NEW YORK STATE OF OPPORTUNITY. Department of Health

ANDREW M. CUOMO
Governor

HOWARD A. ZUCKER, M.D., J.D.
Acting Commissioner

SALLY DRESLIN, M.S., R.N.
Executive Deputy Commissioner

New York State FEDM – Proficiency Testing Program

TO: Laboratory Directors

CATEGORY: Fetal Defect Markers (FEDM)

MAILOUT: ~~January 27, 2015~~ postponed to February 3, 2015

FROM: Dr. G.J. Mizejewski, Director of FEDM Program

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DUE DATE: February ~~11~~, 2015

Samples:
There are five (5) vials labeled **MS321** to **MS325**, each containing various predetermined amounts of alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3) and Dimeric Inhibin A. Also, five additional vials (**AF 321 to AF 325**) containing AFP in amniotic fluid have also been included. In addition, five extra vials **FT321 to FT325** containing human chorionic gonadotropin (hCG) and PAPP-A are added for **mandatory** testing if you offer First Trimester testing. Please analyze for all of those markers tested in your laboratory the same way as you would with a patient sample. If your lab is also measuring Amniotic fluid AFP, you are also required to measure those samples provided. Maternal serum samples are in human-derived serum base, sterile filtered and dispensed. Please **keep refrigerated** until use, but do ***not*** freeze. Before analyzing, make sure samples are mixed completely.

Reporting of Results:
All laboratories **must** submit their proficiency testing results electronically through the electronic proficiency testing reporting system (**EPTRS**) on the Department's Health Commerce System (HCS). The HCS is a secure website and requires all users to obtain an account ID in order to access the HCS and EPTRS application. The portal's URL is https://commerce.health.state.ny.us. Questions regarding the entry and submission of proficiency test results or the account application process can be directed to clepeptrs@health.state.ny.us. If your laboratory does not have an HCS account, you must request one as soon as possible before the next PT event by contacting the Clinical Laboratory Evaluation Program at 518-486-5410.

For help with logins, password problems and reactivating HCS accounts, contact the Commerce Account Management Unit (CAMU) at (866) 529-1890.

Results must be reported for all 5, 10 or 15 Maternal Sera and/or Amniotic fluid samples. Please enter your mass unit results in the spaces provided with one or two decimals accordingly. If a result exceeds your analytical range, indicate this with a "less than (<)"or "greater than (>)" sign if similar results from patient samples are reported in the same manner. If such samples are routinely retested after dilution, you may do so provided the result is identified accordingly. Select the instrument and reagent/kit used for each analyte using the drop-down menus. Please note that the risk factor and further action (not graded) for each of the samples has also been placed in the EPTRS. All applicable fields must be completed. Missing entries will result in a failing grade for the missing results.

If CLEP is contacted for permission to submit results via paper, this request may be approved under extenuating circumstances. However, the lack of active HCS accounts, the lack of submission roles, or the lack of Internet access will not excuse a laboratory from having to submit results electronically. Without such approval, mailed or faxed proficiency test results will not be accepted. Note that such approvals will not be given on the due date! If you have any questions, please call Ms. Helen Ling at (518) 474-0036.

Special Instructions:
In order to achieve uniformity among our labs in reporting gestational age results, please report gestational week in "**decimal weeks (weeks + day/7)**" for the maternal serum samples.

Example: 18,3 weeks in the Ultrasound dating means 18 weeks + 3 days or 18.4 weeks (18 weeks + 3/7 weeks) ***not*** ~~**18.3**~~, i.e. **18.4 should be reported**

Note: We recommend the use of LMP (ultrasound dating when available) in calculating the gestational age, please note that the use of EDD is not an accepted standard of patient care.

Caution:
All human derived specimens should be handled as biohazard materials using Universal Precautions.

Only extra correspondence and/or information about **new kits** may be mailed to**:**

Fetal Defect Markers Proficiency Testing c/o Helen Ling
Wadsworth Center
Empire State Plaza, Room E610
PO BOX 509
Albany, NY 12201-0509

Please let us know immediately if you do not receive the samples in satisfactory condition by calling Ms. Helen Ling at (518) 474-0036 or emailing at helen.ling@health.ny.gov

DUE DATE: Results must be submitted electronically before 11:59 PM of February ~~11~~ 18, 2015.
*Test results will not be evaluated if the results are **submitted** after the due date and a Failing Grade will be assigned.*

The next Proficiency Test mail-out for **2015** has been tentatively scheduled for:

Ship-out date	**Due date**
May 5, 2015	May 20, 2015
September 1, 2015	September 16, 2015

The exact Proficiency Test schedule are posted at: **http://www.wadsworth.org/labcert/clep/PT/ptindex.html**

Second Trimester Demographic Data:

***Note: MS321 and MS325** are the serum sample matched to the amniotic fluid sample **AF321 and AF325, respectively**. (Dating by ultrasound)

Specimen	Maternal Date of Birth	Race[1] W,B,H,A	Maternal Weight (lbs)	IDD[2] Presence	Gravida	Parity	LMP[3]	Draw Date
MS 321	**1/29/1990**	**W**	**135**	**None**	**1**	**0**	**9/5/2014**	**1/23/2015**
MS 322	1/28/1994	A	120	None	3	2	9/12/2014	1/23/2015
MS 323	1/29/1992	W	155	None	3	1	9/5/2014	1/23/2015
MS 324	1/30/1985	H	200	None	2	1	9/26/2014	1/23/2015
MS 325	**1/30/1986**	**W**	**150**	**None**	**1**	**0**	**10/10/2014**	**1/23/2015**

Specimen	GA[4]
AF 321	**20.0**
AF 322	18.7
AF 323	16.0
AF 324	19.7
AF 325	**15.0**

[1]Race: W = White, not of Hispanic origin; B = Black, not of Hispanic origin; H = Hispanic; A = Asian
[2]IDD = Insulin-Dependent Diabetic
[3]LMP = Last Menstrual Period
[4]GA = Gestational Age in Decimal Weeks

New York State Fetal Defect Markers Proficiency Test, FEDM PT, January 2015

PFI __ __ __ __ (1) **Lab Name and address** _____

Date samples obtained __ __ /__ __ /__ __ **Analyzed** __ __ / __ __ / __ __ (2) _____

Due Date: **February 18, 2015** _____

_____

Analyte Second Trimester Maternal Serum	**Analytical results**					Instrument code*	Reagent code*
	Vial **MS321**	Vial **MS322**	Vial **MS323**	Vial **MS324**	Vial **MS325**		
Gestational Age (weeks)	__ __.__ (3)	__ __.__ (4)	__ __.__ (5)	__ __.__ (6)	__ __.__ (7)		
MS AFP (ng/ml)	__ __ __.__ (8)	__ __ __.__ (9)	__ __ __.__ (10)	__ __ __.__ (11)	__ __ __.__ (12)	__ __ __ (13)	__ __ __ (14)
MS AFP MoM	__ __.__ __ (15)	__ __.__ __ (16)	__ __.__ __ (17)	__ __.__ __ (18)	__ __.__ __ (19)		
MS uE3 (ng/ml)	__ __.__ __ (20)	__ __.__ __ (21)	__ __.__ __ (22)	__ __.__ __ (23)	__ __.__ __ (24)	__ __ __ (25)	__ __ __ (26)
MS uE3 MoM	__ __.__ __ (27)	__ __.__ __ (28)	__ __.__ __ (29)	__ __.__ __ (30)	__ __.__ __ (31)		
MS hCG Please Check: _Total(IU/ml)/ _freeβ (mIU/ml)	__ __ __.__ (32)	__ __ __.__ (33)	__ __ __.__ (34)	__ __ __.__ (35)	__ __ __.__ (36)	__ __ __ (37)	__ __ __ (38)
MS hCG Total or Freeβ MoM	__ __.__ __ (39)	__ __.__ __ (40)	__ __.__ __ (41)	__ __.__ __ (42)	__ __.__ __ (43)		
MS Dimeric Inhibin A (pg/ml)	__ __ __.__ (44)	__ __ __.__ (45)	__ __ __.__ (46)	__ __ __.__ (47)	__ __ __.__ (48)	__ __ __ (49)	__ __ __ (50)
MS Dimeric Inhibin A MoM	__ __.__ __ (51)	__ __.__ __ (52)	__ __.__ __ (53)	__ __.__ __ (54)	__ __.__ __ (55)		
Neural Tube Screen 1 = positive, 0 = negative	___ (56)	___ (57)	___ (58)	___ (59)	___ (60)	NTD Based on: ❑ MoM cut-off ❑ Risk cut-off	←
Trisomy 21 Screen 1 = positive, 0 = negative	___ (61)	___ (62)	___ (63)	___ (64)	___ (65)	Based on: ❑ Quad ❑ Triple	←
Trisomy 18 Screen 1 = positive, 0 = negative	___ (66)	___ (67)	___ (68)	___ (69)	___ (70)		

New York State Fetal Defect Markers Proficiency Test, FEDM PT, January 2015

Amniotic Fluid	Vial **AF321**	Vial **AF322**	Vial **AF323**	Vial **AF324**	Vial **AF325**	Instrument code*	Reagent code*
AF AFP (µg/ml)	__ __ __.__ 71	__ __ __.__ 72	__ __ __.__ 73	__ __ __.__ 74	__ __ __.__ 75	__ __ __ 76	__ __ __ 77
AF AFP MoM	__ __.__ __ 78	__ __.__ __ 79	__ __.__ __ 80	__ __.__ __ 81	__ __.__ __ 82		
Interpretation **1 = elevated w/ Ache indicated 0 =Normal**	___ 83	___ 84	___ 85	___ 86	___ 87	Please indicate the Cut-off → MoM value used for interpretation	_____

***codes are on P. 4**

Risk Assessment Ratio (1:n) and Further Action	**MS321**	**MS322**	**MS323**	**MS324**	**MS325**	**Risk (MoM) Cut-off (white, Black, IDDM)**
NTD Risk (or MoM)						**White**_____ **Black**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling, NIPT=noninvasive prenatal testing						**IDDM white**_____ **IDDM black**_____
Trisomy 21 Risk by Quad						**White**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling, NIPT=noninvasive prenatal testing						**Black**_____ **IDDM**_____
Trisomy 21 Risk by Triple						**White**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling, NIPT=noninvasive prenatal testing						**Black**_____ **IDDM**_____
Trisomy 18 Risk						**White**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling, NIPT=noninvasive prenatal testing						**Black**_____ **IDDM**_____
Indicate software company used to calculate risk	_ αlpha	_ Benetech PRA		_ RMA	_other_____	

We, the undersigned, attest that the findings provided were produced in this laboratory from the analysis of proficiency test samples which were introduced into the routine workflow of the laboratory and analyzed using protocols and procedures which are (or which will be) routinely applied to **clinical** specimens. We further attest that the laboratory did not engage in any form of communication with individuals outside of our laboratory regarding the proficiency test and/or results obtained therefrom. **The laboratory director or the authorized assistant director who holds a CQ in Fetal Defect Markers must sign this form (stamps are not acceptable). If the director does not hold a CQ in this category, then the assistant director holding the appropriate CQ must sign. Do not forget to add your CQ codes; these are required for proper tracking of your results. Forms without all the required information will be returned. Failure to submit the required signatures will result in a score of zero.**

Analyst _____ **Laboratory director** _____ **CQ code**__ __ __ __ __ __

Analyst _____ **Assistant director** _____ **CQ code**__ __ __ __ __ __

(Please print and sign your names)

New York State Fetal Defect Markers Proficiency Test, FEDM PT, January 2015

First Trimester Demographic Data:

Sample	Date of Birth	Race (B,W,H)	NT[1] (mm)	M. Wt (lbs)	LMP[3]	**CRL[4] (mm)**	US[2]/ Draw Date
FT 321	1/1/1986	H	1.08	160	11/3/2014	**48**	1/23/2015
FT 322	1/1/1990	W	2.90	150	10/31/2014	**53**	1/23/2015
FT 323	1/1/1994	A	1.10	105	11/7/2014	**45**	1/23/2015
FT 324	1/1/1989	H	1.40	140	10/27/2014	**59**	1/23/2015
FT 325	1/1/1996	W	1.60	130	10/24/2014	**69**	1/23/2015

[1]NT = Nuchal Translucency [2]US = Ultrasound [3]LMP = Last Menstrual Period [4]CRL = Crown Rump Length

First Trimester Maternal Serum	Vial **FT 321**	Vial **FT 322**	Vial **FT 323**	Vial **FT 324**	Vial **FT 325**	Instrument code*	Reagent code*
FT Gestational Age (weeks)	__ __.__ 88	__ __.__ 89	__ __.__ 90	__ __.__ 91	__ __ .__ 92		
FT NT MoM	__ .__ __ 93	__ .__ __ 94	__ .__ __ 95	__ .__ __ 96	__ .__ __ 97		
FT hCG Please Check: _Total(IU/ml)/ _freeβ (mIU/ml)	__ __ __.__ 98	__ __ __.__ 99	__ __ __.__ 100	__ __ __.__ 101	__ __ __.__ 102	__ __ __ 103	__ __ __ 104
FT hCG Total or Freeβ MoM	__ __.__ __ 105	__ __.__ __ 106	__ __.__ __ 107	__ __.__ __ 108	__ __.__ __ 109		
FT PAPP-A Please Check: _ mIU/ml _ng/ml	__ __ __.__ 110	__ __ __.__ 111	__ __ __.__ 112	__ __ __.__ 113	__ __ __.__ 114	__ __ __ 115	__ __ __ 116
FT PAPP-A MoM	__ __.__ __ 117	__ __.__ __ 118	__ __.__ __ 119	__ __.__ __ 120	__ __.__ __ 121		
FT Trisomy 21 Screen 1 = positive, 0 = negative	__ 122	__ 123	__ 124	__ 125	__ 126		
FT Trisomy 18 Screen 1 = positive, 0 = negative	__ 127	__ 128	__ 129	__ 130	__ 131		

Risk Assessment Ratio (1:n)and Further Action	**FT321**	**FT322**	**FT323**	**FT324**	**FT325**	**Risk Cut-off (white, Black, IDDM)**
Trisomy 21 Risk by First Trimester						**White**_____ **Black**_____ **IDDM**_____
R=Repeat, U=Ultrasound, A=Amnio, G=Genetic Counseling, C=CVS , NIPT=noninvasive prenatal testing NFA=NoFurtherAction						
Trisomy 18 Risk by First Trimester						**White**_____ **Black**_____ **IDDM**_____
R=Repeat, U=Ultrasound, A=Amnio, G=Genetic Counseling, NIPT=noninvasive prenatal testing NFA=NoFurtherAction						
Indicate software company used to calculate risk	_ αlpha	_ Benetech PRA		_ RMA	_other_____	

Instrument codes:

Abbott AxSym	ABB
Abbott Architect	ABH
Automatic (Robotic) Pipetting Station with or and Microplate Reader	APM
Bayer/Siemens Technicon Immuno-1	TNM
Siemens (Chiron) ACS-180	COS
Siemens ADVIA-Centaur	COB
Beckman Access/2	BCX
Beckman Unicel Dxl	BCU
Beckman Array	BCA
Siemens Diagnostic Dimension Rxl	DUD
Siemens Diagnostic MARK V with or and Microplate Reader	DPC
Qiagen Plato 3000 with or and Microplate Reader	QPM
Siemens Diagnostic Products Immulite	DPB
Siemens Diagnostic Products Immulite 2000	DPD
Siemens Diagnostic Products Immulite 2500	DPF
Trinity Biotech Nexgen	TBN
Microplate Reader (OD Reading for ELISA)	MPR
DSL Ario	DSA
DSL DSX with or and Microplate Reader	DSX
DSL Plato	DSP
UV/Vis Spectrophotometer	UVA
Gamma Counter	GAA
Rocket Immuno-Electrophoresis	RCE
P E Wallac Delfia	WAD
Analyzer/Instrument not shown, **specify on form**	ZZZ

Reagent/kit codes:

Abbott AFP Mono/Poly	AB1
Abbott AFP Mono/Mono	AB2
Abbott hCG	AB3
Abbott βhCG	AB4
AnshLabs	AN1
Siemens (formerly Bayer)	BA1
Siemens (formerly Chiron)	CO1
Beckman Coulter	BC1
Beckman Coulter *new* 5th IS Total hCG only	**BC2**
Siemens Diagnostic (Dade Behring)	DA1
Beckman Coulter, DSL ELISA (formerly Diagnostic Systems Lab EIA)	DS1
Diagnostic Systems Lab liquid RIA	DS2
Diagnostic Systems Lab solid RIA	DS3
DiaSorin-Clinical Assays	DC1
GenWay	GW1
Siemens Diagnostic (DPC) Coat-A-Count	DP1
Siemens DPC Immulite, Immulite 2000 or Immulite 2500	DP5
In-House	IH1
P E Wallac Delfia kit	PE1
Reagent/Kit not listed, **specify on form****	ZZZ

**If an instrument and/or reagent you are using are not listed please provide us with the information, so that we can include it in the future. If you do not perform an assay leave the fields empty. No special codes are needed to indicate that an assay is not performed.

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March 16, 2015

Dear Laboratory Director,

Attached you will find a summary and critique of the Proficiency Testing mail-out from February 3, 2015 (date was changed from January 27 due to weather), 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.

Yours sincerely,

Gerald J. Mizejewski

Gerald J. Mizejewski, Ph.D.
Assistant Director, Fetal Defect Markers Section
Clinical Laboratory Evaluation Program

Fetal Defect Marker Proficiency Test Mailout[1]
March 2015

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

Samples *n = 25	Sample #	MS 321	MS 322	MS 323	MS 324	MS 325
	Gestational Age (weeks)	20.0	19.0	20.0	17.0	15.0
Maternal Race	Ethnic Group	White	Asian	White	Hispanic	White
Maternal Weight	Pounds (lbs)	135	120	155	200	150
Maternal Age	Years	25	21	23	30	29
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	206.7 ± 11.1	61.6 ± 2.3	244.4 ± 15.2	50.5 ± 2.7	17.3 ± 1.0
	MOM ± Std. Dev.	3.34 ± 0.26	1.07 ± 0.09	4.30 ± 0.34	1.62 ± 0.10	0.61 ± 0.04
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	2.24 ± 0.19	1.18 ± 0.08	1.43 ± 0.10	0.90 ± 0.06	0.34 ± 0.03
	MOM ± Std. Dev.	1.13 ± 0.15	0.69 ± 0.08	0.76 ± 0.12	0.93 ± 0.09	0.54 ± 0.12
human Chorionic Gonadotropin (hCG)	Mean IU/ml ± Std. Dev.	41.7 ± 4.2	20.8 ± 2.4	23.9 ± 2.4	29.6 ± 3.0	96.4 ± 8.0
	MOM ± Std. Dev.	1.99 ± 0.37	0.78 ± 0.09	1.21 ± 0.26	1.27 ± 0.31	2.17 ± 0.44
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	198.6 ± 12.7	169.6 ± 10.8	211.9 ± 14.7	140.2 ± 7.7	264.8 ± 15.6
	MOM ± Std. Dev.	1.00 ± 0.10	0.89 ± 0.10	1.14 ± 0.10	0.95 ± 0.07	1.41 ± 0.10
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(+) (100%)	(-) (100%)	(+) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	G = 84% U = 96% A = 88%	NFA	G = 84% U = 100% A = 88%	NFA	NFA
	NTD Risk 1 in	33	6,680	15	2100	7315
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	G = 92% U = 58% A = 83% N = 17%
	Risk Est. 1 in	7,500	4,750	7,250	5,785	36
2. Quad Test	Pos. (+) or Neg. (-)	(-) (96 %)	(-) (96 %)	(-) (96 %)	(-) (92 %)	(+) (96 %)
	Recommended Action **	NFA	NFA	NFA	NFA	G = 92% U = 64% A = 84% N = 12%
	Risk Est. 1 in	25,000	13,000	20,000	20,000	52
Trisomy-18 Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	10,000	9,000	10,000	10,000	5,000

*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 N = 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 = 25 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 321 Wk 20.0	This specimen was obtained from a 25 year old White woman (Gravida = 1; Parity = 0) in her 20th week of gestation with a body weight of 135 lbs. Her sample screened positive for NTD, and her aneuploidy screen was negative for Down syndrome. Further actions were recommended as: genetic counseling, 84%; ultrasound, 96%; amniocentesis, 88%. This sample was paired to an amniotic fluid specimen (MOM = 1.47) which was in the high normal range.
MS 322 Wk 19.0	This specimen was obtained from a 21 year old Asian woman (Gravida = 3, Parity = 2) in her 19th week of gestation with a body weight of 120 lbs. She had no family history of reproductive complications. Her sample screened negative for NTD, and her aneuploidy screens were also negative for both Trisomy-18 and Trisomy-21. The MS322 sample was not paired to an amniotic fluid specimen.
MS 323 Wk 20.0	This specimen was obtained from a 23 year old White woman (Gravida = 3; Parity = 1) in her 20th week gestation with a body weight of 155 lbs. She had a pre-existing autoimmune disease and personal history of pregnancy loss (see critique). Her sample was a positive screen for NTD (100% consensus; MOM = 4.30). 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, 84%; ultrasound, 100%; and amniocentesis, 88%. The MS323 specimen had no amniotic fluid counterpart.
MS 324 Wk 17.0	This specimen was obtained from a 30 year old Hispanic woman (Gravida = 2, Parity = 1) in her 17th week of gestation with a body weight of 200 lbs. She had no personal history of pregnancy complications and her specimen resulted in a negative screen for NTD with no body weight or ethnic correction indicated. The labs agreed that both Trisomy screens were negative. Specimen MS324 was not paired with an amniotic fluid specimen.
MS 325 Wk 15.0	This specimen was obtained from a 29 year old White woman (Gravida = 1, Parity = 0) in her 15th week gestation with a body weight of 150 lbs. She had a family (siblings) history of pregnancy complications. Her specimen screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100% Triple, 96% Quad). Recommendations for further action from labs reporting a positive T21 quad screen were: genetic counseling, 92%; ultrasound, 64%; amniocentesis, 84% and noninvasive prenatal testing, 12%; while labs reporting a positive triple test recommended genetic counseling, 92%; ultrasound 58%; and amniocentesis, 83% and noninvasive prenatal testing, 17%. Specimen MS325 resulted in a negative T18 screen in 100% of the participating labs. This sample was paired to an amniotic fluid specimen which also had a low AFAFP level (MOM = 0.39).

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

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

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

2) AMNIOTIC FLUID AFP (NTD-analysis):

n=19; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 321 Wk 20.0	AFP = 9.3 ± 0.8 µg/ml MOM = 1.47 ± 0.15	The AF321 sample was targeted for a negative (high normal) screen AFAFP value in the upper gestational age screening range. All labs reported this specimen as a screen negative AFAFP value. The AF321 specimen was paired with an elevated maternal serum sample (MOM = 3.34).
AF 322 Wk 18.7	AFP = 3.0 ± 0.4 µg/ml MOM = 0.35 ± 0.07	The AF322 sample was targeted for a screen negative AFAFP value in the upper gestational age range. All labs reported this specimen as a screen negative AFAFP value. The AF322 specimen was not paired with a maternal serum sample.
AF 323 Wk 16.0	AFP = 10.2 ± 0.9 µg/ml MOM = 0.74 ± 0.08	The AF323 sample was targeted as an NTD negative specimen in the routine gestational age screening range. All labs categorized AF323 as a negative NTD screen. This specimen had no maternal serum counterpart.
AF 324 Wk 19.7	AFP = 6.5 ± 0.5 µg/ml MOM = 0.92 ± 0.10	The AF324 sample was targeted for normal AFAFP value in the upper gestational age range. All labs called AF324 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 325 Wk 15.0	AFP = 7.0 ± 0.6 µg/ml MOM = 0.39 ± 0.12	The AF325 sample was targeted for a reduced AFAFP value in the routine gestational age range. All labs called AF325 a negative screen for AFAFP specimen. The AFAFP sample was matched to maternal serum specimen MS325 whose AFP level was also low (MOM = 0.61).

II. Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *n = 16	**Sample #**	**FT 321**	**FT 322**	**FT 323**	**FT 324**	**FT 325**
	Gestational Age (weeks)	11.5	11.9	11.2	12.4	13.0
Maternal Race	Ethnic Group	Hispanic	White	Asian	Hispanic	White
Maternal Weight	Pounds (lbs)	160	150	105	140	130
Maternal Age	Years	29	25	21	26	19
Fetal Physical Measurements	Crown Rump Length (mm)	48	53	45	59	69
	NT Thickness (mm)	1.08	2.90	1.10	1.40	1.60
	NT – MOM ± Std. Dev.	0.90 ± 0.05	2.22 ± 0.14	0.97 ± 0.06	0.96 ± 0.06	0.95 ± 0.06
Human Chorionic Gonadotropin (hCG) Total	Mean IU/mL ± Std. Dev.	97.5 ± 13.9	196.7 ± 19.9	93.1 ± 12.6	73.9 ± 12.5	73.8 ± 7.4
	MOM ± Std. Dev.	0.99 ± 0.09	2.05 ± 0.14	0.70 ± 0.08	0.77 ± 0.06	0.84 ± 0.07
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	2258.5 ± 742.1	1195.2 ± 372.3	1928.8 ± 660.3	2404.8 ± 765.0	2293.3 ± 1242.4
	MOM ± Std. Dev.	3.81 ± 1.29	1.65 ± 0.57	2.39 ± 0.84	2.51 ± 0.83	1.70 ± 0.99
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(+) (100%)	(-) (100%)	(-) (93%)	(-) (93%)
	Recommended Action **	NFA	G = 93% U = 47% A = 60% C = 53% N = 27%	NFA	NFA[#]	NFA[#]
	Risk Estimate 1 in	19,000	77	20,000	15,000	15,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	NFA	NFA	NFA
	Risk Estimate 1 in	10,000	4,690	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 = 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.1 below. [#]Consensus of labs reporting the trisomy 21 screen as negative.

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:

n = 16 all-lab Consensus Values.

Sample#	Summary Comments:
FT 321 Wk 11.5	This specimen was obtained from a 29 year old Hispanic woman with a body weight of 160 lbs. Her gestational age at the time of screening was 11.5 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 FT321 risk estimate for Trisomy-21 was 1 in 19,000 and the Trisomy-18 risk was 1 in 10,000.
FT 322 Wk 11.9	This specimen was procured from a 25 year old White woman of average body weight (150 lbs). Her gestational age at the time of screening was 11.9 weeks. She had no prior history of any pregnancy complications. This FT specimen was screen positive for Trisomy-21 with 100% of testing labs reporting an elevated risk. Recommendations for further action from labs were: genetic counseling, 93%; ultrasound, 47%; amniocentesis, 60%, CVS, 53% and noninvasive prenatal testing, 27%. The FT322 risk estimate for Trisomy-21 was 1 in 77, and the Trisomy-18 risk was 1 in 4,650.
FT 323 Wk 11.2	This specimen was obtained from a 21 year old Asian woman of average body weight (105 lbs.). Her gestational age at the time of screening was 11.2 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative with an all-lab consensus of 100%. The FT323 risk estimate for Trisomy-21 was 1 in 20,000, and the Trisomy-18 risk was 1 in 10,000.
FT 324 Wk 12.4	This specimen came from a 26 year old Hispanic woman with a body weight of 140 lbs. Her gestational age at the time of screening was 12.4 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 FT324 was 1 in 15,000, and the Trisomy-18 risk was 1 in 10,000. All but one lab were in agreement with both screen assessments.
FT 325 Wk 13.0	This specimen was procured from a 19 year old White woman of average body weight (130 lbs.). Her gestational age at the time of screening was 13.0 weeks. She had no prior family history of pregnancy complications or adverse outcomes. This FT specimen was screen negative for Trisomy-21 and all but one testing lab were in agreement. The FT325 risk estimate for Trisomy-21 was 1 in 15,000, while the Trisomy-18 risk was 1 in 10,000.

III. Critique and Commentary:

A) Second Trimester Maternal Serum and Amniotic Fluid:

In general, the all-lab results were consistent with the targeted values for the NTD and the Trisomy Screens for risks and outcomes. The Caucasian maternal serum sample **MS321** was targeted as a screen positive specimen for NTD (Figs. 2a and 3) and was matched to a high normal **AF321** sample (Fig. 2b). All labs agreed that specimen **MS321** was screen positive for NTD and all but one lab agreed that it screened negative for both Trisomy screens using both the triple and quad tests (Figs 4-6). The risk assessment for NTD in MS321 was 1 in 33. As a follow-up, a polyacrylamide gel electrophoresis is indicated and should be performed to demonstrate the absence or presence of a diagnostic Ache band, which would confirm an NTD. The maternal serum MOM levels for MS321 were: MSAFP MOM = 3.34; MSuE3 MOM = 1.13; MShCG MOM = 1.99; MSDIA MOM = 1.00. It may be of interest that elevated level of MSAFP together with elevated MShCG have been reported to predict complicated pregnancy outcomes such as Trisomy-18, Monosomy-16, Klinefelter's syndrome, and miscarriage.

Sample **MS325** was obtained from a white woman with a prior family history of pregnancy complications. The fetal defect marker MOM values for this specimen (MSAFP MOM = 0.61, MSuE3 MOM = 0.54, MShCG MOM = 2.17, DIA-MOM = 1.41) presented the canonical profile T21 of low MSAFP and low MSuE3, together with elevated MShCG and MSDIA (Fig. 1) resulting in a positive Down Syndrome screen with which all but one lab agreed (100% by triple and 96% by quad test). In addition, the matched **AF325** specimen was low in AFP (MOM value = 0.39). The median T21 risk was 1 in 36 by triple test and 1 in 52 by quad test (Figs. 4, 5). It is interesting that the triple test risk was greater than the quad risk, possibly due to the low MSDIA value. The recommended further actions for the sample **MS325** were genetic counseling, 92%; ultrasound, 64%; amniocentesis, 84% and noninvasive prenatal testing, 12%, from labs performing the quad screen; and genetic counseling, 92%; ultrasound, 58% amniocentesis 83%, and Noninvasive prenatal testing 17% from labs performing the triple screen.

Two other specimens, **MS322** and **MS324**, produced negative screens for NTD, T21, and T18, with no corrections for body weight or race being indicated.

The **MS323** specimen at 20 weeks presents an interesting case involving highly elevated levels of MSAFP, low or normal MSuE3, and normal MShCG and MSDIA levels; this profile resulted in a positive screen for NTD and a negative screen for T21 (Figs. 4, 5) and T18 (Fig 6). The NTD risk assessment for MS323 was 1 in 15. The NTD

follow-up actions recommended for specimen **MS323** were genetic counseling, 84%; ultrasound, 100%; amniocentesis, 88%. Sample **MS323** was modeled after several literature case reports of pregnant women with myasthenia gravis disease (MGD) that exhibited similar levels of 2nd trimester biomarkers for NTD (1-3). Prior to their present pregnancy, some of the women with MGD in these case studies had not experienced complicated pregnancies and had delivered normal term infants. Although the women had been counseled on the effects of medications for MGD taken prior to and during early pregnancy, the women chose to continue gestation and underwent further testing which included ultrasound and MGD-related tests including serum autoantibody assays. Some of the patients in these studies of autoimmune MGD had been treated with prednisone and corticosteroids prior to their pregnancy. All women in these studies had pre-existing MGD upon presentation at the first obstetrician's visit, and most delivered infants with few signs of fetal abnormalities. All patients had experienced periods of remissions and relapses during and following term pregnancy. None delivered a baby with NTD. Thus, MGD during pregnancy can result in a false positive screen for NTD.

Myasthenia Gravis disease is a chronic autoimmune-mediated neuromuscular transmission disorder acquired during late teenage years and young adulthood (8). Most patients have their onset of disease between the ages of 20 and 30 years of age. At least 30-40 people per million worldwide are reported to have MGD (9). This autoimmune neuro-inflammatory disease is two times more prevalent in women than in men (10). The majority of women with MGD are of child-bearing age and MGD is diagnosed in 1 in 20,000 pregnancies in the USA.

MGD is a chronic neuromuscular transmission disorder manifested in skeletal, but not smooth muscle, which produces muscular weakness and fatigue (11). Antibodies are produced against the nicotinic acetylcholine receptor (AchR) at the neuromuscular junction (12). The disease is highly influenced by pregnancy proteins, hormonal factors, and anti-inflammatory agents which appear to serve as neuroprotective factors on the immune induction and effector phases of this neuromuscular disease (13). Thus, changes in circulating pregnancy hormones and soluble factors (estrogens, progesterone, prolactin, growth factors, cytokines, etc.) are thought to have protective effects involving the neuromuscular junction which underlies the pathology of MGD. Such soluble factors will be discussed in more detail below. The underlying pathology involves maternal autoimmune IgG antibodies which bind to the alpha-subunit of the AchR, which prevent or obstruct nerve transmission in the mother. Such IgG autoantibodies are capable of crossing the placenta and entering the fetal compartment.

During pregnancy MGD takes two major forms, a period of notable reduction of MGD symptoms (remission) in the second and third trimester followed by an exacerbation of disease (relapses) in the postpartum period of the mother before returning to pre-pregnancy disease status (14). Past and recent data continue to support the conclusion that long-term MGD development is not worsened, but may actually be somewhat lessened in mothers during pregnancy. In a study of 531 pregnant women with MGD, remissions occurred in 30% in the second and third trimesters, while 39% had relapses throughout pregnancy and 30% in the postpartum period (15). Moreover, investigators found a decrease in relapses in the latter trimesters of pregnancy but increases in relapse occurrence during the first three months following delivery (16). There appears to be protective factors produced and secreted during pregnancy that cause the disease to be less active in some cases. These soluble factors appear to suppress the humoral and/or the cellular immune response systems. Several such factors have been proposed which include the triple test biomarkers of AFP, uE3, and hCG as discussed below (17).

The impact of MGD in most pregnancies is generally small, although some adverse effects on the fetus have been reported. For most MGD patients their disease has no deleterious impact on their ability to conceive, deliver, or on fetal status and well-being (18), and pregnancy had no impact on the long-term progressive course of the MGD or the likelihood of secondary progression of MGD (20). Thus, pregnancy and childbirth in MGD have not been associated with maternal long-term disability and show no effects on fertility and family planning. However, occasionally, there can result in fetal respiratory dysfunction, myocardial damage, thyroid dysfunction, growth retardation, low birth weight/prematurity, and polyhydraminos (19). Other adverse pregnancy outcomes in MGD patients include: spontaneous and induced abortion (12%); stillbirths (2%); neonatal deaths (5%); Cesarean delivery and preterm births (5%), whereas, positive factors include: less pain and shortened hours in labor (21).

Biomarkers of the triple test for prenatal screening have been implicated in the protection of pregnant women with MGD. The estrogens, especially estriol levels, have been shown to increase both in animal models of MGD and in non-pregnant patients with the autoimmune disorder (23). The increased levels of estrogen were found to be correlated with high levels of estrogen nuclear receptors and increases is blood mononuclear cells, thymocyte, monocyte, and lymphocyte populations (24). The influence of estrogens may be involved in the fluctuations of symptoms in women with MGD and in rats with experimental MGD. However, in non-pregnant MGD patients treated with pregnancy levels of uE3, amelioration of disease was not evident even with excessive amounts of estrogen (25). Estrogen treatment was utilized in MGD patients because it had been previously demonstrated that estrogens increased the content of acetylcholine in certain organs in some experimental animal models (26). Since the pathological basis of MGD is that of insufficient utilizable acetylcholine at the motor end plate, an attractive supposition was that pregnancy hormones may increase available quantities of acetylcholine at the nerve-muscle junction (13).

Alpha-fetoprotein (AFP) is a tumor-associated fetal biomarker present in fetal and maternal serum during pregnancy. AFP has a long history as an immunomodulatory agent and is known to either enhance or inhibit the immune response at various times. Recombinant human AFP has been reported to reduce autoimmune-induced visceral organ and neuroinflammation and to increase apoptosis of activated immune cells by reducing access to BCL-2-related apoptotic pathways and increasing the expression of FAS-related (CD95) ligands (27). Furthermore, AFP can increase

both FOXP3 expression in lymph nodes and T-reg cell numbers in certain autoimmune disorders (28). AFP has been extensively studied in animal models of MGD and was found effective in treating and preventing disease induction. AFP at physiological levels exerts significant immunosuppressive effects on T-cells *in vitro* and to enhance the induction of suppressor T-cells (29). Overall, several investigators have shown a beneficial effect of AFP on the course of MGD both in rat models and in human patients (30, 31). Pregnancy fluids enriched with AFP have also been shown to inhibit *in vitro* interaction of antibodies to AchE receptor binding to the AchE receptor itself (30). Removal of AFP from such fluids nullified this latter autoantibody effect. Purified AFP has further been reported to inhibit the phytohemagglutinin (PHA) mitogen reaction induced in the proliferative response of lymphocytes in experimental models of MGD (31). During third trimester human pregnancy, maternal serum AFP levels gradually increase, peak at 30-32 weeks, then proceed to decline; this is the gestational period in which many MGD patients undergo disease remission. Thereafter, maternal serum AFP levels decrease to low nanogram levels at postpartum when most patient relapses were found to occur. Transitory MGD occurs in some newborns following delivery when AFP levels abruptly decrease (32).

In further studies, lab animals receiving intravenous injections of AFP failed to develop experimental MGD following disease induction (33). Also, animals with established experimental MGD showed clinical improvements in response to purified AFP injections in which anti-AchE receptor antibody production was suppressed (32, 34). Rats immunized with Torpedo eel AchE receptor develop MGD and show both early acute and late phase chronic disease similar to humans with MGD; in these instances both phases can be prevented by injections of purified AFP (28). Not only did AFP treatments result in reduced clinical and electromyographic MGD manifestations, AFP also decreased the serum autoantibody titers against AchE receptors (2). In premature and small-for-gestational age (SGA) infants, the levels of AFP remain high as compared to average birth weight newborns; coincidentally, premature and SGA newborns are more common in patients with MGD; thus, high AFP might lessen the symptoms of transitory MGD in some neonates (35).

Human chorionic Gonadotropin (hCG) represents a key component in prenatal screening in the first and second trimesters. HCG is a naturally occurring, immunomodulating agent that is highly expressed in pregnancy and contributes to improvements in other autoimmune diseases such as multiple sclerosis and systemic lupus. The precise mechanism of hCG mediated immune modulation in autoimmune disease is not known. Studies in non-pregnant women with MGD have shown a Gonadotropin-resistant ovarian failure syndrome due to auto-antibody production against the gonadotropins FSH and LH (36). HCG is a surrogate homolog of the luteinizing hormone (LH). Such women have circulating anti-Gonadotropin antibodies as well as peripheral blood lymphocyte subsets directed against the Gonadotropins and to TSH (37). Women with premature ovarian failure during MGD showed high levels of FSH and LH, but low serum estrogens (38). Thus, endocrine disturbances of the hypothalamic-pituitary-gonad axis have been evaluated by means of trophic hormone immunoassays in MGD patients (39). Results indicated that LH, FSH and TSH levels in MGD patients were significantly higher in non-pregnant MGD patients than in controls.

The immune system during pregnancy develops a state of immunocompetence *in utero* and an immunotolerant state in the mother whose body is adapting to the baby as a uterine allograft. For MGD patients in pregnancy, several immunobiological changes are seen to occur (40). First, a number of soluble factors normally increase markedly and then drastically fall following birth; these include estriol, hCG and AFP. In MGD patients such factors could serve to modulate shifts in cytokine levels, decreases in number of adhesion molecules, modulation of antigen presentation in dendritic cells, and modulation of the numbers of subsets of T-cell populations which contribute to decreased immune responses (41). Second, significant enhancement of both humoral and cell-mediated immune responses are seen to occur. Third, immunoprotective soluble factors are produced, which coincide with remissions in the second and third trimesters. Fourth, few if any myasthenic effects are produced in the fetus. However, a few neonates may experience a transitory MGD and display some minimal forms of MGD symptoms (32).

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

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in bar-graph format (Figs. 7-10). All participating labs used either a Beckman UNICEL/Access/2 or Siemens Immulite method. As shown in Figs. 7A-7D, MS-AFP and AF-AFP mass measurements among the individual kits mostly agreed. The exception was Siemens Immulite (DPD/DP5) in amniotic fluid, which reflected values that were 20% lower than those from the Beckman methods. When the kit specific uE3 MOMs were compared, values from Siemens DPC Immulite 2000/2500 ranged nearly 20% higher than those from the Beckman kits, although there was little difference in the actual mass values (Fig. 8A and 8B). The 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 5th generation kits (BCU/BC2; BCX BC2) were about the same as those from the original Beckman kits (BCU/BC1;BCX/BC1), but differed from the Siemens Immulite 2000 results that were 15% higher. This difference was increased rather than eliminated by the conversion to MOM values (Fig. 10B).

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

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

D) 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 =1ng/ml was used.

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

E) First Trimester Screening Software Utilized:

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

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

Therapeutic use of AFP in liver tumors (cont'd)

Butterfield et al have recently reported on a vaccination study of 10 patients investigated for use of AFP as a tumor rejection antigen for hepatomas [10]. The patients, serum AFP(+) and expressing HLA-A2-1 antigens, contained AFP-MHC peptide-pulsed autologous DCs. Following vaccination, there occurred increased frequencies of circulating AFP-specific T cells and IFNgamma-producing T cells. Before vaccination, the HCC patients showed increased frequencies of circulating AFP-specific CD8(+) T cells consisting of naïve, effector, central, and memory phenotypes which were not observed throughout the vaccination study. Further, CD8 phenotype and cytokine responses did not correlate with serum AFP levels. Assessment of CD4(+) T cell responses by ELISA and multi-cytokine assay also did not detect any spontaneous CD4 T cell responses. Thus, these data indicated the existence of an expanded pool of differentiated CD8 cytotoxic T cells in many hepatoma patients which were mostly non-functional and that a detectable CD4 T cell helper response was lacking in the AFP peptide-vaccinated subjects.

In the summation of AFP-epitope studies to date, there is a continuing need of alternative therapies for liver cancer; hence, immunotherapy is an attractive mode of treatment due to its high specificity and sensitivity. Activation of hepatoma-specific immune responses can be achieved by targeting strategies which utilize tumor-associated antigens. AFP is an obvious choice of an oncofetal protein target since it is specifically synthesized by hepatoma cells. Even though AFP is secreted, its fragmented-peptides can be processed by APCs and presented to CD4+ and CD8+ T cells in the context of MHC class-I and class-II antigens. Human trials have already begun using AFP-derived peptides in adjuvant and AFP-peptides pulsed onto the surface of autologous dendritic cells (Table 2). Although AFP is a normal "self" antigen which might induce autoimmunity, a recent report using animal models have failed to demonstrate such effects upon histopathological examination [37]. Although no clinical manifestations were observed in human trials of stage-II hepatoma patients, immunological responses to AFP peptides were in fact demonstrated. AFP peptide epitopes were immunogenic *in vivo* and were able to induce the generation of antigen-specific T cells even in hepatoma patients exhibiting very high serum AFP levels. Follow-up trials further employed AFP peptide-pulsed autologous DCs and 60% of the patients showed MHC directed AFP-specific T cell population increases including IFNgamma-secreting T cells [8, 39]. Meta-analysis of the data demonstrated that the immunological activity of an AFP-based human vaccine showed promise as an immunotherapeutic treatment modality [5, 8, 10]. Such trials, testing AFP in immune-based interventions in hepatoma patients, have indicated that the tumor-associated AFP immune response could indeed serve to impact the recurrence and survival of hepatoma patients in the future.

Concluding remarks

The correlation of elevated AFP levels with liver distress and adverse hepatic outcomes has been known since the 1970s. Even though the quantitative serum levels of AFP did not always correlate with increasing size of liver-derived tumors, the use of AFP as a tumor marker for hepatomas has not abated to the present day in spite of critical assertions to the contrary [35]. Its popularity as a fetal-associated tumor marker increased dramatically in the 1970 and 1980s and achieved prominence in the postoperative monitoring of HCCs and germ cell tumors. With each passing decade, various physiological roles of AFP have been unveiled, but only few attempts were made to merge those functions with the many and varied immunological-based hepatoma therapies being reported. Hence, recent research findings that small AFP-derived peptidic fragments could mount an immune response in the context of a MHC class-I antigen response was a landmark discovery. Still prominent is the long association of AFP with various regulatory cytokine activities which is beginning to emerge into greater prominence. In the future, we can also expect the role of AFP in maintaining the fetus as an allograft in the mother's body to become more clear as its relationship to the cytokines, NK cells, and toll-like receptors are unraveled. The role of T-helper and cytotoxic T cell interaction in the fetal and the neoplastic state also looms on the brink of new and exciting discoveries. Finally, the use of AFP-derived MHC epitopes as tumor rejection antigens directed against hepatomas lies at the threshold of increased clinical therapeutic utility [10].

Table 2 A compilation of preclinical and clinical trial studies employing alpha-fetoprotein peptide epitopes as vaccination agents in plasmid-based and pulsed dendritic cell strategies

Study or trial type	Test subjects	AFP peptide testing agents	Study outcome or response	Comment and/or conclusions	Author and year
Preclinical; immunizations	Mice, human cell lines	AFP plasmid vectors	AFP is a tumor rejection antigen	Established rational for AFP gene therapy	Vollmer et al. 1999 [37]
Preclinical; human donor use	HLA-A2.1 positive donors, human cell lines	AFP-derived peptide segment AA 542 induction	Demostrates Ag binding cytotoxicity, IFN-Gamma	AFP-reactive T cell clones not deleted; A2.1 restricted epitope detected	Butterfield et al. 1999 [6]
Preclinical	Mice, human cell lines	HAFP derived peptide AA 542	Fine specificity of HAFP peptide determined	AA modification affects MHC binding and responses	Meng et al. 2000 [26]
Preclinical; vaccinations	HLA-A. 0201 donors, human cell lines; transgenic mice	74 computer generated AFP peptide segments identified	T cells recognize AFP epitopes in cytotoxicity and cytokine Assays	Immunodominant and subdominant epitopes determined in mouse spleen	Butterfield et al. 2001 [7]
Preclinical; DNA vaccines	Transgenic mice; human cell lines	AFP-engineered dendritic cell vaccines; plasmid DNA	Elicits Th.1-type AFP-specific cells, protective immunity	Cell-free mode of immunization best for large scale vaccinations	Meng et al. 2001 [25]
Clinical trials; phase-I	6 patients enrolled, HLA-A. 0201 positive	AFP-peptide epitopes AA137, AA158, AA325, AA542	All patients generated T cell responses to most peptides	Human T cell repertoire recognizes AFP as MHC Class-I; high SAFP levels found[a]	Butterfield et al. 2003 [9]
Preclinical; human informed consent	HLA-A. 0201 donors; human cell lines	4 immunodominant and 10 subdominant peptides	AFP peptide activated T cells detected and expanded	Both dominant and subdominant AAs activate high avidity T cells	Liu et al. 2006 [23]
Clinical trials; phase I and II	16 patients enrolled, 10 patients fully treated, (HCC +)	Subdermal vaccinations using AA137, AA158, AA325, AA542	Demonstrable patient expanded T cell responses	Human T cell repertoires are capable of responding to AFP-pulsed dendritic cells	Butterfield et al. 2006 [8]
Clinical study (Japan)	38 HLA-A24 positive patients	AFP-peptides AA357, AA403, AA414, AA424, AA434 employed	AFP epitopes recognized in advanced stage hepatomas, tumor pathology	Identification of 5 new AFP epitopes for hepatoma immunotherapy	Mizukoshi et al. 2006 [33]
Clinical trials; phase-I and II	10 patients; vaccinations (pre & post analysis)	AFP-peptides AA137, AA158, AA325, AA542 employed	Expanded pool of CD8 T cells detected	Many CD8 T cell non-functional cells detected; CD4 cell lacking[a]	Butterfield et al. 2007 [10]
Clinical trials review	6 patients, stage IVa, IVb 10 patients, Stage III & IV	AFP peptides in montamide adjuvant used	Partial response; complete response analysis recorded	AFP peptide epitopes were immunogenic *in vivo* and stimulated T cell responses	Butterfield et al. 2007 [8, 10]

Study type = preclinical, clinical trials
Test subjects = mice (normal and transgenic), human cell lines (CML-K562, Hep62, B95-8, BB7.2, W6132, lymphoma lines), donors and patients
AA amino acid sequence derived from human AFP

[a] No correlations with elevated serum AFP (SAFP) levels were found

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

(1) Source: Arch Gynecol Obstet. 2014 Dec 30.

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.

(2) 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

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.

(3) Source: Prenat Diagn. 2015 Jan;35(1):90-6

Title: First and second trimester maternal serum markers in pregnancies with a vanishing twin

Authors: Huang T, Boucher K, Aul R, Rashid S, Meschino WS

Abstract: OBJECTIVE: The aim of this study was to assess the concentration of the first and second trimester maternal serum markers in pregnancies with a vanishing twin.
METHODS: This is a retrospective case-control study of pregnancies screened for Down syndrome in one Ontario center. Singleton pregnancies with ultrasound evidence of a vanishing twin were identified, and each was matched with five normal singleton controls for ethnicity, maternal age, gestational age, and blood sampling date. The median MoM of the first and second trimester serum markers was compared between cases and controls. The differences were assessed using the Mann-Whitney U-test.
RESULTS: The study included 174 pregnancies that had a vanishing twin. Compared with control pregnancies, pregnancy associated plasma protein A increased by 21% ($p = 0.0026$), alpha-fetoprotein (AFP) increased by 10% ($p < 0.0001$), and dimeric inhibin A (DIA) increased by 13% ($p = 0.0470$) in pregnancies with a vanishing twin. Unconjugated oestriol and total human chorionic Gonadotropin were not significantly changed in these pregnancies.
CONCLUSIONS: Pregnancy associated plasma protein A is not an adequate marker for pregnancies with a vanishing twin. The impact of elevated AFP on risk estimation is offset by that of DIA to certain extent. Further studies are needed to establish an adequate adjustment method for AFP and DIA to improve the accuracy of screening results for these pregnancies.

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

(1) Source: Clin Perinatol. 2014 Sep;41(3):605-18

Title: Nephrotic and nephritic syndrome in the newborn

Authors: Rheault MN

Abstract: Glomerular disorders in infancy can include nephrotic and nephritic syndromes. Congenital nephrotic syndrome (CNS) is most commonly caused by single gene mutations in kidney proteins, but may also be due to congenital infections or passive transfer of maternal antibodies that target kidney proteins. Prenatal findings of increased maternal serum α-fetoprotein and enlarged placenta suggest CNS. Neonatal nephritis is rare; its causes may overlap with those of CNS and include primary glomerulonephritis, systemic disease, infections, and transplacental transfer of maternal antibodies. These syndromes in the neonate can cause significant morbidity and mortality, making urgent diagnosis and treatment necessary.

(2) Source: Int J Clin Exp Pathol. 2014 Jul 15;7(8):5302-7

Title: Placental mesenchymal dysplasia: a case of a normal-appearing fetus with intrauterine growth restriction

Authors: Li H, Li L, Tang X, Yang F, Yang KX

Abstract: In this paper, we described a placenta with vesicular lesions in a 23-year-old woman (1-gravid) who visited our hospital at 13 weeks of gestation on prenatal routine examination. Ultrasound

findings showed multiple vesicular lesions which gradually increased as the pregnancy advanced, and a live normal-appearing fetus which was confirmed of IUGR at 30 weeks of gestation in her uterus. Throughout gestation, the maternal serum β-human chorionic gonadotropin level keeps normal, but the serum alpha-fetoprotein was higher than average. The patient delivered an 1800-g female without obvious anomalies at 35 weeks 5 days of gestation due to premature rupture of membrane. The diagnosis of placental mesenchymal dysplasia was determined on the pathological examination and androgenetic/biparental mosaicism in the placenta was identified by immunohistochemical staining of p57kip2.

(3) Source: Pediatr Dermatol. 2015 Jan;32(1):138-40

Title: Aplasia cutis congenita in a setting of fetus papyraceus associated with small fetal abdominal circumference and high alpha-fetoprotein and amniotic acetylcholinesterase

Authors: Mazza JM, Klein JF, Christopher K, Silverberg NB

Abstract: Fetus papyraceus is the fetal death of one or more fetuses in a multiparous pregnancy. The surviving infants can experience extensive aplasia cutis in an H-shaped distribution over the flanks and abdomen as a consequence of the loss of their fetal sibling. We report the case of a monochorionic, diamniotic pregnancy complicated by a single fetal death at 13 weeks of gestational age. Aplasia cutis of the surviving twin was suggested in utero by three criteria: high amniotic and maternal alpha-fetoprotein, detectable acetylcholinesterase, and small abdominal circumference on prenatal ultrasound. This constellation of findings in the setting of fetus papyraceus can be an indicator of aplasia cutis in the surviving fetus.

C) News of Note: Abstracts of New Markers:

(1) Source: Obstet Gynecol. 2015 Feb;125(2):448-52

Title: Alpha-fetoprotein as a tool to distinguish amniotic fluid from urine, vaginal discharge, and semen

Authors: Mor A, Tal R, Haberman S, McCalla S, Irani M, Perlman J, Seifer DB, Minkoff H

Abstract: OBJECTIVE: To estimate whether alpha-fetoprotein (AFP) can be used to distinguish amniotic fluid absorbed in sanitary pads from other similarly absorbed substances (semen, urine, and normal vaginal discharge).
METHODS: A prospective cohort study. Urine and amniotic fluid specimens were collected from 52 pregnant women admitted for labor. Semen specimens were collected from 17 men undergoing infertility evaluation. Alpha-fetoprotein concentrations were measured directly from urine, amniotic fluid, and semen and from pads instilled with samples from these specimens. Alpha-fetoprotein concentrations were also measured from pads absorbed with normal vaginal discharge collected from 27 pregnant women.
RESULTS: Alpha-fetoprotein levels in amniotic fluid (245.38±21.03 ng/mL, n=52) were significantly higher than those measured in maternal urine (0.84±0.17 ng/mL, n=52, P<.001), or semen (1.52±0.35 ng/mL, n=17, P<.001). The same trend was seen when AFP was extracted from pads: amniotic fluid levels (19.44±1.98 ng/mL, n=52) were significantly higher than those of urine (undetectable, n=52), semen (undetectable, n=17), or normal vaginal discharge (0.53±0.16 ng/mL, n=27, P<.001). Receiver operator characteristic curve analysis demonstrated 96.2% sensitivity and 100% specificity for distinguishing the presence of amniotic fluid from normal vaginal discharge on sanitary pads (cutoff 3.88 ng/mL, area under the curve 0.99).
CONCLUSION: When the diagnosis of rupture of membranes is in doubt, AFP levels can assist in differentiating amniotic fluid from other bodily fluids. A method that utilizes sanitary pads and an assay for AFP quantification may be an accurate and convenient way to confirm the diagnosis of rupture of membranes.

(2) Source: Am J Perinatol. 2014 Dec 17

Title: Early-Onset Severe Preeclampsia by First Trimester Pregnancy-Associated Plasma Protein A and Total Human Chorionic Gonadotropin

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

Abstract: OBJECTIVE: This study aims to evaluate the relationship between early-onset severe preeclampsia and first trimester serum levels of pregnancy-associated plasma protein A (PAPP-A) and total human chorionic gonadotropin (hCG).
STUDY DESIGN: The association between early-onset severe preeclampsia and abnormal levels of first trimester PAPP-A and total hCG in maternal serum were measured in a sample of singleton pregnancies without chromosomal defects that had integrated prenatal serum screening in 2009 and 2010 (n = 129,488). Logistic binomial regression was used to estimate the relative risk (RR) of early-onset severe preeclampsia in pregnancies with abnormal levels of first trimester PAPP-A or total hCG as compared with controls.
RESULTS: Regardless of parity, women with low first trimester PAPP-A or high total hCG were at increased risk for early-onset severe preeclampsia. Women with low PAPP-A (multiple of the median [MoM] ≤ the 10th percentile in nulliparous or ≤ the 5th percentile in multiparous) or high total hCG (MoM ≥ the 90th percentile in nulliparous or ≥ the 95th percentile in multiparous) were at more than a threefold increased risk for early-onset severe preeclampsia (RR, 4.2; 95% confidence interval [CI], 3.0-5.9 and RR, 3.3; 95% CI, 2.1-5.2, respectively).
CONCLUSION: Routinely collected first trimester measurements of PAPP-A and total hCG provide unique risk information for early-onset severe preeclampsia.

(3) Source: Eur J Obstet Gynecol Reprod Biol. 2014 Oct;181:89-94

Title: Predictive value of combined serum biomarkers for adverse pregnancy outcomes

Authors: Cohen JL, Smilen KE, Bianco AT, Moshier EL, Ferrara LA, Stone JL

Abstract: OBJECTIVE: To determine if a combination of first and second trimester serum biomarkers (pregnancy-associated plasma protein A (PAPP-A), free βhCG, and maternal serum alpha-fetoprotein (msAFP)) may be utilized to develop a predictive model for adverse pregnancy outcomes.
STUDY DESIGN: We conducted a retrospective analysis including all women who delivered at our institution between 2007 and 2010. We estimated the area under the ROC curve (AUC) to compare predictive abilities of PAPP-A, free βhCG, and msAFP singularly, and in combination for adverse pregnancy outcomes. We sought to predict the risks of preeclampsia, preterm delivery (PTD, <37 weeks gestational age) and low birth weight (LBW, <2500g). Using logistic regression analysis, we created models that controlled for maternal age, race, parity, body mass index, and histories of chronic hypertension and tobacco use.
RESULTS: The final sample included 2199 women. Determining the AUC and optimal cutoff probability values for each of the biomarkers, we found that for PTD and LBW, the combination of all three biomarkers was most predictive, while for preeclampsia the combination of msAFP and PAPP-A was most predictive. The AUC of the three biomarker combination to detect adverse pregnancy outcomes are as follows: LBW 67%, PTD 72%, and preeclampsia 77%. We created race-specific logistic regression models to predict the risk probabilities. To illustrate, the predictive probability for a 33-year-old African American, nullipara with a BMI of 50, chronic hypertension, tobacco use, PAPP-A 0.3, msAFP 2.0 and free βhCG 0.98 MOMs are: PTD 59%, LBW 61% and Preeclampsia 91%.
CONCLUSION: The combination of biomarkers currently utilized in Down syndrome screening may also be used to predict additional adverse pregnancy outcomes. Further studies are needed to determine optimal maternal and fetal surveillance, if and when increased risks are identified.

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

(1) Source: Am J Hum Genet. 2015 Jan 8;96(1):162-9

Title: CRB2 Mutations Produce a Phenotype Resembling Congenital Nephrosis, Finnish Type, with Cerebral Ventriculomegaly and Raised Alpha-Fetoprotein

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

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

(2) Source: J Trauma Acute Care Surg. 2014 Sep;77(3):510-3

Title: Elevated maternal serum α-fetoprotein after minor trauma during pregnancy may predict adverse fetal outcomes

Authors: Tanizaki S, Maeda S, Matano H, Sera M, Nagai H, Kawamura S, Ishida H

Abstract: BACKGROUND: We evaluated the relationship between minor trauma during pregnancy and elevated maternal serum α-fetoprotein level.
METHODS: This is a retrospective review of pregnant patients admitted to Fukui Prefectural Hospital with trauma during a 10-year period. Charts were reviewed for maternal age, gestational age, injury characteristics, Injury Severity Score, the presence of abdominal pain, systolic pressure and heart rate on arrival, fetal hemoglobin level, and maternal serum α-fetoprotein (MSAFP) concentration on arrival.
RESULTS: Fifty-one pregnant patients with any trauma were treated at Fukui Prefectural Hospital. All patients were hemodynamically stable and had minor trauma. An adverse pregnancy outcome occurred in three patients (5%). One patient's fetus had a left kidney injury. Intrauterine fetal death occurred in two patients. The time from injury to fatal death was 180 minutes in one patient and 18 hours in the other patient. The mean ± SD fetal hemoglobin was 0.57% ± 0.88%. The mean ± SD MSAFP was 511 ng/mL ± 1,263 ng/mL. Three patients with adverse pregnancy outcome had a high MSAFP of greater than 1,000 ng/mL.
CONCLUSION: High level of MSAFP may be a predictor of poor fetal outcome following trauma during pregnancy regardless of the severity of the trauma or the mother's hemodynamic status.
LEVEL OF EVIDENCE: Epidemiologic study, level V.

(3) Source: Iran J Reprod Med. 2013 Feb;11(2):127-32

Title: Investigating association between second trimester maternal serum biomarkers and pre-term delivery

Authors: Sehat Z, Goshetasbi A, Taheri Amin M

Abstract: BACKGROUND: Considering the effect of preterm delivery in morbidity and mortality of newborns, its precaution and prevention is so important.
OBJECTIVE: To investigate the association between second trimester maternal serum biomarkers (Human Chorionic Gonadotropin, Alpha-fetoprotein, Non-conjugated estrogen, Inhibin A) and pre-term delivery.
MATERIALS AND METHODS: This is a historical cohort study that has been performed for 700 pregnant women, clients of Nilou Lab in the second trimester of pregnancy to take the Quad Marker test between March to September 2008. The information of mothers having required conditions to enter to study has been registered and after delivery, they called again to be interviewed. These data sets using statistical tests: chi-square test and Roc Curve was analysis.
RESULTS: There is a direct relationship between preterm delivery and increase of Alpha-fetoprotein (p=0.011) and inhibin A (p=0.03) serum level and. Also, there is an inverse relationship between the non-conjugated estrogen (p=0.002) serum level and preterm delivery. Moreover, there is not any relationship between the increase human chorionic gonadotropin (p=0.68) serum level and preterm delivery.
CONCLUSION: The increase in the Alpha-fetoprotein and Inhibin A and decrease in Non-conjugated estrogen serum levels in the second trimester of pregnancy lead to enhance the probability of preterm delivery. Moreover, if the current study is done with higher samples and different sampling environment, it may have different results.

E) Abstracts of New Assay Methodologies:

(1) Source: Anal Chim Acta. 2015 Jan 1;853:228-33

Title: Immunosensor based on carbon nanotube/manganese dioxide electrochemical tags

Authors: Tu MC, Chen HY, Wang Y, Moochhala SM, Alagappan P, Liedberg B

Abstract: This article reports on carbon nanotube/manganese dioxide (CNT-MnO2) composites as electrochemical tags for non-enzymatic signal amplification in immunosensing. The synthesized CNT-MnO2 composites showed good electrochemical activity, electrical conductivity and stability. The electrochemical signal of CNT-MnO2 composites coated glassy carbon electrode (GCE) increased by nearly two orders of magnitude compared to bare GCE in hydrogen peroxide (H2O2) environment. CNT-MnO2 composite was subsequently validated as electrochemical tags for sensitive detection of α-fetoprotein (AFP), a tumor marker for diagnosing hepatocellular carcinoma. The electrochemical immunosensor demonstrated a linear response on a log-scale for AFP concentrations ranging from 0.2 to 100 ng mL(-1). The limit of detection (LOD) was estimated to be 40 pg mL(-1) (S/N=3) in PBS buffer. Further measurements using AFP spiked plasma samples revealed the applicability of fabricated CNT-MnO2 composites for clinical and diagnostic applications.

(2) Source: World J Methodol. 2014 Dec 26;4(4):219-31

Title: Methodical and pre-analytical characteristics of a multiplex cancer biomarker immunoassay

Authors: Hermann N, Dreßen K, Schildberg FA, Jakobs C, Holdenrieder S

Abstract: AIM: To test the methodical and pre-analytical performance of a new multiplex cancer biomarker panel using magnetic beads.
METHODS: The MILLIPLEX(®) MAP Human Circulating Cancer Biomarker Magnetic Bead Panel 1 comprises the tumor markers carcinoembryonic antigen, alpha-fetoprotein, total prostate-specific antigen, cancer antigen 15-3, cancer antigen 19-9, cancer antigen 125, cytokeratine 19-fragment, β-human chorionic gonadotropin, human epididymis protein 4, osteopontin, prolactin, the cell death and angiogenesis markers soluble Fas, soluble Fas-ligand, tumor necrosis factor related apoptosis-inducing ligand, vascular endothelial growth factor and the immunological markers interleukin-6 (IL-6), IL-8, tumor necrosis factor-α, transforming growth factor α, fibroblast growth factor-2, macrophage migration inhibitory factor, leptin, hepatocyte growth factor, and stem cell factor. We determined intra- and inter-assay imprecision as well as dilution linearity using quality controls and serum pools. Furthermore, the stability of the 24 biomarkers examined in this panel was ascertained by testing the influence of different storage temperatures and time span before centrifugation.
RESULTS: For all markers measured in the synthetic internal quality controls, the intra-assay imprecision ranged between 2.26% and 9.41%, while for 20 of 24 measured markers in the physiological serum pools, it ranged between 1.68% and 12.87%. The inter-assay imprecision ranged between 1.48%-17.12% for 23 biomarkers in synthetic, and between 4.59%-23.88% for 18 biomarkers in physiological quality controls. Here, single markers with very low concentration levels had increased imprecision rates. Dilution linearity was acceptable (70%-130% recovery) for 20 biomarkers. Regarding pre-analytical influencing factors, most markers were stable if blood centrifugation was delayed or if serum was stored for up to 24 h at 4 °C and 25 °C after centrifugation. Comparable results were obtained in serum and plasma for most markers. However, great changes were observed for single markers.
CONCLUSION: MILLIPLEX(®) MAP Human Circulating Cancer Biomarker Magnetic Bead Panel 1 assay is a stable and precise method for detection of most biomarkers included in the kit. However, single markers have to be interpreted with care.

(3) Source: Int J Cancer. 2014 Dec 20

Title: Multicenter analysis of soluble Axl reveals diagnostic value for very early stage hepatocellular carcinoma

Authors: Reichl P, Fang M, Starlinger P, Staufer K, Nenutil R, Muller P, Greplova K, Valik D, Dooley S, Brostjan C, Gruenberger T, Shen J, Man K, Trauner M, Yu J, Gao CF, Mikulits W

Abstract: If diagnosed at early stages, patients with hepatocellular carcinoma (HCC) can receive curative therapies, whereas therapeutic options at later stages are very limited. Here, we addressed the potential of soluble Axl (sAxl) as a biomarker of early HCC by analyzing levels of sAxl in 311 HCC and 237 control serum samples from centers in Europe and China. Serum concentrations of sAxl were significantly increased in HCC (18.575 ng/mL) as compared to healthy (13.388 ng/mL) or cirrhotic (12.169 ng/mL) controls. Receiver operating characteristic curve analysis of sAxl in very early stage HCC patients (BCLC 0) showed an area under the curve (AUC) of 0.848, with a sensitivity of 76.9% and a specificity of 69.2%. α-Fetoprotein (AFP)-negative HCC patients displayed an AUC of 0.803, with sensitivity and specificity of 73% and 70.8%. Combination of sAxl and AFP improved diagnostic accuracy to 0.936 in very early HCC patients and to 0.937 in all HCC. Differential diagnosis of very early HCC versus liver cirrhosis showed a combined performance for sAxl and AFP of 0.901 with a sensitivity of 88.5% and a specificity of 76.7%. Furthermore, sAxl levels failed to be elevated in primary ovarian, colorectal and breast carcinomas as well as in secondary hepatic malignancies derived from colon. In summary, sAxl outperforms AFP in detecting very early HCC as compared to healthy or cirrhotic controls and shows high diagnostic accuracy for AFP-negative patients. sAxl is specific for HCC and suggested as a biomarker for routine clinical use.

F) Special Abstract Selection:

(1) Source: Prenat Diagn. 2014 Feb;34(2):168-71

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

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

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

(2) Source: Obstet Gynecol Sci. 2014 May;57(3):223-7

Title: A meningomyelocele with normal intracranial signs on ultrasound and false-negative amniotic fluid alpha-fetoprotein and acetylcholinesterase

Authors: Yoon CH, Kang SK, Jin CH, Park MS, Rho JH

Abstract: Neural tube defects are the major targets of prenatal diagnoses, along with Down syndrome. Prenatal diagnosis of spina bifida is possible at second trimester of gestation through α-fetoprotein and acetylcholinesterase biochemistry assays and ultrasound. In particular, the discovery of characteristic intracranial signs on ultrasound leads to a very high diagnosis rate. However, it is rare for spina bifida to present without intracranial signs while also showing normal values of maternal serum α-fetoprotein, amniotic fluid α-fetoprotein, and acetylcholinesterase. In our hospital, a fetus with spina bifida was delivered at 37+5 weeks' gestation by cesarean section, and was continually followed up over 2 years to date.

(3) Source: Am J Obstet Gynecol. 2013 Apr;208(4):303.e1-7

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

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

Abstract: OBJECTIVE: To assess maternal-fetal outcomes in pregnancies associated with persistently elevated second-trimester maternal serum alpha-fetoprotein.
STUDY DESIGN: A retrospective cohort study in 658 patients with maternal serum alpha-fetoprotein ≥2.5 multiple of median, performed at routine Down syndrome screening. Maternal serum alpha-fetoprotein was assayed a second time in 341 of them. Outcomes were recorded in all cases.
RESULTS: The group with unexplained maternal serum alpha-fetoprotein persistently ≥2.5 multiple of median was associated with more pregnancy complications 37 of 92 (40.2%) as fetal death, preeclampsia, intrauterine growth restriction, and congenital nephrotic syndrome, compared

with the group with maternal serum alpha-fetoprotein that returned to a normal level 37 of 226 (16.4%) (P < .001).
CONCLUSION: When maternal serum alpha-fetoprotein returns to a normal level on a second assay, the risk of adverse outcome significantly decreases, but these pregnancies are still at risk of complications and therefore need close surveillance. Repeat maternal serum alpha-fetoprotein assay allows identification of patients who should be offered amniocentesis to evaluate the risk of nephrotic syndrome and epidermolysis bullosa. Alpha-fetoprotein should be monitored in pregnancies associated with unexplained high maternal serum alpha-fetoprotein. A management strategy based on ultrasound examination, second maternal serum alpha-fetoprotein assay and amniocentesis is proposed to improve prenatal counseling and management of such pregnancies. However, a prospective study remains necessary to evaluate it.

(4) Source: Am J Obstet Gynecol. 2014 Aug;211(2):144.e1-9

Title: Association between maternal characteristics, abnormal serum aneuploidy analytes, and placental abruption

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

Abstract: OBJECTIVE: The objective of the study was to examine the association between placental abruption, maternal characteristics, and routine first- and second-trimester aneuploidy screening analytes.
STUDY DESIGN: The study consisted of an analysis of 1017 women with and 136,898 women without placental abruption who had first- and second-trimester prenatal screening results, linked birth certificate, and hospital discharge records for a live-born singleton. Maternal characteristics and first- and second-trimester aneuploidy screening analytes were analyzed using logistic binomial regression.
RESULTS: Placental abruption was more frequent among women of Asian race, age older than 34 years, women with chronic and pregnancy-associated hypertension, preeclampsia, preexisting diabetes, previous preterm birth, and interpregnancy interval less than 6 months. First-trimester pregnancy-associated plasma protein-A of the fifth percentile or less, second-trimester alpha fetoprotein of the 95th percentile or greater, unconjugated estriol of the fifth percentile or less, and dimeric inhibin-A of the 95th percentile or greater were associated with placental abruption as well. When logistic models were stratified by the presence or absence of hypertensive disease, only maternal age older than 34 years (odds ratio [OR], 1.4; 95% confidence interval [CI], 1.0-2.0), pregnancy-associated plasma protein-A of the 95th percentile or less (OR, 1.9; 95% CI, 1.2-3.1), and alpha fetoprotein of the 95th percentile or greater (OR, 2.3; 95% CI, 1.4-3.8) remained statistically significantly associated for abruption.
CONCLUSION: In this large, population-based cohort study, abnormal maternal aneuploidy serum analyte levels were associated with placental abruption, regardless of the presence of hypertensive disease.

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,
January 2015
Summary of Second Trimester Results

	MS 321	MS 322	MS 323	MS 324	MS 325
Gestational Age All Lab Mean:					
Mean	20.0	19.0	20.0	17.0	15.0
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	20.0	19.0	20.0	17.0	15.0
mean-3*SD	20.0	19.0	20.0	17.0	15.0
N	25	24	24	24	25

	MS 321	MS 322	MS 323	MS 324	MS 325
MS AFP All Lab Mean:					
mean	206.7	61.6	244.4	50.5	17.3
SD	11.1	2.3	15.2	2.7	1.0
%CV	5.4%	3.8%	6.2%	5.4%	5.7%
mean+3SD	240.1	68.6	289.9	58.8	20.2
mean-3SD	173.2	54.6	198.9	42.3	14.3
N	25	25	25	25	25
median	207.1	61.5	242.3	50.0	17.3
mean/all kit median	0.99	1.00	1.01	1.01	1.01
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	203.7	61.4	242.1	50.0	17.1
SD	8.6	2.4	14.0	2.8	1.0
%CV	4.2%	4.0%	5.8%	5.7%	6.0%
mean + 3SD	229.5	68.7	284.1	58.5	20.2
mean - 3SD	177.9	54.0	200.0	41.5	14.0
N	16	16	16	16	16
Median	206.0	61.0	240.9	49.3	17.2
mean/All kit median	0.97	1.00	1.00	1.00	1.00
MS AFP Beckman Access/2 (BCX/BC1) mean:					
mean	213.3	62.6	254.3	52.2	17.8
SD	14.1	2.2	18.1	2.3	0.7
%CV	6.6%	3.5%	7.1%	4.5%	4.1%
mean+3SD	255.6	69.2	308.6	59.3	20.0
mean-3SD	170.9	56.0	200.0	45.2	15.6
N	6	6	6	6	6
median	211.2	62.7	252.1	52.2	17.4
mean/all kit median	1.02	1.02	1.05	1.04	1.04
MS AFP Siemens Immulite 2000 (DPD/DP5) mean:					
mean	209.0	60.2	233.0	48.7	16.9
N	2	2	2	2	2
mean/all kit median	1.00	0.98	0.96	0.97	0.99
MS AFP kit average:					
mean	208.7	61.4	243.1	50.3	17.3
SD	4.8	1.2	10.7	1.8	0.5
all kit median	209.0	61.4	242.1	50.0	17.1

	MS 321	MS 322	MS 323	MS 324	MS 325
MS AFP MoM All Lab Mean:					
mean	3.34	1.07	4.30	1.62	0.61
SD	0.26	0.09	0.34	0.10	0.04
%CV	7.8%	8.3%	7.9%	6.4%	7.1%
mean+3SD	4.12	1.33	5.33	1.93	0.73
mean-3SD	2.56	0.80	3.28	1.31	0.48
N	25	25	24	24	25
All Median	3.30	1.07	4.20	1.63	0.61
mean/all kit median	1.00	1.00	1.00	1.01	1.01
MS AFP MoM Beckman Unicel (BCU/BC1) mean:					
Mean	3.33	1.07	4.32	1.60	0.60
SD	0.18	0.08	0.30	0.15	0.03
%CV	5.3%	7.1%	6.8%	9.4%	5.7%
mean + 3SD	3.86	1.30	5.21	2.05	0.70
mean - 3SD	2.80	0.84	3.44	1.15	0.50
N	16	16	15	16	16
Median	3.30	1.08	4.18	1.63	0.61
mean/all kit median	1.00	1.00	1.00	1.00	1.00
MS AFP MoM Beckman Access/2 (BCX/BC1) mean:					
Mean	3.46	1.11	4.45	1.67	0.64
SD	0.43	0.12	0.40	0.07	0.05
%CV	12.4%	10.7%	9.0%	4.2%	7.6%
mean + 3SD	4.75	1.46	5.65	1.88	0.78
mean - 3SD	2.17	0.75	3.25	1.45	0.49
N	6	6	6	6	6
Median	3.43	1.09	4.47	1.64	0.63
mean/all kit median	1.04	1.03	1.03	1.04	1.06
MS AFP MoM Siemens Immulite 2000 (DPD/DP5) mean:					
Mean	3.14	0.97	3.83	1.46	0.54
N	2	2	2	2	2
mean/all kit median	0.94	0.90	0.89	0.91	0.90
MS AFP MoM kit average:					
mean	3.31	1.05	4.20	1.58	0.59
SD	0.16	0.07	0.33	0.11	0.05
all kit median	3.33	1.07	4.32	1.60	0.60

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	MS 321	MS 322	MS 323	MS 324	MS 325
MS uE3 All Lab Mean:					
mean	2.24	1.18	1.43	0.90	0.34
SD	0.19	0.08	0.10	0.06	0.03
%CV	8.3%	6.9%	7.1%	6.6%	10.3%
mean+3SD	2.80	1.43	1.74	1.08	0.44
mean-3SD	1.68	0.94	1.13	0.72	0.23
N	24	24	24	24	24
mean/all kit median	1.02	1.02	1.01	0.98	0.97
MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	2.20	1.16	1.41	0.88	0.33
SD	0.13	0.08	0.10	0.06	0.03
%CV	5.7%	6.8%	7.3%	7.1%	9.0%
mean+3SD	2.58	1.40	1.72	1.07	0.41
mean-3SD	1.82	0.92	1.10	0.69	0.24
N	16	16	16	16	16
mean/all kit median	1.00	1.00	1.00	0.96	0.93
MS uE3 Beckman Access/2 (BCX/BC1) mean:					
mean	2.46	1.26	1.50	0.94	0.37
SD	0.14	0.05	0.09	0.03	0.02
%CV	5.6%	3.9%	5.8%	3.5%	6.1%
mean+3SD	2.87	1.40	1.76	1.04	0.43
mean-3SD	2.04	1.11	1.24	0.84	0.30
N	6	6	6	6	6
mean/all kit median	1.12	1.08	1.06	1.02	1.05
MS uE3 Siemens Immulite/2000 (DPD/DP5 or 6) mean:					
Mean	1.96	1.13	1.40	0.92	0.35
N	2	2	2	2	2
mean/all Kit Median	0.89	0.97	0.99	1.00	1.00
MS uE3 kit average:					
mean	2.20	1.18	1.44	0.91	0.35
SD	0.25	0.07	0.06	0.03	0.02
all kit median	2.20	1.16	1.41	0.92	0.35

	MS 321	MS 322	MS 323	MS 324	MS 325
MS uE3 MoM All Lab Mean:					
Mean	1.13	0.69	0.76	0.93	0.54
SD	0.15	0.08	0.12	0.09	0.12
%CV	13.3%	11.0%	16.0%	9.8%	21.8%
mean+3SD	1.58	0.91	1.13	1.20	0.89
mean-3SD	0.68	0.46	0.40	0.66	0.19
N	24	23	23	22	24
mean/all kit Median	0.97	0.94	0.96	0.94	0.96
MS uE3 MoM Beckman Unicel (BCU/BC1) Mean:					
Mean	1.09	0.67	0.79	0.89	0.51
SD	0.13	0.08	0.24	0.14	0.07
%CV	11.8%	11.9%	30.8%	15.6%	14.3%
mean+3SD	1.48	0.91	1.53	1.30	0.72
mean-3SD	0.70	0.43	0.06	0.47	0.29
N	16	16	16	16	16
mean/all kit Median	0.93	0.92	1.00	0.90	0.90
MS uE3 MoM Beckman Access/2 (BCX/BC1) Mean:					
Mean	1.17	0.73	0.74	0.99	0.56
SD	0.10	0.05	0.05	0.07	0.06
%CV	8.9%	6.8%	7.4%	6.6%	10.8%
mean+3SD	1.48	0.88	0.90	1.18	0.74
mean-3SD	0.86	0.58	0.58	0.79	0.38
N	6	6	6	6	6
mean/all kit Median	1.00	1.00	0.93	1.00	1.00
MS uE3 MoM Siemens Immulite/2000 (DPD/DP5 or 6) Mean:					
Mean	1.34	0.89	1.02	1.33	0.73
N	2	2	2	2	2
mean/all kit Median	1.15	1.22	1.28	1.34	1.29
MS uE3 MoM kit average:					
mean	1.20	0.76	0.85	1.07	0.60
SD	0.13	0.11	0.15	0.23	0.11
all kit median	1.17	0.73	0.79	0.99	0.56

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	MS 321	MS 322	MS 323	MS 324	MS 325
MS hCG All Lab mean:					
mean	41.7	20.8	23.9	29.6	96.4
SD	4.2	2.4	2.4	3.0	8.0
%CV	10.1%	11.8%	9.8%	10.2%	8.3%
mean+3SD	54.3	28.1	30.9	38.6	120.4
mean-3SD	29.1	13.5	16.8	20.6	72.4
N	24	24	24	24	24
mean/all kit median	0.98	0.98	0.99	0.97	0.93
MS hCG Beckman Unicel (BCU/BC1 or 2) mean:					
mean	40.4	19.9	23.2	28.4	93.0
SD	3.5	1.6	1.7	1.9	5.4
%CV	8.8%	7.9%	7.2%	6.6%	5.8%
mean+3SD	51.0	24.7	28.2	34.1	109.2
mean-3SD	29.8	15.2	18.2	22.8	76.9
N	17	17	17	17	17
median	40.7	20.1	23.3	29.0	93.1
mean/All kit median	0.95	0.94	0.96	0.93	0.90
MS hCG Beckman Access/2 (BCX/BC1 or 2) mean:					
mean	42.6	21.3	24.1	30.5	103.2
SD	2.9	1.4	1.5	1.7	8.1
%CV	6.8%	6.6%	6.1%	5.4%	7.8%
X+3SD	51.3	25.5	28.5	35.4	127.4
X-3SD	33.9	17.1	19.7	25.5	78.9
N	5	5	5	5	5
median	42.1	21.8	23.8	29.6	105.6
mean/All kit median	1.03	1.03	1.02	1.03	1.05
MS hCG Siemens Immulite 2000 (DPD/DP5) mean:					
mean	50.1	26.8	29.6	37.1	108.1
N	2	2	2	2	2
mean/all kit median	1.16	1.19	1.20	1.19	1.00
MS hCG kit average:					
mean	44.4	22.7	25.6	32.0	101.4
SD	5.1	3.6	3.4	4.5	7.7
all kit median	42.6	21.3	24.1	30.5	103.2

	MS 321	MS 322	MS 323	MS 324	MS 325
MS hCG MoM All Lab Mean:					
mean	1.99	0.78	1.21	1.27	2.17
SD	0.37	0.09	0.26	0.31	0.44
%CV	18.4%	11.9%	21.4%	24.1%	20.1%
mean+3SD	3.08	1.06	1.99	2.19	3.48
mean-3SD	0.89	0.50	0.43	0.35	0.86
N	24	22	24	24	24
mean/All Kit Median	1.03	1.00	1.03	1.07	1.00
MS hCG MoM Beckman Unicel (BCU/BC1 or 2) mean:					
mean	1.94	0.78	1.17	1.16	2.05
SD	0.21	0.10	0.17	0.12	0.19
%CV	11.0%	13.0%	14.2%	10.3%	9.3%
X+3SD	2.58	1.08	1.67	1.52	2.63
X-3SD	1.30	0.47	0.67	0.80	1.48
N	17	17	17	16	17
median	1.90	0.73	1.16	1.18	2.05
mean/All kit median	1.00	0.99	1.00	0.98	0.95
MS hCG MoM Beckman Access/2 (BCX/BC1 or 2) mean:					
mean	1.78	0.78	1.09	1.19	2.16
SD	0.26	0.06	0.18	0.11	0.56
%CV	14.5%	8.2%	16.8%	8.9%	25.8%
X+3SD	2.58	1.08	1.67	1.52	2.63
X-3SD	1.30	0.47	0.67	0.80	1.48
N	5	5	5	5	5
median	1.79	0.77	1.10	1.22	2.50
mean/All kit median	0.92	1.00	0.93	1.00	1.00
MS hCG MoM Siemens Immulite 2000 (DPD/DP5) mean:					
mean	2.94	1.40	1.87	1.95	3.24
N	2	2	2	2	2
mean/All kit median	1.51	1.68	1.58	1.57	1.55
MS hCG MoM kit average:					
mean	2.22	0.99	1.37	1.43	2.49
SD	1.22	0.57	0.77	0.80	1.35
all kit median	1.94	0.78	1.17	1.19	2.16

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	MS 321	MS 322	MS 323	MS 324	MS 325
MS Inhibin A all lab mean:					
Mean	198.6	169.6	211.9	140.2	264.8
SD	12.7	10.8	14.7	7.7	15.6
%CV	6.4%	6.4%	7.0%	5.5%	5.9%
mean + 3SD	236.9	202.1	256.1	163.3	311.7
mean- 3SD	160.4	137.1	167.6	117.2	217.9
N	25	25	25	25	25
All Lab Median	197.0	168.1	211.0	139.3	264.0
mean/all kit median	1.00	1.00	1.00	0.99	1.00
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	196.2	168.8	211.2	137.3	263.6
SD	6.8	10.3	14.2	6.4	12.3
%CV	3.5%	6.1%	6.7%	4.7%	4.6%
mean + 3SD	216.6	199.8	254.0	156.5	300.3
mean- 3SD	175.8	137.8	168.5	118.1	226.8
N	16	16	16	16	16
Kit median	195.8	168.0	207.4	137.0	263.3
mean/all kit median	0.98	0.99	1.00	0.97	0.99
MS Inhibin A Beckman Access/2 (BCX/BC1) mean:					
Mean	203.0	171.0	213.0	145.4	267.1
SD	19.2	12.2	16.4	7.3	21.0
%CV	9.4%	7.1%	7.7%	5.0%	7.9%
mean + 3SD	260.5	207.5	262.3	167.3	330.2
mean- 3SD	145.5	134.4	163.7	123.5	204.0
N	9	9	9	9	9
Kit median	209.2	174.8	219.2	144.8	273.4
mean/All kit median	1.02	1.01	1.00	1.03	1.01
MS Inhibin A kit average:					
mean	199.6	169.9	212.1	141.4	265.3
SD	4.8	1.6	1.2	5.7	2.5
all kit median	199.6	169.9	212.1	141.4	265.3

	MS 321	MS 322	MS 323	MS 324	MS 325
MS Inhibin A MoM All Lab mean:					
mean	1.00	0.89	1.14	0.95	1.41
SD	0.10	0.10	0.10	0.07	0.10
%CV	9.5%	10.9%	9.2%	7.1%	7.3%
mean+3SD	1.28	1.18	1.45	1.16	1.72
mean-3SD	0.71	0.60	0.82	0.75	1.10
N	25	25	25	25	25
mean/all kit median	1.00	1.00	1.01	1.00	1.00
MS Inhibin A MoM Beckman Unicel (BCU/BC1) mean:					
Mean	0.99	0.89	1.16	0.95	1.42
SD	0.09	0.11	0.11	0.07	0.10
%CV	8.6%	12.0%	9.7%	7.8%	7.0%
mean + 3SD	1.25	1.20	1.50	1.18	1.72
mean- 3SD	0.74	0.57	0.82	0.73	1.12
N	16	16	16	16	16
Kit Median	0.99	0.87	1.09	0.93	1.39
mean/all kit median	1.00	1.00	1.03	1.00	1.01
MS Inhibin A MoM Beckman Access (BCX/BC1) mean:					
Mean	1.00	0.89	1.10	0.95	1.40
SD	0.12	0.08	0.08	0.06	0.11
%CV	11.5%	9.4%	7.3%	6.1%	8.1%
mean + 3SD	1.35	1.14	1.34	1.12	1.74
mean- 3SD	0.66	0.64	0.86	0.78	1.06
N	9	9	9	9	9
Kit Median	0.99	0.89	1.09	0.95	1.42
mean/all kit median	1.00	1.00	0.97	1.00	0.99
MS Inhibin A MoM kit average:					
mean	1.00	0.89	1.13	0.95	1.41
SD	0.01	0.01	0.04	0.00	0.01
all kit median	1.00	0.89	1.13	0.95	1.41

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	AF321	AF322	AF323	AF324	AF325
AF AFP All Lab mean :					
mean	9.3	3.0	10.2	6.5	7.0
SD	0.8	0.4	0.9	0.5	0.6
%CV	9.1%	15.0%	8.7%	8.1%	8.2%
mean+3SD	11.8	4.3	12.8	8.1	8.7
mean-3SD	6.7	1.6	7.5	4.9	5.3
N	19	19	19	19	17
All kit median	9.3	3.1	9.9	6.4	6.9
mean/all kit mean	1.00	0.97	1.03	1.01	1.02
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	9.1	3.1	10.2	6.4	7.0
SD	0.8	0.3	0.9	0.4	0.6
%CV	8.3%	8.6%	9.0%	6.9%	8.6%
X+3SD	11.3	3.9	12.9	7.7	8.8
X-3SD	6.8	2.3	7.4	5.1	5.2
N	13	13	13	13	13
median	8.8	3.1	10.3	6.5	7.1
mean/all kit median	0.98	1.00	1.03	1.00	1.01
AF AFP Beckman Access/2 (BCX/BC1) mean:					
Mean	9.3	3.2	9.9	6.2	6.9
SD	1.2	0.2	0.7	0.6	0.4
%CV	12.6%	6.6%	7.3%	8.9%	5.9%
X+3SD	12.77	3.79	12.06	7.82	8.08
X-3SD	5.8	2.5	7.7	4.5	5.7
N	3	3	3	3	3
median	9.5	3.1	9.7	5.9	6.8
mean/all kit median	1.00	1.03	1.00	0.96	1.00
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	9.9	1.9	9.8	7.1	1.3
N	2	2	2	2	2
mean/all kit median	1.06	0.62	0.98	1.11	0.18
AF AFP kit average:					
mean	7.0	2.0	7.5	4.9	3.8
SD	0.4	0.7	0.2	0.5	3.3
all kit median	9.3	3.1	9.9	6.4	6.9

	AF321	AF322	AF323	AF324	AF325
AF AFP MoM All Lab Mean:					
mean	1.47	0.35	0.74	0.92	0.39
SD	0.15	0.07	0.08	0.10	0.12
%CV	10.4%	19.7%	10.9%	10.4%	31.6%
mean+3SD	1.93	0.56	0.98	1.21	0.76
mean-3SD	1.01	0.14	0.50	0.64	0.02
N	19	19	19	19	19
All median	1.52	0.37	0.75	0.92	0.43
mean/all kit median	0.97	0.95	0.99	1.00	0.91
AF AFP MoM Beckman Unicel(BCU/BC1) mean:					
Mean	1.44	0.36	0.77	0.90	0.44
SD	0.17	0.05	0.08	0.10	0.06
%CV	12.1%	12.8%	10.3%	10.7%	12.6%
X+3SD	1.96	0.50	1.00	1.20	0.61
X-3SD	0.92	0.22	0.53	0.61	0.27
N	13	13	13	13	13
median	1.50	0.36	0.79	0.90	0.44
mean/all kit median	0.94	1.00	1.13	1.00	1.20
AF AFP MoM Beckman Access (BCX/BC1) mean:					
Mean	1.53	0.39	0.66	0.96	0.37
SD	0.03	0.02	0.06	0.07	0.02
%CV	2.1%	5.9%	8.3%	7.3%	4.7%
X+3SD	1.62	0.46	0.83	1.17	0.42
X-3SD	1.43	0.32	0.50	0.75	0.32
N	3	3	3	3	3
median	1.54	0.38	0.66	0.96	0.36
mean/all kit median	1.00	1.09	0.98	1.06	1.00
AF AFP MoM DPC Immulite 2000 (DPD/DP5) mean:					
Mean	1.53	0.20	0.68	0.90	0.08
N	2	2	2	2	2
mean/all kit median	1.00	0.54	1.00	0.99	0.20
AF AFP MoM kit average:					
mean	1.50	0.32	0.70	0.92	0.30
SD	0.05	0.11	0.06	0.03	0.19
all kit median	1.53	0.36	0.68	0.90	0.37

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	FT321	FT322	FT323	FT324	FT325
FT Gestational Age All Lab Mean:					
Mean	11.5	11.9	11.2	12.4	13.0
SD	0.13	0.11	0.14	0.08	0.08
%CV	1.1%	0.9%	1.2%	0.7%	0.6%
mean+3*SD	11.9	12.2	11.6	12.6	13.3
mean-3*SD	11.1	11.6	10.8	12.1	12.8
N	16	16	16	16	16

	FT321	FT322	FT323	FT324	FT325
FT NT MoM All Lab Mean:					
Mean	0.90	2.22	0.97	0.96	0.95
SD	0.05	0.14	0.06	0.06	0.06
%CV	5.9%	6.3%	6.0%	6.3%	6.3%
mean+3*SD	1.06	2.64	1.14	1.15	1.14
mean- 3*SD	0.74	1.80	0.79	0.78	0.77
N	15	15	15	15	15
All Median	0.90	2.20	0.94	0.97	0.96

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	FT321	FT322	FT323	FT324	FT325		FT321	FT322	FT323	FT324	FT325
FT hCG All Lab Mean:						**FT hCG MoM All Lab Mean:**					
mean	97.5	196.7	93.1	73.9	73.8	Mean	0.99	2.05	0.70	0.77	0.84
SD	13.9	19.9	12.6	12.5	7.4	SD	0.09	0.14	0.08	0.06	0.07
%CV	14.2%	10.1%	13.5%	17.0%	10.0%	%CV	9.1%	6.6%	11.9%	8.1%	8.6%
mean+3*SD	139.1	256.5	130.8	111.5	95.9	mean+3*SD	1.27	2.46	0.95	0.96	1.06
mean- 3*SD	55.9	137.0	55.3	36.3	51.6	mean - 3*SD	0.72	1.64	0.45	0.59	0.62
N	15	15	15	15	15	N	12	12	12	12	12
All lab median	93.8	194.9	90.9	70.0	73.3	All lab Median	0.98	2.05	0.70	0.77	0.83
mean/All kit median	0.89	0.94	0.89	0.86	0.95	mean/All kit Median	0.72	0.78	0.64	0.67	0.77
FT hCG Beckman Unicel/Access 2 (BCU or X/BC1 or 2) mean:						**FT hCG MoM Beckman Unicel or Access 2 (BCU or X/BC1 or 2) mean:**					
mean	93.3	191.9	88.9	69.5	72.2	mean	0.99	2.05	0.70	0.77	0.84
SD	8.3	13.9	6.3	4.7	5.7	SD	0.09	0.14	0.08	0.06	0.07
%CV	8.9%	7.2%	7.0%	6.8%	7.9%	%CV	9.1%	6.6%	11.9%	8.1%	8.6%
mean+3SD	118.2	233.6	107.6	83.6	89.4	mean+3SD	1.27	2.46	0.95	0.96	1.06
mean- 3SD	68.4	150.1	70.1	55.4	55.1	mean-3SD	0.72	1.64	0.45	0.59	0.62
N	13	13	13	13	13	N	12	12	12	12	12
median	92.3	191.6	88.5	69.9	72.5	median	0.98	2.05	0.70	0.77	0.83
mean/All kit median	0.86	0.91	0.85	0.81	0.93	mean/All kit median	0.72	0.78	0.64	0.67	0.77
FT hCG DPC Immulite 2000(DPD/DP5) mean:						**FT hCG MoM DPC Immulite2000 (DPD/DP5) mean:**					
mean	124.9	228.4	120.4	102.5	83.7	mean	1.77	3.24	1.49	1.55	1.33
N	2	2	2	2	2	N	2	2	2	2	2
mean/All kit median	1.14	1.09	1.15	1.19	1.07	mean/All kit median	1.28	1.22	1.36	1.33	1.23
FT hCG kit average:						**FT hCG MoM kit average:**					
mean	109.1	210.1	104.6	86.0	78.0	mean	1.38	2.65	1.09	1.16	1.08
SD	22.4	25.8	22.3	23.3	8.1	SD	0.55	0.84	0.56	0.55	0.35
all kit median	109.1	210.1	104.6	86.0	78.0	all kit median	1.38	2.65	1.09	1.16	1.08

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

	FT321	FT322	FT323	FT324	FT325
FT PAPP-A All Lab Mean:					
Mean	2258.5	1195.2	1928.8	2404.8	2293.3
SD	742.1	372.3	660.3	765.0	1242.4
%CV	32.9%	31.1%	34.2%	31.8%	54.2%
mean + 3SD	4484.7	2312.0	3909.8	4699.9	6020.5
mean- 3SD	32.2	78.4	-52.2	109.6	-1433.8
N	15	15	15	15	15
All Lab Median	2341.0	1271.2	2045.0	2543.2	1766.3
mean/All kit median	0.96	0.95	0.94	0.94	1.25
FT PAPP-A Beckman Unicel(BCU/BC1) Mean:					
Mean	2343.4	1261.6	2045.1	2571.4	1838.5
SD	205.6	104.5	181.5	222.3	118.9
%CV	8.8%	8.3%	8.9%	8.6%	6.5%
mean + 3SD	2960.3	1575.1	2589.5	3238.2	2195.1
mean - 3SD	1726.5	948.1	1500.6	1904.7	1481.9
N	10	10	10	10	10
Kit Median	2396.3	1273.0	2048.7	2552.1	1824.7
mean/All kit median	1.00	1.00	1.00	1.00	1.00
***FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:**					
Mean	3750.0	1855.5	3105.5	3632.8	6269.5
N	2	2	2	2	2
mean/All kit median	1.60	1.47	1.52	1.41	3.41
***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	981.1	533.6	756.8	1030.4	1158.6
SD	57.7	58.6	151.0	230.5	221.2
%CV	5.9%	11.0%	20.0%	22.4%	19.1%
mean + 3SD	1154.2	709.4	1209.9	1721.8	1822.1
mean - 3SD	807.9	357.7	303.8	339.1	495.1
N	3	3	3	3	3
Kit Median	1001.0	563.7	817.0	1113.3	1220.9
mean/All kit median	0.42	0.42	0.37	0.40	0.63
FT PAPP-A kit average:					
mean	2358.2	1216.9	1969.1	2411.6	3088.9
SD	1384.5	662.1	1176.2	1308.5	2775.4
all kit median	2343.4	1261.6	2045.1	2571.4	1838.5

	FT321	FT322	FT323	FT324	FT325
FT PAPP-A MoM All Lab Mean:					
Mean	3.81	1.65	2.39	2.51	1.70
SD	1.29	0.57	0.84	0.83	0.99
%CV	33.9%	34.4%	35.4%	33.0%	58.0%
mean + 3SD	7.69	3.36	4.92	4.99	4.66
mean- 3SD	-0.06	-0.06	-0.15	0.03	-1.26
N	15	15	15	15	15
All Lab Median	4.06	1.76	2.64	2.92	1.46
mean/ All kit median	0.89	0.89	0.89	0.88	1.15
FT PAPP-A MoM Beckman Unicel(BCU/BC1) Mean:					
Mean	4.27	1.87	2.68	2.85	1.48
SD	0.63	0.26	0.32	0.28	0.17
%CV	14.7%	13.8%	11.8%	10.0%	11.5%
mean + 3SD	6.15	2.64	3.63	3.70	1.99
mean - 3SD	2.39	1.09	1.73	1.99	0.97
N	10	10	10	10	10
Kit Median	4.22	1.82	2.68	2.94	1.48
mean/All kit median	1.00	1.00	1.00	1.00	1.00
FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:					
Mean	4.81	2.09	3.18	3.07	4.01
N	2	2	2	2	2
mean/All kit median	1.13	1.12	1.19	1.08	2.71
FT PAPP-A MoM (SMR or APM/AN1) Mean:					
Mean	1.81	0.75	0.94	1.14	0.91
N	2	2	2	2	2
mean/ All kit median	0.42	0.40	0.35	0.40	0.61
FT PAPP-A MoM kit average:					
mean	3.63	1.57	2.27	2.35	2.13
SD	1.60	0.72	1.18	1.05	1.65
all kit median	4.27	1.87	2.68	2.85	1.48

Figure 1

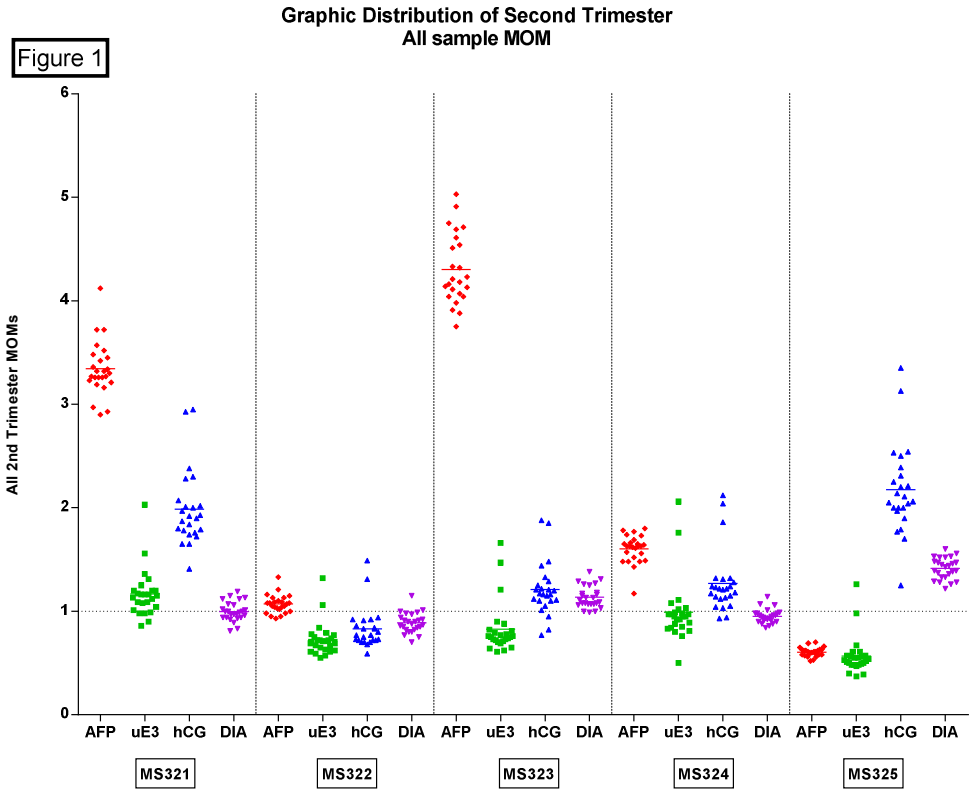


Figure 2a

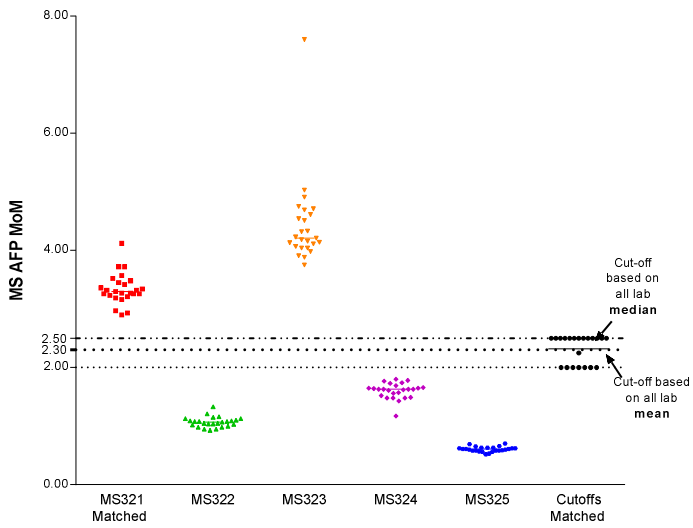


Figure 2b

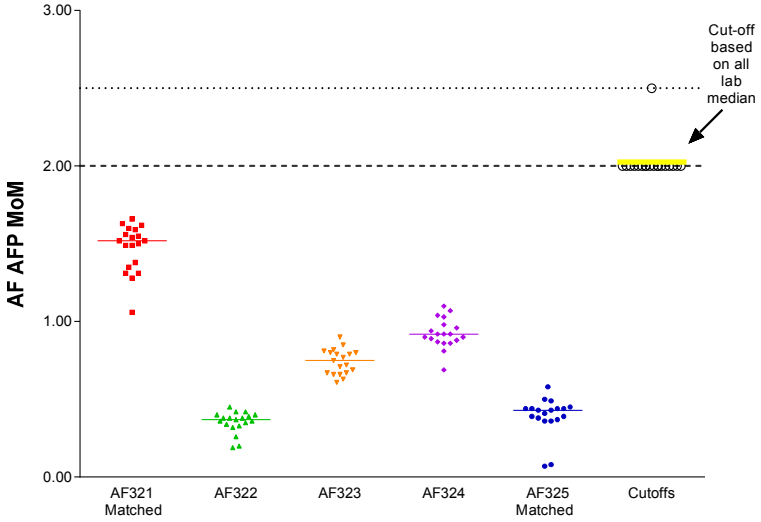


Figure 3

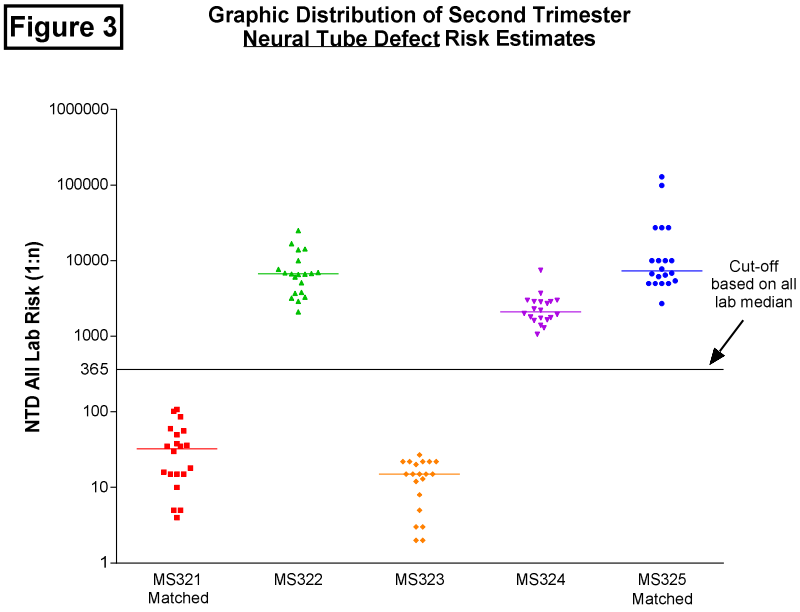


Figure 4

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

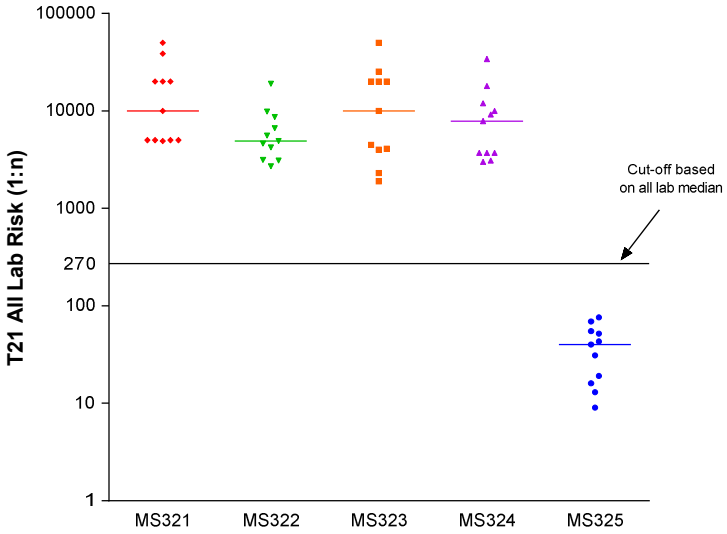


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

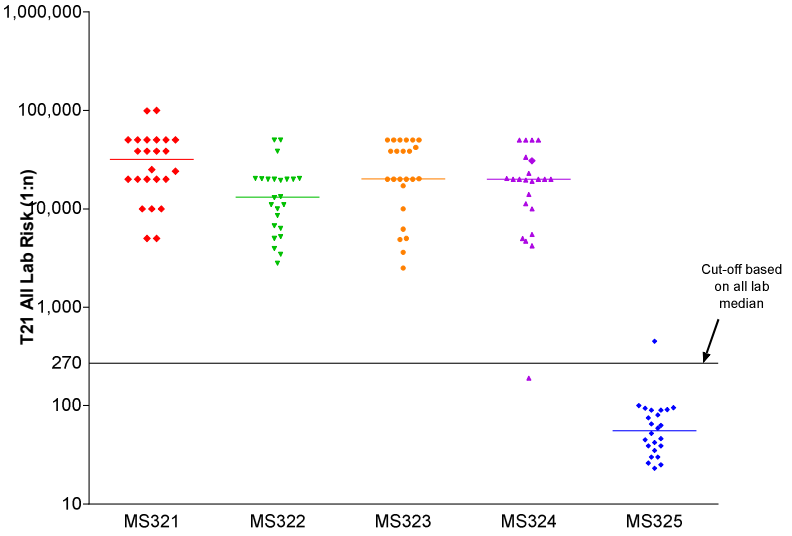
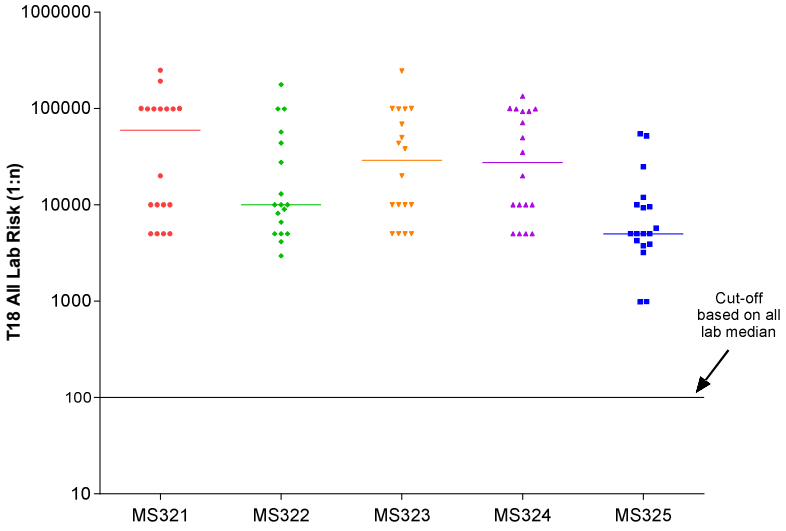


Figure 6

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates



NYS FEDM PT 1/15
Second Trimester

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

Figure 7A

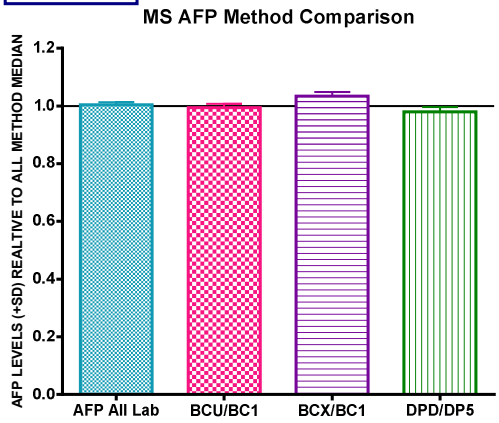


Figure 7B

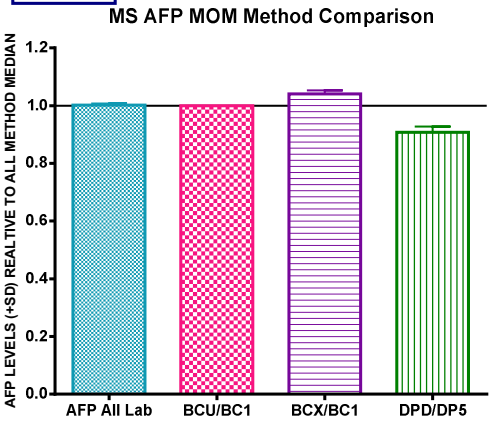


Figure 7C

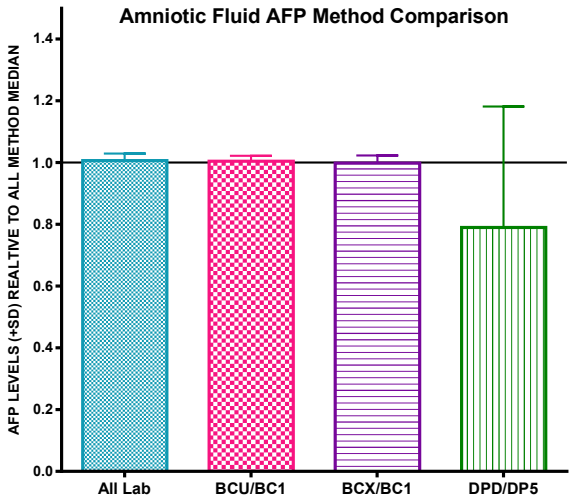
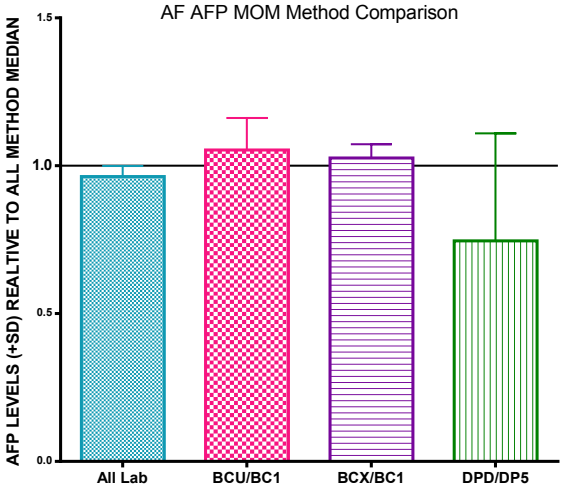


Figure 7D



NYS FEDM PT 1/15
Second Trimester

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

Figure 8A

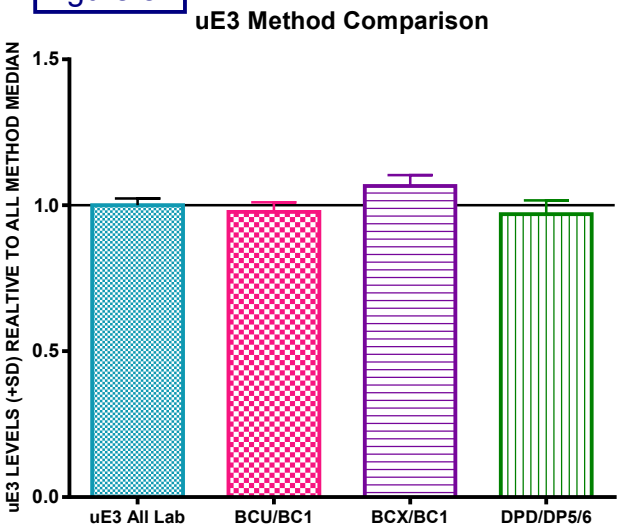


Figure 8B

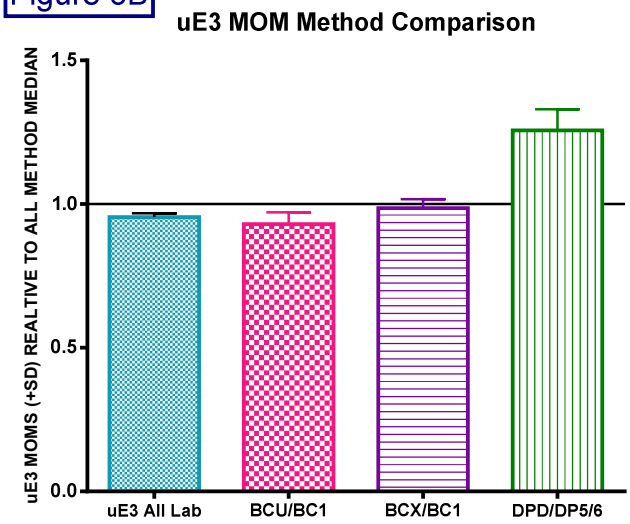


Figure 9A

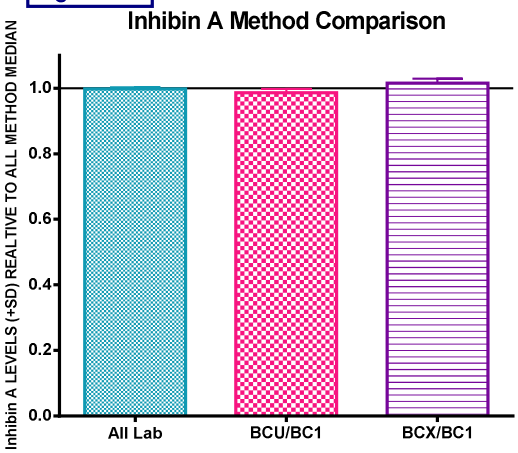


Figure 9B

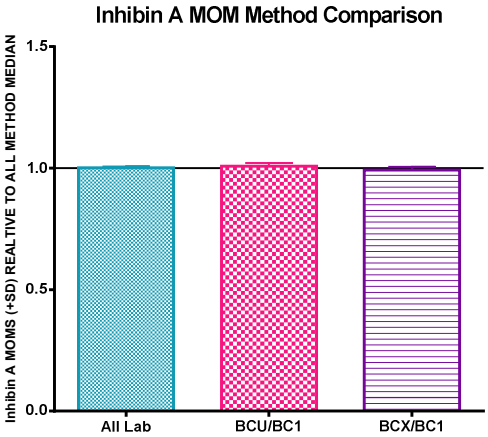


Figure 10A

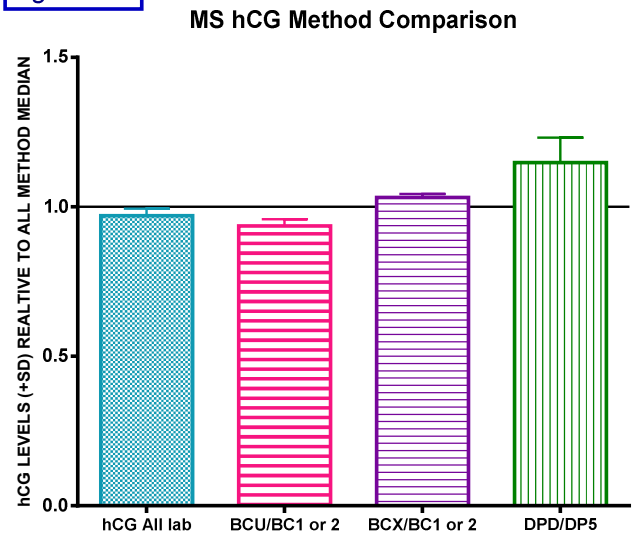
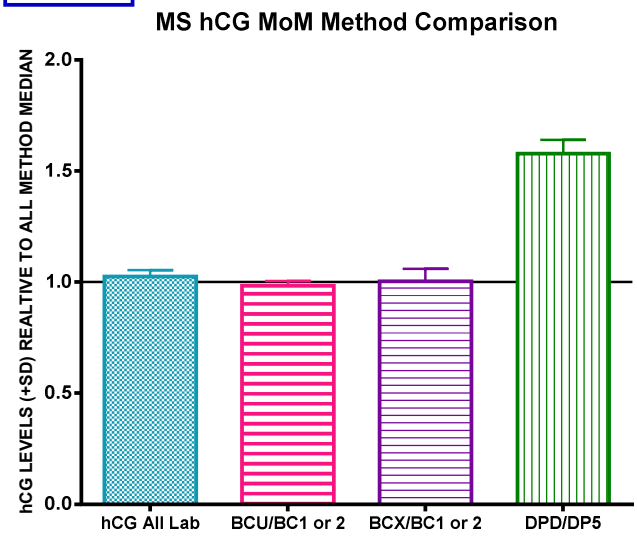


Figure 10B



NYS FEDM PT 1/15
First Trimester

Figure 11A

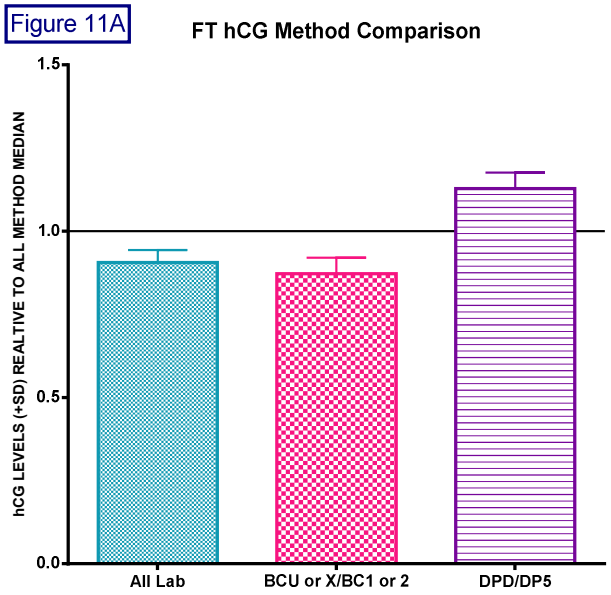


Figure 11B

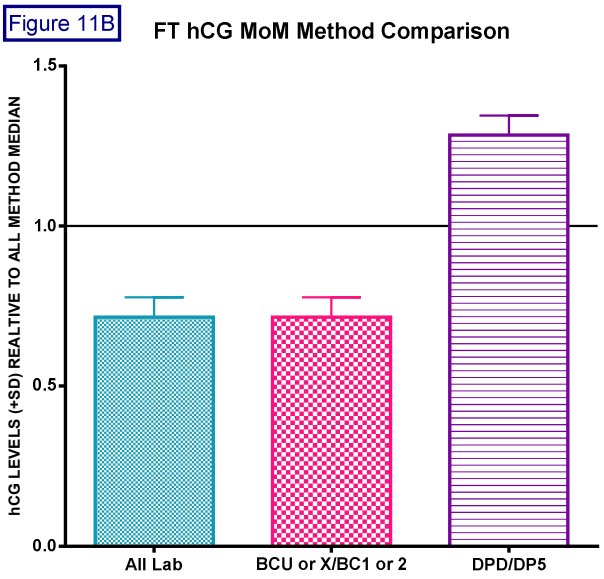


Figure 12A

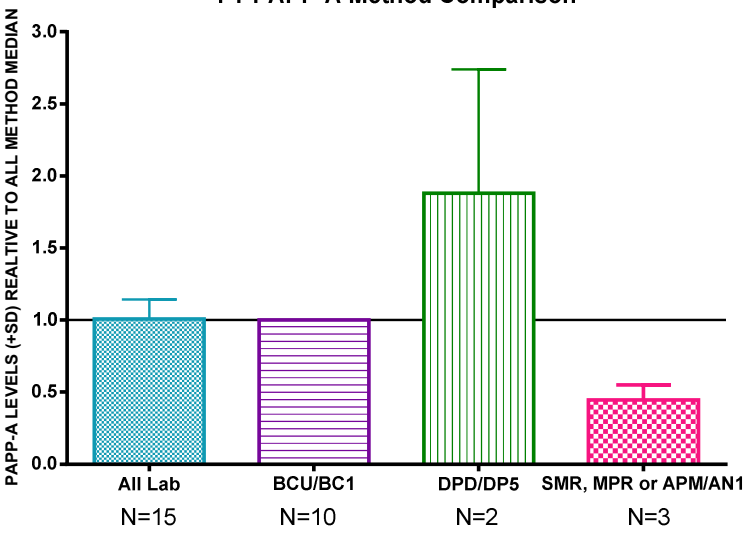
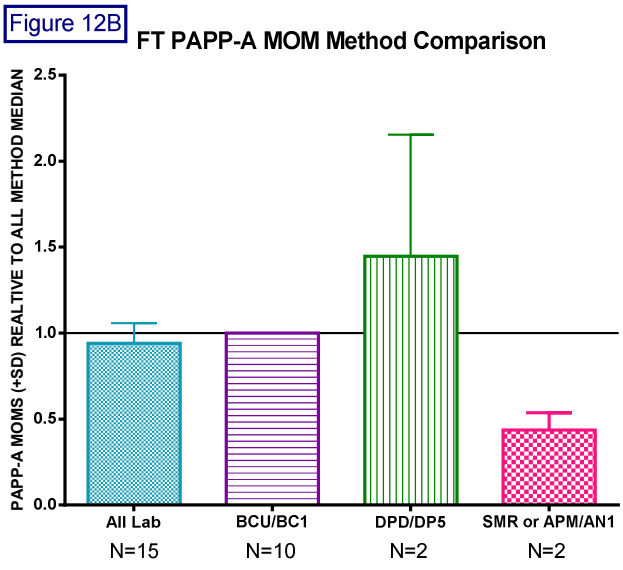


Figure 12B



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

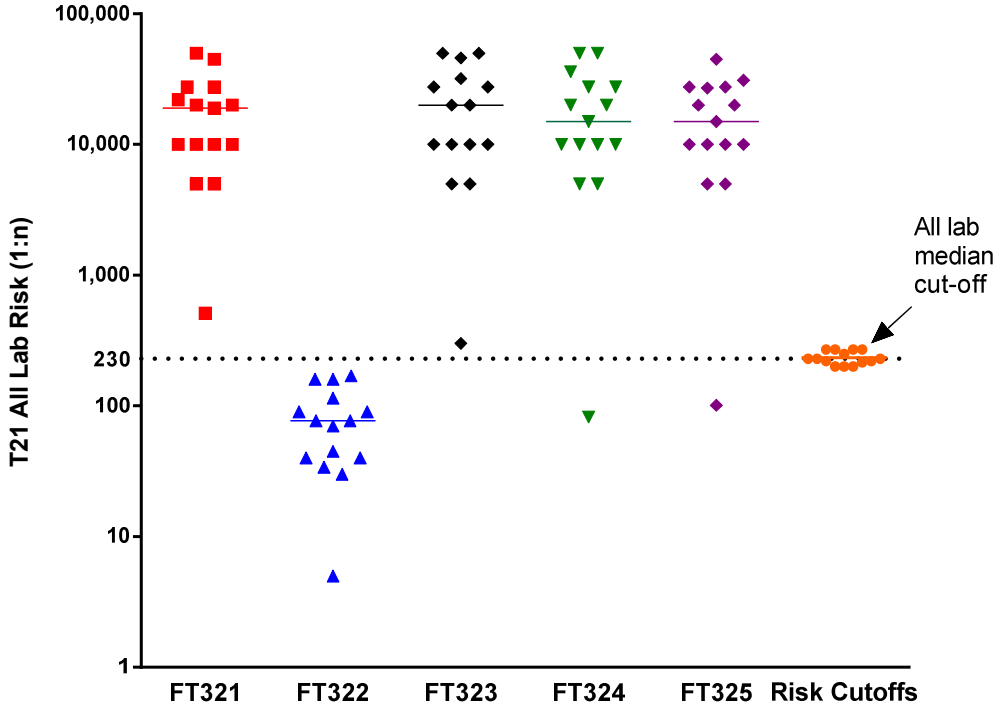


Figure 14

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates