothers with EOPE compared to controls. The combination of all markers with MC

(age, weight, and smoking status) detected 48% of the mothers with EOPE, with a 10% false-positive rate (FPR).

CONCLUSIONS: First-trimester maternal serum levels of PAPPA, fhCGβ, AFP, PlGF, and sTNFR1, together with MC, are predictive of development of subsequent EOPE. These markers, along with MC, form a suitable panel for predicting EOPE.

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

(1) Source: J Clin Med Res. 2015 Jul;7(7):564-5. doi: 10.14740/jocmr2169w.

Title: Trisomy 13 and massive fetomaternal hemorrhage

Authors: Matsui R, Suzuki S, Ito M, Terada Y, Kumasaka S

Abstract: This is the first case report of trisomy 13 complicated by massive fetomaternal hemorrhage (FMH). A pale male infant weighing 2,950 g was delivered with low Apgar scores by emergency cesarean section due to non-reassuring fetal status. The umbilical arterial pH and hemoglobin level were 6.815 and 6.9 g/dL (normal: 13 - 22 g/dL), respectively. The maternal hemoglobin-F and serum alpha-fetoprotein levels were 6.0% (normal: < 1.0%) and 1,150 ng/mL (4.1 multiple of median), respectively. The neonate was diagnosed as having trisomy 13 by a subsequent chromosome examination. In the placenta, massive intervillous thrombosis was observed microscopically. This placental finding has been reported to be associated with both preeclampsia and massive FMH. In addition, the incidence of preeclampsia in pregnancies complicated by trisomy 13 has been reported to be significantly higher than normal karyotype populations. Therefore, the current finding may support the association between trisomy 13 and the incidence of massive FMH.

(2) Source: Int J Clin Exp Pathol. 2015 Feb 1;8(2):2183-5.

Title: Yolk sac tumor of vagina: a case report

Authors: Alhumidi A, Al Shaikh S, Alhammadi A

Abstract: Malignant germ-cell tumors (MGCT) are rare tumors of childhood accounting for less than 3% of pediatric malignancies. Yolk sac (endodermal sinus) tumor is one of the malignant germ cell tumor that usually involves the gonads (ovaries and testes). Its occurrence in the vagina is extremely rare. We report a 6-months old girl presented with a vaginal mass diagnosed as a yolk sac tumor. This diagnosis is confirmed by histopathologic examination, immunehistochemical studies as well as elevated serum alpha fetoprotein (AFP).

(3) Source: Sex Dev. 2015 Jun 3. [Epub ahead of print]

Title: Uniparental Disomy in Somatic Mosaicism 45,X/46,XY/46,XX Associated with Ambiguous Genitalia

Authors: Serra A, Denzer F, Hiort O, Barth TF, Henne-Bruns D, Barbi G, Rettenberger G, Wabitsch M, Just W, Leriche C

Abstract: Disorders of sex development (DSD) affect the development of chromosomal, gonadal and/or anatomical sex. We analyzed a patient with ambiguous genitalia aiming to correlate the genetic findings with the phenotype. Blood and tissue samples from a male patient with penoscrotal hypospadias were analyzed by immunohistochemistry, karyotyping and FISH. DNA was sequenced for the AR, SRY and DHH genes, and further 26 loci in different sex chromosomes

were analyzed by MLPA. The gonosomal origin was evaluated by simple tandem repeat (STR) analysis and SNP array. Histopathology revealed a streak gonad, a fallopian tube and a rudimentary uterus, positive for placental alkaline phosphatase, cytokeratin-7 and c-kit, and negative for estrogen, androgen and progesterone receptors, alpha-inhibin, alpha-1-fetoprotein, β-hCG, and oct-4. Karyotyping showed a 45,X/46,XY mosaicism, yet FISH showed both 46,XX/46,XY mosaicism (gonad and urethral plate), 46,XX (uterus and tube) and 46,XY karyotypes (rudimentary testicular tissue). DNA sequencing revealed intact sequences in SOX9, WNT4, NR0B1, NR5A1, CYP21A2, SRY, AR, and DHH. STR analysis showed only one maternal allele for all X chromosome markers (uniparental isodisomy, UPD), with a weaker SRY signal and a 4:1 ratio in the X:Y signal. Our findings suggest that the observed complex DSD phenotype is the result of somatic gonosomal mosaicism and UPD despite a normal blood karyotype. The presence of UPD warrants adequate genetic counseling for the family and frequent, lifelong, preventive follow-up controls in the patient.

C) News of Note: Abstracts of New Markers:

(1) Source: Ultrasound Obstet Gynecol. 2015 Mar 31. doi: 10.1002/uog.14861

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.

(2) 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.

(3) Source: Arch Gynecol Obstet. 2015 Jul;292(1):81-5. doi: 10.1007/s00404-014-3606-9.

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.

(4) Source: BJOG. 2015 Jun 26. doi: 10.1111/1471-0528.13495

Title: Maternal characteristics and mid-pregnancy serum biomarkers as risk factors for subtypes of preterm birth

Authors: Jelliffe-Pawlowski LL, Baer RJ, Blumenfeld YJ, Ryckman KK, O'Brodovich HM, Gould JB, Druzin ML, El-Sayed YY, Lyell DJ, Stevenson DK, Shaw GM, Currier RJ

Abstract: OBJECTIVE: To examine the relationship between maternal characteristics, serum biomarkers and preterm birth (PTB) by spontaneous and medically indicated subtypes.

DESIGN: Population-based cohort.

SETTING: California, United States of America.

POPULATION: From a total population of 1 004 039 live singleton births in 2009 and 2010, 841 665 pregnancies with linked birth certificate and hospital discharge records were included.

METHODS: Characteristics were compared for term and preterm deliveries by PTB subtype using logistic regression and odds ratios adjusted for maternal characteristics and obstetric factors present in final stepwise models and 95% confidence intervals. First-trimester and second-trimester serum marker levels were analysed in a subset of 125 202 pregnancies with available first-trimester and second-trimester serum biomarker results.

MAIN OUTCOME MEASURE: PTB by subtype.

RESULTS: In fully adjusted models, ten characteristics and three serum biomarkers were associated with increased risk in each PTB subtype (Black race/ethnicity, pre-existing hypertension with and without pre-eclampsia, gestational hypertension with pre-eclampsia, pre-existing diabetes, anaemia, previous PTB, one or two or more previous caesarean section(s), interpregnancy interval $\geq$ 60 months, low first-trimester pregnancy-associated plasma protein A, high second-trimester α-fetoprotein, and high second-trimester dimeric inhibin A). These risks occurred in 51.6-86.2% of all pregnancies ending in PTB depending on subtype. The highest risk observed was for medically indicated PTB <32 weeks in women with pre-existing hypertension and pre-eclampsia (adjusted odds ratio 89.7, 95% CI 27.3-111.2).

CONCLUSIONS: Our findings suggest a shared aetiology across PTB subtypes. These commonalities point to targets for further study and exploration of risk reduction strategies.

(5) Source: J Perinatol. 2015 Apr 30. doi: 10.1038/jp.2015.40

Title: Maternal serum markers, characteristics and morbidly adherent placenta in women with previa

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

Abstract: OBJECTIVE: To examine associations with morbidly adherent placenta (MAP) among women with placenta previa.

STUDY DESIGN: Women with MAP (cases) and previa alone (controls) were identified from a cohort of 236 714 singleton pregnancies with both first and second trimester prenatal screening,

and live birth and hospital discharge records; pregnancies with aneuploidies and neural tube or abdominal wall defects were excluded. Logistic binomial regression was used to compare cases with controls.

RESULT: In all, 37 cases with MAP and 699 controls with previa alone were included. Risk for MAP was increased among multiparous women with pregnancy-associated plasma protein-A (PAPP-A) ⩾95th percentile (⩾2.63 multiple of the median (MoM); adjusted OR (aOR) 8.7, 95% confidence interval (CI) 2.8 to 27.4), maternal-serum alpha fetoprotein (MS-AFP) ⩾95th percentile (⩾1.79 MoM; aOR 2.8, 95% CI 1.0 to 8.0), and 1 and ⩾2 prior cesarean deliveries (CDs; aORs 4.4, 95% CI 1.5 to 13.6 and 18.4, 95% CI 5.9 to 57.5, respectively).

CONCLUSION: Elevated PAPP-A, elevated MS-AFP and prior CDs are associated with MAP among women with previa.

(6) Source: Zhonghua Fu Chan Ke Za Zhi. 2015 Feb;50(2):101-7.

Title: [The value of maternal first and second trimester serum data of β-hCG, PAPP-A, AFP and uE3 in the prediction of preeclampsia]

Authors: Gu W, Lin J, Hou Y

Abstract: OBJECTIVE: To discover the value of combined maternal first and second-trimester serum β-hCG, pregnancy associated plasma protein A (PAPP-A), alpha-fetoprotein (AFP)and unconjugated estriol (uE3) in the prediction of preeclampsia.

METHODS: A total of 1 805 pregnant women who had antenatal care at International Peace Maternal and Child Health Hospital Affiliated to Shanghai Jiaotong University between April 2012 and June 2013 were selected prospectively by random method. According to the outcome, they were defined as the control group and the preeclampsia group (including mild and severe cases). PAPP-A and β-hCG level were measured at 10-14 gestational weeks. AFP, β-hCG and uE3 were measured at 15-20 gestational weeks. The relevance between the serological indicators and outcomes was analyzed. The value of the indicators was judged by receiver operating characteristic (ROC) and Youden index, and the relevant predictive boundary values were identified.

RESULTS: (1) Among the 1 805 cases, 1 739 women did not have hypertension(the control group), while 66 women had preeclampsia (the preeclampsia group). The incidence of preeclampsia was 3.66% (66/1 805), including 43 mild cases and 23 severe cases. (2) At 10-14 gestational weeks, the mean value of PAPP-A in the control group was (3 972 ± 2 311) mU/L, while in the preeclampsia group it was (2 837 ± 1 849)mU/L. The difference between the two groups had statistical significance ($P < 0.01$). The mean value of β-hCG of the control group was 55(37~83) U/L, while in the preeclampsia group it was (57 ± 35)U/L. There was no statistical significance ($P > 0.05$). PAPP-A, β-hCG and AFP of mild preeclampsia cases were (3 249 ± 1 877) mU/L, (61 ± 38) U/L and (35 ± 11) μg/L respectively, and in severe cases they were(1 758 ± 1 297)mU/L, (47 ± 23)U/L and (47 ± 22)μg/L, respectively. There was statistically significant difference in PAPP-A ($P < 0.05$). (3) At 15-20 gestational weeks, β-hCG, AFP and uE3 in the preeclampsia group were (47 909 ± 31 396)U/L, (38 ± 15)μg/L and (0.98 ± 0.31)μg/L respectively, and in the control group they were (39 267 ± 25 054)U/L, (47 ± 18)μg/L and (1.17 ± 0.39) μg/L, respectively. AFP and uE3 of the preeclampsia group were lower than those in the control group and the difference was statistically significant ($P < 0.05$). However, β-hCG and uE3 of the mild preeclampsia cases and the severe cases had no statistical difference ($P > 0.05$). (4)At 10-14 gestational weeks, PAPP-A demonstrated positive relevance to the newborn weight ($r = 0.068$, $P = 0.011$) and gestational weeks at delivery ($r = 0.057$, $P = 0.048$). At 15-20 weeks, positive relevance was found between AFP and the newborn weight ($r = 0.149$, $P = 0.000$), while negative relevance was found between β-hCG and Apgar scores ($r = -0.085$, $P = 0.024$), and positive relevance was found between uE3 and gestational weeks at delivery ($r = 0.086$, $P =$

0.036). (5) PAPP-A, AFP and uE3 data were used as testing parameters to obtain the boundary values of preeclampsia prediction as follows: PAPP-A 1 831 mU/L, AFP 41 µg/L and uE3 1.04 µg/L. The specificity was 97.82% , 98.54% and 98.80% , respectively. (6) ROC was drawn and Youden index was calculated based on the joint predicative factor of PAPP-A, AFP and uE3. Youden index reached its peak (0.41) when the joint predictive factor was 0.032, meaning that the factor had the highest prediction value. The prediction value of the PAPP-A, AFP and uE3 was 0.032, with the specificity and sensitivity of 98.93% and 70.59%, respectively. The odds ratio was 2.37.

CONCLUSION: Both the individual parameter (PAPP-A, AFP and uE3) and the combined data have prediction value for preeclampsia, but the latter is more effective than any of the single parameter.

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

(1) Source: Prenat Diagn. 2015 Jul;35(7):703-8. doi: 10.1002/pd.4596.

Title: Incorporation of dried blood alpha fetoprotein into traditional first trimester Down syndrome screening service

Authors: Carmichael J, Krantz D, Liu HP, Janik D, Hallahan T

Abstract: OBJECTIVE: The aim of this study was to determine whether incorporation of dried blood alpha fetoprotein (AFP) into first trimester screening using the biochemical markers free Beta human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein A (PAPP-A) can improve screening performance.

METHODS: A retrospective study of 34 Down syndrome and 1185 unaffected dried blood specimens. First trimester dried blood AFP was performed using in-house immunofluorometric time-resolved assay. False positive and detection rates were determined from modeling.

RESULTS: The multiple of the median in Down syndrome cases was 0.73. At a fixed 5% false positive rate, incorporating AFP into a free Beta hCG, PAPP-A, and nuchal translucency protocol adds 2% detection resulting in detection rates of 92% to 94% depending on the gestational age of the blood draw. At a fixed 90% detection rate, AFP reduced the false positive rate by 1.0 to 1.6 percentage points depending on gestational age. Using a cutoff of 1/1000, the combination of free beta hCG, PAPP-A, AFP, and nuchal translucency achieved a detection rate of 96% with a false positive rate of 8.4% to 9.9%. Adding in nasal bone increased detection to 98% while reducing false positive rates to 4.1% to 4.7%.

CONCLUSION: Inclusion of dried blood AFP into traditional first trimester screening improves detection while optimizing contingent protocols so that cell-free fetal DNA testing may be offered in a more cost effective manner.

(2) Source: Anal Bioanal Chem. 2015 Jun 24. [Epub ahead of print]

Title: Novel chemiluminescent imaging microtiter plates for high-throughput detection of multiple serum biomarkers related to Down's syndrome via soybean peroxidase as label enzyme

Authors: Zhao F, Chai D, Lu J, Yu J, Liu S

Abstract: Novel chemiluminescent (CL) imaging microtiter plates with high-throughput, low-cost, and simple operation for detection of four biomarkers related to Down's syndrome screening were developed and evaluated. To enhance the sensitivity of CL immunosensing, soybean peroxidase

(SBP) was used instead of horseradish peroxide (HRP) as a label enzyme. The microtiter plates were fabricated by simultaneously immobilizing four capture monoclonal antibodies, anti-inhibin-A, anti-unconjugated oestriol (anti-uE3), anti-alpha-fetoprotein (anti-AFP), and beta anti-HCG (anti-β-HCG), on nitrocellulose (NC) membrane to form immunosensing microtiter wells. Under a sandwiched immunoassay, the CL signals on each sensing site of the microtiter plates were collected by a charge-coupled device (CCD), presenting an array-based chemiluminescence imaging method for detection of four target antigens in a well at the same time. The linear response to the analyte concentration ranged from 0.1 to 40 ng/mL for inhibin-A, 0.075 to 40 ng/mL for uE3, 0.2 to 400 ng/mL for AFP, and 0.4 to 220 ng/mL for β-HCG. The proposed microtiter plates possessed high-throughput, good stability, and acceptable accuracy for detection of four antigens in clinical serum samples and demonstrated potential for practical applicability of the proposed method to Down's syndrome screening. Graphical Abstract Schematic evaluation of the microtiter plater for simultaneous detection of the four biomarkers.

(3) Source: Int J Nanomedicine. 2015 Mar 19;10:2219-28. doi: 10.2147/IJN.S76200.

Title: Sensitive electrochemical immunosensor based on three-dimensional nanostructure gold electrode

Authors: Zhong G, Lan R, Zhang W, Fu F, Sun Y, Peng H, Chen T, Cai Y, Liu A, Lin J, Lin X

Abstract: A sensitive electrochemical immunosensor was developed for detection of alpha-fetoprotein (AFP) based on a three-dimensional nanostructure gold electrode using a facile, rapid, "green" square-wave oxidation-reduction cycle technique. The resulting three-dimensional gold nanocomposites were characterized by scanning electron microscopy and cyclic voltammetry. A "sandwich-type" detection strategy using an electrochemical immunosensor was employed. Under optimal conditions, a good linear relationship between the current response signal and the AFP concentrations was observed in the range of 10-50 ng/mL with a detection limit of 3 pg/mL. This new immunosensor showed a fast amperometric response and high sensitivity and selectivity. It was successfully used to determine AFP in a human serum sample with a relative standard deviation of <5% (n=5). The proposed immunosensor represents a significant step toward practical application in clinical diagnosis and monitoring of prognosis.

(4) Source: Sci Rep. 2015 Apr 24;5:9939. doi: 10.1038/srep09939.

Title: A sensitive label-free amperometric immunosensor for alpha-fetoprotein based on gold nanorods with different aspect ratio

Authors: Zhou C, Liu D, Xu L, Li Q, Song J, Xu S, Xing R, Song H

Abstract: A simple and accurate label-free amperometric immunosensor for α-fetoprotein (AFP) detection is developed based on gold nanorods (GNRs) with different aspect ratio and compared with gold particles (GNPs). The positively charged GNRs and GNPs due to the surface immobilized cetyltrimethyl ammonium bromide (CTAB) can adsorb the negatively charged AFP antibody (Ab) directly. The presence of the GNRs not only enhanced the immobilized amount of biomolecules, but also improved the electrochemical properties of the immunosensor. With the aid of GNRs, the electrochemical signal was greatly enhanced in comparison with GNPs. Under optimal conditions, the proposed immunosensor could detect AFP in a linear range from 0.1 to 200 ng/mL with a detection limit of 0.04 ng/mL (signal-to-noise ratio = 3), and it also possessed good reproducibility and storage stability. Moreover, the detection of AFP in five human serum samples also showed satisfactory accuracy. The proposed methodology was potentially attractive for clinical immunoassay.

E) Abstracts of New Assay Methodologies:

(1) Source: Ann Clin Lab Sci. 2015 May;45(3):264-9.

Title: Screening High Abundance of Peptide for Making Examination Possible in Human Urine

Authors: Fu G, Liu N, Chu L, Zhang M

Abstract: OBJECTIVE: We aim to explore and identify the high abundance of peptides in urine.

METHODS: Random urine samples from 30 healthy individuals were purified by weak cationic-exchange magnetic beads (MB-WCX) and then analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Then the generated mass spectra of peptides were handled by ClinPro-Tools2.1 bioinformatics software and the high abundance of urinary peptide was filtered. Subsequently, the amino acid sequences of highly expressed peptides were identified by a nano-liquid chromatography-tandem mass spectrometry and the corresponding protein names were found by Sequest search.

RESULTS: There were 159 urinary peptides of which mass to charge ratios (m/z) varied between 1,000 and 10,000 in the 30 healthy samples. Only peaks with average intensity >600 and the frequency >50% in the whole group were filtered. There were 15 peptides passed though the threshold and they were identified as fragments of fibrinogen alpha chain precursor, vitronectin precursor, inter-alpha-trypsin inhibitor heavy chain H4, complement component 3, prothrombin, apolipoprotein C-II, and alpha-fetoprotein.

CONCLUSION: These urinary peptides could be used for further research on urine.

(2) Source: Fertil Steril. 2015 Jun 18. pii: S0015-0282(15)00353-2. doi: 10.1016/j.fertnstert.2015.04.044. [Epub ahead of print]

Title: Changes in antimüllerian hormone levels in early pregnancy are associated with preterm birth

Authors: Stegmann BJ, Santillan M, Leader B, Smith E, Santillan D

Abstract: OBJECTIVE: To determine the association of preterm birth with antimüllerian hormone (AMH) levels both in isolation and in combination with other markers of fetoplacental health commonly measured during integrated prenatal screening (IPS) for aneuploidy.

DESIGN: Retrospective case-control study.

SETTING: Not applicable.

PATIENT(S): Pregnant women in Iowa who elected to undergo IPS and who subsequently delivered in Iowa, including women giving birth at <37 weeks' gestation and controls who delivered at ≥37 weeks' gestation.

INTERVENTION(S): None.

MAIN OUTCOME MEASURE(S): Probability of a preterm birth.

RESULT(S): Second trimester AMH levels were not associated with preterm birth, either independently or after controlling for other markers of fetoplacental health. The AMH difference was not associated with preterm birth when modeled alone, but a statistically significant association was found after adjusting for maternal serum α-fetoprotein (MSAFP) and maternal weight change between the first and second trimesters. After stratifying the model by MSAFP

level, most of the risk for preterm birth was identified in women with an MSAFP >1 multiple of the median and who had a stable or rising AMH level in early pregnancy.

CONCLUSION(S): A lack of decline in the AMH level in early pregnancy can be used to identify women with a high probability for preterm birth, especially when MSAFP levels are >1 multiple of the median. Monitoring changes in the AMH level between the first and second trimesters of pregnancy may help identify women who would benefit from interventional therapies such as supplemental progesterone.

(3) Source: Mol Med Rep. 2015 Sep;12(3):3359-64. doi: 10.3892/mmr.2015.3888. Epub 2015 Jun 3.

Title: Overexpression of miR-199b-5p inhibits Ewing's sarcoma cell lines by targeting CCNL1

Authors: Li W, Li Y, Guo J, Pan H, Zhang Y, Wang X

Abstract: MicroRNAs (miRNAs) are known to regulate the expression of a variety of genes, which are important in the development of several types of tumor, including Ewing's sarcoma (ES), at the post-transcriptional level. Although previous studies have identified that the expression of miRNA-199b-5p was downregulated in various types of tumor, the expression levels of miR-199b-5p in ES cells remain to be elucidated. The mechanism underlying ES via the miRNA pathway remains to be elucidated. The present study demonstrated that miR-199b-5p was an important regulator in ES cells and its expression was downregulated in ES originated A673/TC252 cells. The ES cell lines, A673 and TC252, were transfected with an miR-199b-5p mimic to overexpress the levels of this miRNA. This forced expression of miR-199b-5p suppressed the cell proliferation and invasion, arrested cell cycle progression, and promoted cell apoptosis. Furthermore, CCNL1 was identified by bioinformatic software as a potential target gene of miR-199b-5p. Following this, the present study identified CCNL1 as a direct target of miR-199b-5p in ES cells. Taken together, the present study established a functional link between ES, miR-199b-5p and CCNL1, and suggested that miR-199b-5p acts as a tumor suppressor and may be of diagnostic and therapeutic importance for human ES.

F) Special Abstract Selection:

(1) Source: J Obstet Gynaecol Can. 2014 Oct;36(10):927-42.

Title: Prenatal screening, diagnosis, and pregnancy management of fetal neural tube defects

Authors: Wilson RD; SOGC Genetics Committee, Wilson RD, Audibert F, Brock JA, Campagnolo C, Carroll J, Cartier L, Chitayat D, Gagnon A, Johnson JA, Langlois S, MacDonald WK, Murphy-Kaulbeck L, Okun N, Pastuck M; Special Contributors, Popa V; Society of Obstetricians and Gynaecologists of Canada

Abstract: OBJECTIVE: To provide obstetrical and genetic health care practitioners with guidelines and recommendations for prenatal screening, diagnosis, and obstetrical management of fetal open and closed neural tube defects (OCNTD).

OPTIONS: This review includes prenatal screening and diagnostic techniques currently being used for the detection of OCNTD including maternal serum alpha fetoprotein screening, ultrasound, fetal magnetic resonance imaging, and amniocentesis.

OUTCOMES: To improve prenatal screening, diagnosis, and obstetrical management of OCNTD while taking into consideration patient care, efficacy, cost, and care procedures.

EVIDENCE: Published literature was retrieved through searches of PubMed or MEDLINE, CINAHL, and The Cochrane Library in November, 2013, using appropriate controlled vocabulary and key words (e.g., prenatal screening, congenital anomalies, neural tube defects, alpha fetoprotein, ultrasound scan, magnetic resonance imaging). Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies published in English from 1977 to 2012. Searches were updated on a regular basis and incorporated in the guideline to November 30, 2013. Grey (unpublished) literature was identified through searching the websites of health technology assessment and health technology-related agencies, clinical practice guideline collections, clinical trial registries, and national and international medical specialty societies. An online survey of health care practitioners was also reviewed.

VALUES: The quality of evidence in this document was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care (Table).

BENEFITS, HARMS, AND COSTS: This review will provide health care practitioners with a better understanding of the available prenatal screening methods for OCNTD and the benefits and risks associated with each technique to allow evidenced-based decisions on OCNTD screening, diagnosis, and obstetrical management.

(2) Source: Eur J Obstet Gynecol Reprod Biol. 2015 Jun;189:13-8. doi: 10.1016/j.ejogrb.2015.03.016. Epub 2015 Mar 23.

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.

VI. Potentially helpful website connections/locations:

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

2) www.healthopedia.com/alpha-fetoprotein

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

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

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

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

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

	MS 331	MS 332	MS 333	MS 334	MS 335
Gestational Age All Lab Mean:					
Mean	18.0	17.0	20.0	16.0	20.0
SD	0.00	0.00	0.00	0.00	0.00
%CV	0.0%	0.0%	0.0%	0.0%	0.0%
mean+3*SD	18.0	17.0	20.0	16.0	20.0
mean-3*SD	18.0	17.0	20.0	16.0	20.0
N	24	23	23	23	24

	MS 331	MS 332	MS 333	MS 334	MS 335
MS AFP All Lab Mean:					
mean	23.5	40.7	217.3	34.6	193.7
SD	2.0	3.3	19.3	3.1	18.9
%CV	8.5%	8.2%	8.9%	8.9%	9.8%
mean+3SD	29.5	50.7	275.1	43.8	250.4
mean-3SD	17.5	30.8	159.4	25.3	137.0
N	24	24	24	24	24
median	23.25	40.2	213.6	34.1	194.25
mean/all kit median	1.01	1.02	1.01	1.02	0.98
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	22.9	39.9	211.4	33.8	186.3
SD	0.9	2.3	8.8	1.4	14.1
%CV	4.1%	5.7%	4.2%	4.1%	7.6%
mean + 3SD	25.7	46.7	238.0	38.0	228.5
mean - 3SD	20.1	33.0	184.9	29.6	144.1
N	15	15	15	15	15
Median	23.0	39.7	212.7	34.0	181.6
mean/All kit median	0.99	1.00	0.98	1.00	0.95
MS AFP Beckman Access/2 (BCX/BC1) mean:					
mean	23.2	39.9	214.9	33.7	197.0
SD	1.7	2.5	15.0	2.7	11.1
%CV	7.4%	6.2%	7.0%	7.9%	5.6%
mean+3SD	28.4	47.4	259.8	41.7	230.3
mean-3SD	18.1	32.4	169.9	25.7	163.7
N	6	6	6	6	6
median	22.8	39.7	217.8	33.2	195.9
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS AFP Siemens Immulite 2000 (DPD/DP5) mean:					
mean	28.8	48.9	269.0	42.5	238.0
N	2	2	2	2	2
mean/all kit median	1.24	1.23	1.25	1.26	1.21
MS AFP kit average:					
mean	25.0	42.9	231.8	36.6	207.1
SD	3.3	5.2	32.3	5.0	27.3
all kit median	23.2	39.9	214.9	33.8	197.0

	MS 331	MS 332	MS 333	MS 334	MS 335
MS AFP MoM All Lab Mean:					
mean	0.54	1.03	3.86	0.99	3.45
SD	0.03	0.11	0.51	0.12	0.28
%CV	4.8%	11.0%	13.3%	12.5%	8.0%
mean+3SD	0.62	1.38	5.40	1.36	4.28
mean-3SD	0.46	0.69	2.33	0.62	2.63
N	22	24	24	24	23
All Median	0.54	1.02	3.80	0.97	3.45
mean/all kit median	0.97	0.99	1.00	0.98	0.94
MS AFP MoM Beckman Unicel (BCU/BC1) mean:					
Mean	0.54	1.04	3.70	0.98	3.40
SD	0.03	0.07	0.21	0.06	0.26
%CV	4.9%	6.8%	5.5%	6.6%	7.8%
mean + 3SD	0.62	1.25	4.32	1.17	4.19
mean - 3SD	0.46	0.83	3.09	0.78	2.61
N	15	15	15	15	15
Median	0.53	1.02	3.80	0.97	3.39
mean/all kit median	0.97	0.99	0.96	0.97	0.93
MS AFP MoM Beckman Access/2 (BCX/BC1) mean:					
Mean	0.56	1.05	3.85	1.01	3.66
SD	0.08	0.14	0.64	0.10	0.56
%CV	14.6%	13.2%	16.6%	10.4%	15.3%
mean + 3SD	0.80	1.46	5.76	1.32	5.34
mean - 3SD	0.31	0.63	1.94	0.69	1.98
N	6	6	6	6	6
Median	0.54	1.01	3.69	1.01	3.48
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS AFP MoM Siemens Immulite 2000 (DPD/DP5) mean:					
Mean	0.62	1.14	4.29	1.18	3.97
N	2	2	2	2	2
mean/all kit median	1.11	1.09	1.11	1.17	1.08
MS AFP MoM kit average:					
mean	0.57	1.07	3.95	1.05	3.67
SD	0.04	0.05	0.30	0.11	0.28
all kit median	0.56	1.05	3.85	1.01	3.66

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

	MS 331	MS 332	MS 333	MS 334	MS 335		MS 331	MS 332	MS 333	MS 334	MS 335
MS uE3 All Lab Mean:						**MS uE3 MoM All Lab Mean:**					
mean	0.48	0.74	1.87	0.56	1.21	Mean	0.37	0.68	0.96	0.70	0.63
SD	0.06	0.09	0.13	0.06	0.10	SD	0.06	0.08	0.13	0.09	0.07
%CV	12.0%	12.4%	6.9%	10.2%	8.6%	%CV	16.2%	12.0%	14.0%	12.2%	10.6%
mean+3SD	0.65	1.02	2.26	0.73	1.52	mean+3SD	0.55	0.93	1.37	0.96	0.83
mean-3SD	0.30	0.46	1.48	0.39	0.90	mean-3SD	0.19	0.44	0.56	0.44	0.43
N	23	23	23	23	23	N	23	23	23	23	23
mean/all kit median	1.02	1.01	1.00	1.00	1.01	mean/all kit Median	0.93	0.97	1.02	1.01	0.98
MS uE3 Beckman Unicel (BCU/BC1) mean:						**MS uE3 MoM Beckman Unicel (BCU/BC1) Mean:**					
Mean	0.46	0.74	1.87	0.56	1.20	Mean	0.35	0.66	0.94	0.69	0.62
SD	0.05	0.08	0.11	0.05	0.08	SD	0.04	0.08	0.07	0.08	0.04
%CV	10.9%	11.1%	6.0%	9.7%	6.9%	%CV	11.3%	12.7%	7.6%	11.5%	7.2%
mean+3SD	0.62	0.98	2.21	0.72	1.45	mean+3SD	0.47	0.91	1.16	0.93	0.75
mean-3SD	0.31	0.49	1.53	0.40	0.95	mean-3SD	0.23	0.41	0.73	0.46	0.48
N	15	15	15	15	15	N	15	15	15	15	15
mean/all kit median	1.00	1.00	1.00	1.00	1.00	mean/all kit Median	0.89	0.94	1.00	1.00	0.96
MS uE3 Beckman Access/2 (BCX/BC1) mean:						**MS uE3 MoM Beckman Access/2 (BCX/BC1) Mean:**					
mean	0.53	0.80	1.93	0.59	1.31	Mean	0.40	0.73	0.94	0.70	0.65
SD	0.03	0.05	0.10	0.04	0.04	SD	0.04	0.06	0.12	0.06	0.06
%CV	5.6%	6.1%	5.4%	6.2%	2.8%	%CV	10.5%	8.7%	12.5%	8.6%	9.5%
mean+3SD	0.61	0.95	2.24	0.69	1.41	mean+3SD	0.52	0.92	1.29	0.88	0.83
mean-3SD	0.44	0.65	1.62	0.48	1.20	mean-3SD	0.27	0.54	0.58	0.52	0.46
N	6	6	6	6	6	N	6	6	6	6	6
mean/all kit median	1.13	1.09	1.03	1.04	1.09	mean/all kit Median	1.00	1.04	0.99	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	0.40	0.61	1.67	0.46	1.01	Mean	0.42	0.71	1.19	0.77	0.73
N	2	2	2	2	2	N	2	2	2	2	2
mean/all Kit Median	0.86	0.82	0.89	0.82	0.84	mean/all kit Median	1.05	1.00	1.26	1.11	1.12
MS uE3 kit average:						**MS uE3 MoM kit average:**					
mean	0.46	0.71	1.82	0.54	1.17	mean	0.39	0.70	1.02	0.72	0.66
SD	0.06	0.10	0.14	0.07	0.15	SD	0.03	0.04	0.14	0.04	0.06
all kit median	0.46	0.74	1.87	0.56	1.20	all kit median	0.40	0.71	0.94	0.70	0.65

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	MS 331	MS 332	MS 333	MS 334	MS 335
MS hCG All Lab mean:					
mean	49.5	26.8	19.2	32.6	20.0
SD	5.4	3.1	1.9	3.7	2.0
%CV	10.8%	11.5%	10.0%	11.3%	9.9%
mean+3SD	65.6	36.1	25.0	43.6	26.0
mean-3SD	33.4	17.5	13.5	21.5	14.1
N	23	23	23	23	23
mean/all kit median	0.99	1.00	1.00	1.01	1.00
MS hCG Beckman Unicel (BCU/BC1 or 2) mean:					
mean	49.8	26.8	19.2	32.4	20.0
SD	3.3	1.6	0.7	2.1	0.8
%CV	6.6%	6.0%	3.7%	6.6%	3.9%
mean+3SD	59.6	31.6	21.3	38.7	22.3
mean-3SD	40.0	21.9	17.1	26.0	17.6
N	16	16	16	16	16
median	49.2	26.5	19.2	32.8	20.0
mean/All kit median	1.00	1.00	1.00	1.00	1.00
MS hCG Beckman Access/2 (BCX/BC1 or 2) mean:					
mean	53.8	29.8	21.4	36.5	22.1
SD	1.7	1.0	0.5	1.5	1.2
%CV	3.1%	3.4%	2.5%	4.1%	5.4%
X+3SD	58.8	32.9	23.0	41.0	25.7
X-3SD	48.7	26.7	19.8	31.9	18.6
N	5	5	5	5	5
median	54.1	29.6	21.5	36.3	22.1
mean/All kit median	1.25	1.30	1.27	1.29	1.26
MS hCG Siemens Immulite 2000 (DPD/DP5) mean:					
mean	36.2	19.2	14.4	24.3	15.1
N	2	2	2	2	2
mean/all kit median	0.77	0.78	0.80	0.81	0.80
MS hCG kit average:					
mean	46.6	25.3	18.3	31.1	19.1
SD	9.2	5.5	3.6	6.2	3.6
all kit median	49.8	26.8	19.2	32.4	20.0

	MS 331	MS 332	MS 333	MS 334	MS 335
MS hCG MoM All Lab Mean:					
mean	1.99	0.88	0.92	0.87	1.00
SD	0.16	0.08	0.09	0.11	0.10
%CV	8.0%	9.1%	9.6%	12.3%	10.2%
mean+3SD	2.47	1.12	1.19	1.19	1.30
mean-3SD	1.52	0.64	0.65	0.55	0.69
N	23	23	23	22	23
mean/All Kit Median	0.99	1.02	0.99	1.01	1.00
MS hCG MoM Beckman Unicel (BCU/BC1 or 2) mean:					
mean	2.01	0.87	0.93	0.86	1.01
SD	0.14	0.05	0.07	0.07	0.09
%CV	6.9%	6.0%	7.1%	7.7%	8.5%
X+3SD	2.42	1.02	1.13	1.06	1.27
X-3SD	1.59	0.71	0.73	0.66	0.75
N	16	16	16	16	16
median	2.00	0.86	0.93	0.86	1.02
mean/All kit median	1.00	1.00	1.00	1.00	1.01
MS hCG MoM Beckman Access/2 (BCX/BC1 or 2) mean:					
mean	2.03	0.94	0.93	1.02	1.00
SD	0.20	0.14	0.12	0.23	0.12
%CV	9.9%	14.5%	12.6%	22.8%	12.1%
X+3SD	2.63	1.35	1.28	1.72	1.36
X-3SD	1.42	0.53	0.58	0.32	0.64
N	5	5	5	5	5
median	1.96	0.94	0.94	1.03	1.04
mean/All kit median	1.01	1.08	1.00	1.19	1.00
MS hCG MoM Siemens Immulite 2000 (DPD/DP5) mean:					
mean	1.79	0.84	0.83	0.79	0.90
N	2	2	2	2	2
mean/All kit median	0.94	1.01	1.03	1.08	1.04
MS hCG MoM kit average:					
mean	1.94	0.88	0.90	0.89	0.97
SD	0.98	0.44	0.45	0.46	0.49
all kit median	2.01	0.87	0.93	0.86	1.00

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	MS 331	MS 332	MS 333	MS 334	MS 335
MS Inhibin A all lab mean:					
Mean	409.9	188.2	264.7	165.0	286.5
SD	25.5	11.9	15.8	20.0	19.6
%CV	6.2%	6.3%	6.0%	12.1%	6.8%
mean + 3SD	486.5	224.1	312.0	224.9	345.3
mean- 3SD	333.2	152.4	217.4	105.1	227.8
N	24	24	24	24	24
All Lab Median	406.2	186.8	261.2	167.4	281.0
mean/all kit median	1.01	1.00	1.01	0.99	1.01
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	403.6	184.8	262.6	167.3	284.1
SD	21.4	7.7	14.7	8.8	17.0
%CV	5.3%	4.2%	5.6%	5.3%	6.0%
mean + 3SD	467.8	208.0	306.8	193.7	335.3
mean- 3SD	339.4	161.6	218.3	140.9	233.0
N	15	15	15	14	15
Kit median	397.0	185.0	263.1	167.9	279.8
mean/all kit median	0.99	0.99	1.00	1.00	1.00
MS Inhibin A Beckman Access/2 (BCX/BC1) mean:					
Mean	411.9	189.9	263.4	167.2	284.0
SD	16.1	10.6	10.8	5.1	14.2
%CV	3.9%	5.6%	4.1%	3.0%	5.0%
mean + 3SD	460.3	221.6	295.9	182.4	326.7
mean- 3SD	363.5	158.3	230.9	152.0	241.3
N	8	8	8	8	8
Kit median	407.1	192.0	260.2	167.3	282.1
mean/All kit median	1.01	1.01	1.00	1.00	1.00
MS Inhibin A kit average:					
mean	407.7	187.4	263.0	167.2	284.1
SD	5.9	3.6	0.6	0.1	0.1
all kit median	407.7	187.4	263.0	167.2	284.1

	MS 331	MS 332	MS 333	MS 334	MS 335
MS Inhibin A MoM All Lab mean:					
mean	2.49	1.10	1.40	1.04	1.56
SD	0.21	0.08	0.13	0.09	0.15
%CV	8.3%	7.1%	9.2%	9.0%	9.6%
mean+3SD	3.11	1.34	1.79	1.32	2.01
mean-3SD	1.87	0.87	1.02	0.76	1.11
N	24	24	24	23	24
mean/all kit median	1.00	0.99	1.01	1.02	1.01
MS Inhibin A MoM Beckman Unicel (BCU/BC1) mean:					
Mean	2.45	1.08	1.40	1.00	1.57
SD	0.21	0.08	0.13	0.16	0.15
%CV	8.4%	7.6%	9.3%	16.2%	9.6%
mean + 3SD	3.06	1.33	1.79	1.48	2.02
mean- 3SD	1.83	0.84	1.01	0.51	1.12
N	15	15	15	15	15
Kit Median	2.42	1.08	1.38	1.01	1.54
mean/all kit median	0.98	0.98	1.01	0.98	1.02
MS Inhibin A MoM Beckman Access (BCX/BC1) mean:					
Mean	2.55	1.14	1.38	1.04	1.52
SD	0.21	0.06	0.10	0.10	0.15
%CV	8.3%	5.6%	7.5%	9.6%	9.7%
mean + 3SD	3.18	1.33	1.69	1.34	1.96
mean- 3SD	1.92	0.95	1.07	0.74	1.08
N	8	8	8	8	8
Kit Median	2.42	1.08	1.38	1.01	1.54
mean/all kit median	1.02	1.02	0.99	1.02	0.98
MS Inhibin A MoM kit average:					
mean	2.50	1.11	1.39	1.02	1.54
SD	0.07	0.04	0.01	0.03	0.03
all kit median	2.50	1.11	1.39	1.02	1.54

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	AF331	AF332	AF333	AF334	AF335
AF AFP All Lab mean :					
mean	8.1	10.1	9.8	5.8	20.9
SD	0.7	0.6	0.7	0.5	1.7
%CV	8.6%	5.7%	6.9%	8.0%	8.1%
mean+3SD	10.2	11.9	11.8	7.2	25.9
mean-3SD	6.0	8.4	7.8	4.4	15.8
N	18	18	18	18	18
All kit median	8.1	10.0	9.8	5.9	21.1
mean/all kit mean	1.00	1.01	1.00	0.98	0.99
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	8.1	10.0	9.6	5.7	20.4
SD	0.8	0.5	0.6	0.4	1.6
%CV	9.8%	5.1%	6.4%	7.3%	7.9%
X+3SD	10.5	11.5	11.4	6.9	25.2
X-3SD	5.7	8.4	7.8	4.4	15.6
N	12	12	12	12	12
median	8.1	10.0	9.5	5.6	20.4
mean/all kit median	1.00	0.99	0.98	0.96	0.96
AF AFP Beckman Access/2 (BCX/BC1) mean:					
Mean	8.2	10.0	9.8	5.9	21.1
SD	0.5	0.6	0.3	0.2	1.2
%CV	6.1%	5.7%	3.1%	2.9%	5.7%
X+3SD	9.70	11.74	10.70	6.42	24.76
X-3SD	6.7	8.3	8.9	5.4	17.5
N	3	3	3	3	3
median	8.2	10.2	9.8	5.8	20.7
mean/all kit median	1.01	1.00	1.00	1.00	1.00
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	7.8	10.6	10.7	6.5	22.6
N	2	2	2	2	2
mean/all kit median	0.95	1.06	1.09	1.09	1.07
AF AFP kit average:					
mean	6.0	7.7	7.5	4.5	16.0
SD	0.2	0.3	0.6	0.4	1.1
all kit median	8.1	10.0	9.8	5.9	21.1

	AF331	AF332	AF333	AF334	AF335
AF AFP MoM All Lab Mean:					
mean	0.87	1.40	0.87	0.74	3.28
SD	0.09	0.14	0.09	0.10	0.42
%CV	10.3%	10.2%	10.7%	13.2%	12.7%
mean+3SD	1.14	1.82	1.14	1.03	4.53
mean-3SD	0.60	0.97	0.59	0.45	2.03
N	18	18	18	18	18
All median	0.87	1.36	0.84	0.73	3.29
mean/all kit median	1.00	1.03	1.03	1.01	1.00
AF AFP MoM Beckman Unicel(BCU/BC1) mean:					
Mean	0.88	1.37	0.87	0.74	3.21
SD	0.10	0.13	0.10	0.09	0.45
%CV	11.2%	9.6%	11.7%	11.9%	14.0%
X+3SD	1.18	1.76	1.17	1.01	4.56
X-3SD	0.59	0.97	0.56	0.48	1.86
N	12	12	12	12	12
median	0.87	1.33	0.83	0.73	3.25
mean/all kit median	1.04	1.00	1.00	1.00	0.95
AF AFP MoM Beckman Access (BCX/BC1) mean:					
Mean	0.85	1.48	0.83	0.65	3.37
SD	0.05	0.17	0.06	0.11	0.40
%CV	5.9%	11.2%	6.9%	16.2%	11.7%
X+3SD	1.00	1.98	1.00	0.97	4.56
X-3SD	0.70	0.98	0.66	0.34	2.18
N	3	3	3	3	3
median	0.85	1.56	0.81	0.67	3.38
mean/all kit median	1.00	1.08	0.95	0.88	1.00
AF AFP MoM DPC Immulite 2000 (DPD/DP5) mean:					
Mean	0.82	1.35	0.92	0.82	3.52
N	2	2	2	2	2
mean/all kit median	0.96	0.99	1.06	1.10	1.04
AF AFP MoM kit average:					
mean	0.85	1.40	0.87	0.74	3.37
SD	0.03	0.07	0.05	0.08	0.16
all kit median	0.85	1.37	0.87	0.74	3.37

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	FT331	FT332	FT333	FT334	FT335
FT Gestational Age All Lab Mean:					
Mean	13.0	11.4	11.1	13.0	12.4
SD	0.05	0.10	0.12	0.06	0.07
%CV	0.4%	0.9%	1.0%	0.5%	0.6%
mean+3*SD	13.1	11.7	11.5	13.2	12.6
mean-3*SD	12.8	11.1	10.8	12.8	12.2
N	15	15	15	15	15

	FT331	FT332	FT333	FT334	FT335
FT NT MoM All Lab Mean:					
Mean	0.92	2.11	1.07	0.92	0.95
SD	0.05	0.12	0.06	0.05	0.05
%CV	5.4%	5.6%	6.1%	5.4%	5.7%
mean+3*SD	1.07	2.46	1.26	1.07	1.11
mean- 3*SD	0.77	1.76	0.87	0.77	0.79
N	14	14	14	14	14
All Median	0.92	2.08	1.07	0.92	0.95

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	FT331	FT332	FT333	FT334	FT335
FT hCG All Lab Mean:					
mean	70.7	201.9	94.2	65.8	74.1
SD	8.9	35.6	14.7	9.6	10.5
%CV	12.7%	17.6%	15.6%	14.6%	14.1%
mean+3*SD	97.6	308.7	138.2	94.6	105.5
mean- 3*SD	43.9	95.0	50.1	36.9	42.7
N	14	14	14	14	14
All lab median	72.3	215.2	97.0	69.5	77.2
mean/All kit median	1.12	1.20	1.16	1.14	1.14
FT hCG Beckman Unicel/Access 2 (BCU or X/BC1 or 2) mean:					
mean	73.8	215.3	99.3	69.0	77.7
SD	4.9	10.5	7.2	5.3	5.6
%CV	6.6%	4.9%	7.3%	7.7%	7.3%
mean+3SD	88.3	246.9	121.0	84.9	94.6
mean- 3SD	59.2	183.7	77.6	53.1	60.7
N	12	12	12	12	12
median	72.9	217.4	99.9	70.5	79.5
mean/All kit median	1.17	1.28	1.22	1.20	1.19
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	52.5	121.2	63.3	46.2	52.7
N	2	2	2	2	2
mean/All kit median	0.83	0.72	0.78	0.80	0.81
FT hCG kit average:					
mean	63.1	168.3	81.3	57.6	65.2
SD	15.1	66.5	25.5	16.2	17.7
all kit median	63.1	168.3	81.3	57.6	65.2

	FT331	FT332	FT333	FT334	FT335
FT hCG MoM All Lab Mean:					
Mean	0.90	1.95	0.84	0.83	0.82
SD	0.06	0.18	0.11	0.07	0.09
%CV	7.1%	9.3%	13.1%	9.0%	11.0%
mean+3*SD	1.08	2.49	1.17	1.05	1.08
mean - 3*SD	0.71	1.40	0.51	0.61	0.55
N	13	13	13	13	13
All lab Median	0.89	1.98	0.85	0.85	0.82
mean/All kit Median	1.00	1.03	0.98	1.01	0.99
FT hCG MoM Beckman Unicel or Access 2 (BCU or X/BC1 or 2) mean:					
mean	0.89	1.97	0.83	0.83	0.81
SD	0.07	0.17	0.12	0.08	0.10
%CV	7.7%	8.8%	14.0%	9.7%	11.9%
mean+3SD	1.10	2.50	1.18	1.08	1.10
mean-3SD	0.69	1.45	0.48	0.59	0.52
N	11	11	11	11	11
median	0.89	2.01	0.85	0.86	0.78
mean/All kit median	1.00	1.05	0.96	1.01	0.98
FT hCG MoM DPC Immulite2000 (DPD/DP5) mean:					
mean	0.90	1.79	0.89	0.82	0.85
N	2	2	2	2	2
mean/All kit median	1.00	0.95	1.04	0.99	1.02
FT hCG MoM kit average:					
mean	0.90	1.88	0.86	0.82	0.83
SD	0.00	0.13	0.04	0.01	0.02
all kit median	0.90	1.88	0.86	0.82	0.83

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	FT331	FT332	FT333	FT334	FT335
FT PAPP-A All Lab Mean:					
Mean	2072.8	2096.7	1308.7	2073.1	1780.6
SD	1287.2	5282.3	916.3	1288.2	1205.4
%CV	62.1%	251.9%	70.0%	62.1%	67.7%
mean + 3SD	5934.3	17943.6	4057.5	5937.8	5396.6
mean- 3SD	-1788.7	-13750.1	-1440.2	-1791.6	-1835.5
N	14	14	14	14	14
All Lab Median	1724.4	629.5	1015.5	1696.9	1432.9
mean/All kit median	1.20	3.36	1.26	1.22	1.23
FT PAPP-A Beckman Unicel(BCU/BC1) Mean:					
Mean	1727.7	623.8	1037.5	1695.0	1450.5
SD	60.6	44.8	56.9	90.0	95.7
%CV	3.5%	7.2%	5.5%	5.3%	6.6%
mean + 3SD	1909.5	758.3	1208.4	1965.0	1737.8
mean - 3SD	1545.9	489.3	866.7	1425.0	1163.3
N	9	9	9	9	9
Kit Median	1730.0	639.0	1017.0	1717.2	1454.4
mean/All kit median	1.00	1.00	1.00	1.00	1.00
***FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:**					
Mean	5039.1	11250.0	3437.5	5058.6	4550.8
N	2	2	2	2	2
mean/All kit median	2.92	18.03	3.31	2.98	3.14
***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	1130.7	413.2	702.8	1217.1	923.8
SD	253.1	103.4	157.3	298.9	278.3
%CV	22.4%	25.0%	22.4%	24.6%	30.1%
mean + 3SD	1890.0	723.5	1174.8	2113.8	1758.6
mean - 3SD	371.3	102.9	230.8	320.3	89.0
N	3	3	3	3	3
Kit Median	1075.0	378.0	637.0	1288.2	818.0
mean/All kit median	0.65	0.66	0.68	0.72	0.64
FT PAPP-A kit average:					
mean	2632.5	4095.7	1725.9	2656.9	2308.4
SD	2105.4	6196.7	1491.7	2093.6	1959.8
all kit median	1727.7	623.8	1037.5	1695.0	1450.5

	FT331	FT332	FT333	FT334	FT335
FT PAPP-A MoM All Lab Mean:					
Mean	1.70	1.18	1.93	1.83	1.80
SD	0.91	0.59	0.96	0.81	0.92
%CV	53.4%	49.8%	50.0%	44.0%	51.3%
mean + 3SD	4.42	2.93	4.82	4.24	4.57
mean- 3SD	-1.02	-0.58	-0.96	-0.59	-0.97
N	14	14	14	14	14
All Lab Median	1.62	1.02	1.65	1.62	1.53
mean/ All kit median	1.10	1.13	1.12	1.11	1.13
FT PAPP-A MoM Beckman Unicel(BCU/BC1) Mean:					
Mean	1.55	1.04	1.71	1.57	1.60
SD	0.27	0.19	0.28	0.15	0.24
%CV	17.3%	17.9%	16.4%	9.3%	14.9%
mean + 3SD	2.35	1.60	2.56	2.00	2.31
mean - 3SD	0.75	0.48	0.87	1.13	0.88
N	9	9	9	9	9
Kit Median	1.65	1.08	1.70	1.61	1.55
mean/All kit median	1.00	1.00	1.00	0.95	1.00
FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:					
Mean	3.59	2.47	4.06	3.63	3.85
N	2	2	2	2	2
mean/All kit median	2.32	2.38	2.37	2.20	2.41
FT PAPP-A MoM (SMR or APM/AN1) Mean:					
Mean	1.01	0.74	1.24	1.65	1.11
N	2	2	2	2	2
mean/ All kit median	0.65	0.71	0.72	1.00	0.69
FT PAPP-A MoM kit average:					
mean	2.05	1.42	2.34	2.28	2.18
SD	1.36	0.93	1.51	1.17	1.46
all kit median	1.55	1.04	1.71	1.65	1.60

Figure 1

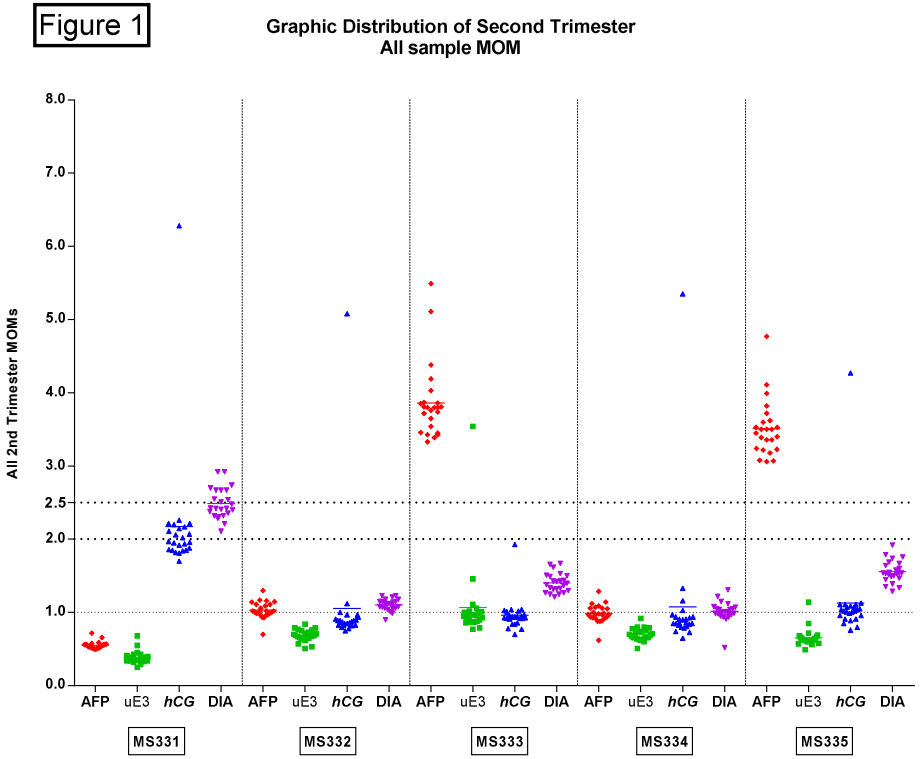


Figure 2a

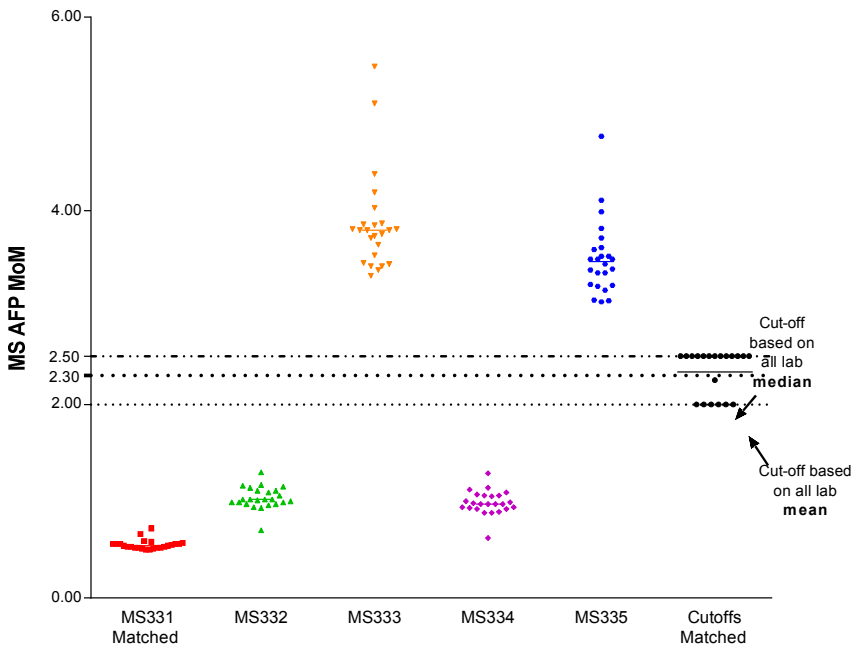


Figure 3

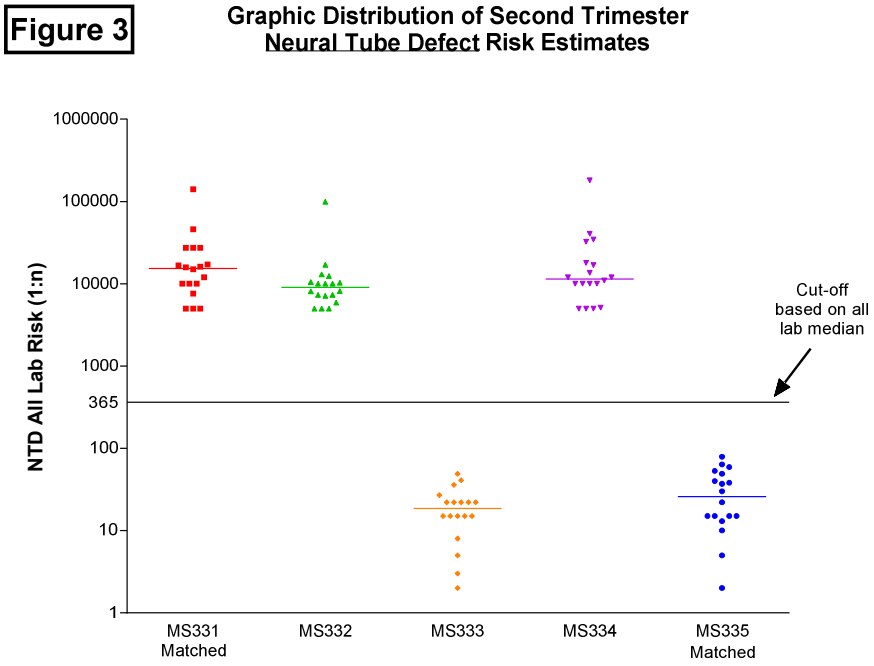


Figure 2b

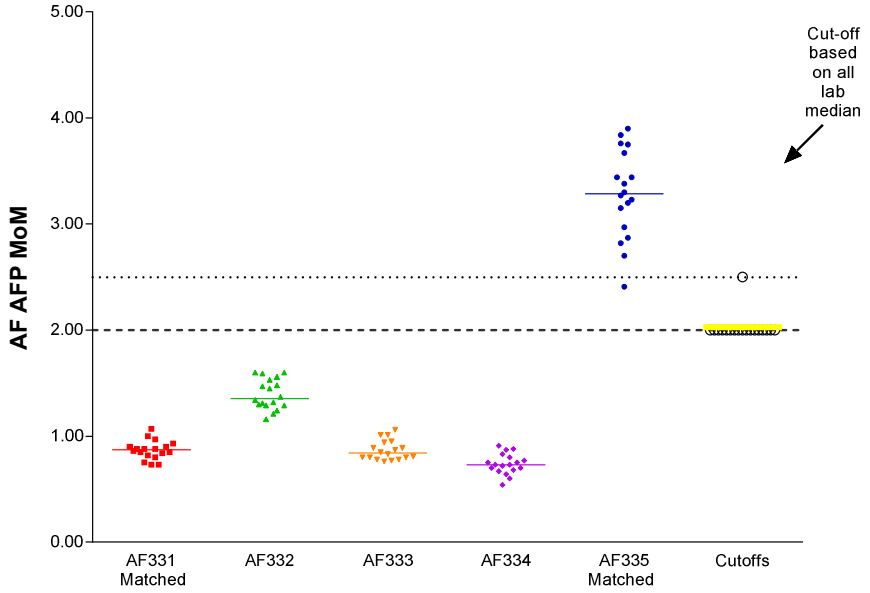


Figure 4

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

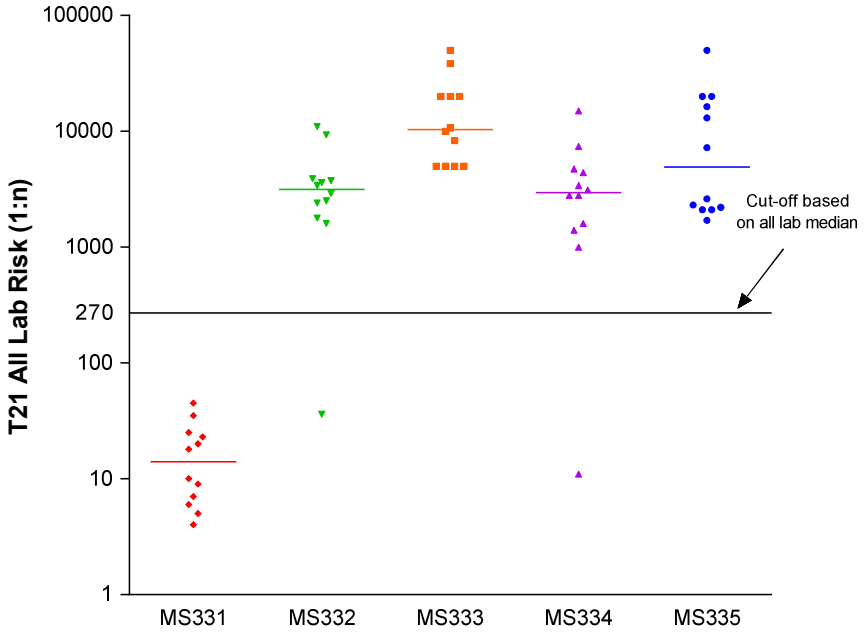


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

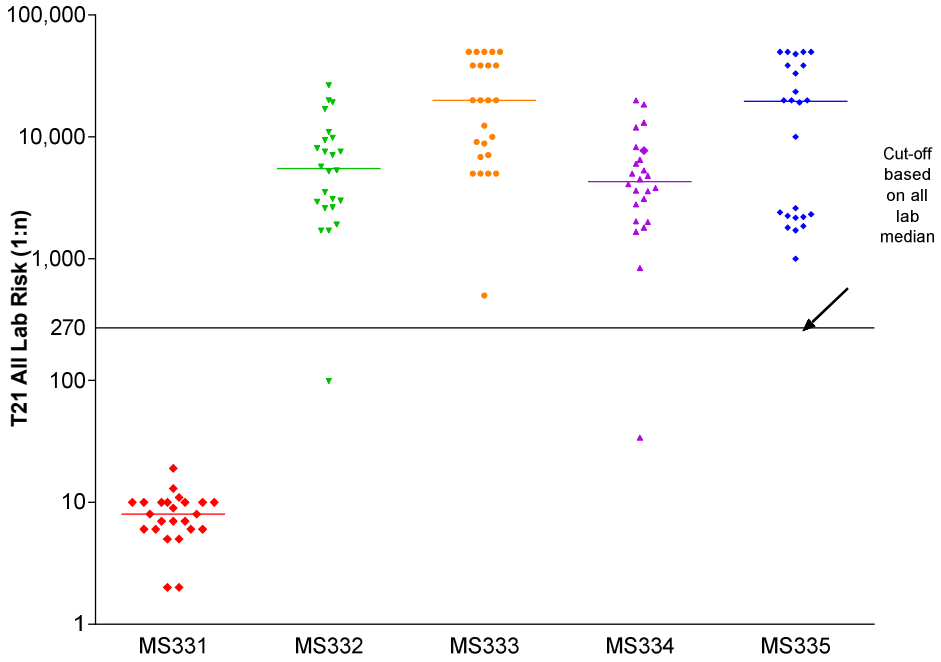
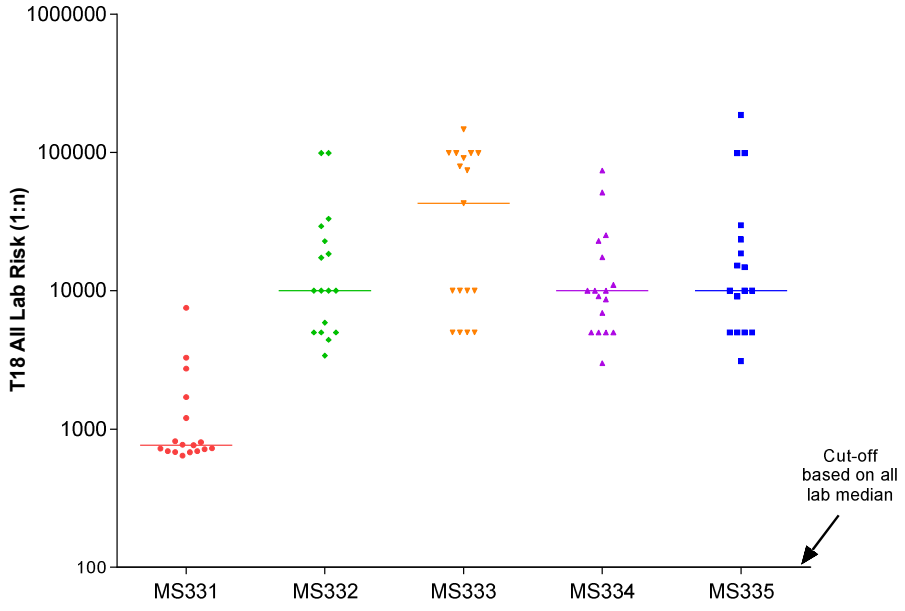


Figure 6

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates



NYS FEDM PT 9/15
Second Trimester

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

Figure 7A

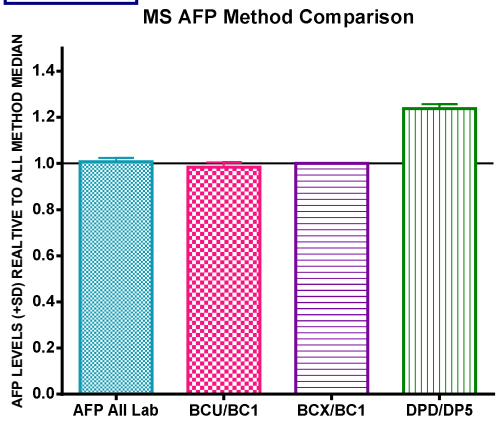


Figure 7B

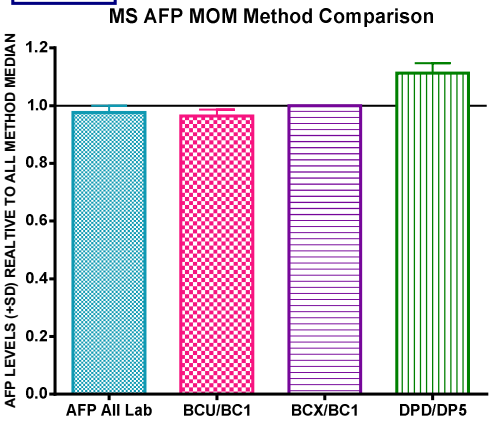


Figure 7C

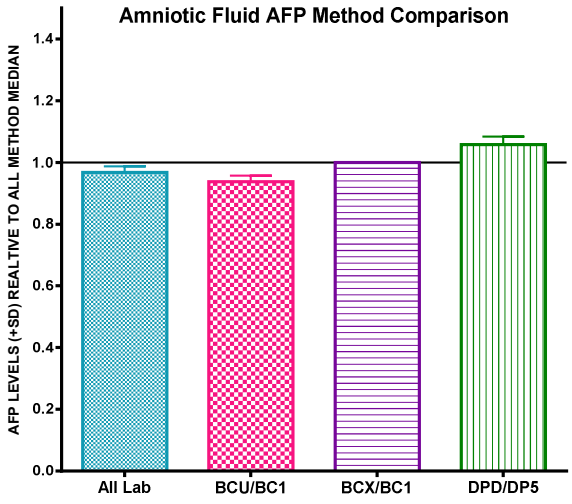
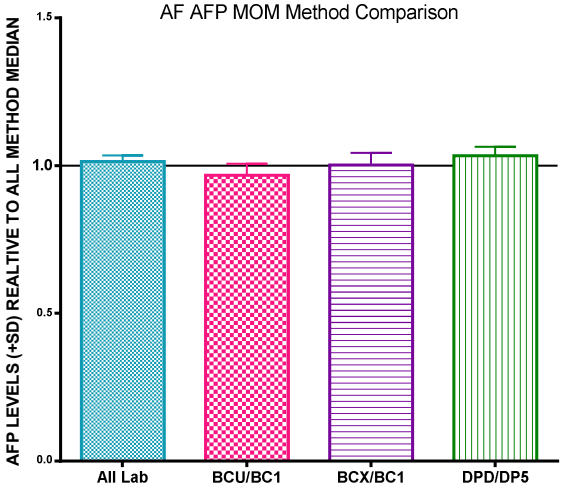


Figure 7D



NYS FEDM PT 9/15
Second Trimester

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

Figure 8A

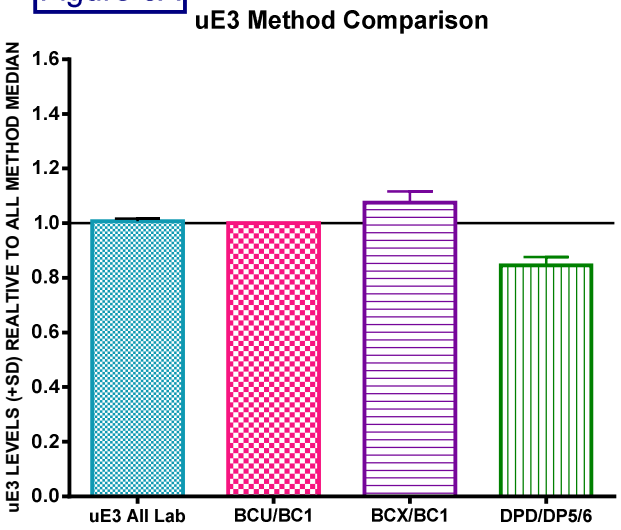


Figure 8B

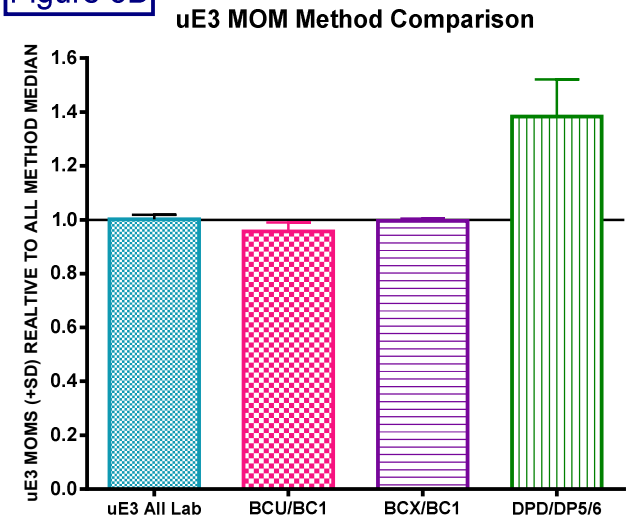


Figure 9A

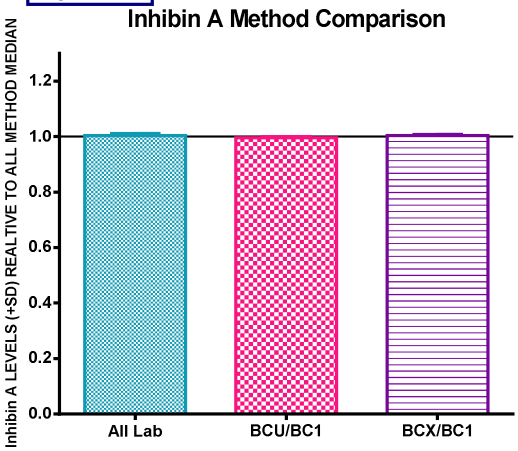


Figure 9B

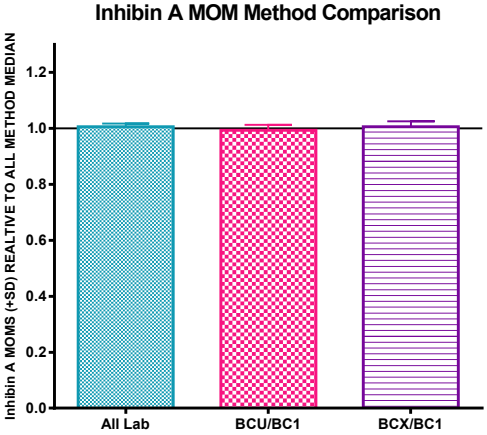


Figure 10A

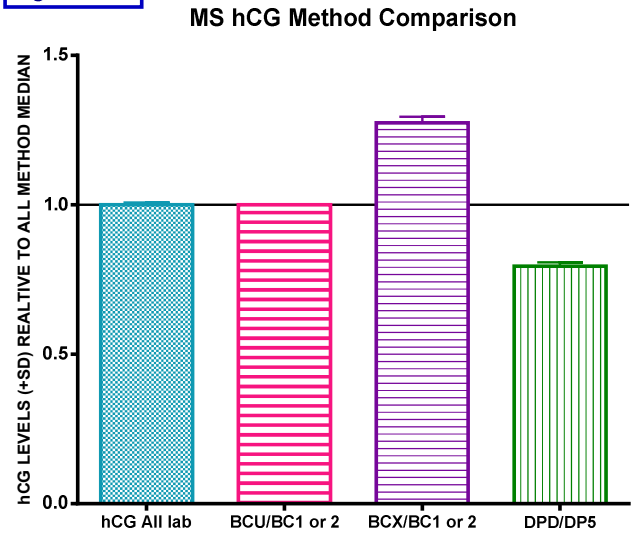
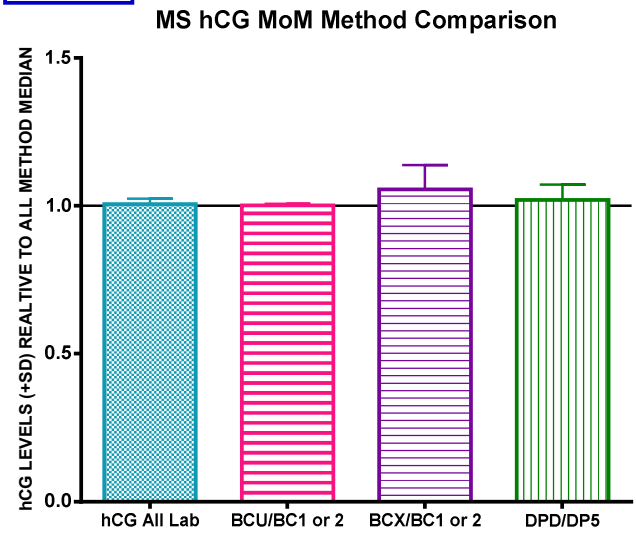


Figure 10B



NYS FEDM PT 9/15
First Trimester

Figure 11A

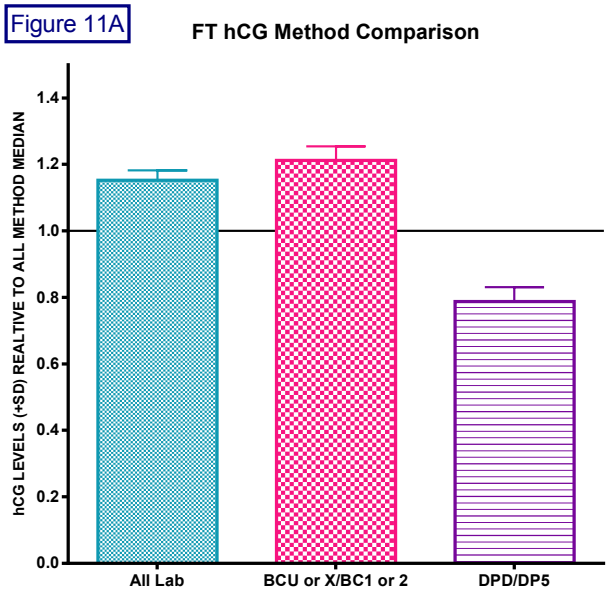


Figure 11B

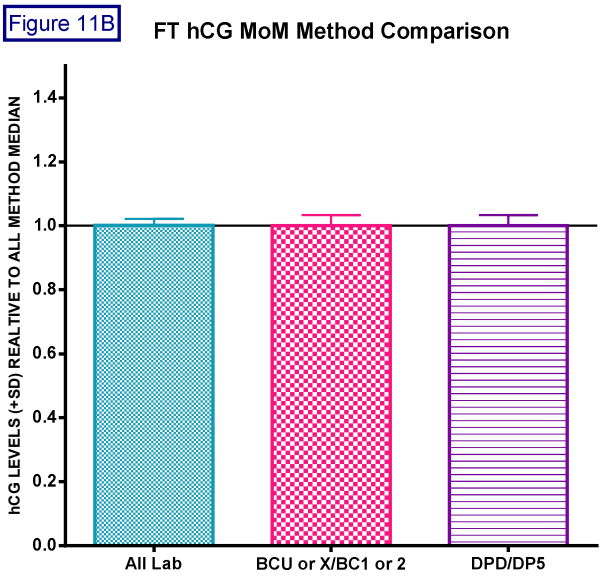


Figure 12A

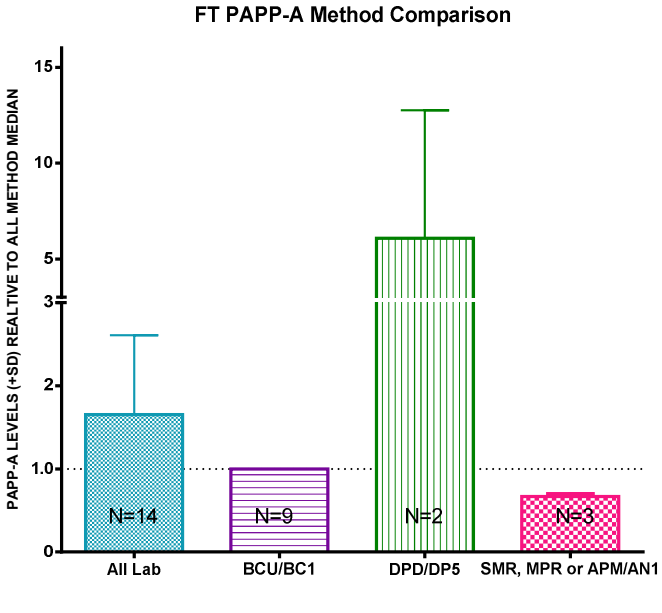
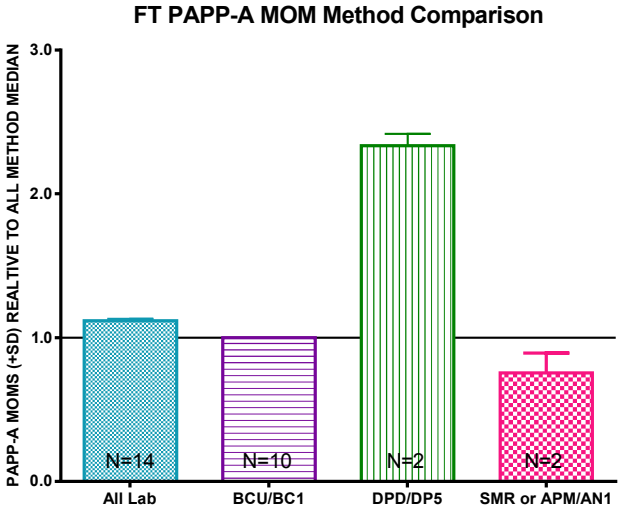


Figure 12B



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

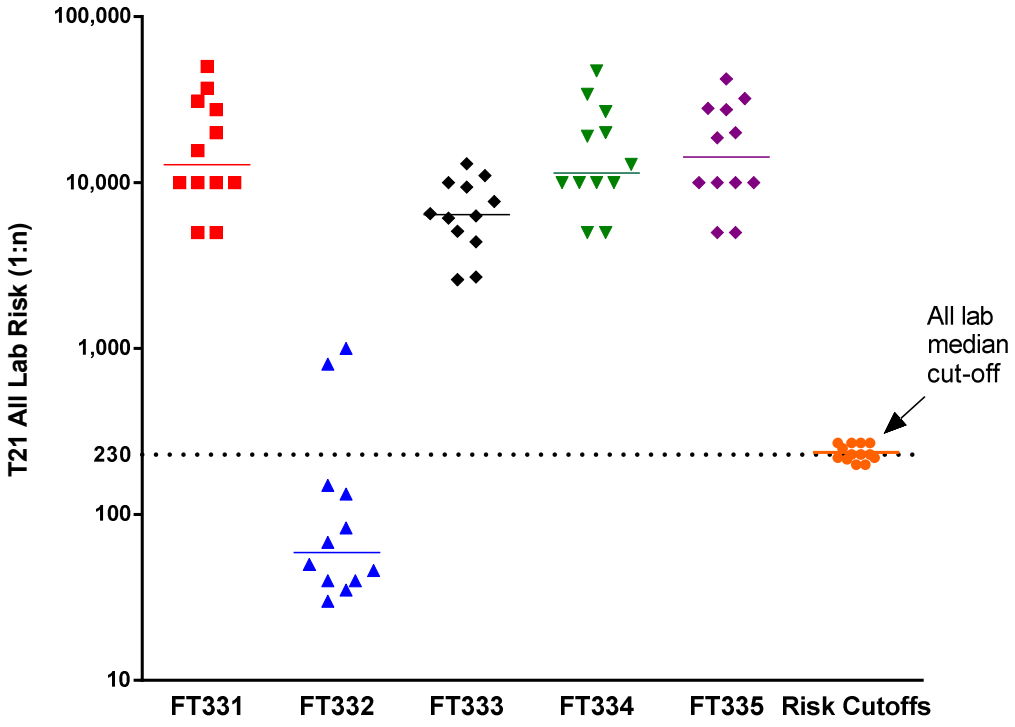


Figure 14

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates