

Fetal Defect Marker Proficiency Test Mailout October, 2009

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from Sept. 15, 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 241	MS 242	MS 243	MS 244	MS 245
	Gestational Age (weeks)	15	17	19	16	18
Maternal Race	Ethnic Group	Black	White	Asian	White	Hispanic
Maternal Weight	Pounds (lbs)	160	145	200	140	150
Maternal Age	Years	18	29	25	33	38
Alpha-Fetoprotein (AFP)	Mean ng/ml \pm Std.Dev.	29.10 \pm 2.18	93.36 \pm 6.46	53.91 \pm 2.97	15.15 \pm 1.13	44.74 \pm 3.50
	MOM \pm Std.Dev.	0.93 \pm 0.09	2.38 \pm 0.22	1.28 \pm 0.12	0.43 \pm 0.04	1.02 \pm 0.10
Unconjugated Estriol (uE3)	Mean ng/ml \pm Std.Dev.	0.82 \pm 0.30	1.33 \pm 0.66	1.93 \pm 0.96	0.42 \pm 0.12	1.68 \pm 0.78
	MOM \pm Std.Dev.	0.80 \pm 0.19	0.74 \pm 0.12	0.76 \pm 0.09	0.33 \pm 0.12	0.77 \pm 0.09
human Chorionic Gonadotrophin (hCG)	Mean IU/ml \pm Std.Dev.	27.12 \pm 3.14	20.73 \pm 1.70	16.32 \pm 1.37	53.39 \pm 5.29	33.28 \pm 3.04
	MOM \pm Std.Dev.	0.68 \pm 0.10	0.85 \pm 0.09	1.05 \pm 0.13	1.75 \pm 0.20	1.62 \pm 0.19
Dimeric Inhibin-A (DIA)	Mean pg/ml \pm Std.Dev.	140.60 \pm 17.75	146.92 \pm 17.58	196.57 \pm 24.49	198.66 \pm 20.86	354.23 \pm 33.30
	MOM \pm Std.Dev.	0.79 \pm 0.09	0.87 \pm 0.12	1.29 \pm 0.19	1.12 \pm 0.14	2.12 \pm 0.26
Neural Tube Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (B) (51.7%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Further Action G,U,A	NFA	G = 25% U = 25% A = 25%	NFA	NFA	NFA
	NTD Risk 1 in	10,000	320	3600	10,000	8520
Trisomy-21 Screen (Positive, Negative) percent 1. <u>Triple test</u>	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (100%)	Pos. (+) (100%)
	Recommended Action**	NFA	NFA	NFA	G = 86% U = 71% A = 79%	G = 93% U = 86% A = 86%
	Risk Est. 1 in	6,560	3,700	3,600	16	138
2. <u>Quad Test</u>	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (100%)	Pos. (+) (100%)
	Recommended Action**	NFA	NFA	NFA	G = 74% U = 67% A = 74%	G = 81% U = 81% A = 78%
	Risk Est. 1 in	11,550	11,000	3,710	25	68
Trisomy-18 Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg.(-) (100%)	Neg. (-) (100%)
	Risk Est. 1 in	9,500	8,600	10,000	428	5,800

*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 \pm 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 = 31 all-lab Consensus Values.

<u>Sample #</u>	<u>Summary Comments (Mock specimens):</u>
MS 241 Wk 15.0	This specimen was obtained from an 18 year old black woman (Gravida = 1, Parity = 0) in her 15 th week gestation with a body weight of 160 lbs. She had no personal history of pregnancy loss. Her specimen, a second pregnancy sample, was negative for NTD (100% consensus); however, a race correction was indicated. Her screen was also negative for both Trisomies with all labs in agreement. No recommendations of further action were submitted for the MS241 sample. The MS241 specimen had no amniotic fluid counterpart.
MS 242 Wk 17.0	This specimen was obtained from a 29 year old white woman (Gravida = 2, Parity = 0) in her 17 th week gestation with a body weight of 145 lbs. She had a family history of pregnancy complications. Her specimen, a second pregnancy sample, was a borderline negative screen for NTD (51.7% consensus; MOM=2.38). 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, 25%, ultrasound, 25% and amniocentesis, 25%. The MS242 specimen had an amniotic fluid counterpart which was also elevated (MoM=2.82).
MS 243 Wk 19.0	This specimen was procured from a 25 year old, Asian woman (Gravida = 3, parity = 2) in her 19 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 with a body weight correction indicated. The labs were also in agreement that both trisomy consensus screens were negative (100%). Specimen MS243 was not paired with an amniotic fluid sample.
MS 244 Wk 16.0	This specimen was obtained from a 33 year old white woman (Gravida = 2, parity = 1) in her 16 th week gestation with a body weight of 140 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, 74%; ultrasound, 67%; and amniocentesis, 74%; while the triple tests were: genetic counseling, 86%; ultrasound 71%, and amniocentesis, 79%. Specimen MS244 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 = 0.67).
MS 245 Wk 18.0	This specimen was obtained from a 38 year old hispanic woman (Gravida = 2, parity = 0) in her 18 th week gestation with a body weight of 130 lbs. She had no family history of pregnancy complications or adverse outcomes, and her aneuploidy screen was positive for Trisomy-21 and negative for Trisomy-18. Recommendation of further action for the T21 screen was reported from the participating labs (see critique). This specimen was not paired with an amniotic fluid specimen.

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 241 Wk 15.0	AFP= 10.00 ± 1.90 µg/ml MOM= 1.28 ± 0.21	The AF241 sample was targeted for normal AFAFP value in the routine gestational age range. All labs called AF241 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 242 Wk 17.0	AFP= 33.10 ± 5.41 µg/ml MOM= 2.82 ± 0.38	The AF242 sample was targeted for a screen positive AFAFP value in the routine gestational age range. All labs reported this specimen as a screen positive AFAFP value. The AF242specimen was paired with maternal serum sample MS242 (MoM= 2.38) which was also elevated.
AF 243 Wk 19.0	AFP= 8.10 ± 1.31 µg/ml MOM= 1.26 ± 0.20	The AF243 sample was targeted for a negative NTD screen for AFAFP in the upper 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 244 Wk 16.0	AFP= 9.60 ± 1.31 µg/ml MOM= 0.67 ± 0.08	The AF244 sample was targeted as an NTD negative screen in the routine gestational age screening range. All labs categorized AF244 as a negative NTD screen specimen and it had a maternal serum counterpart, MS244 (MoM= 0.43) which showed reduced levels of AFP.
AF 245 Wk 18.0	AFP= 13.00 ± 2.10 µg/ml MOM= 1.65 ± 0.24	The AF245 sample was targeted for a non-elevated AFAFP value in the routine gestational age range. Most labs called AF245 a normal MOM AFAFP specimen. This AFAFP sample was not matched to a maternal serum specimen.

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples *N = 16	Sample #	FT 241	FT 242	FT 243	FT244	FT245
	Gestational Age (weeks)	12.4	12.0	11.5	13.1	10.9
Maternal Race	Ethnic Group	Asian	White	Hispanic	White	Black
Maternal Weight	Pounds (lbs)	130	155	145	150	135
Maternal Age	Years	35	21	26	29	32
Nuchal Translucency (NT)-Associated Measurements	Crown Rump Length (mm)	59	55	48	69	40
	NT Thickness (mm)	1.40	2.90	1.08	1.50	1.10
	NT - MOM	0.98 ± 0.11	2.17 ± 0.24	0.91 ± 0.10	0.91 ± 0.09	1.08 ± 0.12
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL	65.90	139.46	76.88	65.57	77.42
	± Std. Dev.	± 11.16	± 25.22	± 15.74	±10.09	±12.97
	MOM	0.93	2.08	1.05	1.10	0.89
	± Std. Dev.	± 0.23	± 0.60	± 0.38	±0.35	±0.15
Pregnancy-Associated Plasma Protein-A (PAPP-A)	Mean mIU/mL	2.44	1.09	2.03	2.92	1.91
	± Std. Dev.	± 1.22	± 0.62	± 1.09	± 1.41	± 0.98
	MOM	1.26	0.76	1.66	1.35	1.97
	± Std. Dev.	± 0.47	± 0.36	± 0.71	±0.45	±0.94
Trisomy-21 Screen (Positive/Negative) percent	Pos (+) or Neg. (-)	Neg. (100%)	Pos. (100%)	Neg. (100%)	Neg. (100%)	Neg. (100%)
	Recommended Action	NFA	G = 80% U = 36% C = 47%	NFA	NFA	NFA
	Risk Estimate	3,500	19	10,000	10,000	10,000
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	823	10,000	10,000	10,000

