

Fetal Defect Marker Proficiency Test Mailout June, 2009

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from May 5, 2009 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. Please review and sign your evaluation. Retain the signed packet 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 = 30	Sample #	MS 236‡	MS 237‡	MS 238	MS 239	MS 240
	Gestational Age (weeks)	18	18	16	16	19
Maternal Race	Ethnic Group	Black	White	White	Hispanic	White
Maternal Weight	Pounds (lbs)	150	200	175	130	150
Maternal Age	Years	20	35	30	25	28
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std.Dev.	49.52 ± 3.45	50.03 ± 3.03	97.86 ± 6.59	83.18 ± 5.83	30.13 ± 2.20
	MOM ± Std.Dev.	1.19 ± 0.13	1.61 ± 0.17	3.24 ± 0.24	2.31 ± 0.18	0.59 ± 0.04
Unconjugated Estriol (uE3)	Mean ng/ml ± Std.Dev.	1.68 ± 0.71	1.82 ± 0.90	1.25 ± 0.54	1.28 ± 0.59	0.86 ± 0.31
	MOM ± Std.Dev.	0.83 ± 0.25	0.91 ± 0.23	0.98 ± 0.30	0.95 ± 0.34	0.35 ± 0.13
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std.Dev.	20.74 ± 1.82	20.68 ± 1.74	28.05 ± 2.36	26.85 ± 2.24	39.35 ± 3.87
	MOM ± Std.Dev.	0.93 ± 0.11	1.18 ± 0.14	1.04 ± 0.11	0.84 ± 0.08	2.12 ± 0.29
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std.Dev.	149.85 ± 16.65	164.43 ± 20.74	142.57 ± 13.64	157.31 ± 17.69	427.16 ± 25.88
	MOM ± Std.Dev.	0.92 ± 0.12	1.14 ± 0.17	0.90 ± 0.10	0.87 ± 0.11	2.47 ± 0.26
Neural Tube Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (82.8%)	Pos. (+) (100%)	Pos. (B) (51.7%)	Neg. (-) (100%)
	Further Action G,U,A	NFA	NFA	G = 59% U = 79% A = 72%	G = 21% U = 31% A = 24%	NFA
	NTD Risk 1 in	3180	449	57	350	8950
Trisomy-21 Screen (Positive, Negative) percent 1. <u>Triple test</u>	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	G = 75% U = 69% A = 69%
	Risk Est. 1 in	3,200	1,100	5,900	7,000	31
2. <u>Quad Test</u>	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	G = 71% U = 64% A = 71%
	Risk Est. 1 in	10,000	2,300	18,000	16,000	10
Trisomy-18 Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Risk Est. 1 in	10,000	10,000	10,000	10,000	1,365

*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 Down positive/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) Second Trimester Maternal Serum Analytes:

A. Narrative Evaluation of Second Trimester Screening Results:

N = 30 all-lab Consensus Values.

<u>Sample #</u>	<u>Summary Comments (Mock specimens):</u>
MS 236 Wk 18.0	This specimen was obtained from a 20 year old diabetic black woman (Gravida = 1, Parity = 0) in her 18 th week gestation with a body weight of 150 lbs. She had no personal history of pregnancy loss. Her specimen, a second pregnancy sample, was negative for NTD (100% consensus); however, race and diabetic corrections were indicated. Her screen was also negative for both Trisomies with all labs in agreement. No recommendations of further action were submitted for the MS236 sample. The MS236 specimen had no amniotic fluid counterpart.
MS 237 Wk 18.0	This specimen was procured from a 35 year old, diabetic white woman (Gravida = 3, parity = 2) in her 18 th week gestation with a body weight of 200 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 (83% consensus); however, 17% reported a positive screen due to the diabetic correction. The labs were also in agreement that both trisomy consensus screens were negative (100%). Specimen MS237 was not paired with an amniotic fluid sample.
MS 238 Wk 16.0	This specimen was obtained from a 30 year old white woman (Gravida = 2, Parity = 1) in her 16 th week gestation with a body weight of 175 lbs. A correction factor for borderline body weight may be indicated. She had a family history of pregnancy complications. Her specimen, a third pregnancy sample, was a positive screen for NTD (100% consensus; MOM=3.24). Her screen was negative for both Trisomies with all labs in agreement. Recommendations of further action from labs performing the NTD screen were: genetic counseling, 59%, ultrasound, 79% and amniocentesis, 72%. The MS238 specimen had no amniotic fluid counterpart.
MS 239 Wk 16.0	This specimen was obtained from a 25 year old hispanic woman (Gravida = 1, parity = 0) in her 16 th week gestation with a body weight of 130 lbs. She had no family history of pregnancy complications or adverse outcomes. Her sample screened borderline negative for NTD and her aneuploidy screen was negative for both Trisomy-21 and for Trisomy-18. Recommendations of further action from 51% of the labs reporting a NTD borderline screen were: repeat, 21% genetic counseling, 21%, ultrasound, 31% and amniocentesis, 24%. No recommendation of further action for the T21 screen was reported from the participating labs. This specimen was not paired with an amniotic fluid specimen.
MS 240 Wk 18.0	This specimen was obtained from a 28 year old white woman (Gravida = 3, parity = 1) in her 19 th week gestation with a body weight of 150 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD; however, her aneuploidy screen was positive for Trisomy-21 (100%). Recommendations of further action from labs performing the T21 quad screen were: genetic counseling, 71%; ultrasound, 64%; and amniocentesis, 71%; while the triple tests were: genetic counseling, 75%; ultrasound 69%, and amniocentesis, 69%. Specimen MS240 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 = 0.89).

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

Instructional Note:

This notice regards the demographic data provided for the mock patients in the FEDM program. For the sake of this program, it will be understood that gravida indicates the pregnant status of a woman and parity is the state of having given birth to a completed term infant or infants. Thus, a gravida = n, indicates number (n) of times pregnant including the present one; a gravida = 2 indicates that the woman 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=31; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 236 Wk 19.0	AFP= 6.20 ± 0.70 µg/ml MOM= 0.79 ± 0.06	The AF236 sample was targeted for normal AF AFP value in the upper gestational age range. All labs called AF236 a non-elevated specimen for NTD. This AF AFP sample was not matched to a maternal serum specimen.
AF 237 Wk 18.0	AFP= 8.30 ± 1.61 µg/ml MOM= 0.85 ± 0.12	The AF237 sample was targeted for a screen negative AF AFP value in the routine gestational age range. All labs reported this specimen as a screen negative AF AFP value. The AF237 specimen was not paired with a maternal serum sample.
AF 238 Wk 17.0	AFP= 10.40 ± 1.10 µg/ml MOM= 0.89 ± 0.07	The AF238 sample was targeted for a negative NTD screen for AF AFP in the routine gestational age screening range. All labs categorized this as an NTD screen non-elevated specimen. This sample was not coupled to a maternal serum specimen.
AF 239 Wk 20.0	AFP= 5.00 ± 0.60 µg/ml MOM= 0.79 ± 0.07	The AF239 sample was targeted as an NTD negative screen in the upper gestational age screening range. All labs categorized AF239 as a negative NTD screen specimen and it had no maternal serum counterpart.
AF 240 Wk 19.0	AFP= 7.01 ± 0.80 µg/ml MOM= 0.89 ± 0.07	The AF240 sample was targeted for a low AF AFP value in the upper gestational age range. Most labs called AF240 a normal MOM AF AFP specimen. This AF AFP sample was matched to a maternal serum specimen, MS240, which was low (MOM = 0.59) and screened positive for T21.

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples N = 16	Sample #	FT 236	FT 237	FT 238	FT239	FT240
	Gestational Age (weeks)	11.0	13.0	11.4	11.9	12.4
Maternal Race	Ethnic Group	Hispanic	White	Black	Asian	White
Maternal Weight	Pounds (lbs)	135	150	145	155	130
Maternal Age	Years	28	30	25	21	35
Physical Measurements of the Fetus	Crown Rump Length (mm)	42	67	47	53	60
	NT Thickness (mm)	1.10	1.50	1.09	2.90	1.30
	NT - MOM	1.02 ± 0.16	0.93 ± 0.12	0.91 ± 0.12	2.23 ± 0.26	0.89 ± 0.11
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL	75.90	58.01	74.00	209.41	68.52
	± Std. Dev.	± 9.11	± 5.80	± 7.51	±25.71	±7.81
Pregnancy-Associated Plasma Protein-A (PAPP-A)	MOM	0.91	0.91	0.95	2.98	0.94
	± Std. Dev.	± 0.12	± 0.10	± 0.12	±0.46	±0.13
Trisomy-21 Screen (Positive/Negative) percent	Mean mIU/mL	1.87	3.10	2.10	1.16	2.71
	± Std. Dev.	± 0.96	± 1.53	± 1.06	± 0.56	± 1.36
Trisomy-21 Screen (Positive/Negative) percent	MOM	1.98	1.56	1.77	0.96	1.48
	± Std. Dev.	± 0.98	± 0.65	± 0.94	±0.44	±0.62
Trisomy-21 Screen (Positive/Negative) percent	Pos (+) or Neg. (-)	Neg. (100%)	Neg. (100%)	Neg. (100%)	Pos. (100%)	Neg. (100%)
	Recommended Action	NFA	NFA	NFA	G = 100% U = 47% C = 60%	NFA
	Risk Estimate	10,000	10,000	10,000	19	6,300
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	Neg. (100%)	Neg. (100%)	Neg. (100%)	Neg. (100%)	Neg. (100%)
	Recommended Action	NFA	NFA	NFA	NFA	NFA
	Risk Estimate	10,000	10,000	10,000	1,284	10,000

(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

1) First Trimester Maternal Sera Only:

B. Narrative Evaluation of First Trimester Screening Results:

N = 16 all-lab Consensus Values.

<u>Sample#</u>	<u>Summary Comments:</u>
FT 236 Wk 11.0	This specimen was obtained from a 28 year old hispanic woman of average body weight (135 lbs.). Her gestational age at time of screening was 11.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs were in agreement. The FT236 risk estimate for Trisomy-21 was 1 in 10,000, while the all-lab Trisomy-18 risk was 1 in 10,000 (negative screen). All labs were in agreement that FT236 was a negative screen for a Trisomy-21 and T18 pregnancy.
FT 237 Wk 13.0	This specimen was obtained from a 30 year old white woman of medium body weight (150 lbs). Her gestational age at time of screening was 13.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs were in agreement. The FT237 risk estimate for Trisomy-21 was 1 in 10,000 while the Trisomy-18 risk was 1 in 10,000.
FT 238 Wk 11.4	This specimen was obtained from a 25 year old black woman of average body weight (145 lbs.). Her gestational age at time of screening was 11.4 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs were in agreement. The FT238 risk estimate for Trisomy-21 was 1 in 10,000; while the all-lab Trisomy-18 risk was 1 in 10,000 (negative screen). All labs were in agreement that FT238 was a negative screen for Trisomy-21 and Trisomy-18.
FT 239 Wk 11.9	This specimen was procured from a 21 year old asian woman of average body weight (155 lbs.). Her gestational age at time of screening was 11.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen positive for T21 and all testing Labs were in agreement (see Critique). The FT239 risk estimate for Trisomy-21 was 1 in 19, while the Trisomy-18 risk was 1 in 1,284.
FT 240 Wk 12.4	This specimen was procured from a 35 year old white woman with a body weight of 130 lbs. Her gestational age at time of screening was 12.4 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative for T21 and T18. The risk estimate for FT240 was 1 in 6,300, and the T18 risk was 1 in 10,000. All labs were in agreement with both FT240 aneuploid screen assessments.

III. Critique and Commentary:

A) Fetal Defect Proficiency Test Mail out 5/5/09 of Second Trimester Maternal Serum and Amniotic Fluid Values:

In general, the all-lab results of the targeted values for the NTD and the Trisomy Screen were consistent with the goals of our projected target values, risks, and outcomes. As displayed in the second trimester tables, maternal serum samples MS238 and MS239 were targeted as elevated and borderline specimens for NTD (Figs. 1 and 3), respectively, and no AF-AFP specimens were matched to these specimens. Thus, specimens MS238 and MS239 were determined to be screen positive and borderline positive for NTD, but negative for Trisomy-21 (T21), and Trisomy-18 (T18). For the MS238 specimen, the NTD screen resulted in a 1 in 57 all-lab risk for open neural tube defects (ONTD), while MS239 resulted in a 1 in 350 all lab NTD screen; while MS238 achieved 100% NTD screen consensus, MS239 failed to achieve consensus (51%). The NTD-related recommended action for specimen MS238 was genetic counseling, 59%; ultrasound; 79%; and amniocentesis, 72%, while MS239 was genetic counseling 21%; ultrasound 31%; and amniocentesis 24%. Sample MS240, a T21 screen positive specimen, was obtained from a white woman with a prior (sibling) history of pregnancy problems. The T21 MOM results for specimen MS240 (MSAFP-MOM = 0.59, MSuE3-MOM = 0.34, MShCG-MOM = 2.12, DIA-MOM = 2.47) were all in accordance with a T21 positive screen; thus, all labs classified this specimen as a T21 positive screen with all recommending further action. The MS240 sample (from a 28 year old woman) produced a risk from the quad test (1 in 10) which was greater than that expected from the maternal age alone (1 in 800). Finally, samples MS236 and MS237 produced negative screens for NTD, Trisomy-21, and Trisomy 18; however, corrections for diabetic status and race were indicated for MS236, while only IDD was recommended for MS237. Regarding the diabetic MS237 specimen, note in Table 1 that nearly 17% of the labs classified this sample as a positive NTD screen due to the diabetic cutoff median MOM levels of 1.90 (see Fig-1). The age-specific risk of the MS240 sample for T21 was 1 in 800.

Specimen MS240, together with a matched AF-AFP sample, deserves further comment in that the MSAFP specimen was T21 screen positive (100%; MSAFP MOM = 0.59) and the paired AF-AFP sample was normal (MOM = 0.89) (Figs. 1 & 2). This mock patient had a sibling history of pregnancy loss; thus, paired maternal MS and AF samples had been obtained at time of amniocentesis. Since her positive MSAFP MOM was accompanied by a low AF-AFP MOM, this was consistent with a T21

positive screen. No demographic correction factors were indicated for the MS240 screen assessment. The MS240 specimen produced a positive Trisomy-21 (T21) result with both the triple and quad testing platforms. Further action recommended for the T21 screen was determined as genetic counseling, 75%, ultrasound (US), 69% and 69% amniocentesis (AM) for labs using the triple screen; while genetic counseling 71%, US 64% and AM, 71% was recorded for labs employing the quad screen. The recommended action on MS240 was consistent with the severity of the similar risk ratio assessments of 1 in 31 from the triple test versus a 1 in 10 risk from the quad test. Note from the point distribution graphs comparing the triple with the quad test (Figs. 5 and 6) that the MS240 point cluster in the quad assay was nearly the same but slightly lower and tighter (note scale difference) than the MS240 cluster in the triple test. As seen with previous specimens, the quad test signaled a slightly higher risk (difference) for Down syndrome than the triple test; however, both screens resulted in very high risks for Down syndrome. At the time of the assay, the sample was accompanied by an amniotic fluid (AF) specimen due to prior family pregnancy complications in sibling-related pregnancies. The AFAFP in this sample was normal (MOM = 0.89). The amniotic fluid cells of this mock patient were subjected to subsequent genotyping and indeed indicated the presence of a Trisomy-21. The MS240 specimen produced a negative screen for Trisomy-18.

Specimen MS238 invites further comment in that the sample was NTD screen positive (100%; MOM=3.24). This mock patient had no prior history of pregnancy loss or complications and a paired amniotic fluid was not available for analysis at time of venipuncture specimen collection. This MS specimen represented a 16 week gestational age sample, thus, an amniocentesis and ultrasound would be indicated. An elevated MSAFP screening value, by itself is not diagnostic of a neural tube defect; however, it is a powerful indicator to pursue subsequent diagnostic testing in order to eliminate the possibility of an open NTD. Subsequent Stage-II ultrasound, AFAFP, and amniocentesis genotyping of MS238 were normal in this mock patient; in addition a fetal-maternal bleed was ruled out and a diagnostic band following ache electrophoresis was absent. Thus, this screened specimen was deemed an unexplained elevated MSAFP specimen. Furthermore, the specimen MS238 displayed a novel biomarker profile in its screen results. The MSAFP was elevated (MOM = 3.24), the uE3 level was at the median (MOM = 0.98), the MShCG was normal (1.04 MOM), and the DIA (MOM = 0.90) was slightly low. In view of these results, most of the participating laboratories called MS238 a positive screen NTD, and screen negative for T21 and T18, and most labs suggested NTD further actions. The presence of an unexplained elevated AFP in a somewhat unique maternal serum profile prompts one to consider conditions where AFP predicts poor outcomes, such as preterm birth (see suggested ref. #35). Interestingly, the MS238 specimen was obtained from a 30 year old Caucasian patient and adverse and complicated outcomes had been a part of her personal family history.

Specimen MS239 is also of interest in that the sample was an NTD borderline negative screen (48%; MOM=2.31). This mock patient had no prior history of pregnancy loss or complications, and a paired amniotic fluid was not available for analysis at time of specimen collection. This MS specimen represented a 16 week gestational age sample and an amniocentesis and ultrasound would have indeed been indicated. A subsequent amniocentesis was performed as a result of this screen and a second MS-specimen was obtained at time of amniocentesis. However, an elevated MSAFP screening value by itself is not diagnostic of a neural tube defect; thus, a repeat MS sample and Ache are powerful indicators as diagnostic testing agents to eliminate or confirm the possibility of an open NTD. Subsequent Stage-II ultrasound, Ache, AFAFP, and amniocentesis genotyping were subsequently found to be normal in this mock patient; however, a slight fetal-maternal bleed was detected. Thus, this screened specimen was deemed an elevated MSAFP specimen due to fetal-maternal bleed but an NTD was ruled out. The specimen MS238 did display an unusual biomarker profile in its screen results. Although the MSAFP was elevated (MOM = 2.31) the uE3 level was low/normal (MOM = 0.85), MShCG was low normal (0.84 MOM) as was the DIA (MOM = 0.87). However, some of the participating laboratories had called MS238 a borderline negative screen NTD accompanied by negative screen for T21 and T18. Only 51% of the labs suggested further NTD actions (see above). Interestingly, the MS239 specimen had been obtained from a 25 year old Hispanic patient and adverse and complicated outcomes had not been a part of her family history.

Unexplained elevated MSAFP as in MS239, other than neural tube defects, have been related to various pregnancy structural complications; however, some can also result from misdating LMPs and/or placental structural problems, such as a fetomaternal bleed. Adverse pregnancy outcomes relative to an elevated maternal serum AFP levels (MOM = >2.5) usually involve placental dysfunction and/or birth defects accompanied by sonographic adverse findings and growth retardation. Elevated AFP levels have long been used as an indicator for perinatal distress conditions such as bilateral renal agensis, placental abruptia/acctia, pre-eclampsia, intra-uterine growth retardation (IUGR), and fetal demise (Ref #3 below).

Fetomaternal transfusion (fetomaternal bleed or hemorrhage) can occur spontaneously or can contribute to variable increases in MSAFP levels following invasive procedures such as chorionic villus sampling (CVS), fetal cordocentesis or percutaneous umbilical blood sampling (PUBS), amniocentesis, (AMN) and therapeutic/elective abortion. (1-6) A fetomaternal bleed (FMB) can be detected by methods such as the Kleihauer-Betke Test, acid-elution technique, identification of fetal red cells and hemoglobin, and measurement of MSAFP levels. (3, 7, 8) The use of MSAFP levels as an indicator of FMB is remarkable in that as little as 0.05 to 0.10 ml volume of AFP can result in a 20 – 40% elevation of MSAFP levels following CVS and AMN procedures. (9, 10, 11) This is due largely to the steep concentration gradient of nanogram concentrations in the maternal serum versus microgram levels of AFP in amniotic fluid, and milligram levels in the fetus. Raised levels of MSAFP have been associated with late first trimester transdominal CVS but not transcervical CVS; however, neither procedure interferes nor comprizes subsequent measurement of MSAFP in second trimester NTD screening programs. (11, 12, 16) Following PUBS procedures, MSAFP values were reported to increase 30 to 40% affecting up to 30% of the patients undergoing this procedure. (5, 10, 13) Although FMB occurances alone have produced fetal hydrops (excessive accumulation in fetal fluid compartments) in some instances, there were no significant associations with adverse pregnancy outcomes such as preterm delivery, intrauterine

growth retardation, and intrauterine fetal death. (14) In cases of therapeutic and/or elective abortions, 30% of the women experienced significant elevations in MSAFP levels (60 – 70% increase) 48 hours following the procedure especially with the use of RU-486 (5, 14, 15): Following amniocentesis, increases in MSAFP levels (from 15 to 25 ugs) have also been reported in as many as 15 – 20% of the patients (3, 4, 11). The FMBs have not been found to be associated with the site of placentation in the uterine wall, but have been correlated with increased incidences of placental sonolucencies and interfacial placental complications at time of ultrasound (10, 15, 16). Finally, FMB can threaten pregnancy outcome by the increased trafficking of fetal cells that could affect fetomaternal blood incompatibility. The degree of FMB following abortion, for example, is considered sufficient to cause RBC antigen sensitization in rhesus-negative women and anti-D prophylaxis must be administered to such women to avoid rhesus iso-immunization (16). In cases of amniocentesis, as little as 0.1 ml rhesus-positive fetal blood is enough to cause iso-immunization in mothers; thus, immunoglobulin-anti-D is administered to all such rhesus negative mothers. (5, 7, 13)

The MSAFP references listed below refer to the text discussed in the preceding paragraph:

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6. Brezinka C, Hagens AM, Wladimiroff JW, Los FJ: Fetal ductus venosus flow velocity waveforms and maternal serum AFP before and after first-trimester transabdominal chorionic villus sampling. *Prenat Diagn.* 1995, 15:699-703.
7. Gilbert WM, Scioscia AL: Spontaneous fetal-maternal hemorrhage resulting in hydrops and elevated maternal serum alpha-fetoprotein levels. *J Ultrasound Med.* 1991, 10:645-648.
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13. Smidt-Jensen S, Lundsteen C, Lind AM, Dinesen K, Philip J: Transabdominal chorionic villus sampling in the second and third trimesters of pregnancy: chromosome quality, reporting time, and feto-maternal bleeding. *Prenat Diagn.* 1993, 13:957-969.
14. Christmas JT, Vanner LV, Daniels RM, Bodurtha JN, Hays PM, Redwine FO: The effect of fetomaternal bleeding on the risk of adverse pregnancy outcome in patients with elevated second-trimester maternal serum alpha-fetoprotein levels. *Am J Obstet Gynecol.* 1994, 171:315-319; discussion 319-320.
15. Chard T, Olajide F, Kitau M: Changes in circulating alphafetoprotein following administration of mifepristone in first trimester pregnancy. *Br J Obstet Gynaecol.* 1990, 97:1030-1032.
16. Downing GJ, Kilbride HW, Yeast JD: Nonimmune hydrops fetalis caused by a massive fetomaternal hemorrhage associated with elevated maternal serum alpha-fetoprotein levels. A case report. *J Reprod Med.* 1990, 35:444-446.

B) Assay Kit Performance:

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, and Inhibin A) are presented in a bar-graph format (Figures 7-10) for each of the five MS samples. As shown in the MSAFP graph, AFP mass measurements among the individual kits largely agreed, although Siemens/Bayer ADVIA-Centaur was slightly higher, and DPC Immulite was marginally lower for some samples. For uE3, the mean/all kit median for Diagnostic Systems Lab hovered about 1.0 (see Fig. 8A); however, labs employing DPC Immulite 2500 or Immulite 2000 yielded values achieving 1.2 to 1.7 times higher than the mean/all kit median (see dotted line). In contrast, Beckman Access/2 and Unicel measured uE3 values 40 to 45% lower than the mean/all kit median of 1.0. However, if the MS uE3 MOMs are plotted in a method comparison as mean/all kit median, the kit differences are normalized except for the Diagnostic Systems Lab liquid radioactive assay (see Figures 8A and 8B). Regarding the hCG kits (see Fig. 9), the Siemens/Bayer ADVIA-Centaur /ACS-180, and Beckman Access/2 yielded similar mean hCG values hovering about and above the 1.0 mean/all kit median value, while Beckman Unicel and DPC Immulite or Immulite 2000 kits demonstrated 3% to 5% lower values. In order to enhance uniformity among the various kits employed to measure hCG, we

incorporate intact recombinant (total) hCG analyte into our PT specimens. Finally, the method comparison of Inhibin-A is displayed in Fig. 10 for the Beckman Access/2 or Unidel versus the Diagnostic Systems Lab (DSL) assay platforms. Beckman kits were equal to or marginally higher and DSL was 15 to 20% lower than the 1.0 mean/all kit median value. Labs lacking peer group companions and in-house assays will be deemed non-gradable (NG) for individual analyte groups as the situation dictates.

The bar graph in Figure 11 is provided to display kit performances among the amniotic fluid (AF-AFP) test samples. As shown in the amniotic fluid bar graph, overall kit performance slightly wavered about the 1.0 mean/all kit median mark except for sample AF237. The AF237 variation outlier (1.38% higher) for Siemens/Bayer can be explained by a small kit user sample size (N=3). Overall Siemens/Bayer ADVIA-Centaur/ACS-180 and Abbott-AxSYM kits were 5 - 10% higher (except for sample AF237) Beckman Unidel and Beckman Access/2, were about 10% - 20% lower than the 1.0 mean/all-kit median. Interestingly, DPC Immulite was lower about the 1.0 mean and was lower still in sample AF237. Finally, please be advised that these specimens are derived from actual AF samples, and therefore these results are directly relevant to patient screening.

C) Second Trimester Screening Software Utilized:

For informational purposes, it was deemed of interest to provide the software package usage by our participating laboratories. The alpha and Benetech software packages were each used by 24% of the labs respectively; Robert Maciel (RMA) software was employed by 34%, while in-house software comprised 14%, and 3% of labs used programs classified as "other" which are proprietary software packages.

D) First Trimester Screen:

Five first trimester maternal serum mock samples are now and will be included in all future mailouts in order to survey and assess New York State licensed laboratories concerning participation and assay capabilities in first trimester Down syndrome screening. All laboratories that are **validation-approved** and presently performing 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), and draw date. Crown-rump length (CRL) measurements, race, and maternal body weight have also been included in the case histories to better evaluate all-lab participant NT information requirements.

As demonstrated in the FT table 2 (Section – II) above, the all lab measurement of the 11.0 week Hispanic FT236 specimen for total hCG resulted in a mass mean of 75.90 ± 9.11 , with a non-elevated MOM = 0.91. Furthermore, the all-lab mass mean for PAPP-A was 1.87 ± 0.96 mIU/ml with a MOM = 1.98. The all-lab T21 risk assessment was 1 in 8,000 for the FT236 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT236 sample was screen negative (See Figure 14 risk distribution). The risk cut-off level for Hispanics ranges from 200 to 270 among the participating labs. Thus, the FT236 sample resulted in a 100% all lab T21 negative screen assessment. No further action was indicated. Finally, the FT236 specimen also screened negative for T18 (1 in 10,000) using a cutoff of 1 in 100.

In the FT237 Caucasian sample, the gestational age all-lab mean was reported as 13.0 weeks. Assay measurements for FT237 resulted in an all-lab total hCG mass measurement of 58.01 ± 5.80 based on two present methods, Beckman Access/2 or Unidel and Siemens/DPC Immulite or 2000, while the all-lab PAPP-A mass assessments were 3.10 ± 1.53 obtained from the Diagnostic Systems Lab and Siemens/DPC Immulite kits (see Figs. 12 and 13). The first trimester all-lab trisomy-21 consensus for FT237 was screen negative, with a risk of 1 in 10,000. As observed in the FT table 2 above (see Section – II), the all-lab measurement for FT237 of total hCG resulted in a MOM value of 0.91 and the all-lab MoM mean for PAPP-A was 1.56. However, it should be reminded that two methods were employed for PAPP-A measurements, the Diagnostic Systems kit (N=10) and the DPC Immulite kit (N=5) (see Fig. 13A for mean/all kit medians). These two methods measure PAPP-A quite differently; the DPC Immulite PAPP-A assay mass mean for FT237 was 5.59 mIU/ml versus Diagnostic Systems Lab's 2.33 mIU/ml and these differences largely remained even after normalization to MOM values (see assay kit performance section D. 1.). Regardless of these results, all the labs agreed that the FT237 sample was screen negative for Trisomy 21 (See Fig. 14 point distribution). Since PAPP-A measurement for first trimester Down syndrome is associated with low MOM values, higher PAPP-A MOMs together with normal hCG levels would be consistent with a screen negative outcome. The all-lab T18 risk assessment for FT237 was 1 in 10,000; hence, the FT237 specimen resulted in a negative screen for Trisomy-18.

As shown in the above First Trimester table 2 (Section-II) for the FT238 Afro-American specimen, the gestational age all-lab mean was reported as 11.4 weeks. Assay measurements from FT-screening participating laboratories resulted in an all-lab total hCG mass measurement of 74.00 ± 7.51 IU/ml based on the two methods above; while the all-lab PAPP-A mass assessment was 2.10 ± 1.06 mIU/ml. The first trimester all-lab Trisomy-21 screen consensus for FT238 was negative. The all-lab FT trisomy-21 risk assessment was 1 in 10,000. As observed in the FT table above (Table 2, Section – II) the all lab measurement of total hCG for sample FT238 achieved a MOM value of 0.95. In comparison, the all-lab MoM for PAPP-A was 1.77, a normal value. Despite the assay differences in the PAPP-A DPC mass mean values compared to the Diagnostic Systems Lab means, all labs agreed that the FT238 sample was screen negative for Trisomy 21 (See Fig. 14 risk distribution). The FT238 specimen also resulted in a negative screen for Trisomy-18 with a risk assessment of 1 in 10,000.

As displayed in the FT table 2 (Section – II) above, the all lab measurement of the 12.0 week Asian FT239 specimen for total hCG resulted in a mass mean of 209.41 IU/ml ± 25.71 , with an elevated MOM = 2.98. Furthermore, the all-lab mass mean for PAPP-A was 1.16 ± 0.56 mIU/ml with a MOM = 0.65 ± 0.32 . The all-lab T21 risk assessment was 1 in 19 for the FT239 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT238 sample was screen positive (See Figure 14

risk distribution) demonstrating a high hCG MOM = 2.98, a low PAPP-A MOM = 0.96, and an increased NT MOM = 2.23. The risk cut-off level for Asians ranges from 200 to 270 among the participating labs. Since analyte MOM measurements for the first trimester Down syndrome screen detection are associated with raised NT, low PAPP-A, and high hCG MOMs, the FT238 results were indeed consistent with a T21 positive screen. Thus, the FT239 sample resulted in a 100% all lab T21 positive screen assessment. Further actions by the labs included genetic counseling, 100%; ultrasound, 47%; and amniocentesis/CVS = 28 – 32%. Finally, the FT239 specimen screened negative for T18 (1 in 1,287) using a cutoff of 1 in 100.

As demonstrated in the FT table 2 (Section-II) above for the Caucasian FT240 specimen, the gestational age all-lab mean was reported as 12.4 weeks. Assay measurements from FT-screening participating laboratories resulted in the all-lab total hCG mass measurement of 68.52 ± 7.81 IU/ml based on the two methods, while the all-lab PAPP-A mass assessment was 2.71 ± 1.36 mIU/ml. The first trimester all-lab trisomy-21 screen consensus for the FT240 specimen was negative (100%). The all-lab FT Trisomy-21 risk assessment was 1 in 6,300. As observed in the table (Table 2, Section – II), the all lab measurement of total hCG MOM for FT240 produced a value of 0.94. In comparison, the all-lab MOM mean for PAPP-A resulted in 1.48. Even with the result of assay differences in the PAPP-A DPC kit mass mean compared to Diagnostic Systems Labs, all labs agreed that the FT240 sample was screen negative for Trisomy 21. (See Fig. 14 risk distribution). The FT240 specimen also resulted in a negative screen for Trisomy-18 with an all-lab risk assessment of 1 in 10,000.

D. 1.) First Trimester Assay kit Performance:

The performance of the kits used for first trimester maternal serum analytes (hCG and PAPP-A) are presented in a bar-graph format (Figures 12, 13) for each of the five FT samples. As shown in the total FT hCG kit graph, hCG measurement between the two kits were somewhat the same, with Beckman Unicel/Access kit measuring slightly higher than the DPC kits and DPC being about 5% lower. In contrast, results from the two PAPP-A kits varied widely with the mean/all kit median values from Diagnostic Systems Lab (DSL) being less than half of those obtained with DPC Immulite or Immulite 2000 kit. This observation did not change when FT PAPP-A MOMs were plotted as a method comparison of mean/all kit median (see figure 13A and 13B). This brings into question how the normative data were obtained and utilized and the nature of the algorithms used to calculate T21 risk.

E) First Trimester Screening Software Utilized:

For informational purposes, it was deemed of interest to provide the software package usage by our participating laboratories. The alpha and Benetech software packages were each used by 27% and 20% of the labs respectively; Robert Maciel (RMA) software was employed by 33%, while in-house software comprised 20% of labs. None of the labs used programs classified as “other” which are proprietary software packages.

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Abstracts

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

(1) Title: Impact of antiphospholipid biology in maternal Down syndrome screening

Source: *Prenatal Diagnosis.* 29(5):481-8, 2009

Authors: Brochet C. Morin N. Coudert M. Vauthier-Brouzes D. Costedoat-Chalumeau N. Bernard M.

Abstract: AB OBJECTIVE: Women with antiphospholipid (aPL) biology present obstetric complications. The alpha-fetoprotein (AFP) serum levels of these patients are higher than in general population. Because AFP is involved in the calculation of the risk of trisomy 21 (T21), we studied the effect of AFP variations in the presence of aPL during T21 screening. METHODS: The study group (aPL group) was comprised of 64 pregnancies in women with aPL antibodies. The control group was comprised of 21 655 pregnancies included in the national program for routine Down syndrome (DS) screening by maternal serum markers [human chorionic gonadotrophin (hCG) and AFP] between 14 + 0 and 18 + 6 weeks of gestation. RESULTS: AFP values, converted in logarithm of multiples of the median (MoM), were significantly higher in the aPL group (0.03 vs 0.10; p = 0.018). After a matricial transformation of AFP MoM and hCG MoM in the aPL group, new T21 risks presented a median of one in 1665 versus one in 2574 (p < 0.0001 with a rank-sign test). CONCLUSION: Our results highlight the fact that in the presence of aPL antibodies, the calculated risk of T21 is underestimated. Therefore, clinicians should interpret the screening borderline results in aPL patients with caution.

(2) Title: Screening for placental insufficiency in high-risk pregnancies: is earlier better?.

Source: *Placenta.* 29(12):1034-40, 2008

Authors: Costa SL. Proctor L. Dodd JM. Toal M. Okun N. Johnson JA. Windrim R. Kingdom JC.

Abstract: AB OBJECTIVE: To compare a profile of placental function between the first and second trimesters in pregnancies at high risk of adverse perinatal outcomes attributable to placental insufficiency. STUDY DESIGN: Prospective cohort study in 61 singleton pregnancies. Uterine artery Doppler and placental morphology (shape and texture) were determined at 11-13(+6) weeks and at 18-23(+6) weeks. First trimester (pregnancy-associated placental protein-A [PAPP-A]) and second trimester (total hCG and alpha fetoprotein [AFP]) serum biochemistry were determined. The two screening periods were compared for the prediction of a range of severe adverse perinatal outcomes (intrauterine growth restriction [IUGR], abruption, severe pre-eclampsia/HELLP syndrome, delivery < 32 weeks, or stillbirth). RESULTS: Adverse perinatal outcomes occurred in 14 (23%) women; 3 (4.9%) losses < 20 weeks, 2 (3.3%) stillbirths > 20 weeks, 4 (6.6%) IUGR, 7 (11.5%) severe pre-eclampsia/HELLP syndrome, and 10 (16.4%) deliveries < 32 weeks. Abnormal second trimester placental morphology was significantly associated with adverse outcome [+LR: 3.6, 95% CI: 1.3-8.5; -LR: 0.63, 95% CI: 0.36-0.93; p=0.025], as was > or = 1 abnormal second trimester tests [+LR: 5.9, 95% CI: 1.6-24; -LR: 0.68, 95% CI: 0.59-0.89; p=0.005] or > or = 2 abnormal second trimester tests [+LR: 3.6, 95% CI: 1.3-7.7; -LR: 0.58, 95% CI: 0.27-0.94; p=0.035]. No combination of first trimester tests significantly predicted severe adverse perinatal outcomes. A study sample size of 822 women with similar high-risk characteristics would be needed

in order to refute the conclusion that present methods of first trimester screening are not inferior to second trimester screening for severe placental insufficiency ($p=0.05$, power 80%, z-test). CONCLUSIONS: In clinically high-risk pregnancies, prediction of adverse perinatal outcomes using placental function testing is more effective in the second compared with the first trimester.

(3) Title: Fetal chromosomal abnormalities: antenatal screening and diagnosis

Source: American Family Physician. 79(2):117-23, 2009

Authors: Anderson CL. Brown CE.

Abstract: AB Pregnant women of all ages should be offered screening and invasive diagnostic testing for chromosomal abnormalities before 20 weeks' gestation. New developments in screening methods have increased the number of options for patients. Diagnostic options include chorionic villus sampling in the first trimester and amniocentesis in the second trimester. Screening options in the first trimester include nuchal translucency testing in combination with measurement of pregnancy-associated plasma protein A and human chorionic gonadotropin. Nuchal translucency testing alone is not as effective. Screening options in the second trimester include serum screening using triple or quadruple screening, and ultrasonography. Patients may also choose a combination of first- and second-trimester screening in an integrated, stepwise sequential, or contingent sequential fashion. These options include an analysis of pregnancy-associated plasma protein A, with or without nuchal translucency testing, in combination with quadruple screening. An integrated test with nuchal translucency testing is the most effective method for women who present in the first trimester. If nuchal translucency testing is unavailable, the maternal serum-integrated test is safest and most effective. For women who do not present until the second trimester, the quadruple screen is recommended. Comprehensive counseling should be available to all pregnant women. Specific screening tests will depend on availability of the procedure and patient preference.

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

(1) Title: A case of umbilical cord hemangioma: doppler studies and review of the literature.

Source: European Journal of Obstetrics, Gynecology, & Reproductive Biology. 144(1):8-14, 2009

Authors: Papadopoulos VG. Kourea HP. Adonakis GL. Decavalas GO.

Abstract: AB Hemangiomas of the umbilical cord are extremely rare benign vascular tumors, not always detected prenatally. They have been associated with increased alpha-fetoprotein (AFP), hydramnios, congenital anomalies, and increased perinatal mortality. Impaired umbilical circulation has been proposed as the predisposing factor for fetal compromise. We report a case of an antenatally detected umbilical cord hemangioma with one artery crossing the tumor, and we reviewed the literature. Close surveillance with Doppler flow studies of the umbilical vessels were carried out throughout the pregnancy. All indices were normal, except from the intra-tumoral part of the umbilical artery under discussion that showed increasing resistance from 32 weeks onwards. Our review confirmed the reported association with increased AFP and hydramnios. The placental end of the cord was the preferred site of location, and the umbilical artery the commonest vessel of origin. Association with cutaneous vascular malformations, and single umbilical artery were assessed.

(2) Title: Neonatal presentation of a rare metabolic liver disease.

Source: Tropical Gastroenterology. 29(4):229-31, 2008

Authors: David JJ. Tullu MS. Rathi P. Sawalakhe N. Ghildiyal RG.

Abstract: AB Tyrosinemia is a rare paediatric metabolic liver disorder. A 15-days-old neonate born of a third degree consanguineous marriage presented with jaundice due to tyrosinemia, which progressed to fatal hepatic encephalopathy. The diagnosis was based on very high alpha-fetoprotein level, with urine aminoacidogram revealing tyrosine spot and liver biopsy depicting cirrhosis. Very early neonatal presentation and rapid progression were the unusual features of this case.

(3) Title: Clinical experience with infantile hepatic hemangioendothelioma.

Source: World Journal of Surgery. 33(3):597-602, 2009

Authors: Moon SB. Kwon HJ. Park KW. Yun WJ. Jung SE.

Abstract: AB BACKGROUND: Infantile hepatic hemangioendothelioma (IHHE) is a rare disorder with only a few series reported in the medical literature. We reviewed our treatment experience with IHHE over 17 years. METHODS: A retrospective analysis of patients with IHHE between 1991 and 2008 was performed. RESULTS: Sixteen patients (median age 30 days) with IHHE were identified. A palpable abdominal mass was the most common presentation. All except two cases could be diagnosed radiologically. Thirteen patients had a unilobar single tumor and three patients had bilobar disease. Nine patients with symptoms and a resectable tumor underwent complete resection. Three patients with symptoms and unresectable tumor underwent medical treatment with steroids and interferon. Four asymptomatic patients were closely observed. Overall, 14 patients were cured and 1 patient died of postoperative bleeding. One patient is still on medication, and the tumor has greatly decreased in size. Two patients with bilobar disease showed elevated levels of serum alpha-fetoprotein at presentation. Histopathology confirmed type 1 IHHE in all of the 10 specimens. CONCLUSIONS: The presence of clinical symptoms is a key element determining the treatment options. In symptomatic patients, primary surgical resection should be considered whenever feasible.

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

(1) Title: Determination of trace alpha-fetoprotein variant by affinity adsorption solid substrate-room temperature phosphorimetry.

Source: Luminescence. 24(1):15-22, 2009

Authors: Liu JM. Liu ZB. Li XL. Li ZM. Huang XM. Hong FS. Lin WN. Chen F.

Abstract: AB The 3.5-generation dendrimers (3.5G-D)-porphyrin (P) dual luminescent molecule (3.5G-D-P) was used to label concanavalin agglutinin (Con A); the product of the reaction is 3.5G-D-P-Con A. A new method for the determination of trace AFP-V by affinity adsorption solid substrate-room temperature phosphorimetry (AA-SS-RTP) was established, based on the room temperature phosphorescence (RTP) property of the product on polyamide membrane (PAM) substrate and the specific affinity adsorption (AA) reaction between 3.5G-D-P-Con A and alpha-fetoprotein variant (AFP-V), which caused the RTP of the system to be sharply enhanced, the DeltaIp was linearly correlated to the content of AFP-V. The sensitivity of the method was obviously high. It could accurately detect the content of AFP-V in serum. The results were tallied well with those obtained by the ELISA method.

(2) Title: An electrochemical biosensor for alpha-fetoprotein based on carbon paste electrode constructed of room temperature ionic liquid and gold nanoparticles.

Source: Talanta. 78(3):1148-54, 2009

Authors: Ding C. Zhao F. Ren R. Lin JM.

Abstract: AB A novel and effective electrochemical immunosensor for the rapid determination of alpha-fetoprotein (AFP) based on carbon paste electrode (CPE) consisting of room temperature ionic liquid (RTIL) N-butylpyridinium hexafluorophosphate (BPPF(6)) and graphite. The surface of the CPE was modified with gold nanoparticles for the immobilization of the alpha-fetoprotein antibody (anti-AFP). By sandwiching the antigen between anti-AFP on the CPE modified with gold nanoparticles and the secondary antibody, polyclonal anti-human-AFP labeled with horseradish peroxidase (HRP-labeled anti-AFP), the immunoassay was established. The concentration of AFP was determined based on differential pulse voltammetry (DPV) signal, which was generated in the reaction between O-aminophenol (OAP) and H(2)O(2) catalyzed by HRP labeled on the sandwich immunosensor. AFP concentration could be measured in a linear range of 0.50-80.00 ng mL(-1) with a detection limit of 0.25 ng mL(-1). The immunosensor exhibited high sensitivity and good stability, and would be valuable for clinical assay of AFP.

(3) Title: Novel automated pulse immunoassay for human alpha-fetoprotein.

Source: Annals of Clinical Biochemistry. 46(Pt 2):117-22, 2009

Authors: Iwata K. Kanayama Y. Sahara T. Karube I. Inaba N. Fujino S. Kawano M. Sakurabayashi I.

Abstract: AB BACKGROUND: To improve current alpha-fetoprotein (AFP) assays, which are expensive and time-consuming, a specific AFP reagent has been developed for practical use in our newly developed high-speed, highly sensitive pulse immunoassay (PIA) system, in which a latex immunoagglutination reaction is carried out under a high-frequency pulse voltage, leading to an enhanced immunological reaction. METHODS: We evaluated the assay performance (reproducibility, sensitivity, dilution linearity, interference) of the newly developed automated AFP PIA compared with the current AFP assay. RESULTS: Using pooled serum samples, the within-run reproducibility resulted in a correlation variation of 3.6-4.7%. The AFP assay detection limit was determined to be 2.5 microg/L. Linear sequential dilution was found up to nearly 700 microg/L. Even up to an AFP concentration of 1.0 g/L, the prozone phenomenon was not observed. Free and conjugated bilirubin, haemolytic haemoglobin, chyle and rheumatoid factor did not show any test interference. Using AFP-positive serum samples from 114 patients, the correlation between our PIA and a chemiluminescence immunoassay resulted in an excellent correlation coefficient of 0.994. CONCLUSIONS: The performance of AFP reagents in the PIA device shows that the system has excellent speed and equal sensitivity and specificity compared with the most highly sensitive conventional method. Our PIA system thus appears ready for use in the clinical diagnosis setting.

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

1) Title: Lectinomics I. Relevance of exogenous plant lectins in biomedical diagnostics.

Source: Biologia. 64(1):1-19, 2009

Authors: Mislovicova D. Gemeiner P. Kozarova A. Kozar T.

Abstract: AB This review focuses on utilization of plant lectins as medical diagnostic reagents and tools. The lectin-related diagnostic is aimed at detection of several diseases connected to alteration of the glycosylation profiles of cells and at identification of microbial and viral agents in clinical microbiology. Certain lectins, proposed for or used as diagnostic tools could even recognize those cellular determinants, which are not detected by available antibodies. Broad information is presented on the lectinomics field, illustrating that lectin diagnostics might become practical alternative to antibody-based diagnostic products. In addition, the rising trend of lectin utilization in biomedical diagnostics might initiate a development of innovative methods based on better analytical technologies. Lectin microarray, a rapid and simple methodology, can be viewed as an example for such initiative. This technology could provide simple and efficient screening tools for analysis of glycosylation patterns in biological samples (cellular extracts, tissues and the whole cells), allowing thus personalized detection of changes associated with carbohydrate-related diseases.

(2) Title: The impact of crown-rump length measurement error on combined Down syndrome screening: a simulation study.

Source: Ultrasound in Obstetrics & Gynecology. 33(5):506-11, 2009

Authors: Salomon LJ. Bernard M. Amarsy R. Bernard JP. Ville Y.

Abstract: AB OBJECTIVE: To evaluate the impact of a 5-mm error in the measurement of crown-rump length (CRL) in a woman undergoing ultrasound and biochemistry sequential combined screening for Down syndrome. METHODS: Based on existing risk calculation algorithms, we simulated the case of a 35-year-old-woman undergoing combined screening based on nuchal translucency (NT) measurement and early second-trimester maternal serum markers (human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) expressed as multiples of the median (MoM)). Two measurement errors were considered (+ or - 5 mm), for four different CRLs (50, 60, 70 and 80 mm), with five different NT measurements (1, 1.5, 2, 2.5 and 3 mm) in a patient undergoing biochemistry testing at 14 + 4, 15, 16, 17 or 18 weeks' gestation. Four different values for each maternal serum marker were tested (1, 1.5, 2 and 2.5 MoM for hCG, and 0.5, 0.8, 1 and 1.5 MoM for AFP), leading to a total of 3200 simulations of the impact of measurement error. In all cases the ratio between the risk as assessed with or without the measurement error was calculated (measurement error-related risk ratio (MERR)). RESULTS: Over 3200 simulated cases, MERR ranged from 0.53 to 2.14. In 586 simulations (18.3%), it was < 0.66 or > 1.33. Based on a risk cut-off of 1/300, women would have been misclassified in 112 simulations (3.5%). This would go up to 33 (27.5%) out of the 120 simulations in women with 'borderline' risk, with 1.5 MoM for hCG and 0.5 MoM for AFP, and NT measurement of 1 or 2mm. CONCLUSION: Down syndrome screening may be highly sensitive to measurement errors in CRL. Quality control of CRL measurement should be performed together with quality control of NT measurement in order to provide the highest standard of care.

(3) Title: Maternal serum markers of placental damage in uncomplicated dichorionic and monochorionic pregnancies in comparison with monochorionic pregnancies complicated by severe twin-to-twin transfusion syndrome and the response to fetoscopic laser ablation.

Source: [Eur J Obstet Gynecol Reprod Biol.](#) 2009

Authors: [Fox CE](#), [Pretlove SJ](#), [Chan BC](#), [Mahony RT](#), [Holder R](#), [Kilby MD](#).

Abstract: OBJECTIVE: Twin-to-twin transfusion syndrome (TTTS) is a morbid perinatal condition associated with abnormal placentation and is treated by fetoscopic laser ablation (FLA). We assessed basal maternal serum alpha-fetoprotein (MSAFP) and free beta-human chorionic gonadotrophin (f-betaHCG) in uncomplicated dichorionic (DC) and monochorionic (MC) twin pregnancies and a cohort of MC twin pregnancies complicated by severe TTTS. Changes in MSAFP and f-betaHCG post-FLA were measured as markers of placental coagulation. STUDY DESIGN: In a prospective case-cohort study, MC twins complicated by TTTS (n=23) were studied. A cohort of uncomplicated DC (n=12) and MC (n=6) twin pregnancies, which were appropriately grown for gestation with normal liquor volumes were also studied. Using solid phase, two site fluorimetric assays, both MSAFP and f-betaHCG from uncomplicated and complicated cohorts were measured. Samples were taken, prior to FLA then at intervals after the procedures (6h, 24h and 1 week). RESULTS: The median multiples of median (MoM) were not significantly different in uncomplicated DC twin pregnancies for MSAFP 1.85 (95% CI 1.62-2.34) or fbetaHCG 1.66 (95% CI 1.21-2.04) compared to uncomplicated MC twin pregnancies (MSAFP 1.40 (95% CI 1.16-2.58) and fbetaHCG 1.70 (95% CI 0.32-3.35)). However, the median MSAFP MoM in MC twin pregnancies complicated by severe TTTS was increased (MSAFP 3.10 (95% CI 2.67-4.43); p<0.05) with a more significant increase being noted in median fbetaHCG (MoM 5.75 (95% CI 5.22-9.12); p<0.0001) compared to uncomplicated twin pregnancies. Post-FLA, the median MSAFP increased significantly at 6h by 445% (636.65U/ml (95% CI 616-1216.9U/ml)) and remained elevated at 1 week (553.4U/ml (95% CI 203.7-3020.8U/ml; p=0.001)). No significant difference in median fbetaHCG was noted post-FLA (p=0.36). This rise in MSAFP appears unrelated to the number of placental anastomoses coagulated or the total energy used. Also, in the small cohort in which amniodrainage alone was performed no rise in MSAFP was noted. CONCLUSIONS: MSAFP and fbetaHCG are increased in TTTS indicating an association with abnormal placentation. Post-FLA, a significant rise in MSAFP was noted for up to a week post-coagulation. This was not noted after amniodrainage.

E). Special Abstract Selection:

1) Title: Maternal and fetal serum transformed alpha-fetoprotein levels in normal pregnancy.

Source: Journal of Obstetrics & Gynaecology Research. 35(2):271-276, 2009

Authors: Gonzalez-Bugatto F. Foncubierta E. Bailen MD. Illanes S. Hervias-Vivancos B. Bartha JL.

Abstract: AB To evaluate transformed alpha-fetoprotein (t-AFP) (a new molecular conformation of alpha-fetoprotein) levels in maternal serum and fetal serum in normal pregnancy. Prospective longitudinal study. Fifty pregnant women were studied in two groups: 25 were evaluated in each trimester of pregnancy and near term (12, 20, 32 and 36 weeks) and the other 25 were evaluated at the time of planned cesarean section at term. In the first group, maternal serum t-AFP was measured and in the second group, maternal and fetal serum t-AFP were analyzed. Maternal serum t-AFP levels (medians) were 14.73 ng/mL in the first trimester, 28.29 ng/mL in the second trimester, 30.45 ng/mL in the early third trimester and 8.06 ng/mL in late pregnancy. t-AFP levels were significantly higher in maternal than in fetal serum (P < 0.001). There were no significant correlations between AFP and t-AFP levels in maternal versus fetal serum. t-AFP increases during pregnancy until the early third trimester and then falls before delivery. t-AFP levels are higher in maternal than in fetal serum which suggests that native AFP is transformed to t-AFP either in the mother or in the placenta.

2) Title: Maternal Overweight and Obesity and the Risk of Congenital Anomalies A Systematic Review and Meta-analysis.

Source: JAMA. 301(6):636-650, 2009

Authors: Stothard KJ. Tennant PWG. Bell R. Rankin J.

Abstract: AB Context Evidence suggests an association between maternal obesity and some congenital anomalies. Objective To assess current evidence of the association between maternal overweight, maternal obesity, and congenital anomaly. Data Sources MEDLINE, EMBASE, CINAHL, and Scopus (January 1966 through May

2008) were searched for English- language studies using a list of keywords. Reference lists from relevant review articles were also searched. Study Selection Observational studies with an estimate of prepregnancy or early pregnancy weight or body mass index (BMI) and data on congenital anomalies were considered. Of 1944 potential articles, 39 were included in the systematic review and 18 in the meta- analysis. Data Extraction and Synthesis Information was extracted on study design, quality, participants, congenital anomaly groups and subtypes, and risk estimates. Pooled odds ratios (ORs) comparing risk among overweight, obese, and recommended- weight mothers (defined by BMI) were determined for congenital anomaly groups and subtypes for which at least 150 cases had been reported in the literature. Results Pooled ORs for overweight and obesity were calculated for 16 and 15 anomaly groups or subtypes, respectively. Compared with mothers of recommended BMI, obese mothers were at increased odds of pregnancies affected by neural tube defects (OR, 1.87; 95% confidence interval [CI], 1.62- 2.15), spina bifida (OR, 2.24; 95% CI, 1.86- 2.69), cardiovascular anomalies (OR, 1.30; 95% CI, 1.12- 1.51), septal anomalies (OR, 1.20; 95% CI, 1.09- 1.31), cleft palate (OR, 1.23; 95% CI, 1.03- 1.47), cleft lip and palate (OR, 1.20; 95% CI, 1.03- 1.40), anorectal atresia (OR, 1.48; 95% CI, 1.12- 1.97), hydrocephaly (OR, 1.68; 95% CI, 1.19-2.36), and limb reduction anomalies (OR, 1.34; 95% CI, 1.03- 1.73). The risk of gastroschisis among obese mothers was significantly reduced (OR, 0.17; 95% CI, 0.10- 0.30). Conclusions Maternal obesity is associated with an increased risk of a range of structural anomalies, although the absolute increase is likely to be small. Further studies are needed to confirm whether maternal overweight is also implicated.

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) headtoe.apta.org/topic/medtest/hw1663/results.htm
- 4) www.pregnancy-info.net/slpha_feto_protein.html
- 5) www.healthopedia.com/alpha-fetoprotein