NEW YORK
state department of
HEALTH

Nirav R. Shah, M.D., M.P.H.
Commissioner

Sue Kelly
Executive Deputy Commissioner

New York State FEDM – Proficiency Testing Program

TO: Laboratory Directors

CATEGORY: Fetal Defect Markers (FEDM)

MAILOUT: May 10, 2011

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

DUE DATE: May 25, 2011

Samples:
There are five (5) vials labeled **MS266** to **MS270**, 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 266 to AF 270) containing AFP in amniotic fluid have also been included. In addition, five extra vials **FT 266 to FT 270** containing human chorionic gonadotropin (**hCG**) and **PAPP-A** are added for ***optional*** testing. Please note that you do not have an option if you offer First Trimester and or Integrated Testing but the results of **FT 266 to FT 270** will ***not be graded***. 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. Also, please **see attached May 2011 bulletin**.

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 Maternal Sera and/or Amniotic fluid samples; otherwise a zero grade will be applied to the missing data. 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.

Please Turn Over

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 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.

DUE DATE: Results must be submitted electronically before 11:59 PM of May 25, 2011.
*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 2011 has been tentatively scheduled for:

Ship-out date	**Due date**
September 13, 2011	September 28, 2011

Demographic Data:

Specimen	Maternal Date of Birth	Race[1] W,B,H,A	Maternal Weight (lbs)	IDD[2] Presence	Gravida	Parity	LMP[3]	Draw Date	Specimen	GA[4]
MS 266	5/1/1985	W	150	None	3	0	1/7/2011	5/6/2011	AF 266	19.0
MS 267	5/3/1981	H	129	None	2	1	12/24/2010	5/6/2011	AF 267	18.0
MS 268	**4/13/1982**	**B**	**145**	**None**	**4**	**2**	**1/21/2011**	**5/6/2011**	**AF 268**	**15.0**
MS 269	5/2/1986	A	105	None	1	0	12/31/2010	5/6/2011	AF 269	17.0
MS 270	**2/13/1983**	**W**	**135**	**None**	**2**	**0**	**12/17/2010**	**5/6/2011**	**AF 270**	**20.0**

***Note: MS268 and MS270** are the serum sample matched to the amniotic fluid sample **AF268 and AF270, respectively**. (Dating by ultrasound)

[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

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Fetal Defect Marker Proficiency Test Mailout[1]
May 2011

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from May, 2011, for Fetal Defect Markers, which included samples for first and second trimester screening, as well as amniotic fluids. Your laboratory's results and grades are printed on a separate sheet; also included are the grades from the previous two PT events. These will be mailed to you separately. Please review and sign your evaluation. Retain the signed evaluation in your files. You will need it for your next laboratory survey to demonstrate participation in the NYSPT program.

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

Samples *N = 27	**Sample #**	**MS 266**	**MS 267**	**MS 268**	**MS 269**	**MS 270**
	Gestational Age (weeks)	17.0	19.0	15.0	18.0	20.0
Maternal Race	Ethnic Group	White	Hispanic	Black	Asian	White
Maternal Weight	Pounds (lbs)	150	129	145	105	135
Maternal Age	Years	26	30	29	25	28
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std. Dev.	16.80 ± 1.21	44.60 ± 3.90	12.51 ± 1.01	36.40 ± 2.81	126.21 ± 9.80
	MOM ± Std. Dev.	0.43 ± 0.04	0.80 ± 0.09	0.37 ± 0.04	0.66 ± 0.09	2.02 ± 0.22
Unconjugated Estriol (uE3)	Mean ng/ml ± Std. Dev.	1.03 ± 0.15	1.36 ± 0.18	0.34 ± 0.06	1.21 ± 0.14	1.63 ± 0.22
	MOM ± Std. Dev.	1.16 ± 0.40	0.92 ± 0.27	0.61 ± 0.25	0.96 ± 0.26	0.93 ± 0.29
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std. Dev.	10.94 ± 1.03	17.95 ± 2.00	64.17 ± 9.90	19.44 ± 2.28	17.10 ± 1.89
	MOM ± Std. Dev.	0.46 ± 0.04	0.91 ± 0.10	1.55 ± 0.31	0.78 ± 0.11	0.96 ± 0.08
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std. Dev.	154.55 ± 15.68	214.15 ± 24.35	297.58 ± 30.22	132.16 ± 14.96	237.97 ± 21.79
	MOM ± Std. Dev.	0.91 ± 0.13	1.14 ± 0.19	1.56 ± 0.25	0.67 ± 0.12	1.17 ± 0.16
Neural Tube Screen (Positive, Negative) Percent	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (81.5%)
	Further Action G,U,A	NFA	NFA	NFA	NFA	NFA
	NTD Risk 1 in	10,000	8,000	10,000	10,000	550
Trisomy-21 Screen (Positive, Negative) Percent 1. Triple test	Pos. (+) or Neg. (-)	(-) (100%)	(-) (100%)	(+) (71%)	(-) (100%)	(-) (100%)
	Recommended Action**	NFA	NFA	G = 50% U = 57% A = 57%	NFA	NFA
	Risk Est. 1 in	5,000	2,600	50	3,400	5,400
2. Quad Test	Pos. (+) or Neg. (-)	(-) (96%)	(-) (100%)	(+) (96%)	(-) (100%)	(-) (100%)
	Recommended Action **	NFA	NFA	G = 73% U = 81% A = 85%	NFA	NFA
	Risk Est. 1 in	2,555	1,903	31	6,252	20,000
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	4,193	10,000	3,102	10,500	15,000

*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, and A = amniocentesis.
**This percentage is normalized to labs requesting further action. ‡ Insulin Dependent Diabetic pregnancy.

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

1) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:
N = 27 all-lab Consensus Values.

Sample #	Summary Comments (Mock specimens):
MS 266 Wk 17.0	This specimen was obtained from a 26 year old white woman (Gravida = 3, parity = 0) in her 17th week of gestation with a body weight of 150 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD and both Trisomy-18 and Trisomy-21. However, her MShCG and MSAFP samples were extremely low (see critique for further discussion). This sample was not paired to an amniotic fluid specimen.
MS 267 Wk 19.0	This specimen was obtained from a 30 year old Hispanic woman (Gravida = 2, Parity = 1) in her 19th week gestation with a body weight of 129 lbs. She had no family history of pregnancy complications. To date, her pregnancy appeared to follow a favorable course of gestation, and her specimen resulted in a negative screen for NTD with a race correction indicated. The labs were also in agreement that both Trisomy screens were negative. Specimen MS267 was not paired with an amniotic fluid sample.
MS 268 Wk 15.0	This specimen was obtained from a 29 year old Black woman (Gravida = 4, Parity = 2) in her 15th week of gestation with a body weight of 145 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (96%) on the basis of low AFP and uE3, and moderately elevated hCG and inhibin-A levels. Recommendations for further action from labs performing the T21 quad screen were: genetic counseling, 73%, ultrasound, 85% and amniocentesis, 81%; while the triple tests were: genetic counseling, 50%; ultrasound, 57% and amniocentesis, 57%. Specimen MS268 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 = 0.63).
MS 269 Wk 18.0	This specimen was obtained from a 25 year old Asian woman (Gravida = 1, Parity = 0) in her 18th week of gestation with a body weight of 105 lbs. A body weight/race correction may be indicated. She had no personal history of pregnancy loss. Her specimen was negative for NTD and for both Trisomies with all labs in agreement. Thus, no recommendations for further action were noted. This specimen had no amniotic fluid counterpart.
MS 270 Wk 20.0	This specimen was obtained from a 28 year old white woman (Gravida = 2, parity = 0) in her 20th week of gestation with a body weight of 135 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD and her aneuploidy screens were negative for both Trisomy-18 and Trisomy-21. The MS270 sample was paired to an amniotic fluid specimen, which was elevated (AFAFP MOM = 3.03). Please see Critique below for further discussion of samples MS270 and AF270.

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 times pregnant including the present one; a gravida = 2 indicates that the women was pregnant once before in addition to her present pregnancy. Parity = 1 indicates the patient already has one child; however, multiple birth is also considered as a single parity.

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

2) AMNIOTIC FLUID AFP (NTD-analysis):

N=27; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 266 Wk 19.0	AFP = 8.32 ± 0.91 µg/ml MOM = 1.07 ± 0.10	The AF266 sample was targeted for a normal AFAFP value in the upper gestational age range. All labs called AF266 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 267 Wk 15.0	AFP = 12.38 ± 1.73 µg/ml MOM = 1.30 ± 0.11	The AF267 sample was targeted for a negative NTD screen for AFAFP in the routine gestational age screening range. All labs categorized this as an NTD screen negative specimen. This sample was not matched to a maternal serum specimen.
AF 268 Wk 15.0	AFP = 10.60 ± 1.27 µg/ml MOM = 0.63 ± 0.08	The AF268 sample was targeted for a low level AFAFP value in the routine gestational age range. Most labs called AF268 a low MOM AFAFP specimen. This AFAFP sample was matched to maternal serum specimen MS268, which also showed low levels of AFP (MOM = 0.37).
AF 269 Wk 21.0	AFP = 12.36 ± 1.55 µg/ml MOM = 1.07 ± 0.10	The AF269 sample was targeted as an NTD negative screen in the upper gestational age screening range. All labs categorized AF269 as a negative NTD screen specimen. This specimen had no maternal serum counterpart.
AF 270 Wk 20.0	AFP = 19.25 ± 2.30 µg/ml MOM = 3.03 ± 0.31	The AF270 sample was targeted for a screen positive AFAFP value in the upper gestational age range. All labs reported this specimen as a screen positive AFAFP value. The AF270 specimen was paired with maternal serum sample MS270, which was negative (MOM = 2.02). Please see Critique below for further discussion of samples MS270 and AF270.

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 16	**Sample #**	**FT 266**	**FT 267**	**FT 268**	**FT 269**	**FT 270**
	Gestational Age (weeks)	13.0	11.9	11.5	12.5	11.2
Maternal Race	Ethnic Group	Black	Asian	White	White	Hispanic
Maternal Weight	Pounds (lbs)	150	120	125	130	135
Maternal Age	Years	29	30	28	25	28
Nuchal Translucency (NT)-Associated Measurements	Crown Rump Length (mm)	67	53	48	61	45
	NT Thickness (mm)	1.10	1.20	1.20	2.80	1.10
	NT - MOM	0.67 ± 0.04	0.90 ± 0.07	0.98 ± 0.07	1.86 ± 0.14	0.95 ± 0.07
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	60.64 ± 8.51	63.09 ± 9.47	89.77 ± 14.47	142.79 ± 28.56	68.89 ± 11.97
	MOM ± Std. Dev.	0.92 ± 0.12	0.77 ± 0.09	1.07 ± 0.10	1.95 ± 0.27	0.83 ± 0.08
Pregnancy-Associated Plasma Protein–A (PAPP-A)	Mean ng/mL*** ± Std. Dev.	804.66 ± 85.53	609.34 ± 51.54	2094.87 ± 161.54	307.53 ± 41.89	473.15 ± 40.03
	MOM ± Std. Dev.	0.86 ± 0.51	0.88 ± 0.52	3.31 ± 1.50	0.37 ± 0.23	0.93 ± 0.52
Trisomy-21 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(+) (100%)	(-) (100%)
	Recommended Action NFA**	NFA	NFA	NFA	G = 93% U = 47% A = 53% C = 47%	NFA
	Risk Estimate	5,800	5,800	12,000	1 in 6	7,600
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	(-) (100%)	(-) (100%)	(-) (100%)	(-) (80%)	(-) (100%)
	Recommended Action	NFA	NFA	NFA	NFA	NFA
	Risk Estimate	10,000	10,000	10,000	109	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; C = chorionic villus sampling; N = number of labs participating; FT = First Trimester.

**This percentage is normalized to labs requesting further action.

***Results from methods that give IU/ml were converted to ng/ml as described in section D.1 below.

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:
N = 16 all-lab Consensus Values.

Sample#	Summary Comments:
FT 266 Wk 13.0	This specimen was obtained from a 29 year old Black woman of normal body weight (150 lbs.). Her gestational age at the time of screening was 13.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative with all testing Labs in agreement. The FT266 risk estimate for Trisomy-21 was 1 in 5,800, while the Trisomy-18 risk was 1 in 10,000.
FT 267 Wk 11.9	This specimen was obtained from a 30 year old Asian woman of average body weight (120 lbs.). Her gestational age at the time of screening was 11.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative and all testing Labs were in agreement. The FT267 risk estimate for Trisomy-21 was 1 in 5,800, while the Trisomy-18 risk was 1 in 10,000.
FT 268 Wk 11.5	This specimen was obtained from a 28 year old White woman of average body weight (125 lbs). Her gestational age at the time of screening was 11.5 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen negative and all testing Labs were in agreement. The FT268 risk estimate for Trisomy-21 was 1 in 12,000, while the Trisomy-18 risk was 1 in 10,000.
FT 269 Wk 12.5	This specimen was procured from a 25 year old White woman of average body weight (130 lbs.). Her gestational age at the time of screening was 12.5 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. This FT specimen was screen positive for Trisomy-21 and all testing Labs were in agreement (see Critique). The FT269 risk estimate for Trisomy-21 was 1 in 6, while the Trisomy-18 risk was 1 in 109.
FT 270 Wk 11.2	This specimen was procured from a 28 year old Hispanic woman with a body weight of 135 lbs. Her gestational age at the time of screening was 11.2 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative for Trisomy-21 and Trisomy-18. The Trisomy-21 risk estimate for FT270 was 1 in 7,600, and the Trisomy-18 risk was 1 in 10,000. All labs were in agreement with both screen assessments.

III. Critique and Commentary:

A) Second Trimester Maternal Serum and Amniotic Fluid:

In general, the all-lab results were consistent with the targeted values for the NTD and the Trisomy Screens for risks, and outcomes. The Caucasian maternal serum sample **MS270** was targeted as negative specimen for NTD (Figs. 1 and 3) and was matched to an elevated **AF270** sample (Fig. 3 & 4; see discussion below). Most labs (82%) agreed that specimen MS270 was screen negative for NTD and negative for both Trisomy screens, and that AF270 was elevated for AFP (see below). The MS270 sample generated no recommendations for further action. Sample **MS268** was obtained from a black woman with a prior family (sibling) history of pregnancy complications. The T21 MOM results for specimen MS268 (MSAFP-MOM = 0.37, MSuE3-MOM = 0.61, MShCG-MOM = 1.55, DIA-MOM = 1.56) were consistent with a T21 positive screen; thus, most labs (71% triple and 96% quad) classified this specimen as T21 screen positive and recommended the following further action. The T21-related recommended action for MS268 triple screen was genetic counseling, 50%; ultrasound, 57%; and amniocentesis, 57%; while the quad test recommended action was genetic counseling, 73%; ultrasound, 81% and amniocentesis was 85%. The MS268 sample produced a risk from the quad test of 1 in 31 and a triple test risk of 1 in 50. Two other specimens, **MS267**, and **MS269** produced negative screens for NTD, T21, and T18; corrections for body weight were indicated in some instances. The **MS266** specimen is a special case involving reduced levels of MSAFP and MShCG will be discussed below.

Although the **MS270** sample was screen negative for NTD, T21, and T18, the amniotic fluid sample paired with this specimen was problematic. The **AF270** sample was determined to have an elevated AFP value by all participating laboratories. This mock patient had been referred to a tertiary care medical center for an amniocentesis due to a family history of pregnancy complications and poor outcomes in several extended and close family members. The maternal serum sample was obtained prior to the amniocentesis, and following amniocentesis, the post-procedure AF specimen (untainted by color) together with the MS sample was then analyzed at a tertiary care center. The AF270 (but not MS270) sample was determined to be screen positive for NTD. One possible cause of an unexplained elevated AFAFP is due to a fetal bleed from needle penetration during the invasive amniocentesis procedure. Less than 1% contamination of fetal blood into the amniotic fluid is sufficient to cause the AFAFP elevation reported by all participating laboratories. In a real-life situation, a fetal hemoglobin and acetylcholinesterase assays would be indicated. The final outcome in this mock patient showed that level-II diagnostic ultrasound showed no presence of a neural tube defect or any other anomaly and a diagnostic Ache band was lacking following gel electrophoresis. In retrospect, AF270 would be deemed a false positive amniotic fluid sample based on the later diagnostic results possibly due to a fetal-anmiotic fluid bleed.

The specimen **MS268** was designed to represent a positive screen for Down Syndrome with the typical MS profile of low MSAFP, low MSuE3, and elevated MShCG constituting the classical "triple test". Since the year 2000, the addition of maternal serum dimeric inhibin-A (MS-DIA) as the fourth constituent of the quad testing platform has considerably improved the prenatal screen for Down Syndrome. With the addition of MS-DIA in second trimester screening, the detection rate has been found to increase to 75% (from 65%) while maintaining a 5% false positive rate (58). In the case of specimen MS268, the MS-DIA MOM value of 1.56 increased the risk value of 1 in 50 (triple test) to a greater risk of 1 in 31 (quad test). This increased risk screen was further exemplified by the "further actions" reported by the participating laboratories (see Table-1). Moreover, a positive T21 screen for MS268 using the triple test was only reported by 71% of laboratories compared to a 96% consensus positive screen from labs using the quad test.

Although the **MS266** sample was screen negative for NTD, T 21 and T 18, two of the analytes in this specimen were of special interest. The MS266 sample was determined to have both low MSAFP (MOM = 0.43) and low MShCG (MOM = 0.46) values by all participating laboratories. This mock patient had been referred to a tertiary care medical center for a consultation due to a family history of pregnancy complications and poor outcomes in several extended and close family members. The maternal serum sample was then sent to a prenatal biomarker screening and tertiary care center following the consultation but amniocentesis had not yet been performed. The MS266 patient was a suspected candidate for Edward's Syndrome (T18), which denotes an abnormality of extra chromosome 18 material. The final outcome in this mock patient showed a normal karotype and level-II diagnostic ultrasound revealed no presence of trisomy-related defects or any other structural or anatomic anomaly. In retrospect, MS266 with both reduced AFP and hCG was screen negative and was only deemed suspicious for T 18 based on those two analyte results.

Reduced levels of both MSAFP and MShCG in the same specimen in triple and quad testing can be a cause for concern in the screening laboratory. Decreased MOM values in all three analytes of the triple test would classically signal a risk for T18 requiring confirmation of the fetal karyotype from the amniocyte cells. However, specimen MS266 in the present mailout demonstrated normal MSuE3 (MOM=1.0) levels in conjunction with the reduced levels of both MSAFP (MOM=0.43) and MShCG (MOM=0.46). As stated above, such analyte profiles could lead to a false positive screen result of T18. One such report found that false positive T18 results tended to occur in mothers that were heavier in body weight and younger in age (29). The authors of this latter report indicated that such patients were not at increased risk to develop pregnancy complications and that the screening result may be related to an inadequate correction for increased maternal body weight. In fact, maternal body weight adjustments applied to all three analytes of the triple test have been found to considerably reduce the false positive rate for T18 screens without affecting the detection rate (35). In another related study, fetuses afflicted with T18 showed a significantly lower body weight distribution when compared to control fetuses and Down Syndrome (T21) fetuses (30). Interestingly, the corresponding MSAFP MOM values in that particular study were 0.72 for the T21 fetuses and 0.51 for the T18 fetuses. Hence, fetal weight could partially account for the lower MOM levels noted in the MSAFP measurements and possibly the other analytes since MShCG MOMs from T18 pregnancies have been

reported to be as low as 0.15 (36). In some instances where reduced MShCG accompanied low MSAFP, fetal death was reported to occur in the 15th to 16th gestational week period (31). Overall, low MSAFP and MShCG screening levels should prompt ultrasound examinations to check fetal vitality and to rule out hydatidiform molar pregnancies (34). Molar pregnancies are also referred to as gestational trophoblastic disease, hydatidiform mole, or simply as a "mole" of pregnancy.

The clinical significance of isolated low (but not absent) MSAFP has been studied extensively since the 1980's. Other than incorrect gestational age dating, the results derived from large pregnancy cohort studies have concluded that low MSAFP (<0.25 MOM) was associated with fetal loss and accounted for increased fetal wastage in the second trimester (41). However, the landmark discovery in 1984 of low MSAFP levels linked to fetal chromosomal abnormalities laid the foundation for the trisomy prenatal screening programs presently in use (Merkatz et al, 1984, Ref #58). This report provided the impetus for the use of low MSAFP (and later other analytes) for the prenatal detection of fetal autosomal trisomies, especially Down Syndrome and Edward's Syndrome. In a subsequent study of 27,000 pregnancies, researchers confirmed that MSAFP MOMs of <0.82 correlated with the presence of a Down Syndrome fetus (42). A report by Simpson et al then showed that many trisomic fetuses, together with other chromosomal disorders, were identified by the use of MSAFP values at 0.4 MOMs or less in pregnant women from 27 to 31 years old (43). An ultrasonographic study by Nelson et al. subsequently demonstrated that MSAFP values of 0.2 MOM or lower correlated with fetal demise, hydatidiform mole, non-pregnancy, and adverse outcomes in term pregnancies (44). The latter study was also the first to establish that decreased amniotic fluid AFAFP levels (ranging from values 0.09 to 0.41 MOMs) were found to accompany fetuses involving T21 and T18 trisomies (45). In contrast to the pathological conditions of the fetus, a study reported that reduced MSAFP MOMs did not correlate with birth weight, gestational age, arterial cord blood pH, and APGA scores at birth (46). Rather, low MSAFP MOMs were correlated with conditions such as missed abortions, blighted ova, and fetal viability; thus, low AFP is strongly associated with fetal death (47). Finally, a pregnant woman displaying low MSAFP was reported to have a paternal balanced translocation in association with an aneuploid fetus which is addressed below (58).

Although the relationship of low MSAFP with fetal chromosomal abnormalities is now well-established, the cause remains an unexplained phenomenon. Nonetheless, after generating years of prenatal screening results involving low or reduced MSAFP, a growing list of linked autosomal and sex chromosome defects have been compiled. Such chromosome abnormalities (syndromes) include: a) Spondylocostal dysplasia-1, (Jarcho-Levin Syndrome) (48); b) sex chromosome monosomy (49); c) dup (22q) Syndrome (50); d) Brachmann-de Lange Syndrome (51); e) del (15)(q11q13), (Prader-Willi Syndrome) (52); f) mosaicism isochromosome 20q (53); g) 48XXYY Syndrome (54); h) 9q (9q22.3-q31.3) (55); and i) Trisomy-9 (40). These chromosomal abnormalities manifest in syndromes consisting of anatomical defects such as disorganization of skeletal bone elements (Jarcho-Levin); obigohydraminos; intra-uterine growth retardation (IUGR); facial malformations and cardiac defects (dup22q); nuchal cystic hygroma, mental retardation, hirsutism, microbrachycephaly, with limb deformations (Brachmann-de Lange); short bone lengths (Prader-Willi); agenesis of the corpus callosum and deformed facial features (48XXYY); fetal macrocephaly and overgrowth (Gorlin Syndrome) (56), and renal pyelectasis (56).

As stated above, a molar pregnancy is an abnormality of the placenta caused by a problem at the time the egg and sperm are fused at fertilization. When MShCG alone is present in low or reduced levels, it can be associated with a mole, which mimics a healthy pregnancy and shows an excess of XXX or XXY karyotypes (32). Low levels of isolated MShCG alone or in combination with other low level screening analytes have been found in various other chromosomal disorders. The most common is T18 which has been shown to demonstrate MShCG MOMs of 0.05 (36). In one report of multiple T18 cases, 44% of those pregnancies exhibited MShCG MOMs less than 0.25 (37). In other studies, nearly 10% of all chromosomally abnormal fetuses demonstrated MShCG MOM values of less than 0.30; in contrast, oligohydramios and macrosomia (non-genetic defects) pregnancies had higher values ranging between 0.85 to 1.0 MOM (38, 39).

Consistent with reports of low MShCG in association with chromosomal abnormalities, a case report of Trisomy-9 has been described and diagnosed in a pregnancy exhibiting low free beta-hCG levels as a result of a

nondisjunction error in the maternal genome (40). Using ultrasonography, the Trisomy-9 disorder manifested structural anomalies that included intrauterine growth restriction, congenital diaphragmatic hernia, fetal ascites, horseshoe kidney, and low amniotic fluid levels. The skeletal bone anomalies in this disorder included facial dysmorphism and limb deformities. The low MS free beta-hCG levels were associated with the presence of an elevated AFAFP level reflecting an open sacral spina bifida coexistent with a myelomeningocoele. The karyotype of the afflicted fetus was reported to be 47XX+9 representative of a Trisomy-9 (40).

There are instances in some pregnancies where low MShCG levels are present while MSAFP levels are either normal or elevated. In one such study, increased MSAFP levels and decreased MS free beta-hCG levels showed significance in predicting adverse outcomes in fetal conditions such as low birth weight, IUGR, preterm delivery, and stillbirths, displaying risks up to 6-fold that of cases with normal analyte levels (33). Pregnancies that were deemed screen positive for T18 can also exhibit normal MSAFP, together with low hCG, low uE3, and low inhibin-A levels (32). In a separate pregnancy study involving Trisomy-9, a primigravida woman displayed an elevated MSAFP together with a low MS free beta-hCG level (40). In that report, an amniocentesis followed by karyotyping revealed the Trisomy-9 was accompanied by elevated AFAFP levels; this fetus also displayed multiple anatomic abnormalities. In another large study, it was found that women's screens demonstrating variable triple test analyte results (elevated or reduced) were sometimes present with adverse pregnancy outcomes in pregnancies of normal appearing newborns (39). Lastly, it was determined that the second trimester analytes exhibiting high MSAFP and low MShCG levels were useful predictors of congenital heart defects in pregnancies without chromosomal defects (57).

In the clinical setting, it has been recommended that pregnant women with unexplained isolated or combined levels of low AFP, hCG, uE3, or inhibin-A levels in the second trimester should receive normal antenatal care, as this pattern of analytes has not been sufficiently studied concerning adverse perinatal outcomes (54). However, in second trimester screening, low levels of 2 of the 3 analytes in the triple test, and 3 or 4 analytes in the quad test are likely to trigger a screen risk for chromosomal defects such as T18, especially when MShCG and MSuE3 are involved. In comparison, an unexplained low PAPP-A (<0.4 MOM) and/or a low hCG (<0.5 MOM) in the first trimester has been associated with an increased frequency of adverse obstetrical outcomes; however, at present no specific protocols for treatment are available (54).

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

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in a bar-graph format (Figs. 7-10) for each of the five MS samples. As shown in Figs. 7A and 8A, AFP and uE3 mass measurements in serum among the individual kits mostly agreed, although the values from the Siemens DPC Immulite kits were about 10% lower for AFP, and 5-10% higher for uE3 than those obtained **with** Beckman instruments. In contrast, when the kit specific uE3 MOMs were compared, values from Siemens DPC Immulite 2000/2500 and the New Siemens DPC Immulite 2000/2500 ranged from 20 to 50% higher than those from Beckman (Fig. 8B). Regarding the hCG kits (Fig. 9), the two Beckman instruments (Access 2 and UNICEL DXI) yielded similar mean hCG values, while the Siemens DPC Immulite/2000 results were 10-20% lower than those from the other assay platforms. Finally, the method comparison for Inhibin-A displayed in Fig. 10 shows that the results from the Beckman Access/2 or Unicel were similar, whereas the results from the Diagnostic Systems Lab (DSL) assay platform were 20-30% lower.

Interestingly, when the AFP measurements in amniotic fluid were compared, the differences among the various methods seemed somewhat larger than in serum (Fig. 7B). In particular, results from the Abbott Asxym were 15-30% higher, Beckman Unicel DXL instruments were about 5-10% lower, with the results from the other instruments somewhere in between. Since these specimens are derived from actual AF samples, these levels would be comparable to real patient testing**.**

C) Second Trimester Screening Software Utilized:

The alpha and Benetech software packages were each used by 30% and 26%, of the labs, respectively; Robert Maciel (RMA) software was employed by 30%; and in-house and "other" softwares comprised 15%. Labs using programs classified as "other" are presumably proprietary software packages.

D) First Trimester Screen:

Five first trimester maternal serum mock samples were provided in the present mailout. All laboratories that are **validation-approved** and presently perform first trimester Down syndrome screening are **REQUIRED** to test and report screen results; however, the laboratory results will **not** be graded at this time. Those laboratories not presently offering the test, nor planning to implement the test, can request that no further samples be sent to them. The FT sample (FT = first trimester) information provided to participating labs included maternal age, nuchal translucency (NT measurements in millimeters), last menstrual period (LMP), crown-rump length (CRL) measurements, race, maternal body weight, and draw date.

As demonstrated in FT Table 2, Section II, the all lab measurement of the 13.0 week Black **FT266** specimen for total hCG resulted in a mass mean of 60.64 IU/ml ± 8.51, with a MOM of 0.92 (Table 2). Furthermore, the all-lab mass mean for PAPP-A was 804.66 ± 85.53 ng/ml with a MOM of 0.86 ± 0.51. This resulted in an all-lab T21 risk assessment of 1 in 5,800 for the FT266 specimen consistent with a negative screen (Fig. 13). Thus, the FT266 sample resulted in a 100% T21 negative screen assessment.

The all lab measurement of the 11.9 week Asian **FT267** specimen for total hCG resulted in a mass mean of 63.09 ± 9.47 IU/ml, with a MOM of 0.77; the all-lab mass mean for PAPP-A was 609.34 ± 51.54 ng/ml with a MOM of 0.88 ± 0.52; and the all-lab T21 risk assessment was 1 in 5,800. The FT267 sample resulted in a 100% T21 negative screen assessment. No further action was indicated. Finally, the FT267 specimen screened negative for T18 (1 in 10,000) using a cutoff of 1 in 100 (Figs. 13, 14).

In the **FT268** White specimen, the gestational age all-lab mean was reported as 11.5 weeks. Assay measurements for FT268 resulted in an all-lab total hCG mass measurement of 89.77 ± 14.47 IU/ml (MOM = 1.07 ± 0.10), while the all-lab PAPP-A mass assessment was 2094.87 ± 161.54 ng/ml (MOM = 3.31 ± 1.50). All labs agreed that the FT268 sample was screen negative for T21 with a risk assessment of 1 in 12,000 (Fig. 13). The all-lab T18 risk assessment for FT268 was 1 in 10,000; hence, the FT268 specimen resulted in a negative screen for T18 (Fig. 14).

As shown in Table 2 for the **FT269** white specimen, the gestational age all-lab mean was reported as 12.5 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 142.79 ± 28.56 IU/ml (MOM = 1.95 ± 0.27) and an all-lab PAPP-A mass measurement of 307.53 ± 41.89 ng/ml (MOM = 0.37 ± 0.23). The all-lab T21 screen consensus for FT269 was positive with a risk assessment of 1 in 6. Further actions recommended by the labs included genetic counseling, 94%; ultrasound, 47%; and amniocentesis/CVS = 69/50%. Finally, the FT269 specimen screened negative (73%) for T18 (1 in 109) using a risk cutoff of 1 in 100.

For the Hispanic **FT270** specimen, the gestational age all-lab mean was reported as 11.2 weeks. Assay measurements resulted in an all-lab total hCG mass measurement of 68.89 ± 11.97 IU/ml (MOM = 0.83 ± 0.08) while the all-lab PAPP-A mass assessment was 473.15 ± 40.03 ng/ml (MOM = 0.93 ± 0.52). The all-lab FT T21 risk assessment was 1 in 7,600 and all labs agreed that the FT270 sample was negative for T21 (Fig. 13). The FT270 specimen also resulted in a negative screen for T18 with an all-lab risk assessment of 1 in 10,000.

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

In order to compare the new Beckman Access 2/Unicel assays (60% users) for PAPP-A with those of the older Siemens Immulite and DSL assay platforms, a conversion factor was calculated from participating labs from the last five PT mailouts. Since there was a shift in the Siemens slope from the pre-2011 PT samples (FT 241-260), we used only the last 10 data points (FT samples 261-270) to calculate the conversion factor. Beckman Access 2/Unicel (y-axis) data for PAPP-A in ug/ml were plotted (Fig. 15A) versus Siemens Immulite 2000 (x-axis) data in mIU/ml, yielding a linear correlation with an R^2 value of 0.9735 and a slope of 0.158. In Fig. 15B, Beckmann Access2/Unicel PAPP-A values (y-axis) were plotted against DSL PAPP-A values (y-axis), yielding a second degree polynomial correlation with an R^2 value of 0.9948. Using the respective correlation equations allowed us to convert mIU/ml values into ng/ml and to directly compare Beckman Access 2/Unicel PAPP-A mass units of ng/ml to the mIU/mL mass units generated by Siemens Immulite and DSL (Fig. 12A). However, for grading purposes, each lab's results were compared to their own peer group without conversion.

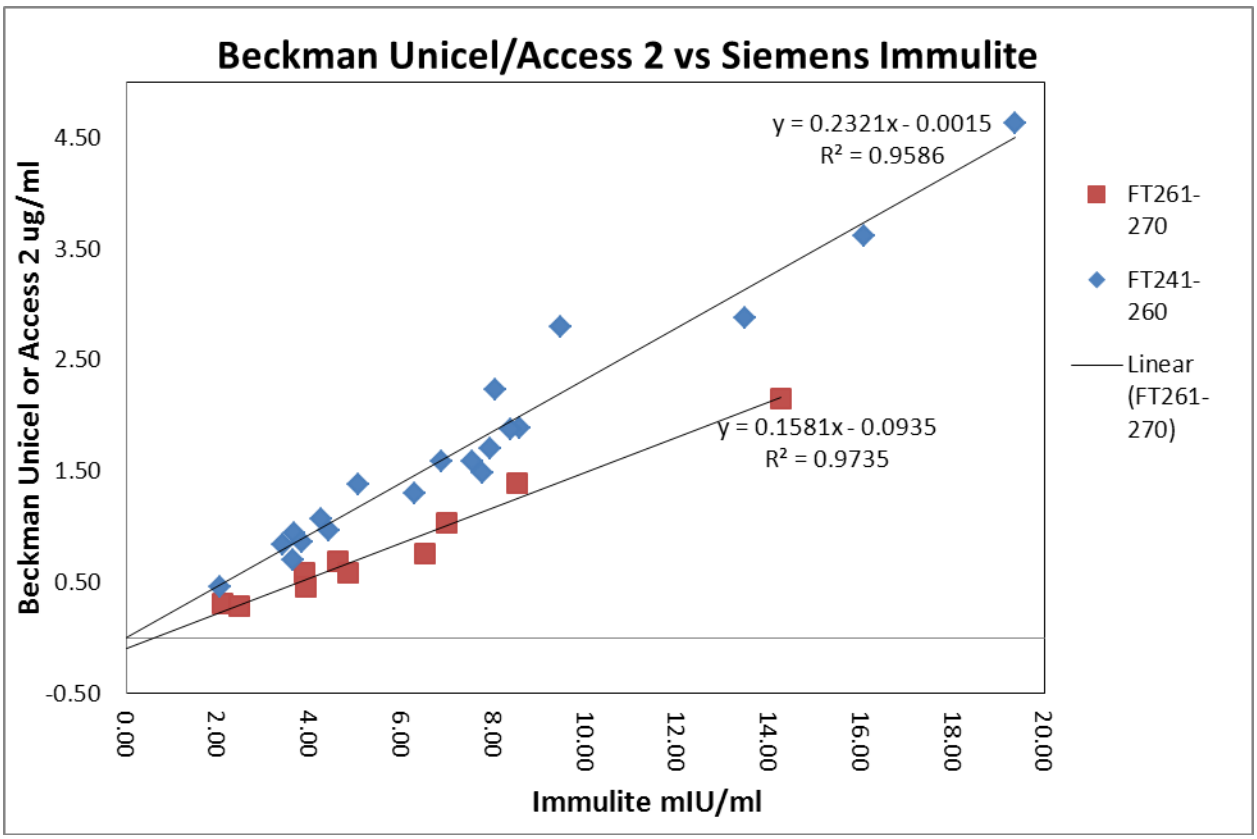


Fig. 15A

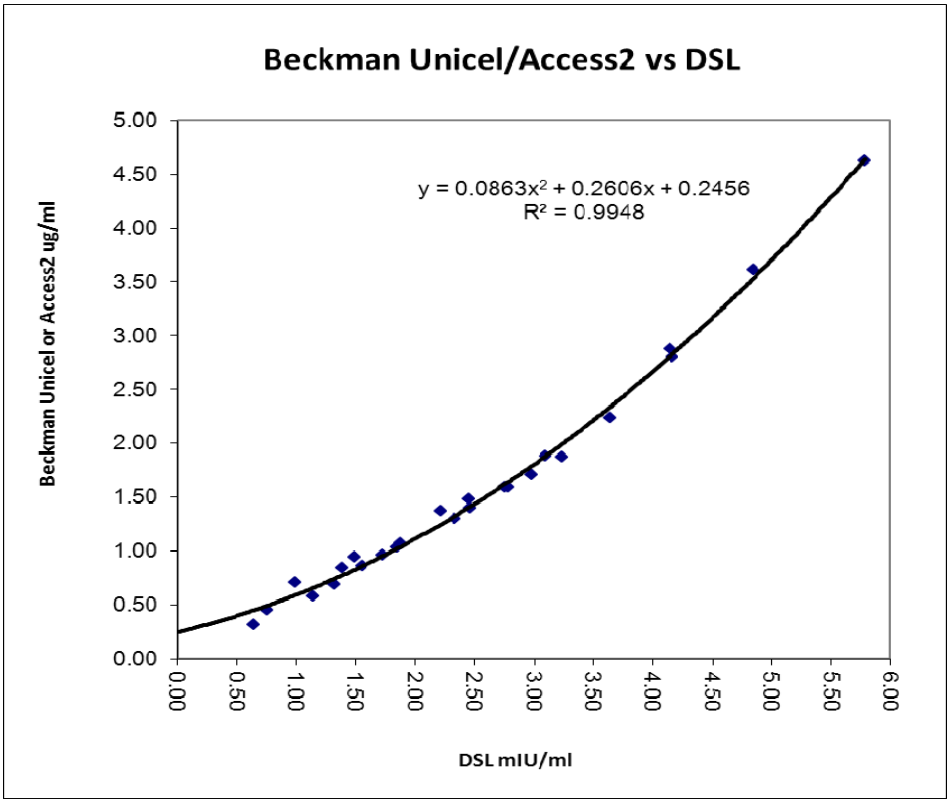


Fig. 15B

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in Figs. 11 and 12 for each of the five FT samples. As shown in Fig. 11, hCG measurements between the two Beckman instruments were similar, while the Siemens Immulite instruments measured approximately 20-30% lower the Beckman Access 2/Unicel instruments. The results from the three PAPP-A kits, when converted to the same mass units, were relatively consistent among each other. In contrast, when the PAPP-A kit MOMs were compared; those from Siemens Immulite were more than double those from DSL and Beckman (Fig. 12B).

E) <u>First Trimester Screening Software Utilized:</u>

The alpha and Benetech software packages were each used by 33% and 13% of the labs, respectively; Robert Maciel (RMA) software was employed by 33%; and in-house software comprised 20%. None of the labs used programs classified as "other" which are proprietary software packages.

<u>G.J. Mizejewski, Ph.D.</u>

New and Related References (Suggested reading):

1. Beta, J., F. E. Bredaki, et al. "Maternal Serum alpha-Fetoprotein at 11-13 Weeks' Gestation in Spontaneous Early Preterm Delivery." Fetal Diagn Ther, 2011.
2. Papoutsis, D., S. Mesogitis, et al. "Partial molar pregnancy with a chromosomically and phenotypically normal embryo: presentation of an extremely rare case and review of literature." J Matern Fetal Neonatal Med, 2011.
3. Jelliffe-Pawlowski, L., R. Baer, et al. "Second trimester serum predictors of congenital heart defects in pregnancies without chromosomal or neural tube defects." Prenat Diagn, 2011.
4. Trabert, B., M. P. Longnecker, et al. "Placental characteristics as a proxy measure of serum hormone and protein levels during pregnancy with a male fetus." Cancer Causes Control, 2011.
5. Tongprasert, F., C. Wanapirak, et al. "Maternal serum hCG, PAPP-A and AFP as predictors of hemoglobin Bart disease at mid-pregnancy." Prenat Diagn, 2011.
6. Ree, P. H., W. B. Hahn, et al. "Early Detection of Preeclampsia Using Inhibin A and Other Second-Trimester Serum Markers." Fetal Diagn Ther, 2011.
7. Sprawka, N., G. Lambert-Messerlian, et al. "Adjustment of maternal serum alpha-fetoprotein levels in women with pregestational diabetes." Prenat Diagn **31**(3): 282-5, 2011.
8. Sikovanyecz, J., E. Horvath, et al. "Fetomaternal transfusion after amniocentesis and cordocentesis." Ir J Med Sci, 2010.
9. Sikovanyecz, J., E. Horvath, et al. "Role of the vascular endothelial growth factor in the inverse relationship between increased nuchal translucency thickness and fetomaternal transfusion." J Perinat Med **39**(2): 157-61, 2010.
10. Knapik, D. and A. Olejek "[Analysis of cervicovaginal fluid in the diagnosis of premature rupture of membranes]." Ginekol Pol **82**(1): 50-5, 2011.
11. Castellotti, B., C. Mariotti, et al. "Ataxia with oculomotor apraxia type1 (AOA1): novel and recurrent aprataxin mutations, coenzyme Q10 analyses, and clinical findings in Italian patients." Neurogenetics, 2011.
12. Tang, J., D. Tang, et al. "A novel strategy for ultra-sensitive electrochemical immunoassay of biomarkers by coupling multifunctional iridium oxide (IrO(x)) nanospheres with catalytic recycling of self-produced reactants." Anal Bioanal Chem, 2011.
13. Thangaratinam, S., J. Langenveld, et al. "Prediction and primary prevention of pre-eclampsia." Best Pract Res Clin Obstet Gynaecol, 2011.
14. Mussa, A., G. B. Ferrero, et al. "Neonatal hepatoblastoma in a newborn with severe phenotype of Beckwith-Wiedemann syndrome." Eur J Pediatr, 2011.
15. Abt, D., M. Meier, et al. "Hereditary persistence of alpha-fetoprotein mimicking testicular cancer in a patient with acute epididymitis." Scand J Urol Nephrol, 2011.
16. Miao, Z. Y., X. Liu, et al. "[First trimester and second-trimester integrated screening for Down's syndrome]." Zhonghua Yi Xue Za Zhi **91**(3): 185-8, 2011.
17. Aoyagi, Y., Y. Tamura, et al. "History and recent progress in evaluation of the fucosylated alpha-fetoprotein fraction." J Gastroenterol Hepatol **26**(4): 615-6, 2011.
18. van Deuren, M., M. Verhagen, et al. "Hereditary persistence of alpha-fetoprotein." Neth J Med **69**(2): 96, 2011.
19. King, C. R. "A novel embryological theory of autism causation involving endogenous biochemicals capable of initiating cellular gene transcription: A possible link between twelve autism risk factors and the autism 'epidemic'." Med Hypotheses **76**(5): 653-60, 2011.
20. Njim, L., A. Moussa, et al. "[Malignant recurrence of "mature" sacrococcygeal teratoma. A clinicopathological study of 4 cases]." Rev Med Liege **66**(1): 25-9, 2011.
21. Abbass, K., M. K. Kaplon, et al. "Elevated Alpha-Fetoprotein Level: A Dilemma." J Gastrointest Cancer, 2011.
22. Nouso, K., Y. Kobayashi, et al. "Prognostic importance of fucosylated alpha-fetoprotein in hepatocellular carcinoma patients with low alpha-fetoprotein." J Gastroenterol Hepatol, 2011.
23. Koh, K. N., M. Park, et al. "Prognostic implications of serum alpha-fetoprotein response during treatment of hepatoblastoma." Pediatr Blood Cancer, 2011.
24. Wang, H., D. Sun, et al. "Electrochemiluminescence immunosensor for alpha-fetoprotein using Ru(bpy)(3)(2+)-encapsulated liposome as labels." Colloids Surf B Biointerfaces **84**(2): 515-9, 2011.
25. Satake, N., M. Chikakiyo, et al. "Gastric cancer with choriocarcinoma and yolk sac tumor components: case report." Pathol Int **61**(3): 156-60, 2011.

26. Braga-Neto, P., L. A. Dutra, et al. "Alpha-fetoprotein as a biomarker for recessive ataxias." Arq Neuropsiquiatr **68**(6): 953-5, 2011.
27. Meng, L., N. Gan, et al. "A three-dimensional, magnetic and electroactive nanoprobe for amperometric determination of tumor biomarkers." Int J Mol Sci **12**(1): 362-75, 2011.
28. Basaran A, Topatan B, Basaran M. The association between preeclampsia and placental disruption induced by chorionic villous sampling. Farina A et al. Prenatal Diagnosis 2010. Prenat Diagn 30:1230-1232.
29. Wenstrom KD, Owen J, Brumfield CG, Davis RO, Dubard M, Garcia T. Significance of a false-positive trisomy 18 multiple-marker screening test. Obstet Gynecol 90:938-942, 1997.
30. Hsu JJ, Hsieh CC, Chiu TH, Soong YK. [The relationship between weights of fetuses with autosomal trisomies and low maternal serum alpha-fetoprotein]. Changgeng Yi Xue Za Zhi 15:33-38, 1992.
31. Baschat AA, Harman CR, Farid G, Chodirker BN, Evans JA. Very low second-trimester maternal serum alpha-fetoprotein: Association with high birth weight. Obstet Gynecol 99:531-536, 2002.
32. Benn PA, Gainey A, Ingardia CJ, Rodis JF, Egan JF. Second trimester maternal serum analytes in triploid pregnancies: correlation with phenotype and sex chromosome complement. Prenat Diagn 21:680-686, 2001.
33. Spencer K. Second-trimester prenatal screening for Down syndrome and the relationship of maternal serum biochemical markers to pregnancy complications with adverse outcome. Prenat Diagn 20:652-656, 2000.
34. Jauniaux E, Bersinger NA, Gulbis B, Meuris S. The contribution of maternal serum markers in the early prenatal diagnosis of molar pregnancies. Hum Reprod 14:842-846, 1999.
35. Barkai G, Chaki R, Sochat M, Goldman B. Human chorionic gonadotrophin and trisomy 18. Am J Med Genet 41:52-53, 1991.
36. Miller CH, O'Brien TJ, Chatelain S, Butler BB, Quirk JG. Alteration in age-specific risks for chromosomal trisomy by maternal serum alpha-fetoprotein and human chorionic gonadotropin screening. Prenat Diagn 11:153-158, 1991.
37. Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn 7:623-630, 1987.
38. Sayin NC, Canda MT, Ahmet N, Arda S, Sut N, Varol FG. The association of triple-marker test results with adverse pregnancy outcomes in low-risk pregnancies with healthy newborns. Arch Gynecol Obstet 277:47-53, 2008.
39. Chen CP, Chern SR, Cheng SJ, Chang TY, Yeh LF, Lee CC, Pan CW, Wang W, Tzen CY. Second-trimester diagnosis of complete trisomy 9 associated with abnormal maternal serum screen results, open sacral spina bifida and congenital diaphragmatic hernia, and review of the literature. Prenat Diagn 24:455-462., 2004.
40. Davenport DM, Macri JN. The clinical significance of low maternal serum alpha-fetoprotein. Am J Obstet Gynecol 146:657-661, 1983.
41. Spencer K, Carpenter P. Screening for Down's syndrome using serum alpha fetoprotein: a retrospective study indicating caution. Br Med J (Clin Res Ed) 290:1940-1943, 1985.
42. Simpson JL, Baum LD, Marder R, Elias S, Ober C, Martin AO. Maternal serum alpha-fetoprotein screening: low and high values for detection of genetic abnormalities. Am J Obstet Gynecol 155:593-597, 1986.
43. Nelson LH, Burton BK, Sowers S. Ultrasonography in patients with low maternal serum alpha-fetoprotein. J Ultrasound Med 6:59-61, 1987.
44. Ashwood ER, Cheng E, Luthy DA. Maternal serum alpha-fetoprotein and fetal trisomy-21 in women 35 years and older: implications for alpha-fetoprotein screening programs. Am J Med Genet 26:531-539, 1987.
45. Simpson JL, Baum LD, Depp R, Elias S, Somes G, Marder R. Low maternal serum alpha-fetoprotein and perinatal outcome. Am J Obstet Gynecol 156:852-862, 1987.
46. Haddow JE, Hill LE, Palomaki GE, Knight GJ. Very low versus undetectable maternal serum alpha-fetoprotein values and fetal death. Prenat Diagn 7:401-406, 1987.
47. Romero R, Ghidini A, Eswara MS, Seashore MR, Hobbins JC. Prenatal findings in a case of spondylocostal dysplasia type I (Jarcho-Levin syndrome). Obstet Gynecol 71:988-991, 1988.
48. Winston YE, Horger EO, 3rd, Van Dorsten JP. Low serum alpha-fetoprotein level and sex chromosome monosomy. Am J Obstet Gynecol 159:186-187, 1988.
49. Johnson MP, Greb A, Goyert G, Drugan A, Qureshi F, Sacks AJ, Evans MI. Midtrimester diagnosis and anomalies in the dup(22q) syndrome: correlation of aneuploidy with low maternal serum alpha-fetoprotein and oligohydramnios. Am J Med Genet 36:94-96, 1990.
50. Bruner JP, Hsia YE. Prenatal findings in Brachmann-de Lange syndrome. Obstet Gynecol 76:966-968, 1990.
51. Le Bris-Quillevere MJ, Riviere D, Pluchon-Riviere E, Chabaud JJ, Parent P, Volant A, Boog G. Prenatal diagnosis of del(15)(q11q13). Prenat Diagn 10:405-411, 1990.

52. Richkind KE, Mahoney MJ, Evans MI, Willner J, Douglass R. Prenatal diagnosis and outcomes of five cases of mosaicism for an isochromosome of 20q. Prenat Diagn 11:371-376, 1991.
53. Nyberg RH, Karhu R, Karikoski R, Simola KO. The 48,XXYY syndrome: a case detected by maternal serum alpha-fetoprotein screening. Prenat Diagn 14:644-645, 1994.
54. Chen CP, Lin SP, Wang TH, Chen YJ, Chen M, Wang W. Perinatal findings and molecular cytogenetic analyses of de novo interstitial deletion of 9q (9q22.3-->q31.3) associated with Gorlin syndrome. Prenat Diagn 26:725-729, 2006.
55. Corteville JE, Dicke JM, Crane JP. Fetal pyelectasis and Down syndrome: is genetic amniocentesis warranted? Obstet Gynecol 79:770-772, 1992.
56. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 148:886-894, 1984.
57. Byrne-Essif K, Hoyme HE. Identification of a balanced translocation carrier by spouse's low maternal serum alpha fetoprotein associated with an aneuploid fetus. J Med Genet 27:728, 1990.
58. Knight GJ, Palomaki GE, Neveux LM, Haddow JE, Lambert-Messerlian GM. Clinical validation of a new dimeric inhibin-A assay suitable for second trimester Down's syndrome screening. J Med Screen 8:2-7, 2001.
59. Mohan A, Asselin J, Sargent IL, Groome NP, Muttukrishna S. Effect of cytokines and growth factors on the secretion of inhibin A, activin A and follistatin by term placental villous trophoblasts in culture. Eur J Endocrinol 145:505-511, 2001.
60. Chand AL, Harrison CA, Shelling AN. Inhibin and premature ovarian failure. Hum Reprod Update 16:39-50, 2010.
61. Welt CK. Regulation and function of inhibins in the normal menstrual cycle. Semin Reprod Med 22:187-193, 2004.
62. Reis FM, Di Blasio AM, Florio P, Ambrosini G, Di Loreto C, Petraglia F. Evidence for local production of inhibin A and activin A in patients with ovarian endometriosis. Fertil Steril 75:367-373, 2001.
63. Fujiwara T, Sidis Y, Welt C, Lambert-Messerlian G, Fox J, Taylor A, Schneyer A. Dynamics of inhibin subunit and follistatin mRNA during development of normal and polycystic ovary syndrome follicles. J Clin Endocrinol Metab 86:4206-4215, 2001.
64. Wenstrom KD, Chu DC, Owen J, Boots L. Maternal serum alpha-fetoprotein and dimeric inhibin A detect aneuploidies other than Down syndrome. Am J Obstet Gynecol 179:966-970, 1998.
65. Menon U, Riley SC, Thomas J, Bose C, Dawnay A, Evans LW, Groome NP, Jacobs IJ. Serum inhibin, activin and follistatin in postmenopausal women with epithelial ovarian carcinoma. BJOG 107:1069-1074, 2000.
66. Petraglia F, Florio P, Luisi S, Gallo R, Gadducci A, Vigano P, Di Blasio AM, Genazzani AR, Vale W. Expression and secretion of inhibin and activin in normal and neoplastic uterine tissues. High levels of serum activin A in women with endometrial and cervical carcinoma. J Clin Endocrinol Metab 83:1194-1200, 1998.

Abstracts

A). Screening Abstract “Picks-of-the-Month”:

(1) Title: Second trimester serum predictors of congenital heart defects in pregnancies without chromosomal or neural tube defects.

Source: Prenat Diagn, 2011, Feb. 24.

Authors: Jelliffe-Pawlowski, L., R. Baer, et al.

Abstract: OBJECTIVE: To compare euploid pregnancies with congenital heart defects (CHDs) to similar pregnancies without CHDs on typically collected second trimester biomarker measurements. METHOD: Second trimester serum levels of alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), and unconjugated estriol were compared for 306 CHD cases and 1224 no-CHD controls drawn from a sample of singleton pregnancies without chromosomal or neural tube defects (NTDs). Logistic regression models were built comparing biomarkers for cases and controls. RESULTS: Regardless of the severity of defect, CHD cases were more likely to have unusually high AFP and/or hCG levels and/or unusually low hCG and/or uE3 levels [odds ratio (OR) 1.8-2.4, 95% confidence intervals (CIs) 1.2-4.0]. Cases with critical CHDs were more than twice as likely to have an AFP multiple of the median (MoM) >/= the 95th percentile and/or an hCG and/uE3 MoM </= the 5th percentile (OR 2.1-3.9, 95% CIs 1.1-7.8). CONCLUSION:

Abnormal levels of specific second trimester maternal serum biomarkers indicated an increased risk for CHDs among this sample of low risk pregnancies. Our data suggest that future efforts aimed at improving CHD detection in low risk pregnancies may benefit from considering serum biomarkers.

(2) Title: Maternal Serum alpha-Fetoprotein at 11-13 Weeks' Gestation in Spontaneous Early Preterm Delivery.

Source: Fetal Diagn Ther, 2011, March 11.

Authors: Beta, J., F. E. Bredaki, et al.

Abstract: Objective: To examine the potential value of maternal serum level of alpha-fetoprotein (AFP) in the first trimester of pregnancy in the prediction of spontaneous early preterm delivery. Methods: Maternal serum concentration of AFP at 11-13 weeks' gestation was measured in a case-control study of singleton pregnancies delivering phenotypically normal neonates, including 33 cases with spontaneous delivery before 34 weeks and 99 matched controls delivering after 37 weeks. The median multiple of the median (MoM) serum AFP in the two outcome groups was compared and the bivariate gaussian distributions were simulated in a previously described screened population of 33,370 pregnancies to estimate the performance of screening for early delivery by a combination of maternal characteristics and obstetric history with serum AFP. Results: In the preterm delivery group compared to the term delivery group, the median serum AFP MoM was higher (1.33 vs. 0.97, p = 0.006). The estimated detection rate of preterm delivery, at a false-positive rate of 10%, from maternal characteristics and obstetric history was 27.5% and this increased to 36.0% with the addition of serum AFP. Conclusions: Measurement of serum AFP at 11-13 weeks improves the prediction of early preterm delivery provided by maternal characteristics and obstetric history.

(3) Title: Early Detection of Preeclampsia Using Inhibin A and Other Second-Trimester Serum Markers.

Source: Fetal Diagn Ther, 2011, Jan. 21.

Authors: Ree, P. H., W. B. Hahn, et al.

Abstract: Objective: The purpose of this study was to determine whether second-trimester maternal serum markers including inhibin A are useful for the detection of preeclampsia. Methods: Between January 2005 and March 2009, we analyzed the data of 4,764 subjects who underwent second-trimester multiple-marker screening for Down syndrome. Serum samples were assayed at 15+0 to 20+6 weeks for maternal serum alpha-fetoprotein (MSAFP), human chorionic gonadotrophin (hCG), unconjugated estriol (uE(3)) and inhibin A. We reviewed all medical records retrospectively, and assessed the relationships of several markers with preeclampsia using logistic regression analysis. Results: The study sample included 41 patients who developed preeclampsia and a control group consisting of the other 4,723 healthy subjects treated between January 2005 and March 2009. There were no significant differences in gestational ages at blood sampling, maternal weights, gravidity and parity between the two groups. However, the mean ages, Apgar scores, gestational age at delivery and neonatal weights were significantly different between the study group and the control group. The levels of markers in the study group were significantly increased compared to the control group, 1.76 +/- 2.68 for inhibin A, 1.18 +/- 0.69 for MSAFP, and 1.62 +/- 1.18 for hCG, but uE(3) did not differ significantly between the two groups. The AUC of inhibin A was 0.715, but the AUC of a three-marker combination model (0.800) was even better. A mid-trimester inhibin A concentration of 1.5 MoM or greater had a sensitivity of 60% and a false-positive rate of 16% for the prediction of preeclampsia. Inhibin A was the best predictor of preeclampsia. Three other markers were reliable predictive markers of preeclampsia. Conclusions: Inhibin A and other second-trimester serum markers may be useful for early detection of preeclampsia. Inhibin A was in fact the most important predictable marker among the

markers we surveyed. The results of this study support those of previous studies, and provide quantified data elucidating the occurrence of preeclampsia.

(4) Title: Maternal serum hCG, PAPP-A and AFP as predictors of hemoglobin Bart disease at mid-pregnancy.

Source: Prenat Diagn, 2011, Feb. 10.

Authors: Tongprasert, F., C. Wanapirak, et al.

Abstract: OBJECTIVE: To evaluate the ability of maternal serum-free beta-human chorionic gonadotrophin (beta-hCG), pregnancy-associated plasma protein-A (PAPP-A), and alpha fetoprotein (AFP) levels in the screening of fetuses with hemoglobin (Hb) Bart's disease among pregnancies at risk. MATERIALS AND METHODS: Pregnancies at risk for fetal Hb Bart's disease scheduled for cordocentesis at 18 to 22 weeks were recruited into the study. Maternal serum-free beta-hCG, PAPP-A, and AFP concentrations were measured before cordocentesis, and the final fetal diagnosis of Hb Bart disease was based on fetal Hb typing using high-performance liquid chromatography. RESULTS: Of 57 recruited pregnancies, 11 had fetal Hb Bart's disease and 46 were unaffected. Maternal serum alpha-fetoprotein (MSAFP) concentrations were significantly higher in women with fetal Hb Bart's disease than those with unaffected fetuses (median 99.53 vs 50.83, $P < 0.001$), whereas the concentrations of free beta-hCG and PAPP-A were not significantly different between the two groups ($P = 0.543$ and 0.777, respectively). CONCLUSION: Second-trimester MSAFP may be clinically a useful screening test for fetal Hb Bart's disease among pregnancies at risk.

B). Case History Screening “picks-of-the-month”:

(1) Title: Partial molar pregnancy with a chromosomically and phenotypically normal embryo: presentation of an extremely rare case and review of literature.

Source: J Matern Fetal Neonatal Med, 2011, March 17.

Authors: Papoutsis, D., S. Mesogitis, et al.

Abstract: We present an extremely rare case of partial molar pregnancy with a chromosomically and phenotypically normal embryo and review of the literature. A 31-year-old nulliparous was referred to us at 30 weeks of gestation due to absence of fetal movements and subsequent ultrasound examination revealed intrauterine demise. Prenatal amniocentesis due to raised maternal serum alpha-fetoprotein had shown a karyotypically normal female embryo and second trimester ultrasound demonstrated no anatomic abnormalities. Upon induction of labor with misoprostol, a phenotypically normal embryo was delivered and the placenta showed intermixed areas of marked hydatidiform villous change and normal parenchyma. Pathologic examination of the placenta confirmed the molar change of placenta. Two are the main theories discussed herein that explain the placental molar changes in singleton pregnancies: confined placental mosaicism (one case reported to date) and placental mesenchymal dysplasia (70 cases reported). Differential diagnosis is based on histopathologic features and genetic analysis of placenta.

(2) Title: Triploidy in a fetus following amniocentesis referred for maternal serum screening test at second trimester.

Source: Indian J Hum Genet **16**(2): 94-6, 2010.

Authors: Bagherizadeh, E., M. Oveisi, et al.

Abstract: Amniocentesis was carried out at 17 weeks gestation in a 27-year-old woman, following an abnormal maternal serum screening (MSS) test. MSS test was carried out primarily to estimate the risk of trisomy for chromosome 21. The maternal serum markers used were alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), and unconjugated estriol (uE3), together with maternal age. The fetus was identified as screen-positive for Edward's syndrome (trisomy 18), with low uE3, normal AFP and hCG levels. The calculated risk for trisomy 18 was more than 1:50. To identify any possible chromosomal abnormality, cytogenetic investigation was carried out on the amniotic fluid sample. The fetus's karyotype showed triploidy with 69, XXX chromosome complement in all the metaphase spreads obtained from three different cultures, using GTG banding technique. Upon termination of the fetus, gross abnormalities indicative of triploidy were present in the fetus.

(3) Title: Neonatal hepatoblastoma in a newborn with severe phenotype of Beckwith-Wiedemann syndrome.

Source: Eur J Pediatr, 2011, March 30.

Authors: Mussa, A., G. B. Ferrero, et al.

Abstract: Beckwith-Wiedemann syndrome is an overgrowth disorder characterized by neonatal macrosomia, abdominal wall defects, macroglossia, renal anomalies, organomegaly, hypoglycemia, and cancer predisposition. Hepatoblastoma is the second most frequent tumor and periodic serum alpha-fetoprotein (alphaFP) dosage is the cornerstone of the tumor surveillance for its early detection. In this report, we describe the outstanding case of a Beckwith-Wiedemann syndrome (BWS) newborn with severe phenotype and paternal chromosome 11 uniparental disomy (UPD11) associated with a high tumor risk. Based on the clinical picture and previous reports, a close monitoring of alphaFP was commenced. The marker was normal immediately after birth, but rapidly raised in 20 days, leading to the diagnosis of an extremely aggressive hepatoblastoma. The latter was successfully treated with pre-surgical reductive chemotherapy, gross total mass resection, and subsequent chemotherapy. Based on this observation, the tumor surveillance routinely suggested every 3 months should be more intense and with closer time intervals in newborns with severe BWS phenotype. We suggest monitoring neonatal alphaFP every 20 days in such cases.

C). News of Note: Abstract of New Markers:

(1) Title: Relationships between cell-free DNA and serum analytes in the first and second trimesters of pregnancy.

Source: Obstet Gynecol **116**(3): 673-8, 2010.

Authors: Vora, N. L., K. L. Johnson, et al.

Abstract: OBJECTIVE: To assess the relationship between first- and second-trimester cell-free DNA levels and maternal serum screening markers. METHODS: First- and second-trimester residual maternal serum samples from 50 women were obtained. First-trimester (pregnancy-associated plasma protein A and beta-hCG) and second-trimester serum analytes (beta-hCG, alpha-fetoprotein, unconjugated estriol, and inhibin A) had been measured at the time of sample receipt. All fetuses were male as confirmed by birth records. Cell-free DNA was extracted and measured by real-time quantitative polymerase chain reaction amplification using glyceraldehyde phosphate dehydrogenase and DYS1 as markers of total DNA and fetal DNA, respectively. Determination of linear associations between first- and second-trimester serum markers and cell-free DNA levels using Pearson correlations was performed. RESULTS: Statistically significant correlations between first-trimester pregnancy-associated plasma protein A multiples of the median and both total (r=0.36, P=.016) and fetal (r=0.41, P=.006) DNA in the first trimester were observed. There were no significant correlations between first-trimester serum human chorionic gonadotropin or

any second-trimester serum marker with DNA levels. CONCLUSION: Correlation between serum pregnancy-associated plasma protein A and first-trimester circulating cell-free fetal and total DNA levels is a novel finding. Pregnancy-associated plasma protein A is a glycoprotein of placental origin, and its correlation to cell-free fetal DNA in maternal serum suggests a common tissue origin through apoptosis of placental cells. However, because pregnancy-associated plasma protein A and cell-free DNA were only marginally correlated and cell-free DNA can be reliably detected in the first trimester, the addition of cell-free DNA to serum screening strategies may be helpful in predicting adverse pregnancy outcome. LEVEL OF EVIDENCE: II.

(2) Title: Is ultrasound alone enough for prenatal screening of trisomy 18? A single centre experience in 69 cases over 10 years.

Source: Prenat Diagn **30**(11): 1094-9, 2010.

Authors: Lai, S., W. L. Lau, et al.

Abstract: OBJECTIVES: To evaluate ultrasound scan and other prenatal screening tests for trisomy 18 in a regional obstetric unit and to review the management approach for women with positive trisomy 18 screening results. METHODS: Prenatal diagnosis databases were accessed to identify fetuses that had confirmed trisomy 18 karyotypes or were at high risk for trisomy 18 on second-trimester biochemical screening or first-trimester combined screening tests over a period of 10 years from 1 September 1997 to 30 September 2007. RESULTS: Sixty-nine women were confirmed to have trisomy 18 fetuses by karyotyping either prenatally (n = 61) or postnatally/post-miscarriage (n = 8) during the study period. The detection rate of ultrasound scan </= 14 weeks and 18 to 21 weeks to detect trisomy 18 was 92.7 and 100%, respectively. A total of 80 and 87% of fetuses had two or more ultrasound abnormalities detected in the </= 14 weeks and 18 to 21 weeks anomaly scans, respectively. Forty-eight women screened positive for trisomy 18 by second-trimester biochemical screening with human chorionic gonadotrophin (hCG) and alpha fetoprotein (AFP). Only one was true positive (positive predictive value = 1/48 or 2%). Eleven women screened positive for trisomy 18 by first-trimester combined screening with nuchal translucency scan and maternal serum for pregnancy-associated plasma protein A (PAPP-A) and hCG between 11 and 13 + 6 weeks. Three were true positive (positive predictive value = 3/11 or 27%). All four cases with positive screening had ultrasound abnormalities. CONCLUSIONS: Ultrasound scan for fetal anomalies is the most effective screening test for trisomy 18. A policy of conservative management for women with positive second-trimester biochemical screening or first-trimester combined screening for trisomy 18 is reasonable in the absence of ultrasound fetal abnormalities. Unnecessary invasive tests can be avoided.

(3) Title: A case of neonatal intrapericardial teratoma. Clinical and pathological findings.

Source: Acta Paediatr, 2011, Feb. 1.

Authors: Laforgia, N., G. Calderoni, et al.

Abstract: Aim: It is of general agreement that complete surgical removal after birth of intrapericardial fetal teratomas is needed, because of the risk of severe cardiovascular and respiratory distress, related to the mass size, location and secondary pericardial effusion. Histological examination generally shows mature aspect of cells and tissues. Methods: We present a case of grade II immature pericardial teratoma, diagnosed in utero and completely removed after birth. Results: Even surgical removal was complete, histological aspects raised the need of long follow-up with serial alpha-fetoprotein determinations. Conclusion: A neonatal grade II immature pericardial teratoma was completely removed after birth. The follow-up of the patient, until 10 months of life, was good with no recurrence of the disease.

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

(1) Title: [Evaluation of a new, microfluidic chip-based immunoassay for measurement of AFP-L3].

Source: Rinsho Byori **58**(12): 1155-61, 2010.

Authors: Sato, S., J. Toyota, et al.

Abstract: PURPOSE: AFP-L3 is an isoform of a-fetoprotein which has a fucosylated carbohydrate chain, and the fraction of AFP-L3/total AFP (AFP-L3%) specifically increases in hepatocellular carcinoma (HCC) patients and is widely used for screening and prognosis of HCC. The newly developed microTAS method which combines microchip electrophoresis and lectin affinity electrophoresis can rapidly provide AFP-L3% and total AFP measurements simultaneously at higher sensitivity. Here, we evaluated the system to know its analytical performance and clinical utility. METHOD: Fully automated immunoanalyzer, microTASWako i30 which utilizes Liquid-phase Binding Assay-Electrokinetic Analyte Transport Assay (LBA-EATA method) as the assay principle was employed for the measurement of total AFP and AFP-L3%. We evaluated detection sensitivity, precision, accuracy, and correlation of the method. RESULTS: The detection sensitivity was 0.3 ng/ml for both AFP-L1 and L3. The accuracy of the assay was 91.3-105.0% for total AFP. The precision of the assay was CV 1.9% at 2 ng/ml of total AFP, and CV 1.3% for 10% of AFP-L3% at 20ng/ml of total AFP. The microTAS method showed good correlation with the lectin affinity electrophoresis (AFP-L3 Test Wako) and the LBA methods (LBA Wako AFP-L3 on LiBASys) methods, giving correlation coefficient (r) of 0.988 and 0.988, respectively. The microTAS immunoreaction assay time and the total assay time including chip preparation were 1 and 9 min, respectively. CONCLUSION: Since the microchip assay is rapid and highly sensitive, it should have better clinical utility than the current methods.

(2) Title: Lens Culinaris Agglutinin-Reactive Fraction of Alpha-Fetoprotein as a Marker of Prognosis and a Monitor of Recurrence of Hepatocellular Carcinoma After Curative Liver Resection.

Source: Ann Surg Oncol, 2011, Feb. 20.

Authors: Zhang, X. F., E. C. Lai, et al.

Abstract: BACKGROUND: The aim of this study was to determine the role of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein (AFP-L3) as a prognostic marker and a monitor marker of recurrence after curative resection of hepatocellular carcinoma (HCC). METHODS: From December 2002 to May 2004, 395 consecutive patients with HCC who underwent curative partial hepatectomy were included in the study. The tumor characteristics and clinical outcomes of patients with positive preoperative and postoperative AFP-L3 were compared with those with negative results. RESULTS: A high ratio of AFP-L3 to total AFP was an indicator of pathologic aggressiveness. Patients with positive preoperative AFP-L3 had significantly earlier recurrence (median time to recurrence 22.0 +/- 2.4 months vs 45.0 +/- 6.9 months, $P < .001$) when compared with those with negative preoperative results. Significantly more patients with continuously positive or negative-turn-positive AFP-L3 results after surgery developed recurrence, particularly distant metastases, when compared with patients with continuously negative AFP-L3 results. The overall and disease-free survivals were significantly shorter in the positive than the negative preoperative AFP-L3 group. The overall and disease-free survivals were significantly shorter in the continuously positive and the negative-turn-positive than the continuously negative postoperative AFP-L3 group. CONCLUSION: Positive preoperative AFP-L3 and continuously positive or negative-turn-positive AFP-L3 results after surgery predicted a more aggressive tumor behavior, higher tumor recurrence, and poorer clinical outcomes. HCC patients with an increased proportion of AFP-L3 to total AFP should be more aggressively treated and closely followed-up.

(3) Title: Electrochemiluminescence immunosensor for alpha-fetoprotein using Ru(bpy)(3)(2+)-encapsulated liposome as labels.

Source: Colloids Surf B Biointerfaces **84**(2): 515-9, 2011.

Authors: Wang, H., D. Sun, et al.

Abstract: In this work, an electrochemiluminescence (ECL) immunosensor for ultrasensitive detection of alpha-fetoprotein (AFP) was fabricated using Ru(bpy)(3)(2+)-encapsulated liposome as the label and electrodeposited gold nanoparticles (GNPs) as the immobilizing support. Great signal amplification was achieved since liposome could encapsulate large amount of reporter molecules and GNPs could provide large active surface. Under optimized conditions, with sandwich type format, a linear range of AFP from 0.005 to 0.2pg/mL and an extremely low detection limit of 0.001pg/mL was obtained, much lower than that in previous reports. The proposed ECL immnuosensor showed high sensitivity, specificity, and good stability, which may open a new door to ultrasensitive detection of proteins in clinical analysis.

E). Special Abstract Selection:

(1) Title: "Prediction and primary prevention of pre-eclampsia."

Source: Best Pract Res Clin Obstet Gynaecol, 2011, March 29.

Authors: Thangaratinam, S., J. Langenveld, et al.

Abstract: Pre-eclampsia is associated with increased maternal and perinatal mortality and morbidity. Early recognition of women at risk of pre-eclampsia will enable the identification of high-risk women who may benefit from enhanced surveillance and prophylaxis. In this chapter, we summarise the accuracy of various tests used to predict the onset of pre-eclampsia and the effectiveness of preventative treatment. The tests used to predict pre-eclampsia include clinical history, examination findings, laboratory and haemodynamic tests. In general, tests in early pregnancy for predicting later development of pre-eclampsia have better specificity than sensitivity, as Body Mass Index greater than 34, alpha-fetoprotein, fibronectin and uterine artery Doppler (bilateral notching) all have specificities above 90%. Only uterine artery Doppler resistance index and combinations of indices have a sensitivity of over 60%. Test such as kallikreinuria not used in clinical practice, has shown high sensitivity above 80%, without compromising specificity, and require further investigation. None of the tests are sufficiently accurate to recommend them for routine use in clinical practice. The various treatment options for preventing pre-eclampsia include pharmacological agents, dietary supplementation and lifestyle modification. Antiplatelet agents, primarily low-dose aspirin, reduce the risk of pre-eclampsia by 10% (RR 0.90, 95% CI 0.84 to 0.97). Calcium effectively prevents pre-eclampsia (RR 0.45, 95% CI 0.31 to 0.65); the beneficial effect being observed in the high-risk group (RR 0.22; 95% CI 0.12 to 0.42) and in the group with low nutritional calcium intake (RR 0.36, 95% CI 0.20 to 0.65). Pharmacological agents, such as low molecular weight heparin, progesterone, nitric oxide donors, anti-hypertensive medication and diuretics are not effective in preventing pre-eclampsia. Dietary supplements, such as magnesium, anti-oxidants, marine oils and folic acid, do not reduce the incidence of pre-eclampsia. Evidence is lacking to support lifestyle preventative interventions for pre-eclampsia, such as rest, exercise and reduced dietary salt intake.

(2) Title: [First trimester and second-trimester integrated screening for Down's syndrome].

Source: Zhonghua Yi Xue Za Zhi **91**(3): 185-8, 2011.

Authors: Miao, Z. Y., X. Liu, et al.

Abstract: OBJECTIVE: To evaluate the effect of first and second-trimester integrated screening so as to provide an efficient screening protocol for Down's syndrome. METHODS: Using the dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA), the freebetahCG (beta human chorionic gonadotropin), PAPP-A (pregnancy associated plasma protein-A) and NT (nuchal translucency) value of type B ultrasound were assayed in the pregnancy serum during the first trimester (11-13W(+6) d) and free betahCG and AFP (alpha fetoprotein) during the second trimester(15-20W(+6) d). By the risk calculation software, the risks during both trimesters and their integrated risk were calculated for each patient respectively. Amniocentesis and venepuncture were employed for diagnosing the high-risk patients (> 1/270). Electronic network follow-up was carried out after delivery. RESULTS: In a total of 4237 pregnant women, 98 were found to carry a high risk during the first trimester, 241 during the second trimester and 101 during the integrated screening respectively. And 2, 3 and 4 cases were diagnosed with Down's symptom at a detection rate of 50%, 75% and 100% and a detection efficiency of 1:50, 1:80 and 1:25 respectively. CONCLUSION: Integrated screening is superior to either the first or second-trimester screening. With a lower false positive rate and a higher detection rate, it reduces the chance of invasive puncture. Advanced type B ultrasonic technology is needed to improve the first-trimester diagnostic efficiency and to develop a better integrated screening protocol.

(3) Title: Placental characteristics as a proxy measure of serum hormone and protein levels during pregnancy with a male fetus.

Source: Cancer Causes Control, 2011, Feb. 19.

Authors: Trabert, B., M. P. Longnecker, et al.

Abstract: OBJECTIVE: In utero exposure to steroid hormones may be related to risk of some cancers such as testicular germ cell tumors (TGCT). To determine whether placental characteristics are good surrogate measures of maternal biomarker levels, we evaluated the correlations in mothers of sons at higher (whites, n = 150) and lower (blacks, n = 150) risk of TGCT. Associations with birth weight were also examined. METHODS: All mothers, participants in the Collaborative Perinatal Project, were primigravidas who gave birth to male singletons. Associations between placental weight and placental thickness and third-trimester biomarker levels were evaluated using linear regression. Partial correlation coefficients for placental characteristics and birth weight were also estimated. RESULTS: Placental weight was positively correlated with alpha-fetoprotein (AFP), sex hormone-binding globulin (SHBG), testosterone, estradiol and estriol in whites, and AFP and estriol in blacks. Placental thickness was not associated with any biomarker. After adjustment for placental weight, birth weight was not correlated with any biomarker. CONCLUSIONS: In these data, placental weight was modestly correlated with third-trimester biomarker level; however, it appeared to be a better surrogate for third-trimester biomarker level than birth weight. Placental thickness had limited utility as a surrogate measure for biomarker levels.

(4) Title: A novel embryological theory of autism causation involving endogenous biochemicals capable of initiating cellular gene transcription: A possible link between twelve autism risk factors and the autism 'epidemic'.

Source: Med Hypotheses **76**(5): 653-60, 2011.

Authors: King, C. R.

Abstract: Human alpha-fetoprotein is a pregnancy-associated protein with an undetermined physiological role. As human alpha-fetoprotein binds retinoids and inhibits estrogen-dependent cancer cell proliferation, and because retinoic acid (a retinol metabolite) and estradiol (an estrogen) can both initiate cellular gene transcription, it is hypothesized here that alpha-fetoprotein functions during critical gestational periods to prevent retinoic acid and maternal estradiol from inappropriately stimulating gene expression in developing brain regions which are sensitive to these chemicals. Prenatal/maternal factors linked to increased autism risk include valproic acid, thalidomide,

alcohol, rubella, cytomegalovirus, depression, schizophrenia, obsessive-compulsive disorder, autoimmune disease, stress, allergic reaction, and hypothyroidism. It will be shown how each of these risk factors may initiate expression of genes which are sensitive to retinoic acid and/or estradiol - whether by direct promotion or by reducing production of alpha-fetoprotein. It is thus hypothesized here that autism is not a genetic disorder, but is rather an epigenetic disruption in brain development caused by gestational exposure to chemicals and/or conditions which either inhibit alpha-fetoprotein production or directly promote retinoic acid-sensitive or estradiol-sensitive gene expression. This causation model leads to potential chemical explanations for autistic brain morphology, the distinct symptomatology of Asperger's syndrome, and the differences between high-functioning and low-functioning autisms with regard to mental retardation, physical malformation, and sex ratio. It will be discussed how folic acid may cause autism under the retinoic acid/estradiol model, and the history of prenatal folic acid supplementation will be shown to coincide with the history of what is popularly known as the autism epidemic. It is thus hypothesized here that prenatal folic acid supplementation has contributed to the post-1980 increase in US autism diagnoses. In addition to explaining the epidemic within the wider retinoic acid/estradiol model of causation, this theory leads to potential explanations for certain genetic findings in autism, autistic regression, and changing trends in autism symptomatology with regard to mental retardation, wheat allergy, and gastrointestinal problems.

VI. Potentially helpful website connections/locations:

1) pregnancy.about.com/cs/afp/a/afptesting.htm

2) health.allrefer.com/health/alpha-fetoprotein-info.html

3) headtotoe.apta.org/topic/medtest/hw1663/results.htm

4) www.pregnancy-info.net/slpha_feto_protein.html

5) www.healthopedia.com/alpha-fetoprotein

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

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

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

9) http://pregnancyandbaby.sheknows.com/pregnancy/baby/Understanding-the-AFP-test-445.

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

Teachings on Alpha-fetoprotein

Vol. 5, Part 2

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

Structural and Functional Aspect of AFP:

Section - I.

A. Structural Variants: Molecular variants of mammalian AFP have been reported in the scientific literature since the 1970s. Some of these earlier variant forms were attributed to carbohydrate microheterogeneity and isoforms associated with varying isoelectric points [Lamerz, 1997] [Ichikawa, 2006]. Later reports demonstrated AFP forms that were genetic isoforms and lectin glycoforms demonstrable by electrophoretic and chromatographic procedures [Taketa, 1998] [Taketa, 1998]. Still other variants were detected following high-pressure liquid chromatography (HPLC) utilizing lectin, heavy metal, and hydrophobic solid phase separation methodology [Mizejewski, 2001]. The advent of monoclonal antibodies permitted the detection and analyses of epitopic domains and subdomains that comprise the overall antigenic determinant sites on AFP [Kang, 2001] [Yakimenko, 2001]. Finally, the discovery and characterization of the molten globule forms (MGF) of AFP have provided a new level of understanding regarding the various folding transition forms of this fetal protein [Uversky, 1997].

Molecular variants of HAFP have further been reported as a result of clinical assays which detected aberrant molecular forms. Several such reports of aberrant AFP molecules first appeared in the clinical cystic fibrosis literature resulting in confusion of the clinical usefulness of AFP for this genetic disorder. In the 1970s and 1980s prior to the development of monoclonal antibodies, polyclonal antibody assays were not as precise and sensitive as today's immunoassays. Such factors resulted in disparate baseline levels of HAFP in the sera of non-disease, normal adult patients which ranged in concentrations from 5-20 ng/ml. In addition, a previously reported cationic form of HAFP has been confirmed to be HAFP complexed with IgM molecules; this cationic form has now been described in several independent laboratories [Beneduce, 2004] [Mizejewski, 1997] [Mizejewski, 2001] [Mizejewski, 2002]. Abberent forms of HAFP have also been detected in the reproductive/and urinary tract in various clinical patients,

and in the sera of human patients (breast cancer, reproductive disorders etc). A non-secreted form of HAFP, lacking the N-terminal signal sequence segment, was recently reported in recombinant AFP studies employing yolk sac tumors [Fukasawa, 2005]. Truncated forms of HAFP (~ 50,000 Daltons) have further been detected in cell cultures comprised of hepatomas, testicular embryonal carcinomas, and breast tumors [Mizejewski, 2002]. Variant forms of HAFP transcripts from non-translated regions of the AFP mRNA have recently been reported in CD34+ hematopoietic progenitor cells derived from mesodermal germ cells [Kubota, 2002]. These latter investigators described two variant forms of HAFP mRNA that are not expressed in mature cells. The variant AFP mRNAs differed from the authentic transcripts by incorporating exons from the 5′-untranslated region of the HAFP gene. The abnormal AFP transcript was found only in bone marrow, thymus, and brain tissue. The various folding intermediate forms of HAFP have recently been investigated using bacterial and yeast recombinant methodology [Yazova, 2003] [Leong, 2006]. The folding of both glycosylated (yeast) and non-glycosylated (E. coli) forms of recombinant HAFP was studied following protein purification from aggregation-prone inclusion bodies. After AFP was denatured, it readily refolded under dilution, redox, reactions and ELISA conditions in both of the recombinant produced AFP forms. In summary, the denaturation of recombinant-derived HAFP was found to be a reversible process independent of its starting source, fatty acid relationship, and glycosylated state.

B. Biological Roles: Determination of the biological roles of mammalian α-fetoprotein (AFP) has been a research objective for many years. Similar to albumin, serum AFP is known to bind and transport a multitude of ligands such as bilirubin, fatty acids, retinoids, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxin, and various drugs [Arsenov, 2001] [Milligan, 1998]. Indeed, AFP has been shown to bind in vitro many substances, some of which serve as ligands for members of the steroid/thyroid nuclear receptor superfamily [Mizejewski, 1993] [Dauphinee, 2002] [Bois-Joyeux, 2000]. However, other ligands that bind to AFP (rodent and human) include metabolic stains, L-tryptophan, warfarin, triazine dyes, phenylbutazone, streptomycin, phenytoin, anilinonaphthaline sulfate, heavy

metals, low carbon-chain alcohols, and polyunsaturated fatty acids [Mizejewski, 2001] [Mizejewski, 1997]. Although the physicochemical and structural properties of HAFP have been extensively described, it was mostly the *in vitro* functional roles that have been confirmed to date. Thus, the physiological properties of HAFP have encompassed mainly ligand carrier/transport functions but modulation of the immune response assays has been widely addressed (see below). Interestingly, it is growth regulation that has recently emerged as an important function of human AFPs as well as in other mammals (see below).

During the last decade, a multitude of studies have established AFP as a regulator of ontogenic and oncogenic growth [Mizejewski, 1997] [Mizejewski, 2001]. In fact, it is the growth-modulating activity that distinguishes AFP from albumin, a major blood protein carrier/transport molecule of the albuminoid gene family. Reports now support the concept that native, full-length AFP is largely a growth-enhancing protein whose overall activity is enacted through a cyclic AMP-protein kinase A activation pathway [Wang, 1998] [Li, 2002]. However, growth is a process that requires fine-tuning for both up-and down-regulation regulation to operate correctly over defined time periods such as pregnancy. Although sustained growth of the fetus is required for full-term pregnancy, the fetus does encounter situations that require periods of temporary or prolonged growth cessation, such as differentiation, transformation, and the prevention of organ/tissue overgrowth [Butterstein, 2003]. Furthermore, the fetus may experience pulses of stress/shock insults in the microenvironment compartments of both the extracellular and intracellular fetal mileux. Thus, fetal growth in a tissue or the extracellular matrix may require a temporary halt until fetal homeostasis is achieved and/or until compensated signal transduction pathways are reestablished via adaptor/scaffold protein-to-protein interactions. Such stress/shock encounters involving AFP include environmental extremes of osmolality, pH, oxygen tension, ischemia, glucose shock, osmotic pressure, anemia, anoxia, and excessively high ligand (steroids, fatty acids, etc) concentrations [Mizejewski, 2001].

The growth regulatory properties of AFP have aroused investigational interest in studies of ontogeneic and oncogenic growth in both cell cultures and animal models. A multitude of reports have

now documented that HAFP is capable of regulating growth in reproductive, hematopoietic, placental, hepatic, inflammatory, and lymphatic cells [Oertel, 2006] [Schnater, 2006] [Mizejewski, 2001] [Butterstein, 1999] [Mizejewski, 2004]. Since the late 1990s, AFP is viewed as a protein associated with modulating cell proliferation, differentiation, regeneration, and transformation in both ontogenetic and oncogenic growth processes [Mizejewski, 1997] [Mizejewski, 2003]. Although an AFP gene knockout in mice resulted in infertile female offspring and not lethality or developmental arrest, [Gabant, 2002] a similar outcome may not be necessarily true for human beings. Although such an investigation cannot be ethically pursued in humans, clinical cases of AFP congenital deficiency have been reported in the literature [Sharony, 2004] [Sharony, 2003]. Patients in such studies were asymptomatic and presented with normal development in their clinical histories.

In its native form, HAFP displays largely growth-enhancing properties, regardless of whether the tissue is of fetal or postnatal origin; it is the ligand-free form of HAFP at physiological dose levels that have been shown to enhance tumor growth [Dudich, 2006] [Uversky, 1997]. HAFP has further been shown to possess pro-angiogenic properties that promote neovascularization and growth in both fetal and tumor tissues [Liang, 2004] [Takahashi, 2004]. Recent findings further indicate that HAFP can also stimulate the expression of certain oncogenes (c-Fos, c-Jun, and n-Ras) which, in turn, enhances the proliferation of human carcinoma cells [Li, 2004]. Finally, HAFP has been shown to functionally impair dendritic cells inducing immune dysfunction and apoptosis of antigen processing cells (APCs) [Um, 2004]. In the latter report, the authors suggested a mechanism by which hepatoma cells could escape immunological surveillance as a result of cells bearing AFP molecules on their cell surfaces.

8. Rozenberg P, Bussieres L, Chevret S, Bernard JP, Malagrida L, Cuckle H, Chabry C, Durand-Zaleski I, Bidat L, Lacroix I, Moulis M, Roger M, Jacquemot MC, Bault JP, Boukobza P, Boccara P, Vialard F, Giudicelli Y, Ville Y. Screening for Down syndrome using first-trimester combined screening followed by second-trimester ultrasound examination in an unselected population. Am J Obstet Gynecol 195: 1379–1387, 2006.
9. Drugan A, Weissman A, Evans MI. Screening for neural tube defects. Clin Perinatol 28:279–287, vii, 2001.
10. Muller F. Prenatal biochemical screening for neural tube defects. Childs Nerv Syst 19:433–435, 2003.
11. Benn PA, Ying J. Preliminary estimate for the second-trimester maternal serum screening detection rate of the 45,X karyotype using alpha-fetoprotein, unconjugated estriol and human chorionic gonadotropin. J Matern Fetal Neonatal Med 15:160–166, 2004.
12. Duric K, Skrablin S, Lesin J, Kalafatic D, Kuvacic I, Suchanek E. Second trimester total human chorionic gonadotropin, alpha-fetoprotein and unconjugated estriol in predicting pregnancy complications other than fetal aneuploidy. Eur J Obstet Gynecol Reprod Biol 110: 12–15, 2003.
13. Sharony R, Amiel A, Bouaron N, Kidron D, Itzhaky D, Fejgin M. Congenital deficiency of alpha-fetoprotein and associated chromosomal abnormality in the placenta. Am J Med Genet A 121:113–117, 2003.
14. Bartha JL, Comino-Delgado R, Arce F, Alba P, Broullon JR, Barahona M. Relationship between alpha-fetoprotein and fetal erythropoiesis. J Reprod Med 44:689–697, 1999.
15. Chen H, Egan JO, Chiu JF. Regulation and activities of alpha-fetoprotein. Crit Rev Eukaryot Gene Expr 7:11–41, 1997.
16. Johnson PJ, Poon TC, Hjelm NM, Ho CS, Blake C, Ho SK. Structures of disease-specific serum alpha-fetoprotein isoforms. Br J Cancer 83: 1330–1337, 2000.
17. Moldogazieva NT, Terent'ev AA, Shaitan KV. [Relationship between structure and function of alpha-fetoprotein: conformational status and biological activity]. Biomeditsinskaia khimiia 51:127–151, 2005.
18. Lazarevich NL. Molecular mechanisms of alpha-fetoprotein gene expression. Biochemistry (Mosc) 65:117–133, 2000.
19. Gillespie JR, Uversky VN. Structure and function of alpha-fetoprotein: a biophysical overview. Biochim Biophys Acta 1480: 41–56, 2000.
20. Terentiev AA, Moldogazieva NT. Structural and functional mapping of alpha-fetoprotein. Biochemistry (Mosc) 71:120–132, 2006.
21. Lamerz R. AFP isoforms and their clinical significance (overview). Anticancer Res 17:2927–2930, 1997.
22. Ichikawa E, Kuriyama S, Yuji J, Masaki T, Uchida N, Nishioka M, Taketa K. Further resolution of alpha-fetoprotein glycoforms by two-dimensional isoelectric focusing and lectin affinity electrophoresis. Electrophoresis 27:3480–3487, 2006.
23. Taketa K. Characterization of sugar chain structures of human alpha-fetoprotein by lectin affinity electrophoresis. Electrophoresis 19:2595–2602, 1998.
24. Taketa K. Multimodal application of lectin affinity electrophoresis of alpha-fetoprotein. Electrophoresis 19:1774–1779, 1998.
25. Mizejewski GJ. Alpha-fetoprotein structure and function: relevance to isoforms, epitopes, and conformational variants. Exp Biol Med (Maywood) 226:377–408, 2001.
26. Kang Y, Matsuura E, Sakamoto T, Sakai M, Nishi S. Analysis of epitopes of mouse monoclonal antibodies against human alpha-fetoprotein. Tumour Biol 22:254–261, 2001.
27. Yakimenko EF, Yazova AK, Goussev AI, Abelev GI. Epitope mapping of human alpha-fetoprotein. Biochemistry (Mosc) 66:524–530, 2001.
28. Uversky VN, Narizhneva NV, Kirschstein SO, Winter S, Lober G. Conformational transitions provoked by organic solvents in beta-lactoglobulin: can a molten globule like intermediate be induced by the decrease in dielectric constant? Fold Des 2:163–172, 1997.

29. Beneduce L, Castaldi F, Marino M, Tono N, Gatta A, Pontisso P, Fassina G. Improvement of liver cancer detection with simultaneous assessment of circulating levels of free alpha-fetoprotein (AFP) and AFP-IgM complexes. Int J Biol Markers 19:155–159, 2004.
30. Mizejewski GJ. Biological role of alpha-fetoprotein in cancer: prospects for anticancer therapy. Expert Rev Anticancer Ther 2: 709–735, 2002.
31. Fukasawa H, Iwamoto H, Hirata S, Shoda T, Yokota S, Nishi S, Hoshi K. Novel human alpha-fetoprotein mRNA isoform lacking exon 1 identified in ovarian yolk sac tumor. J Soc Gynecol Investig 12:456–462, 2005.
32. Kubota H, Storms RW, Reid LM. Variant forms of alpha-fetoprotein transcripts expressed in human hematopoietic progenitors. Implications for their developmental potential towards endoderm. J Biol Chem 277:27629–27635, 2002.
33. Yazova AK, Goussev AI, Christiansen M, Kushlinsky NE, Stogova E, Norgaard-Pedersen B, Abelev GI. Human fetal and tumor alpha-fetoproteins differ in conformationally dependent epitope variants expression. Immunol Lett 85:261–270, 2003.
34. Leong SS, Middelberg AP. The refolding of different alpha-fetoprotein variants. Protein Sci 15:2040–2050, 2006.
35. Arsenov DV, Golubeva MB, Kisel MA, Konoplya NA, Lyubin GS, Kuz'mitskii BB, Strel'chenok OA. Modification of humoral immune response in C57Bl/6 mice with a complex of alpha-fetoprotein and retinoid acid derivatives. Bull Exp Biol Med 132:963–965, 2001.
36. Milligan SR, Khan O, Nash M. Competitive binding of xenobiotic oestrogens to rat alpha-fetoprotein and to sex steroid binding proteins in human and rainbow trout (Oncorhynchus mykiss) plasma. Gen Comp Endocrinol 112:89–95, 1998.
37. Mizejewski GJ. An apparent dimerization motif in the third domain of alpha-fetoprotein: molecular mimicry of the steroid/thyroid nuclear receptor superfamily. Bioessays 15:427–432, 1993.
38. Dauphinee MJ, Mizejewski GJ. Human alpha-fetoprotein contains potential heterodimerization motifs capable of interaction with nuclear receptors and transcription/growth factors. Med Hypotheses 58:453–461, 2002.
39. Bois-Joyeux B, Chauvet C, Nacer-Cherif H, Bergeret W, Mazure N, Giguere V, Laudet V, Danan JL. Modulation of the far-upstream enhancer of the rat alpha-fetoprotein gene by members of the ROR alpha, Reverb alpha, and Reverb beta groups of monomeric orphan nuclear receptors. DNA Cell Biol 19:589–599, 2000.
40. Wang XW, Xu B. Stimulation of tumor-cell growth by alpha-fetoprotein. Int J Cancer 75:596–599, 1998.
41. Li MS, Li PF, Yang FY, He SP, Du GG, Li G. The intracellular mechanism of alpha-fetoprotein promoting the proliferation of NIH 3T3 cells. Cell Res 12:151–156, 2002.
42. Butterstein G, Morrison J, Mizejewski GJ. Effect of alpha-fetoprotein and derived peptides on insulin- and estrogen-induced fetotoxicity. Fetal Diagn Ther 18:360–369, 2003.
43. Oertel M, Menthena A, Chen YQ, Shafritz DA. Properties of cryopreserved fetal liver stem/progenitor cells that exhibit long-term repopulation of the normal rat liver. Stem Cells 24:2244–2251, 2006.
44. Schnater JM, Bruder E, Bertschin S, Woodtli T, de Theije C, Pietsch T, Aronson DC, von Schweinitz D, Lamers WH, Kohler ES. Subcutaneous and intrahepatic growth of human hepatoblastoma in immunodeficient mice. J Hepatol 45:377–386, 2006.
45. Butterstein GM, Mizejewski GJ. Alpha-fetoprotein inhibits frog metamorphosis: implications for protein motif conservation. Comp Biochem Physiol A Mol Integr Physiol 124:39–45, 1999.
46. Mizejewski GJ. Biological roles of alpha-fetoprotein during pregnancy and perinatal development. Exp Biol Med (Maywood) 229: 439–463, 2004.
47. Gabant P, Forrester L, Nichols J, Van Reeth T, De Mees C, Pajack B, Watt A, Smitz J, Alexandre H, Szpirer C, Szpirer J. Alpha-fetoprotein, the major fetal serum protein, is not essential for embryonic development but is required for female fertility. Proc Natl Acad Sci U S A 99:12865–12870, 2002.

48. Sharony R, Zadik I, Parvari R. Congenital deficiency of alpha- fetoprotein. Eur J Hum Genet 12:871–874, 2004.
49. Dudich E, Semenkova L, Dudich I, Denesyuk A, Tatulov E, Korpela T. Alpha-fetoprotein antagonizes X-linked inhibitor of apoptosis protein anticaspase activity and disrupts XIAP-caspase interaction. FEBS J 273:3837–3849, 2006.
50. Uversky VN, Narizhneva NV, Ivanova TV, Tomashevski AY. Rigidity of human alpha-fetoprotein tertiary structure is under ligand control. Biochemistry 36:13638–13645, 1997.
51. Liang OD, Korff T, Eckhardt J, Rifaat J, Baal N, Herr F, Preissner KT, Zygmunt M. Oncodevelopmental alpha-fetoprotein acts as a selective proangiogenic factor on endothelial cell from the fetomaternal unit. J Clin Endocrinol Metab 89:1415–1422, 2004.
52. Takahashi Y, Ohta T, Mai M. Angiogenesis of AFP producing gastric carcinoma: correlation with frequent liver metastasis and its inhibition by anti-AFP antibody. Oncol Rep 11:809–813, 2004.
53. Li MS, Li PF, Chen Q, Du GG, Li G. Alpha-fetoprotein stimulated the expression of some oncogenes in human hepatocellular carcinoma Bel 7402 cells. World J Gastroenterol 10:819–824, 2004.
54. Um SH, Mulhall C, Alisa A, Ives AR, Karani J, Williams R, Bertoletti A, Behboudi S. Alpha-fetoprotein impairs APC function and induces their apoptosis. J Immunol 173:1772–1778, 2004.

Figure 1

Maternal Sera AFP MoM

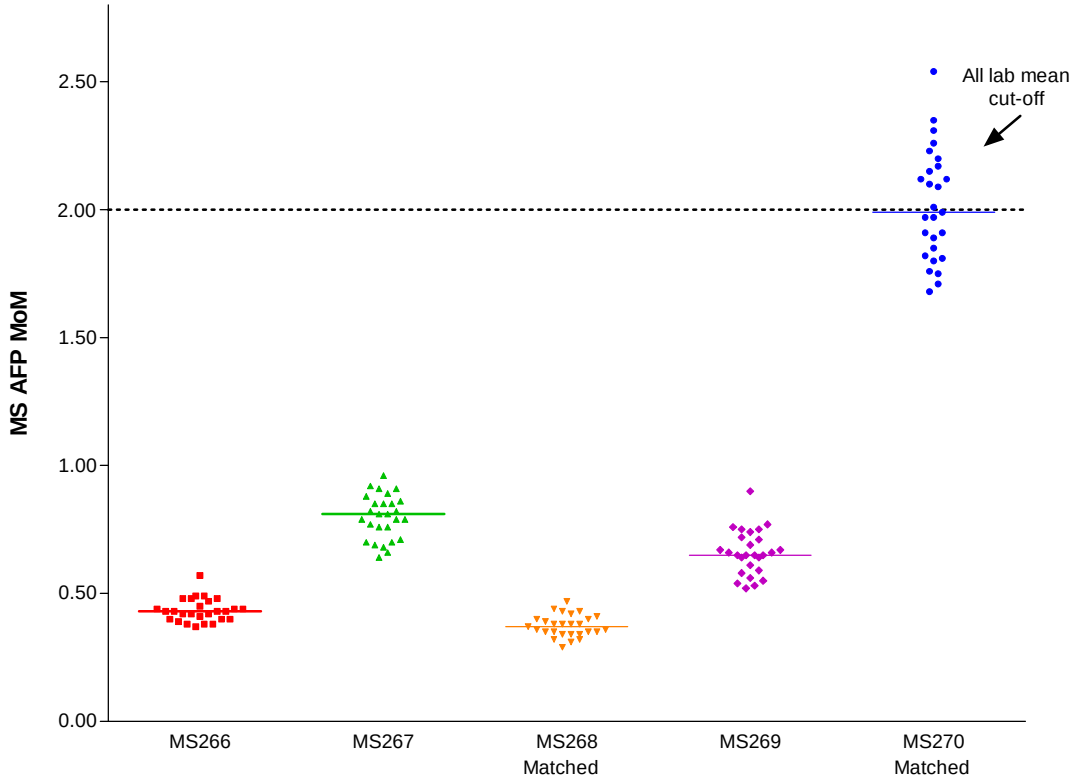


Figure 2

Amniotic Fluid AFP MoM

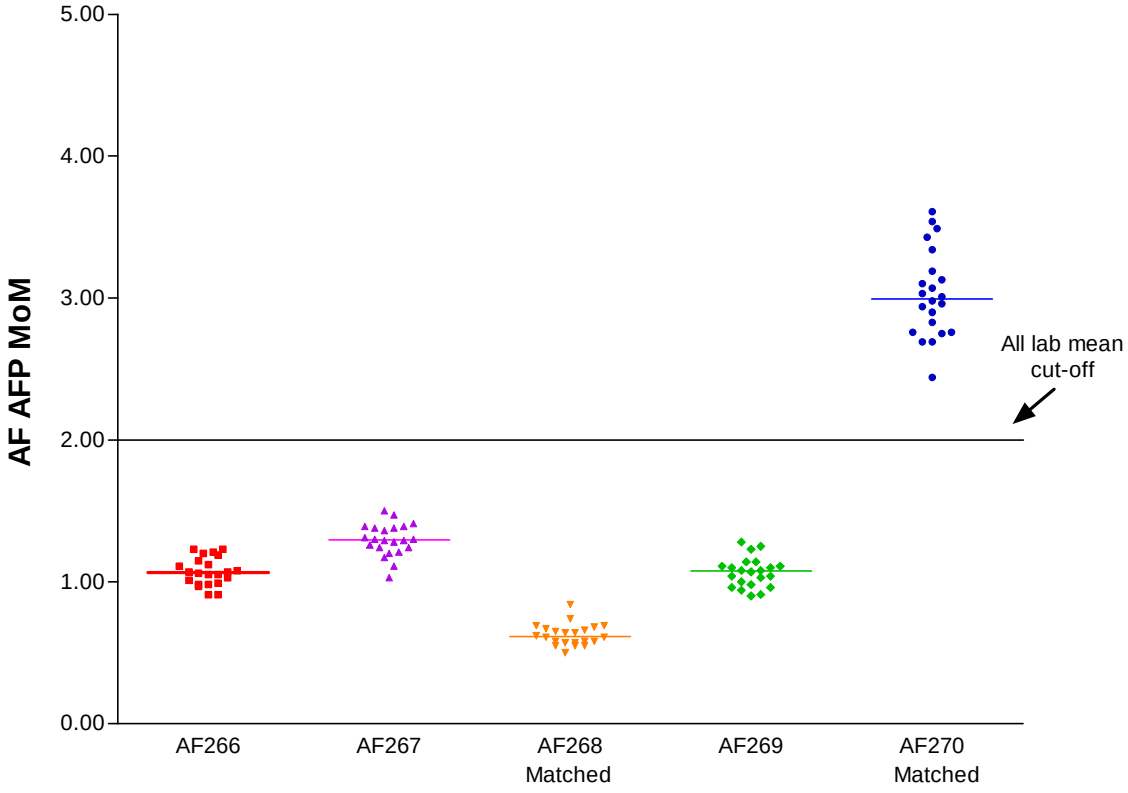


Figure 3

Graphic Distribution of Second Trimester Neural Tube Defect Risk Estimates

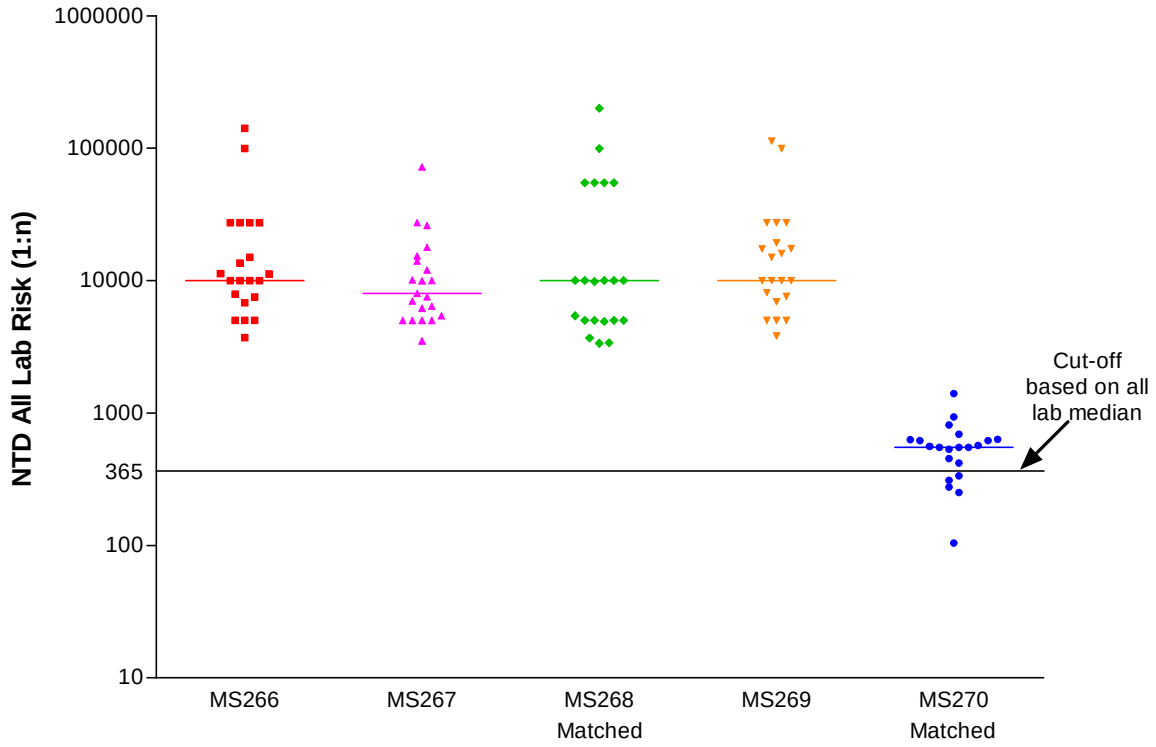


Figure 4

Graphic Distribution of Second Trimester Trisomy 18 Risk Estimates

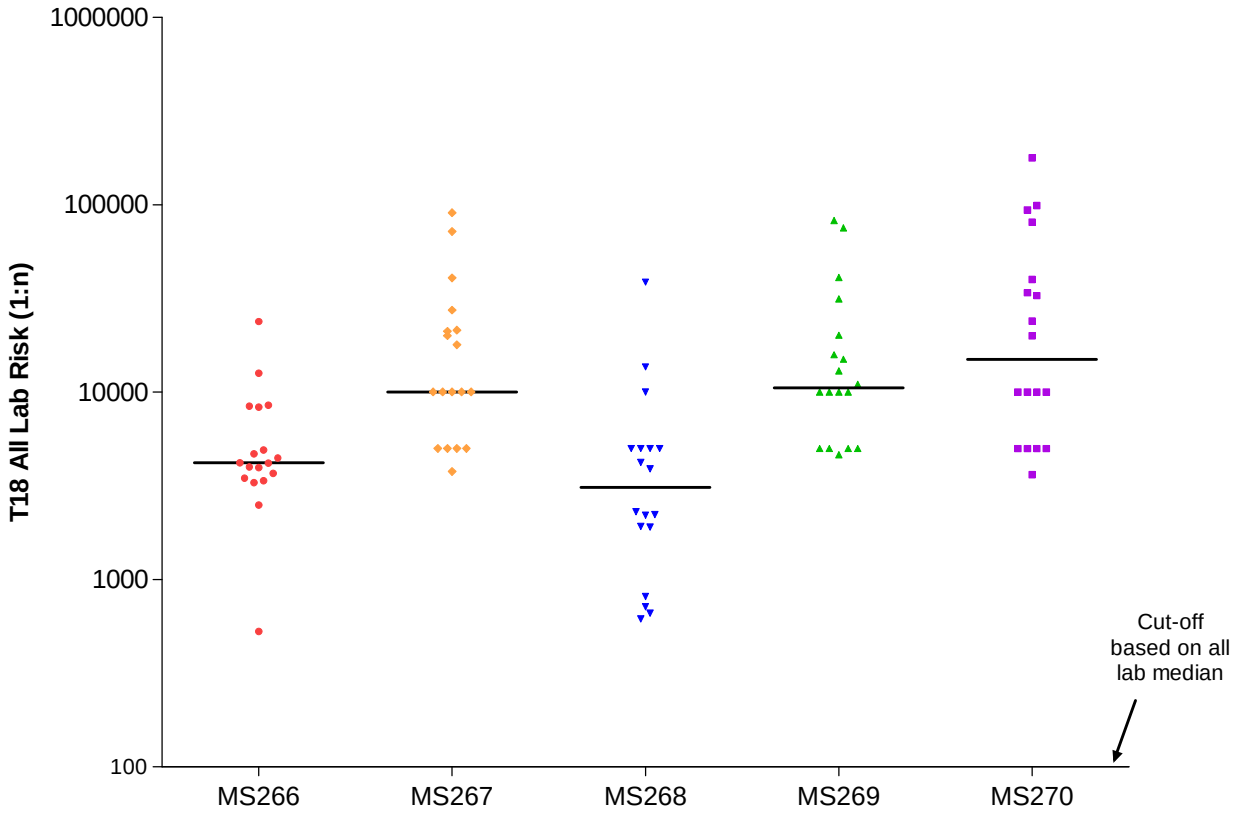


Figure 5

Graphic Distribution of Second Trimester Trisomy 21 Triple Risk Estimates

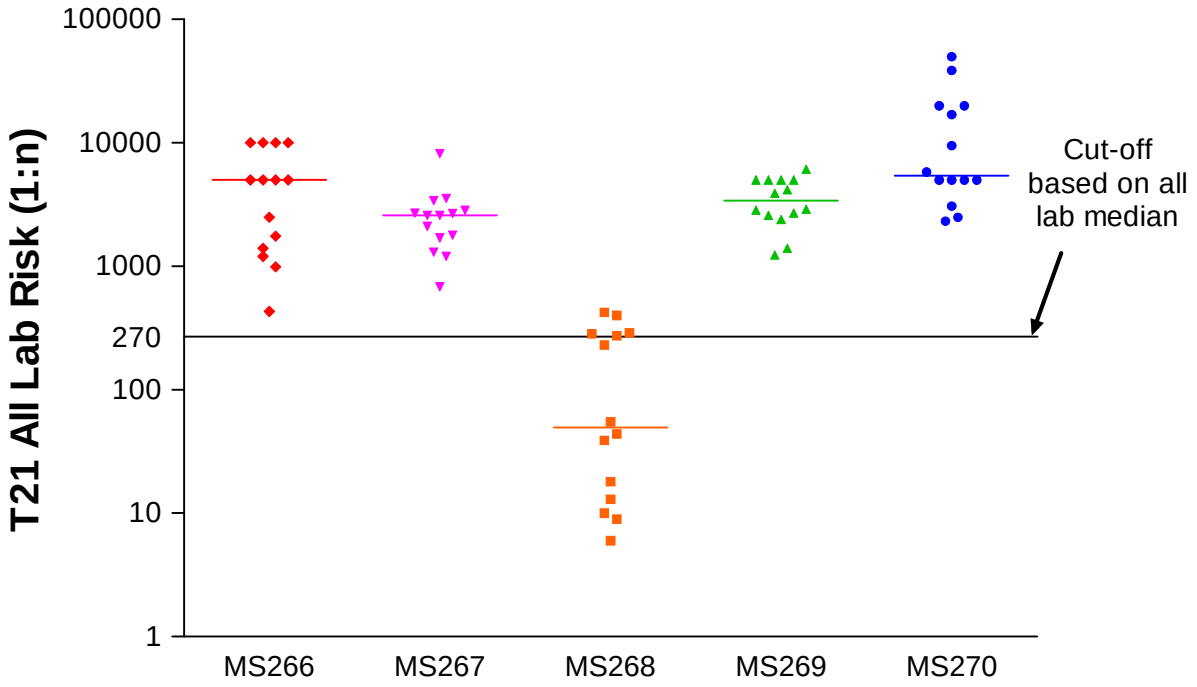


Figure 6

Graphic Distribution of Second Trimester Trisomy 21 Quadruple Risk Estimates

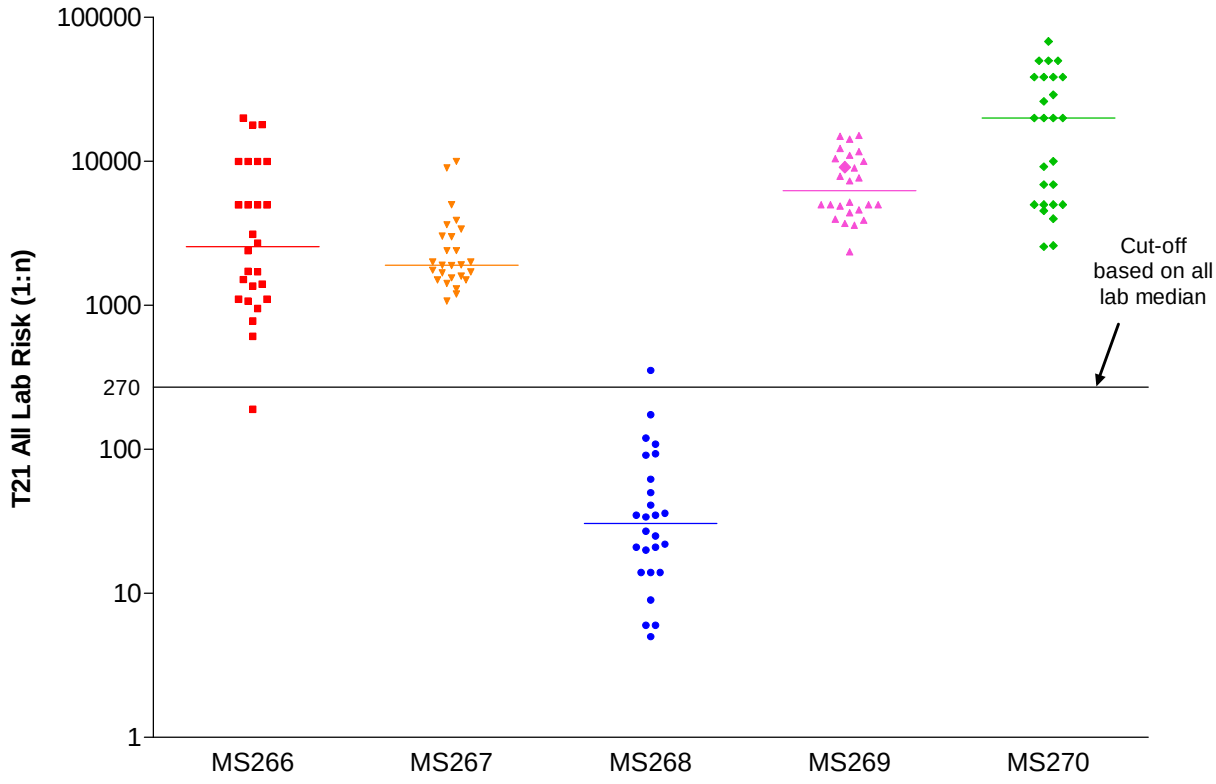


Figure 7A **MS AFP FEDM PT 5/11 Method Comparison**

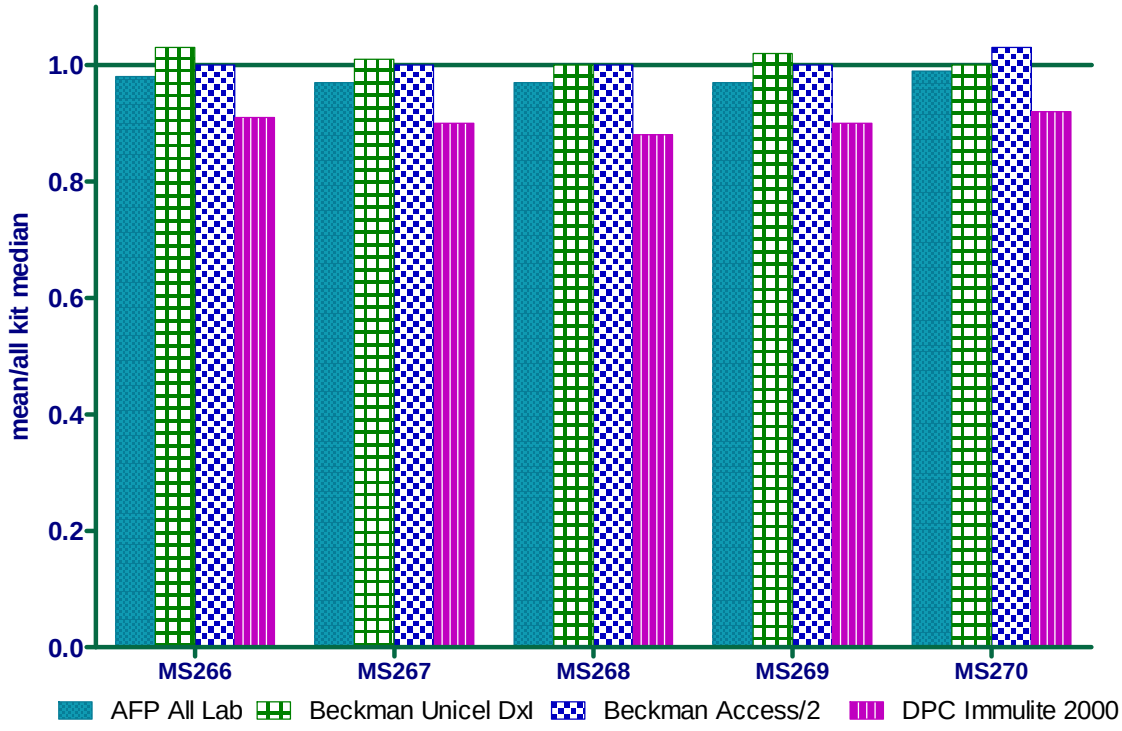


Figure 7B **AF AFP FEDM PT 5/11 Method Comparison**

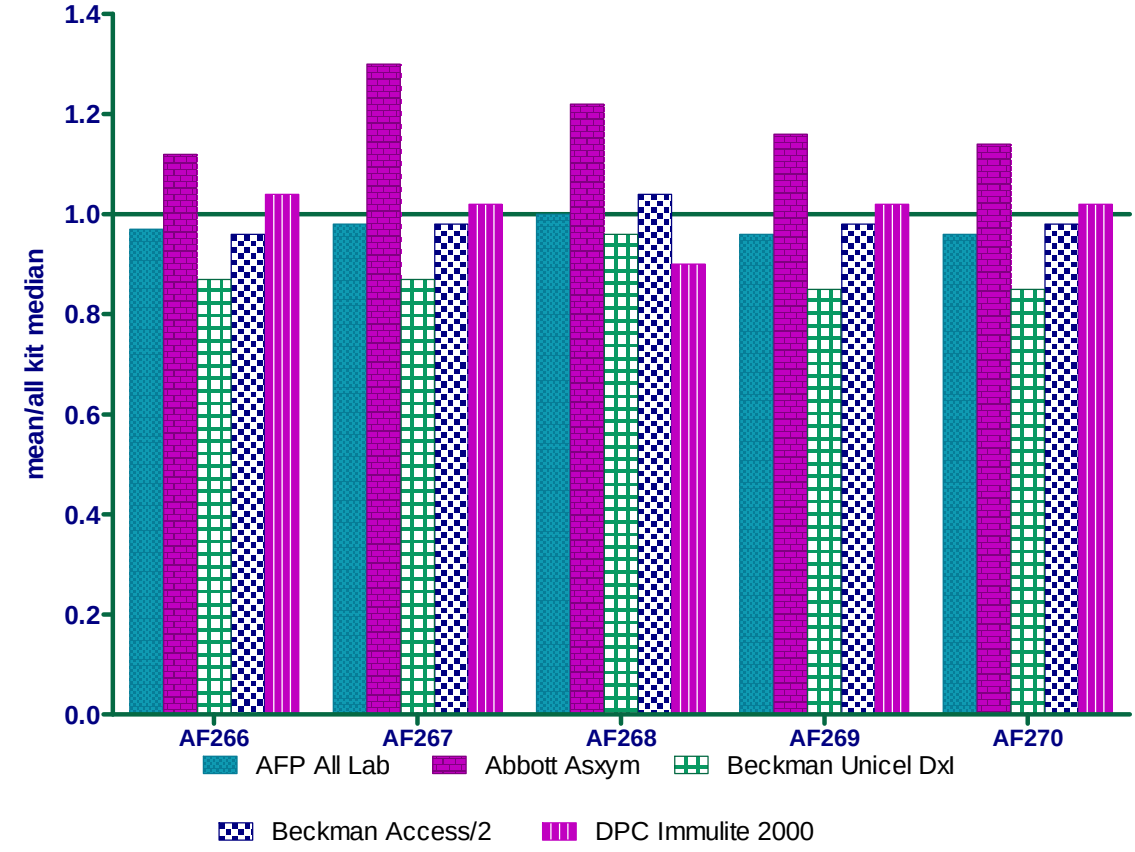


Figure 8A

MS uE3 FEDM PT 5/11 Method Comparison

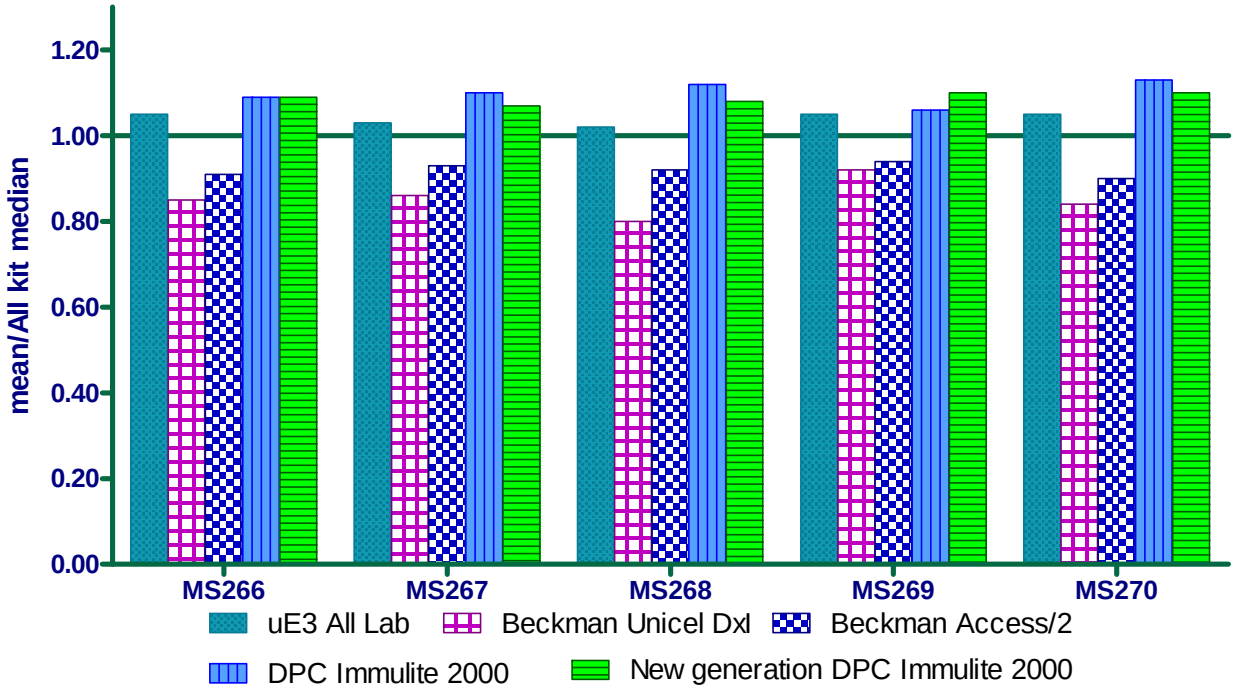


Figure 8 B

MS uE3 MOM FEDM PT 5/11 Method Comparison

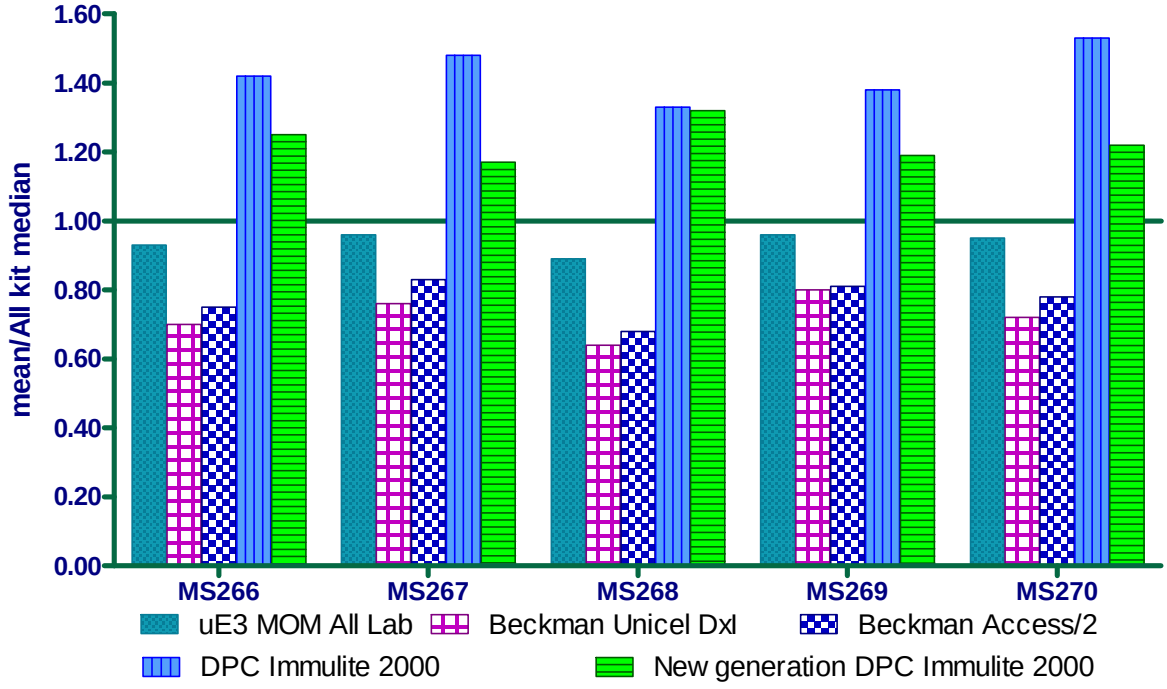


Figure 9

MS hCG FEDM PT 5/11 Method Comparison

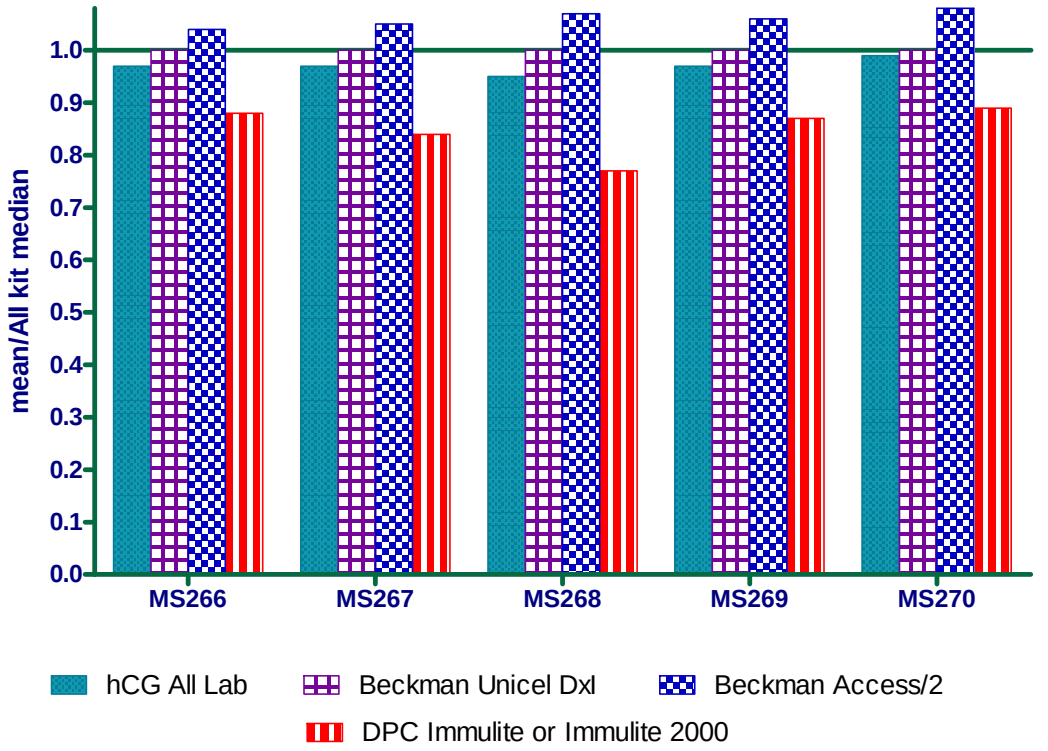


Figure 10

MS Inhibin A FEDM PT 5/11 Method Comparison

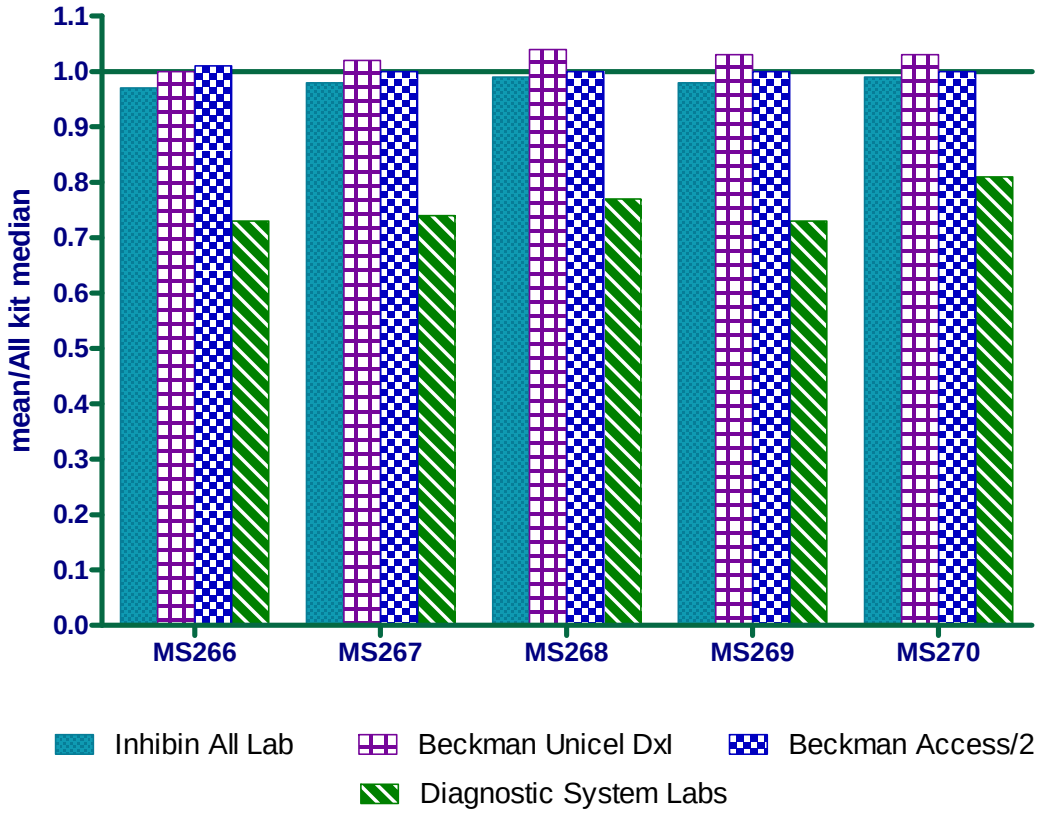


Figure 11

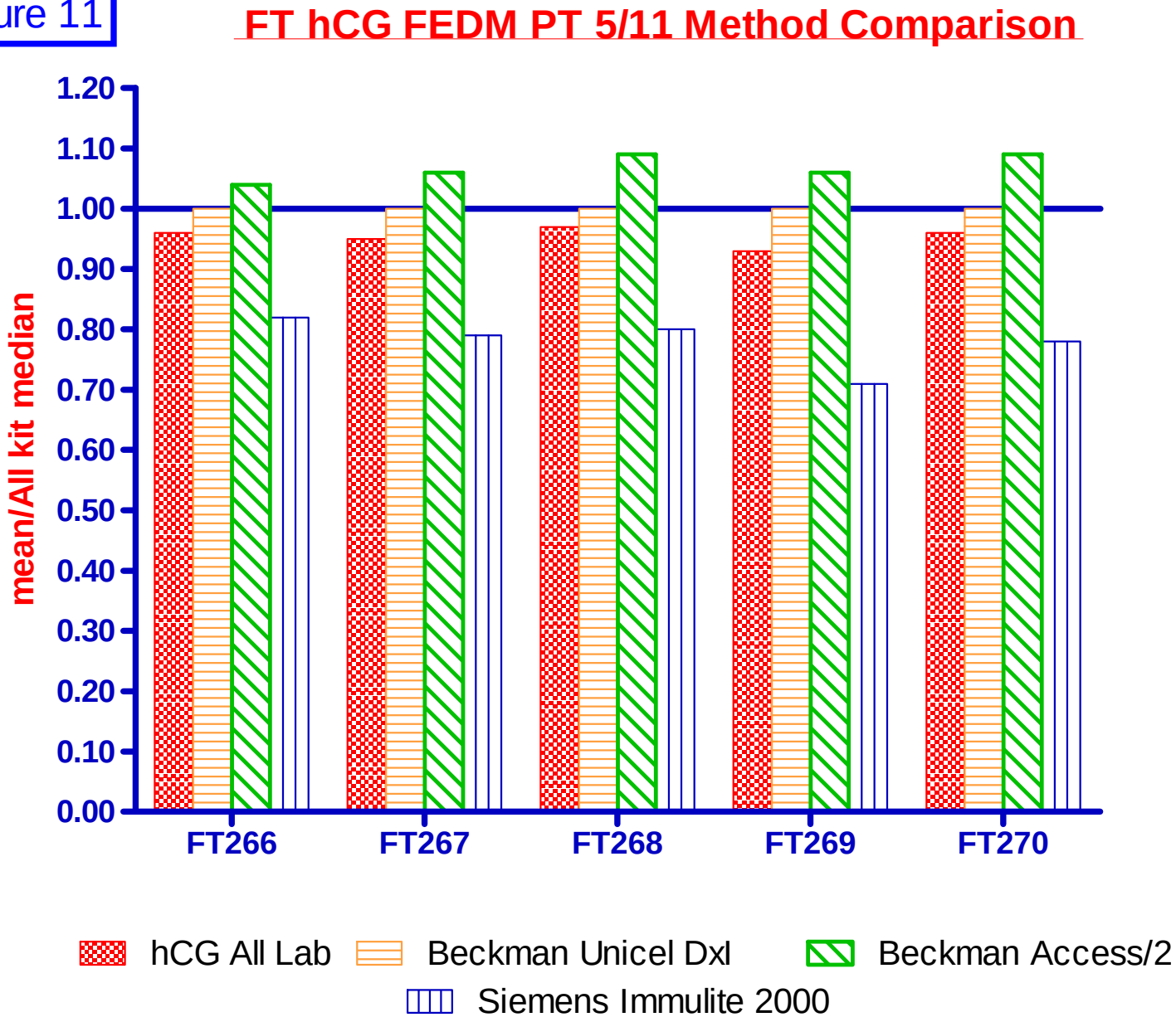
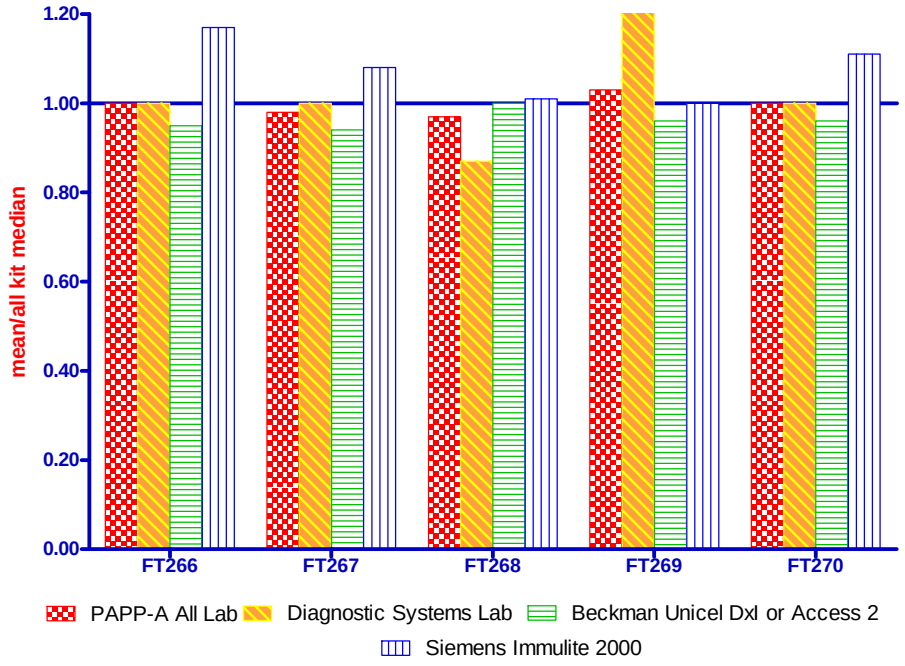

FT hCG FEDM PT 5/11 Method Comparison
mean/All kit median
1.20
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
FT266
FT267
FT268
FT269
FT270
hCG All Lab
Beckman Unicel DxI
Beckman Access/2
Siemens Immulite 2000

Figure 12A

FT PAPP- A FEDM PT 5/11 Method Comparison



*Please note: this graph is derived from converted values due to the difference in mass units used (mIU/ml -> ng/ml)

Figure 12B

FT PAPP- A MOM FEDM PT 5/11 Method Comparison

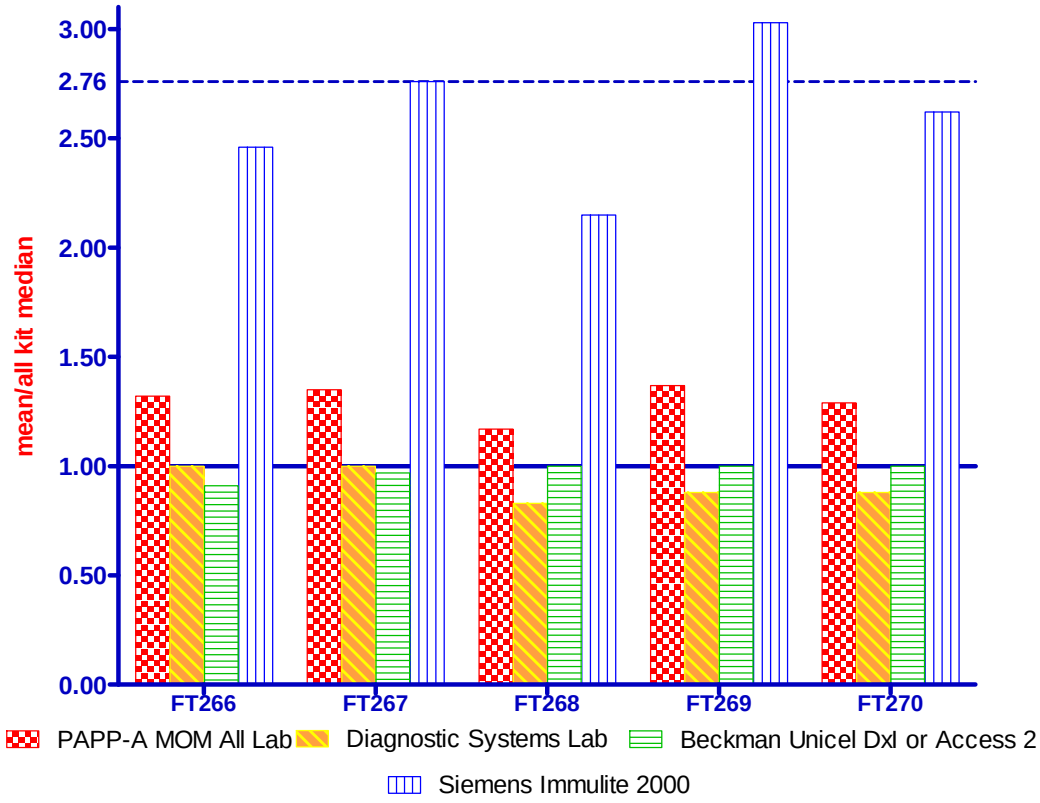


Figure 13

Graphic Distribution of First Trimester
Trisomy 21 Risk Estimates

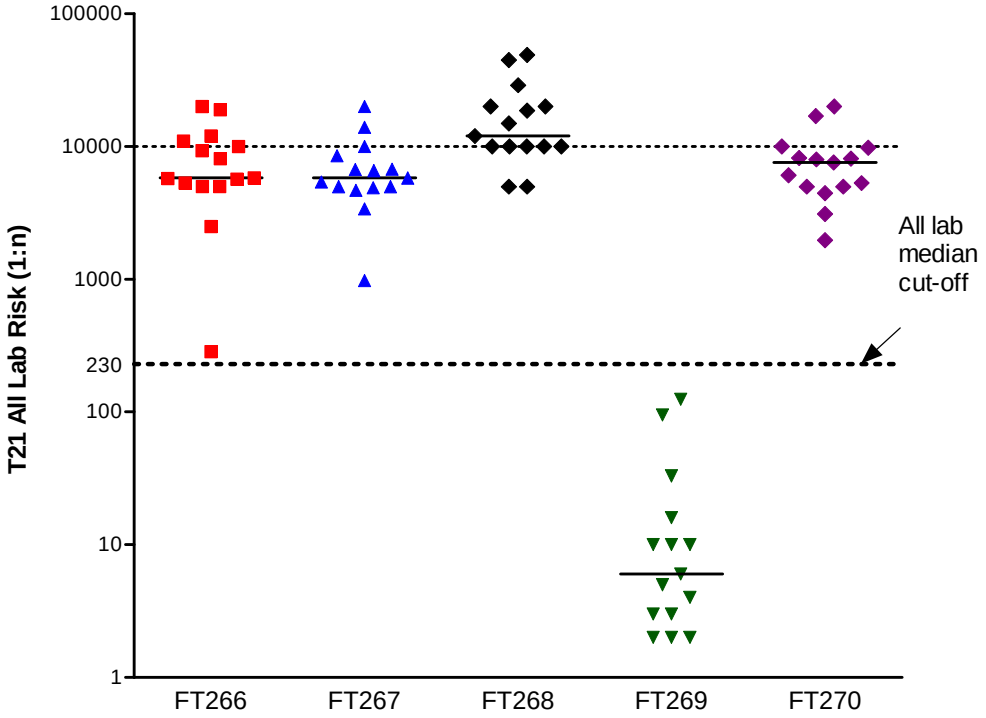
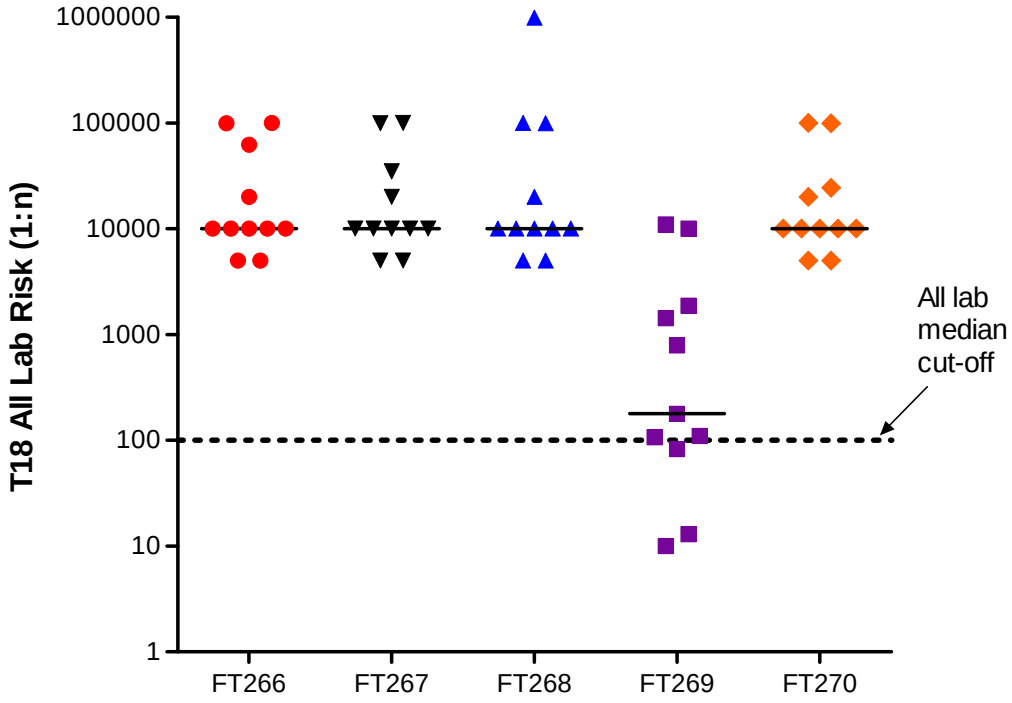


Figure 14

Graphic Distribution of First Trimester
Trisomy 18 Risk Estimates



New York State Fetal Defect Markers Proficiency Test,
May 2011
Summary of Results

	MS 266	MS 267	MS 268	MS 269	MS 270
Gestational Age All Lab Mean:					
Mean	17.0	19.0	15.0	18.0	20.0
SD	0.00	0.00	0.00	0.00	0.00
%CV	0.0%	0.0%	0.0%	0.0%	0.0%
X+3*SD	17.0	19.0	15.0	18.0	20.0
X-3*SD	17.0	19.0	15.0	18.0	20.0
N	27	27	27	27	27

	MS 266	MS 267	MS 268	MS 269	MS 270
MS AFP All Lab Mean:					
mean	16.8	44.6	12.5	36.4	126.2
SD	1.2	3.9	1.0	2.8	9.8
%CV	6.9%	8.8%	7.9%	7.7%	7.8%
mean+3SD	20.3	56.4	15.5	44.9	155.6
mean-3SD	13.3	32.8	9.6	28.0	96.8
N	27	27	27	27	27
median	16.9	45.0	12.8	36.3	128
mean/all kit median	0.98	0.97	0.97	0.97	0.99

	MS 266	MS 267	MS 268	MS 269	MS 270
MS AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	15.6	41.4	11.4	33.5	118.1
SD	0.7	3.6	0.8	1.7	6.2
%CV	4.3%	8.8%	7.3%	5.2%	5.3%
mean+3SD	17.6	52.3	13.9	38.7	136.9
mean-3SD	13.6	30.5	8.9	28.3	99.4
N	8	8	8	8	8
median	15.3	41.1	11.4	33.7	116.0
mean/all kit median	0.91	0.90	0.88	0.90	0.92

	MS 266	MS 267	MS 268	MS 269	MS 270
MS AFP Beckman Unicel (BCU/BC1) mean:					
Mean	17.6	46.5	13.0	38.3	128.0
SD	1.1	4.4	0.8	2.8	12.9
%CV	6.4%	9.4%	6.0%	7.3%	10.1%
mean + 3SD	21.0	59.6	15.3	46.7	166.8
mean - 3SD	14.2	33.4	10.7	29.9	89.1
N	8	8	8	8	8
Median	17.8	47.4	13.1	39.6	131.1
mean/All kit median	1.03	1.01	1.00	1.02	1.00

	MS 266	MS 267	MS 268	MS 269	MS 270
MS AFP Beckman Access (BCX/BC1) mean:					
mean	17.1	46.1	13.0	37.4	132.3
SD	0.7	2.1	0.5	1.7	4.5
%CV	4.4%	4.6%	4.0%	4.4%	3.4%
mean+3SD	19.3	52.4	14.6	42.4	145.8
mean-3SD	14.8	39.7	11.5	32.5	118.7
N	9	9	9	9	9
median	17.3	45.0	13.1	37.0	132.3
mean/all kit median	1.00	1.00	1.00	1.00	1.03

	MS 266	MS 267	MS 268	MS 269	MS 270
MS AFP MoMs All Lab Mean:					
mean	0.43	0.80	0.37	0.66	2.02
SD	0.04	0.09	0.04	0.09	0.22
%CV	8.5%	10.8%	11.5%	13.2%	10.8%
mean+3SD	0.54	1.06	0.50	0.92	2.67
mean-3SD	0.32	0.54	0.24	0.40	1.37
N	26	27	27	27	27

	MS 266	MS 267	MS 268	MS 269	MS 270
MS AFP kit average:					
mean	16.8	44.6	12.5	36.4	126.1
SD	1.0	2.8	0.9	2.6	7.2
all kit median	17.1	46.1	13.0	37.4	128.0

New York State Fetal Defect Markers Proficiency Test,
May 2011
Summary of Results

	MS 266	MS 267	MS 268	MS 269	MS 270
MS uE3 All Lab Mean:					
mean	1.03	1.36	0.34	1.21	1.63
SD	0.15	0.18	0.06	0.14	0.22
%CV	14.2%	13.3%	16.5%	11.7%	13.6%
mean+3SD	1.47	1.90	0.51	1.63	2.30
mean-3SD	0.59	0.82	0.17	0.78	0.97
N	26	26	26	26	26
mean/all kit median	1.05	1.03	1.02	1.05	1.05
MS uE3 Beckman Unicel (BCU/BC1) mean:					
Mean	0.92	1.21	0.29	1.13	1.43
SD	0.12	0.12	0.04	0.14	0.12
%CV	13.3%	9.9%	14.3%	12.5%	8.5%
mean+3SD	1.29	1.58	0.41	1.55	1.80
mean-3SD	0.55	0.85	0.17	0.70	1.07
N	8	8	8	8	8
Median	0.95	1.23	0.30	1.09	1.45
mean/all kit median	0.85	0.86	0.80	0.92	0.84
MS uE3 BeckmanAccess (BCX/BC1) mean:					
mean	0.98	1.32	0.33	1.15	1.55
SD	0.09	0.12	0.03	0.06	0.08
%CV	9.2%	8.8%	8.6%	5.1%	5.0%
mean+3SD	1.25	1.67	0.42	1.33	1.78
mean-3SD	0.71	0.97	0.25	0.97	1.31
N	9	9	9	9	9
median	0.97	1.34	0.33	1.16	1.58
mean/all kit median	0.91	0.93	0.92	0.94	0.90

	MS 266	MS 267	MS 268	MS 269	MS 270
MS uE3 DPC Immulite 2000 (DPD/DP5) mean:					
Mean	1.17	1.55	0.40	1.30	1.93
SD	0.13	0.14	0.03	0.17	0.12
%CV	11.3%	9.0%	6.2%	13.2%	6.1%
mean+3SD	1.57	1.97	0.48	1.82	2.28
mean-3SD	0.78	1.13	0.33	0.79	1.58
N	3	3	3	3	3
Median	1.20	1.54	0.40	1.23	1.90
mean/all kit median	1.09	1.10	1.12	1.06	1.13
MS uE3 New generation DPC Immulite 2000 (DPD/DP6) mean:					
Mean	1.17	1.51	0.39	1.35	1.88
SD	0.07	0.16	0.05	0.08	0.10
%CV	6.1%	10.5%	12.6%	6.1%	5.2%
mean+3SD	1.57	1.97	0.48	1.82	2.28
mean-3SD	0.78	1.13	0.33	0.79	1.58
N	6	6	6	6	6
Median	1.18	1.49	0.40	1.35	1.85
mean/All Kit Median	1.09	1.07	1.08	1.10	1.10
MS UE3 kit average:					
mean	1.06	1.40	0.35	1.23	1.70
SD	0.13	0.16	0.05	0.11	0.24
all kit median	1.08	1.42	0.36	1.23	1.71

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	MS 266	MS 267	MS 268	MS 269	MS 270
MS uE3 MoMs All Lab Mean:					
Mean	1.16	0.92	0.61	0.96	0.93
SD	0.40	0.27	0.25	0.26	0.29
%CV	34.1%	28.9%	40.8%	27.0%	31.7%
X+3SD	2.35	1.71	1.37	1.73	1.81
X-3SD	-0.03	0.12	-0.14	0.18	0.05
N	26	26	26	26	26
mean/All Kit Median	0.93	0.96	0.89	0.96	0.95
MS uE3 MoMs (BCU/BC1) Mean:					
Mean	0.88	0.73	0.44	0.79	0.70
SD	0.16	0.09	0.08	0.13	0.08
%CV	18.1%	12.4%	18.1%	16.6%	10.8%
X+3SD	1.35	0.99	0.68	1.19	0.93
X-3SD	0.40	0.46	0.20	0.40	0.47
N	8	8	8	8	8
mean/All Kit Median	0.70	0.76	0.64	0.80	0.72
MS uE3 MoMs (BCX/BC1) Mean:					
Mean	0.94	0.80	0.47	0.81	0.77
SD	0.08	0.08	0.05	0.08	0.08
%CV	8.7%	9.8%	10.0%	9.9%	10.7%
X+3SD	1.19	1.03	0.61	1.05	1.01
X-3SD	0.70	0.56	0.33	0.57	0.52
N	9	9	9	9	9
mean/All Kit Median	0.75	0.83	0.68	0.81	0.78

	MS 266	MS 267	MS 268	MS 269	MS 270
MS uE3 MoM (DPD/DP5) Mean:					
Mean	1.78	1.41	0.92	1.37	1.49
SD	0.11	0.24	0.19	0.25	0.06
%CV	6.2%	17.2%	20.6%	18.2%	3.7%
X+3SD	2.11	2.14	1.48	2.12	1.66
X-3SD	1.45	0.68	0.35	0.62	1.33
N	3	3	3	3	3
mean/All Kit Median	1.42	1.48	1.33	1.38	1.53
MS uE3 MoM (DPD/DP6) Mean:					
Mean	1.56	1.11	0.91	1.18	1.19
SD	0.31	0.18	0.21	0.16	0.09
%CV	20.0%	15.8%	23.7%	13.8%	7.4%
X+3SD	2.50	1.64	1.55	1.67	1.45
X-3SD	0.62	0.58	0.26	0.69	0.93
N	6	6	6	6	6
mean/All Kit Median	1.25	1.17	1.32	1.19	1.22
MS UE3 MoM kit average:					
mean	1.29	1.01	0.68	1.04	1.04
SD	0.45	0.31	0.26	0.29	0.37
all kit median	1.25	0.95	0.69	1.00	0.98

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	MS 266	MS 267	MS 268	MS 269	MS 270
MS hCG All Lab Mean:					
mean	10.94	17.95	64.17	19.44	17.10
SD	1.03	2.00	9.90	2.28	1.89
%CV	9.4%	11.2%	15.4%	11.7%	11.0%
mean+3SD	14.0	24.0	93.9	26.3	22.8
mean-3SD	7.9	11.9	34.5	12.6	11.4
N	27	27	27	27	27
mean/all kit median	0.97	0.97	0.95	0.97	0.99

	MS 266	MS 267	MS 268	MS 269	MS 270
MS hCG DPC Immulite 2000 (DPD/DP5) mean:					
mean	9.9	15.6	52.1	17.3	15.4
SD	0.8	1.0	5.0	1.8	1.5
%CV	7.7%	6.3%	9.7%	10.7%	9.5%
mean+3SD	12.1	18.6	67.2	22.8	19.7
mean-3SD	7.6	12.7	36.9	11.8	11.0
N	8	8	8	8	8
median	9.8	15.5	52.3	17.2	15.5
mean/all kit median	0.88	0.84	0.77	0.87	0.89

	MS 266	MS 267	MS 268	MS 269	MS 270
MS hCG Beckman Unicel (BCU/BC1) mean:					
mean	11.23	18.56	67.49	19.96	17.30
SD	0.63	1.12	4.56	1.90	1.42
%CV	5.6%	6.0%	6.8%	9.5%	8.2%
mean+3SD	14.23	24.42	91.63	25.78	22.62
mean-3SD	9.06	14.38	52.81	16.35	14.60
N	8	8	8	8	8
median	11.25	18.55	67.25	19.65	17.10
mean/All kit median	1.00	1.00	1.00	1.00	1.00

	MS 266	MS 267	MS 268	MS 269	MS 270
MS hCG Beckman Access (BCX/BC1) mean:					
mean	11.6	19.4	72.2	21.1	18.6
SD	0.9	1.7	6.5	1.6	1.3
%CV	7.4%	8.6%	9.0%	7.5%	7.2%
mean+3SD	14.2	24.4	91.6	25.8	22.6
mean-3SD	9.1	14.4	52.8	16.3	14.6
N	9	9	9	9	9
median	11.9	18.9	73.0	21.1	18.7
mean/all kit median	1.04	1.05	1.07	1.06	1.08

	MS 266	MS 267	MS 268	MS 269	MS 270
MS hCG MoMs All Lab Mean:					
mean	0.46	0.91	1.55	0.78	0.96
SD	0.04	0.10	0.31	0.11	0.08
%CV	8.2%	11.5%	19.8%	14.7%	8.8%
mean+3SD	0.58	1.23	2.48	1.12	1.21
mean-3SD	0.35	0.60	0.63	0.44	0.70
N	26	26	26	26	26

	MS 266	MS 267	MS 268	MS 269	MS 270
MS hCG kit average:					
mean	10.9	17.9	63.9	19.4	17.1
SD	0.9	2.0	10.5	2.0	1.6
all kit median	11.2	18.6	67.5	20.0	17.3

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	MS 266	MS 267	MS 268	MS 269	MS 270
MS Inhibin A all lab mean:					
Mean	154.55	214.15	297.58	132.16	237.97
SD	15.68	24.35	30.22	14.96	21.79
%CV	10.1%	11.4%	10.2%	11.3%	9.2%
mean + 3SD	201.6	287.2	388.3	177.0	303.3
mean- 3SD	107.5	141.1	206.9	87.3	172.6
N	26	26	26	26	26
All Lab Median	156.9	221.8	306.9	134.0	241.6
mean/all kit median	0.97	0.98	0.99	0.98	0.99

	MS 266	MS 267	MS 268	MS 269	MS 270
MS Inhibin A Beckman Access (BCX/BC1) mean:					
Mean	160.2	219.5	300.5	134.9	240.6
SD	4.8	10.4	11.7	5.7	9.7
%CV	3.0%	4.8%	3.9%	4.3%	4.0%
mean + 3SD	174.4	250.8	335.6	152.1	269.6
mean- 3SD	145.9	188.1	265.5	117.7	211.6
N	13	13	13	13	13
median	160.5	221.7	304.0	134.9	243.1
mean/All kit median	1.01	1.00	1.00	1.00	1.00

	MS 266	MS 267	MS 268	MS 269	MS 270
MS Inhibin A Beckman Unicel (BCU/BC1) mean:					
Mean	159.0	223.0	313.2	138.8	247.6
SD	8.1	20.5	21.3	11.3	20.5
%CV	5.1%	9.2%	6.8%	8.1%	8.3%
mean + 3SD	183.4	284.4	377.1	172.7	309.2
mean- 3SD	134.6	161.6	249.2	104.9	186.0
N	10	10	10	10	10
median	156.2	229.3	315.0	139.0	250.2
mean/all kit median	1.00	1.02	1.04	1.03	1.03

	MS 266	MS 267	MS 268	MS 269	MS 270
MS Inhibin A Diagnostic System Labs (DS1) Mean:					
Mean	115.5	161.5	232.9	98.2	194.5
SD	6.9	11.9	31.7	5.4	11.6
%CV	5.9%	7.3%	13.6%	5.5%	6.0%
mean + 3SD	136.1	197.1	328.0	114.3	229.3
mean- 3SD	94.9	125.9	137.7	82.1	159.7
N	3	3	3	3	3
median	116.9	162.3	238.0	97.3	188.0
mean/all kit median	0.73	0.74	0.77	0.73	0.81

	MS 266	MS 267	MS 268	MS 269	MS 270
MS Inhibin A kit average:					
mean	144.9	201.3	282.2	124.0	227.6
SD	25.5	34.5	43.2	22.4	28.8
all kit median	159.0	219.5	300.5	134.9	240.6

	MS 266	MS 267	MS 268	MS 269	MS 270
MS Inhibin A MoM All Lab Mean:					
mean	0.91	1.14	1.56	0.67	1.17
SD	0.13	0.19	0.25	0.12	0.16
%CV	13.8%	16.4%	15.8%	18.4%	13.6%
mean+3SD	1.28	1.70	2.30	1.05	1.65
mean-3SD	0.53	0.58	0.82	0.30	0.69
N	26	26	26	26	26

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	AF 266	AF 267	AF 268	AF 269	AF 270
AF AFP All Lab Mean :					
mean	8.32	12.38	10.60	12.36	19.25
SD	0.91	1.73	1.27	1.55	2.30
%CV	10.9%	14.0%	12.0%	12.6%	11.9%
mean+3SD	11.0	17.6	14.4	17.0	26.1
mean-3SD	5.6	7.2	6.8	7.7	12.3
N	22	22	22	22	22
All kit median	8.6	12.6	10.6	12.8	20.0
mean/All kit mean	0.97	0.98	1.00	0.96	0.96

	AF 266	AF 267	AF 268	AF 269	AF 270
AF AFP DPC Immulite 2000 (DPD/DP5) mean:					
mean	8.9	12.9	9.6	13.1	20.4
SD	0.3	0.9	0.7	0.7	1.3
%CV	3.4%	7.0%	6.9%	5.2%	6.2%
mean+3SD	9.8	15.6	11.6	15.1	24.2
mean-3SD	8.0	10.2	7.6	11.0	16.6
N	5	5	5	5	5
median	8.9	12.6	9.7	12.7	21.1
mean/all kit median	1.04	1.02	0.90	1.02	1.02

	AF 266	AF 267	AF 268	AF 269	AF 270
AF AFP MoMs All Lab Mean:					
mean	1.07	1.30	0.63	1.07	3.03
SD	0.10	0.11	0.08	0.10	0.31
%CV	9.2%	8.7%	12.1%	9.8%	10.1%
mean+3SD	1.37	1.63	0.85	1.38	3.95
mean-3SD	0.78	0.96	0.40	0.75	2.11
N	22	22	22	22	22

	AF 266	AF 267	AF 268	AF 269	AF 270
AF AFP Beckman Unicel (BCU/BC1) mean:					
Mean	7.5	11.0	10.2	10.9	17.1
SD	0.7	1.1	1.2	1.3	1.6
%CV	9.6%	10.0%	11.8%	11.7%	9.2%
X+3SD	10.5	15.2	13.6	16.2	24.9
X-3SD	6.0	9.4	8.5	8.9	14.1
N	7	7	7	7	7
median	7.5	11.5	10.6	10.9	17.0
mean/All kit median	0.87	0.87	0.96	0.85	0.85

	AF 266	AF 267	AF 268	AF 269	AF 270
AF AFP Beckman Access (BCX/BC1) mean:					
mean	8.3	12.3	11.0	12.6	19.5
SD	0.7	1.0	0.8	1.2	1.8
%CV	9.0%	7.8%	7.6%	9.7%	9.2%
mean+3SD	10.5	15.2	13.6	16.2	24.9
mean-3SD	6.0	9.4	8.5	8.9	14.1
N	7	7	7	7	7
median	8.2	12	10.9	12.3	19.1
mean/all kit median	0.96	0.98	1.04	0.98	0.98

	AF 266	AF 267	AF 268	AF 269	AF 270
AF AFP Abbott Axsym (ABB/AB2) mear:					
mean	9.7	16.3	13.0	14.9	22.8
N	2	2	2	2	2
mean/all kit median	1.12	1.30	1.22	1.16	1.14
AF AFP kit average:					
mean	8.6	13.1	11.0	12.9	20.0
SD	0.9	2.3	1.4	1.6	2.4
all kit median	8.6	12.6	10.6	12.8	20.0

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	FT266	FT267	FT268	FT269	FT270
FT Gestational Age All Lab Mean:					
Mean	13.0	11.9	11.5	12.5	11.2
SD	0.12	0.11	0.13	0.10	0.13
%CV	1.0%	0.9%	1.1%	0.8%	1.2%
X+3*SD	13.4	12.2	11.9	12.8	11.6
X-3*SD	12.6	11.6	11.1	12.2	10.8
N	16	16	16	16	16

	FT266	FT267	FT268	FT269	FT270
FT NT MoMs All Lab Mean:					
Mean	0.67	0.90	0.98	1.86	0.95
SD	0.04	0.07	0.07	0.14	0.07
%CV	6.1%	8.2%	7.3%	7.4%	7.8%
X+3SD	0.79	1.13	1.20	2.27	1.18
X- 3SD	0.55	0.68	0.77	1.45	0.73
N	15	15	15	15	15
All Median	0.66	0.88	0.99	1.83	0.96

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	FT266	FT267	FT268	FT269	FT270
FT hCG All Lab Mean:					
mean	60.64	63.09	89.77	142.79	68.89
SD	8.51	9.47	14.47	28.56	11.97
%CV	14.0%	15.0%	16.1%	20.0%	17.4%
X+3SD	86.2	91.5	133.2	228.5	104.8
X-3SD	35.1	34.7	46.4	57.1	33.0
N	15	15	15	15	15
mean/All kit median	0.96	0.95	0.97	0.93	0.96

	FT266	FT267	FT268	FT269	FT270
FT hCG Beckman Unicel (BCU/BC1) mean:					
mean	63.28	66.18	92.68	154.15	71.65
SD	4.48	2.51	8.80	6.72	4.09
%CV	7.1%	3.8%	9.5%	4.4%	5.7%
X+3SD	89.89	90.72	133.25	218.36	106.24
X-3SD	42.34	49.08	67.95	107.67	49.33
N	4	4	4	4	4
median	62.35	66.40	89.90	151.55	70.75
mean/All kit median	1.00	1.00	1.00	1.00	1.00

	FT266	FT267	FT268	FT269	FT270
FT hCG DPC Immulite 2000(DPD/DP5) mean:					
mean	52.0	52.4	74.5	109.4	56.0
SD	3.7	5.3	7.0	16.1	6.5
%CV	7.2%	10.1%	9.4%	14.8%	11.7%
X+3SD	63.2	68.3	95.5	157.9	75.7
X-3SD	40.7	36.6	53.5	61.0	36.4
N	5	5	5	5	5
median	52.0	49.7	72.3	103.0	54.7
mean/All kit median	0.82	0.79	0.80	0.71	0.78

	FT266	FT267	FT268	FT269	FT270
FT hCG Beckman Access (BCX/BC1) mean:					
mean	66.1	69.9	100.6	163.0	77.8
SD	7.9	6.9	10.9	18.4	9.5
%CV	12.0%	9.9%	10.8%	11.3%	12.2%
X+3SD	89.9	90.7	133.2	218.4	106.2
X-3SD	42.3	49.1	68.0	107.7	49.3
N	6	6	6	6	6
median	67.2	69.0	100.4	160.0	74.5
mean/All kit median	1.04	1.06	1.09	1.06	1.09

	FT266	FT267	FT268	FT269	FT270
FT hCG MoMs All Lab Mean:					
Mean	0.92	0.77	1.07	1.95	0.83
SD	0.12	0.09	0.10	0.27	0.08
%CV	12.8%	12.1%	9.2%	13.8%	9.9%
mean+3*SD	1.28	1.05	1.37	2.75	1.08
mean- 3*SD	0.57	0.49	0.78	1.14	0.58
N	14	14	14	14	14
All Median	0.91	0.77	1.07	1.91	0.85

	FT266	FT267	FT268	FT269	FT270
FT hCG kit average:					
mean	60.5	62.8	89.2	142.2	68.5
SD	7.5	9.2	13.4	28.7	11.2
all kit median	63.3	66.2	92.7	154.2	71.7

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	FT266	FT267	FT268	FT269	FT270
FT PAPP-A All Lab Mean:					
Mean	804.66	609.34	2094.87	307.53	473.15
SD	85.53	51.54	161.54	41.89	40.03
%CV	10.6%	8.5%	7.7%	13.6%	8.5%
mean + 3SD	1061.26	763.97	2579.50	433.20	593.23
mean- 3SD	548.06	454.72	1610.24	181.86	353.07
N	15	15	15	15	15
All Lab Median	791.90	592.50	2113.00	300.00	470.31
mean/All kit median	1.00	0.98	0.97	1.03	1.00

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A DPC Immullite 2000 (DPD/DP5) Mean:					
Mean	937.31	672.23	2162.06	297.53	527.31
SD	49.09	28.69	87.07	14.69	46.75
%CV	5.2%	4.3%	4.0%	4.9%	8.9%
X + 3SD	1084.57	758.30	2423.28	341.60	667.55
X - 3SD	790.05	586.16	1900.84	253.46	387.06
N	3	3	3	3	3
Kit Median	940.47	660.64	2167.33	290.68	531.00
mean/All kit median	1.17	1.08	1.01	1.00	1.11

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A Beckman Unicel or Access (BCU or BCX/BC1) Mean:					
Mean	761.19	584.28	2150.50	286.55	455.17
SD	49.53	31.21	114.94	20.41	27.09
%CV	6.5%	5.3%	5.3%	7.1%	6.0%
X + 3SD	909.78	677.90	2495.32	347.78	536.44
X - 3SD	612.59	490.65	1805.68	225.32	373.90
N	9	9	9	9	9
Kit Median	746.0	580.6	2139.4	289.8	453.5
mean/All kit median	0.95	0.94	1.00	0.96	0.96

	FT266	FT267	FT268	FT269	FT270
***FT PAPP-A Diagnostic Systems Lab (DS1) Mean:**					
Mean	802.44	621.66	1860.81	380.49	472.95
SD	63.88	69.91	139.19	16.91	15.50
%CV	8.0%	11.2%	7.5%	4.4%	3.3%
X + 3SD	1.82	1.53	3.60	0.60	0.83
X - 3SD	1.06	0.60	2.54	0.30	0.59
N	3	3	3	3	3
Kit Median	825.49	592.50	1882.80	380.35	470.31
mean/All kit median	1.00	1.00	0.87	1.28	1.00

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A kit average:					
mean	833.64	626.06	2057.79	321.52	485.14
SD	92.12	44.14	170.69	51.36	37.58
all kit median	802.44	621.66	2150.50	297.53	472.95

***Note: The above 2 tables contain converted values (mIU/ml->ng/ml) from equations obtained based on in house correlation data.**
(see critique)

New York State Fetal Defect Markers Proficiency Test,
May 2011
Summary of First Trimester Results

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A MoM All Lab Mean:					
Mean	0.86	0.88	3.31	0.37	0.93
SD	0.51	0.52	1.50	0.23	0.52
%CV	58.8%	59.1%	45.4%	63.1%	56.4%
mean + 3SD	2.38	2.45	7.81	1.06	2.49
mean- 3SD	-0.66	-0.68	-1.20	-0.33	-0.64
N	15	15	15	15	15
All Lab Median	0.70	0.70	2.75	0.27	0.69
mean/ All kit median	1.32	1.35	1.17	1.37	1.29

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A MoM DPC Immulite 2000 (DPD/DP5) Mean:					
Mean	1.75	1.85	6.05	0.81	1.89
SD	0.43	0.31	0.55	0.01	0.36
%CV	24.3%	16.6%	9.1%	1.2%	19.1%
X + 3SD	3.03	2.77	7.71	0.84	2.97
X - 3SD	0.48	0.92	4.39	0.78	0.80
N	3	3	3	3	3
mean/All kit median	2.46	2.76	2.15	3.03	2.62

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A MoM Beckman Unicel or Access (BCU or BCX/BC1) Mean:					
Mean	0.65	0.65	2.82	0.27	0.72
SD	0.10	0.10	0.41	0.03	0.08
%CV	15.6%	15.2%	14.7%	11.4%	11.4%
X + 3SD	0.95	0.95	4.06	0.36	0.97
X - 3SD	0.35	0.36	1.57	0.18	0.47
N	8	8	8	8	8
Kit Median	0.66	0.62	2.70	0.27	0.70
mean/All kit median	0.91	0.97	1.00	1.00	1.00

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A MoM Diagnostic System Labs (DS1) Mean:					
Mean	0.71	0.67	2.35	0.24	0.63
SD	0.09	0.12	0.36	0.04	0.06
%CV	11.9%	18.2%	15.3%	17.1%	9.5%
X + 3SD	0.97	1.03	3.43	0.36	0.81
X - 3SD	0.46	0.31	1.27	0.12	0.45
N	3	3	3	3	3
Kit Median	0.71	0.73	2.25	0.23	0.64
mean/ All kit median	1.00	1.00	0.83	0.88	0.88

	FT266	FT267	FT268	FT269	FT270
FT PAPP-A MoM kit average:					
mean	1.04	1.06	3.74	0.44	1.08
SD	0.62	0.68	2.02	0.32	0.70
all kit median	0.71	0.67	2.82	0.27	0.72

New York State Fetal Defect Markers Proficiency Test, FEDM PT, May 2011

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

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

Due Date: **May 10, 2011** _____

_____

Analyte Second Trimester Maternal Serum	**Analytical results**					Instrument code*	Reagent code*
	Vial **MS266**	Vial **MS267**	Vial **MS268**	Vial **MS269**	Vial **MS270**		
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, May 2011

Amniotic Fluid	Vial **AF266**	Vial **AF267**	Vial **AF268**	Vial **AF269**	Vial **AF270**	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	**MS266**	**MS267**	**MS268**	**MS269**	**MS270**	**Risk (MoM) Cut-off (white, Black, IDDM)**
NTD Risk (or MoM)						**White**_____ **Black**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling						**IDDM white**_____ **IDDM black**_____
Trisomy 21 Risk by Quad						**White**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling						**Black**_____ **IDDM**_____
Trisomy 21 Risk by Triple						**White**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling						**Black**_____ **IDDM**_____
Trisomy 18 Risk						**White**_____
R=Repeat, U=Ultrasound, A=Amnio NFA=NoFurtherAction, G=Genetic Counseling						**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, May 2011

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 266	1/1/1982	B	1.10	150	2/4/2011	**67**	5/6/2011
FT 267	1/1/1981	A	1.20	120	2/11/2011	**53**	5/6/2011
FT 268	1/1/1983	W	1.20	125	2/14/2011	**48**	5/6/2011
FT 269	1/1/1986	W	2.80	130	2/7/2011	**61**	5/6/2011
FT 270	2/1/1983	H	1.10	135	2/18/2011	**45**	5/6/2011

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

First Trimester Maternal Serum	Vial **FT 266**	Vial **FT 267**	Vial **FT 268**	Vial **FT 269**	Vial **FT 270**	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		

Results will *not* be graded. Information will be used for future possible implementation.

Risk Assessment Ratio (1:n)and Further Action	**FT266**	**FT267**	**FT268**	**FT269**	**FT270**	**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 NFA=NoFurtherAction						
Trisomy 18 Risk by First Trimester						**White**_____ **Black**_____ **IDDM**_____
R=Repeat, U=Ultrasound, A=Amnio, G=Genetic Counseling 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
Bayer/Siemens (Chiron) ACS-180	COS
Bayer/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
(DSL ELISA) with Microplate Reader	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
Bayer/Siemens	BA1
Bayer/Siemens (Chiron)	CO1
Beckman Coulter	BC1
Siemens Diagnostic (Dade Behring)	DA1
Diagnostic Systems Lab EIA (DSL ELISA)	DS1
Diagnostic Systems Lab liquid RIA	DS2
Diagnostic Systems Lab solid RIA	DS3
DiaSorin-Clinical Assays	DC1
Siemens Diagnostic (DPC) Coat-A-Count	DP1
Siemens DPC Immulite, Immulite 2000 or Immulite 2500	DP5
New **Siemens DPC Immulite, Immulite 2000 or Immulite 2500 for uE3 only**	**DP6**
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.