*N=total numbers may vary since some labs do not test all analytes. (B) = borderline negative or positive; NFA = no further action; G = genetic counseling; U = ultrasound; 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 241 Wk 12.4	This specimen was obtained from a 35 year old Asian woman of average body weight (130 lbs.). Her gestational age at time of screening was 12.4 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 FT241 risk estimate for Trisomy-21 was 1 in 3,500, while the all-lab Trisomy-18 risk was 1 in 10,000 (negative screen). All labs were in agreement that FT241 was a negative screen for a Trisomy-21 and T18 pregnancy.
FT 242 Wk 12.0	This specimen was procured from a 21 year old White woman of average body weight (155 lbs.). Her gestational age at time of screening was 12.0 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 FT242 risk estimate for Trisomy-21 was 1 in 19, while the Trisomy-18 risk was 1 in 823.
FT 243 Wk 11.5	This specimen was obtained from a 26 year old Hispanic woman of average body weight (145 lbs.). Her gestational age at time of screening was 11.5 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 FT243 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 FT243 was a negative screen for Trisomy-21 and Trisomy-18.
FT 244 Wk 13.1	This specimen was obtained from a 29 year old White woman of medium body weight (150 lbs). Her gestational age at time of screening was 13.1 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 FT244 risk estimate for Trisomy-21 was 1 in 10,000 while the Trisomy-18 risk was 1 in 10,000.
FT 245 Wk 10.9	This specimen was procured from a 32 year old Black woman with a body weight of 135 lbs. Her gestational age at time of screening was 10.9 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative for T21 and T18. The Trisomy-21 risk estimate for FT245 was 1 in 10,000, and the T18 risk was 1 in 10,000. All labs were in agreement with both FT245 aneuploid screen assessments.

III. Critique and Commentary:

A) Fetal Defect Proficiency Test Mail out 9/15/09 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 MS242 was targeted as a borderline elevated specimen for NTD (Figs. 1 and 3) and the AF-242 specimen was matched to this specimen. Hence, specimens MS242 was borderline screen negative for NTD (MOM = 2.38), but negative for Trisomy-21 (T21), and Trisomy-18 (T18). For the MS242 specimen, the NTD screen resulted in a 1 in 320 all-lab risk for open neural tube defects (ONTD), and it only achieved a 52% NTD screen consensus. The NTD-related recommended action for specimen MS242 reflected the borderline screen assessment and was genetic counseling; 25%; ultrasound; 25%; and amniocentesis; 25%. Sample MS244, a T21 screen positive specimen, was obtained from a white woman with a prior (sibling) history of pregnancy problems. Concerning the Down's screen, the T21 MOM results for specimen MS244 (MSAFP-MOM = 0.43, MSuE3-MOM = 0.33, MShCG-MOM = 1.75, DIA-MOM = 1.12) were all in accordance with a T21 positive screen; thus, all labs classified this specimen as a T21 positive screen and all recommended further action. The MS244 sample (from a 33 year old woman) produced a risk from the quad test (1 in 25) which was greater than that expected from the maternal age alone (1 in 420). Finally, samples MS241 and MS243 produced negative screens for NTD, Trisomy-21, and Trisomy 18; however, correction for body weight was indicated for MS243.

Specimen MS244, together with a matched AF244 sample, deserves further comment in that the MSAFP specimen was T21 screen positive (MSAFP MOM = 0.43) and the paired AF AFP sample was correspondingly low (MOM = 0.67) (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. No demographic correction factors were indicated for the MS244 screen assessment. The MS244 specimen results produced a positive Trisomy-21 (T21) screen with both the triple and quad testing platforms. Further action recommended for the T21 screen was determined as genetic counseling, 86%, ultrasound (US) 71%, and 79% amniocentesis (AM) for labs using the triple screen; while genetic counseling 74%, US 67% and AM 74% was recorded for labs employing the quad screen. The recommended action on MS244 was consistent with the severity of the similar risk ratio assessments of 1 in 16 from the triple test versus a 1 in 25 risk from the quad test. Note from the point distribution graphs comparing the triple with the

quad test (Figs. 5 and 6) that the MS244 point cluster in the quad assay closely correlated (note scale difference on figures) with the MS244 cluster in the triple test. As seen with previous mailout specimens, the quad test signaled a slightly different risk (see table 1) for Down syndrome than the triple test; however, both screens resulted in 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 levels in the MS244 sample was also lowered (MOM = 0.67). The amniotic fluid cells of this mock patient were subjected to subsequent genotyping and indeed indicated the presence of a Trisomy-21. The MS242 specimen produced a negative screen for Trisomy-18. The risk of Down syndrome for the MS244 sample was greater than that expected from maternal age alone (1 in 420).

Specimen MS242 invites further comment in that the sample was NTD borderline negative screen (51.7%; MOM=2.38). This 29 year old patient had no prior history of pregnancy loss or complications and a paired amniotic fluid was available for analysis at time of venipuncture specimen collection (AFAFPMOM=2.82). This MS specimen represented a 17 week gestational age sample and both amniocentesis and ultrasound were recommended. Although time would not permit a second sample MS repeat, an MS-specimen had been obtained at time of amniocentesis. 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 and AFAFP levels of MS242 were abnormal in this mock patient; in addition a fetal-maternal bleed was ruled out for AF242, but a diagnostic band following ache electrophoresis was present. Thus, this screened specimen was diagnostically confirmed as an elevated MSAFP specimen screen positive for NTD. All of the participating laboratories called MS242 a positive screen for NTD, and screen negative for T21 and T18, and all suggested NTD further actions.

Specimen MS245 is also of interest in that the sample was NTD negative with a T21 positive screen. Specimen MS245 showed elevated levels of both HCG (MOM=1.62) and DIA (MOM=2.12). 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. Elevated MShCG and MSDIA screening values, by themselves are not indicative of a Down syndrome. However, the combination of the two elevated analytes plus advanced maternal age in the triple/quad tests generated a T21 positive screen in MS245. Her risk due to maternal age (38 years) alone was 1 in 120. Subsequent Stage-II ultrasound, ache, AFAFP, and amniocentesis genotyping were found to be unremarkable in this mock patient. Evidently this mock patient represented a false positive patient at risk for T21 (1 in 68, Quad; 1 in 138, triple). Specimen MS245 had displayed an unusual biomarker profile and advanced maternal age in producing these screen results. Although the MSAFP was normal (MoM= 1.02) and the uE3 level was slightly low (MoM= 0.77), the MShCG was elevated as was the DIA (see above). The omission of DIA in the triple test seemed to lower the risk below that of maternal age only (1 in 120). It was evident that the lower risk in the triple test (1 in 138) versus the quad test (1 in 68) reflected the high percentages seen in the recommended further actions (G=93%, U=86%, and A=86%; triple test; and G=81%, U=81%, and A=78%; quad test). Interestingly, the MS245 specimen had been obtained from a 38 year old Hispanic patient and adverse pregnancy outcomes had not been a part of her family history. As discussed below, elevated hCG and/or DIA have been implicated with multiple pregnancy complications.

Unexplained elevated MShCG and MSDIA, either alone or together, have been related to a variety of pregnancy anatomical complications. However, other obstetrical problems can result from multifetal pregnancies, aneuploidies, and/or placental-maternal interface problems. Adverse pregnancy outcomes relative to elevated maternal serum DIA levels (MOM = >2.5) usually involve placental dysfunction and/or birth defects accompanied by adverse sonographic findings and growth retardation. Elevated DIA levels have also been used as an indicator for conditions of perinatal distress such as multicystic dysplastic kidney, preterm birth, pre-eclampsia, intra-uterine growth retardation (IUGR), Turner Syndrome with hydrops, and multi-fetal reduction (see references below).

Other than Down syndrome, various other pregnancy conditions can contribute to MSDIA levels following gestational events such as loss of a twin, Trisomy 18, low birth weight, and smoking during pregnancy (1-7). DIA is of placental origin, synthesized on the human chromosome 2q region, and is elevated in the first and second trimesters of Down syndrome maternal sera (1). The physiological role of DIA is to inhibit FSH secretion at the level of the pituitary, which contributes to preventing ovulation during pregnancy (8). Mid-trimester DIA alone is a biomarker for both pre-eclampsia (PE) and in some cases, intrauterine growth retardation (9). When DIA is employed in the quad test, a PE detection rate of 35% (5% false positive rate) is achieved (10). When DIA is utilized as a predictor of adverse pregnancy outcome, preterm birth, low birth weight, fetal loss, and fetal demise can be included (4). Elevated DIA is also known to detect multicystic dysplastic kidney, two-vessel umbilical cord, and hydrocele (1). In comparison, MSDIA is reduced (MOM=0.74) in cases of Trisomy-18 (11), but elevated in Turner syndrome with hydrops (12). Regarding single fetal loss, MSDIA is only useful in the third trimester of pregnancy (6). In monitoring the well-being of twin and multiple fetal pregnancies, MSDIA has been found useful in the detection of fetal reduction of one or more fetuses (13). In the prenatal screening for Down syndrome in women with insulin-dependent diabetes mellitus (5), no correction for the MOM value for MSDIA was found to be necessary (MOM=1.03). Finally, the effect of smoking in pregnancy on MSDIA levels has been reported and findings suggested that a need for MOM adjustment of MSDIA should be considered (14).

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

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2. Dugoff L, Cuckle HS, Hobbins JC, Malone FD, Belfort MA, Nyberg DA, Comstock CH, Saade GR, Eddleman KA, Dar P, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME: Prediction of patient-specific risk for fetal loss using maternal characteristics and first- and second-trimester maternal serum Down syndrome markers. *Am J Obstet Gynecol.* 2008, 199:290.e291-296.
3. Chen HJ, Huang LW, Lin YH, Seow KM, Hsieh BC, Hwang JL, Tzeng CR: Midtrimester maternal serum inhibin A levels after multifetal pregnancy reduction. *Prenat Diagn.* 2007, 27:431-434.
4. Dugoff L, Hobbins JC, Malone FD, Vidaver J, Sullivan L, Canick JA, Lambert-Messerlian GM, Porter TF, Luthy DA, Comstock CH, Saade G, Eddleman K, Merkatz IR, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME: Quad screen as a predictor of adverse pregnancy outcome. *Obstet Gynecol* 2005, 106:260-267.
5. Huttly W, Rudnicka A, Wald NJ: Second-trimester prenatal screening markers for Down syndrome in women with insulin-dependent diabetes mellitus. *Prenat Diagn* 2004, 24:804-807.
6. Muttukrishna S: Role of inhibin in normal and high-risk pregnancy. *Semin Reprod Med.* 2004, 22:227-234.
7. Watanabe H, Hamada H, Yamada N, Ogura T, Yasuoka MO, Okuno S, Fujiki Y, Sohda S, Kubo T: Second-trimester maternal pregnancy-associated plasma protein a and inhibin a levels in fetal trisomies. *Fetal Diagn Ther.* 2002, 17:137-141.
8. Lambert-Messerlian GM, Pinar H, Laprade E, Tantravahi U, Schneyer A, Canick JA: Inhibins and activins in human fetal abnormalities. *Mol Cell Endocrinol.* 2004, 225:101-108.
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10. Cuckle H, Sehmi I, Jones R: Maternal serum inhibin A can predict pre-eclampsia. *Br J Obstet Gynaecol.* 1998, 105:1101-1103.
11. Breathnach FM, Malone FD, Lambert-Messerlian G, Cuckle HS, Porter TF, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, Klugman S, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, Tripp T, Bianchi DW, D'Alton ME: First- and second-trimester screening: detection of aneuploidies other than Down syndrome. *Obstet Gynecol.* 2007, 110:651-657.
12. Lambert-Messerlian GM, Saller DN, Jr., Tumber MB, French CA, Peterson CJ, Canick JA: Second-trimester maternal serum inhibin A levels in fetal trisomy 18 and Turner syndrome with and without hydrops. *Prenat Diagn.* 1998, 18:1061-1067.
13. Goodwin KM, Sweeney PJ, Lambert-Messerlian GM, Canick JA: High maternal serum inhibin A levels following the loss of one fetus in a twin pregnancy. *Prenat Diagn.* 2000, 20:1015-1017.
14. Rudnicka AR, Wald NJ, Huttly W, Hackshaw AK: Influence of maternal smoking on the birth prevalence of Down syndrome and on second trimester screening performance. *Prenat Diagn.* 2002, 22:893-897.

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 somewhat lower for some samples. For uE3, the mean/all kit median for Beckman Unicel DxI and Beckman Access/2 hovered around 1.0 (see Fig. 8A); however, labs employing DPC Immulite 2000 or Immulite 2500 yielded values achieving 1.8 to 2.6 times higher than the mean/all kit median (see solid line). In contrast, Fig 8B showed that MOM values from DPC Immulite 2000 or Immulite 2500 were below that of the all kit median (see solid line). Regarding the hCG kits (see Fig. 9), the Beckman Unicel DxI and Access/2 and DPC Immulite or Immulite 2000 yielded similar mean hCG values hovering about and above the 1.0 mean/all kit median value, while Siemens/Bayer ADVIA-Centaur /ACS-180 kits demonstrated 5% to 10% higher 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 Unicel DxI and Access/2 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. Overall, Siemens/Bayer ADVIA-Centaur and Abbott-AxSYM kits were 5 - 10% higher versus Beckman Unicel DxI and Access/2, were about 10% - 20% lower than the 1.0 mean/all-kit median (see solid line). 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 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 have been provided 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 12.4 week Asian FT241 specimen for total hCG resulted in a mass mean of 65.90 ± 11.16 , with a non-elevated MOM = 0.93. Furthermore, the all-lab mass mean for PAPP-A was 2.44 ± 1.22 mIU/ml with a MOM = 1.26. The all-lab T21 risk assessment was 1 in 8,000 for the FT241 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT241 sample was screen negative (See Figure 14 risk distribution) demonstrating normal analyte MOMs. The FT T21 risk cut-off level for Caucasians ranges from 169 to 270 among the participating labs. Thus, the FT241 sample resulted in a 100% all lab T21 negative screen assessment. No further action was indicated. Finally, the FT241 specimen also screened negative for T18 (1 in 756) using a cutoff of 1 in 100.

As displayed in the FT table 2 (Section – II) above, the all lab measurement of the 12.0 week Caucasian FT242 specimen for total hCG resulted in a mass mean of $139.46 \text{ IU/ml} \pm 25.22$, with an elevated MOM = 2.08 ± 0.60 . Furthermore, the all-lab mass mean for PAPP-A was 1.09 ± 0.62 mIU/ml with a MOM = 0.76 ± 0.37 . These results generated a T21 positive screen for specimen FT242 and the all-lab T21 risk assessment was 1 in 19. 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 FT242 results (Fig. 14, B) were indeed consistent with a T21 positive screen. Thus, the FT242 sample resulted in a 100% all lab T21 positive T21 screen assessment. Further actions by the labs included genetic counseling, 80%; ultrasound, 36%; and amniocentesis/ CVS = 47%. Finally, the FT242 specimen screened negative for T18 using a cutoff of 1 in 100 with a risk of 1 in 823.

As shown in the above First Trimester table 2 (Section-II) for the FT243 Hispanic specimen, the gestational age all-lab mean was reported as 11.5 weeks. Assay measurements from FT-screening participating laboratories resulted in an all-lab total hCG mass measurement of 76.88 ± 15.74 IU/ml based on the two methods above; while the all-lab PAPP-A mass assessment was 2.03 ± 1.09 mIU/ml. The first trimester all-lab Trisomy-21 screen consensus for FT243 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 FT243 achieved a MOM value of 1.05 ± 0.38 . In comparison, the all-lab MoM for PAPP-A was 1.66 ± 0.71 , a normal value. All labs agreed that the FT243 sample was screen negative for Trisomy 21 (See Fig. 14 risk distribution). The FT243 specimen also resulted in a negative screen for Trisomy-18 with a risk assessment of 1 in 10,000.

In the FT244 Caucasian sample, the gestational age all-lab mean was reported as 13.1 weeks. Assay measurements for FT244 resulted in an all-lab total hCG mass measurement of 65.57 ± 10.09 based on two present methods, while the all-lab PAPP-A mass assessments were 2.92 ± 1.41 . The first trimester all-lab trisomy-21 consensus for FT244 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 FT244 of total hCG resulted in a MOM value of 1.10 ± 0.35 and the all-lab MoM mean for PAPP-A was 1.35 ± 0.45 . All labs agreed that the FT244 sample was screen negative for Trisomy 21 (See Fig. 14 risk distribution). The all-lab T18 risk assessment for FT244 was 1 in 10,000, hence, the FT244 specimen resulted in a negative screen for Trisomy-18.

As demonstrated in the FT table 2 (Section-II) above for the Black FT245 specimen, the gestational age all-lab mean was reported as 10.9 weeks. Assay measurements from FT-screening participating laboratories resulted in the all-lab total hCG mass measurement of 77.42 ± 12.97 IU/ml based on the two methods, while the all-lab PAPP-A mass assessment was 1.91 ± 0.98 mIU/ml. The first trimester all-lab trisomy-21 screen consensus for the FT245 specimen was negative (100%). The all-lab FT Trisomy-21 risk assessment was 1 in 10,000. As observed in the table (Table 2, Section – II), the all lab measurement of total hCG MOM for FT245 produced a value of 0.89 ± 0.15 . In comparison, the all-lab MOM mean for PAPP-A resulted in 1.97 ± 0.94 . All labs agreed that the FT245 sample was screen negative for Trisomy 21. (See Fig. 14 risk distribution). The FT245 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, 13A and B) 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 DxI/Access/2 kit measuring a slightly higher than the DPC kits. 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. When PAPP-A MOMs were plotted, together with the Beckman Unicel DxI/Access/2, the relationship did not change. Note in plotting the PAPP-

A mass mean/all kit median, that Beckman Unicel DxI/Access/2 kit was omitted due to a difference in reporting units by the manufacturer (Figs. 13A and B).

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 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.

G.J. Mizejewski, Ph.D.

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Abstracts

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

(1) Title: The ability of the quadruple test to predict adverse perinatal outcomes in a high-risk obstetric population.

Source: J Med Screen 2009, 16:55-59

Authors: Lao MR, Calhoun BC, Bracero LA, Wang Y, Seybold DJ, Broce M, Hatjis CG

Abstract: OBJECTIVE: To determine the ability of the quadruple Down's syndrome screening test (quad screen) to predict other adverse perinatal outcomes (APO) in a high-risk obstetric population. SETTING: A tertiary medical centre in West Virginia. METHODS: We retrospectively reviewed 342 obstetric patients with quad screen data from a single clinic. The quad screen included maternal serum levels of alphafetoprotein (AFP), human chorionic gonadotrophin (hCG), unconjugated oestriol (uE(3)), and inhibin A. The risk of APO was compared between patients with at least one abnormal marker versus no abnormal markers and ≥ 2 abnormal markers versus < 2 abnormal markers. Abnormal markers were determined by cut-off values produced by Receiver Operator Characteristic (ROC) curves and the FASTER trial. Unadjusted and adjusted effects were estimated using logistic regression analysis. RESULTS: The risk of having an APO increased significantly for patients with abnormal markers by about three-fold using ROC and two-fold using FASTER trial thresholds. CONCLUSIONS: The quad screen shows value in predicting risk of APO in high-risk patients.

(2) Title: Are tests for predicting pre-eclampsia good enough to make screening viable? A review of reviews and critical appraisal

Source: Acta Obstet Gynecol Scand 2009, 88:758-765

Authors: Cnossen JS, ter Riet G, Mol BW, van der Post JA, Leeflang MM, Meads CA, Hyde C, Khan KS

Abstract: The aim of this article is to review the accuracy of tests purported to be predictive of pre-eclampsia, a major cause of maternal and perinatal mortality and morbidity worldwide. A review of systematic reviews was done. A total of 219 studies were evaluated for the accuracy of 27 tests for predicting pre-eclampsia. Study quality assessment and data abstraction were performed using piloted proformas. Bivariate meta-analyses were used to synthesize data. Levels of sensitivity and specificity were measured. There were deficiencies in many areas of methodology including blinding, test description, and reference standard adequacy. No test had a high level of both sensitivity and specificity of greater than 90%. Where multiple studies were available, only BMI > 34 , alpha-fetoprotein, fibronectin (cellular and total), and uterine artery Doppler (bilateral notching) measurements reached specificity above 90%. Only Doppler (any/unilateral notching, resistance index, and combinations)

measurements were over 60% sensitive. Studies were of variable quality and most tests performed poorly. Further research should focus on tests which offer much higher levels of sensitivity than tests currently available. High sensitivity is a more useful attribute in early detection of pre-eclampsia than specificity because consideration of benefits, harms and costs indicates a much greater preference for minimizing false negatives than false positives, although the ideal would be to avoid both.

(3) Title: Placental size and the prediction of severe early-onset intrauterine growth restriction in women with low pregnancy-associated plasma protein-A

Source: Ultrasound Obstet Gynecol. 2009, 34:274-282.

Authors: Proctor LK, Toal M, Keating S, Chitayat D, Okun N, Windrim RC, Smith GC, Kingdom JC

Abstract: OBJECTIVES: Screening studies for trisomy 21 demonstrate that low maternal serum pregnancy-associated plasma protein-A (PAPP-A) at 11-13 weeks' gestation is associated with stillbirth, intrauterine growth restriction (IUGR) and pre-eclampsia in chromosomally normal fetuses. However, the strength of these associations is too weak to justify screening for these placental insufficiency syndromes. Our objective was to evaluate placental size and uterine artery (UtA) Doppler imaging as second-stage screening tests for women with low PAPP-A. METHODS: We prospectively studied 90 normal singleton pregnancies with first-trimester PAPP-A \leq 0.30 multiples of the median. Maternal serum alpha-fetoprotein (AFP) at 15-18 weeks' gestation, and second-trimester placental size and UtA Doppler indices were assessed as predictors of pregnancy outcome. RESULTS: The risks of IUGR, preterm delivery before 32 weeks' gestation and stillbirth were significantly associated with small placental size (relative risk (RR), 3.96; 95% CI, 2.21-5.98; RR, 3.96; 95% CI, 2.21-5.98; and RR, 6.44, 95% CI, 2.74-14.54, respectively) and elevated AFP (RR, 3.67; 95% CI, 1.78-7.71; RR, 2.48; 95% CI, 1.23-4.94; and RR, 5.14; 95% CI, 1.66-16.85, respectively), but not with abnormal UtA Doppler indices. The combination of elevated AFP and small placental size further increased the risk of IUGR (RR, 4.88; 95% CI, 2.88-5.31), delivery before 32 weeks' gestation (RR, 4.25; 95% CI, 2.38-4.98) and stillbirth (RR, 7.44; 95% CI, 3.04-3.75). CONCLUSIONS: Small placental size and elevated AFP, but not UtA Doppler indices, identify women with low PAPP-A at high risk of IUGR, extreme preterm delivery and stillbirth. These additional screening tests may directly improve perinatal outcomes in women with low PAPP-A.

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

(1) Title: [Infantile testicular tumor (yolk sac tumor): a case report].

Source: Hinyokika Kyo. 2009, 55:367-370

Authors: Muraoka K, Yokonisi T, Matsumoto T, Umemoto S, Shioi K, Komiya A, Tomoda T, Yoshida M, Takase K, Oogo Y, Kobayashi K, Noguchi S, Kishi H

Abstract: A 5-month-old-male was brought to our hospital in April 2004 with left scrotal swelling. His serum alpha-fetoprotein AFP and human chorionic gonadotropin-beta levels were 6,862.9 and $<$ 0.1 ng/ml, respectively. Computed tomography (CT) revealed no metastasis. Left high ligation of testis was performed. Pathological examination demonstrated Yolk sac tumor. He is alive without evidence of recurrence for 53 months postoperatively.

(2) Title: Ataxia with oculomotor apraxia type 2: novel mutations in six patients with juvenile age of onset and elevated serum alpha-fetoprotein.

Source: Neuropediatrics. 2008, 39:347-350

Authors: Bernard V, Stricker S, Kreuz F, Minnerop M, Gillessen-Kaesbach G, Zuhlke C

Abstract: Ataxia with oculomotor apraxia type 2 (AOA2), a neurodegenerative disorder with juvenile to adolescent onset is caused by mutations within the SENATAXIN gene (SETX). We performed molecular analyses in six patients showing clinically an AOA2 phenotype and moderate to significant elevated serum alpha-fetoprotein levels. Sequencing the 24 coding exons and flanking intronic sequences revealed 11 novel DNA variations, including seven unknown missense mutations, a dinucleotide deletion, a four-nucleotide deletion affecting the 5' splice site of exon 22 and two sequence variations, which are considered to be polymorphisms. By molecular testing the clinical diagnosis has been confirmed in all patients.

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

Source: Eur J Obstet Gynecol Reprod Biol. 2009, 144:8-14

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

Abstract: 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.

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

(1) Title: Transformed Alpha-Fetoprotein (t-AFP) Levels in Women with Threatened Preterm Labor.

Source: Gynecol Obstet Invest. 2009, 68:199-204

Authors: Gonzalez-Bugatto F, de Los Angeles Bailen M, Fernandez-Macias R, Fernandez-Deudero A, Hervias-Vivancos B, Bartha JL

Abstract: Background/Aims: To evaluate maternal serum transformed alpha-fetoprotein (MSt-AFP) levels, a new molecular conformation of AFP was used in cases of threatened preterm labor (TPL). Methods: Prospective case-control study. Maternal serum levels of classical AFP and transformed AFP (t-AFP) were compared between 2 groups matched by gestational age: 25 women with TPL and 25 healthy pregnant women as controls. Results: There was no significant difference in classical maternal serum AFP (MSAFP) levels between the 2 groups. In contrast, MSt-AFP levels were significantly lower in cases of TPL than in the control group [7.64 (1.78-29.06) vs. 33.38 (13.80-190.50) ng/ml; p = 0.006]. Similarly, the t-AFP:AFP ratio was also decreased in the TPL group [0.04 (0.004-0.12) vs. 0.16 (0.05-0.80); p = 0.008]. There was no significant correlation between MSAFP and MSt-AFP levels. Conclusions: MSt-AFP levels are decreased in women with TPL.

(2) Title: Novel alpha-fetoprotein-V messenger RNA isoforms in humans.

Source: Reprod Sci. 2009, 16:794-801

Authors: Tagaya H, Fukasawa H, Shoda T, Hoshi K, Hirata S

Abstract: It has been demonstrated that several messenger RNA (mRNA) isoforms have been transcribed from the alpha-fetoprotein (AFP) gene. In rats, it was reported that the novel exon, termed the exon V, exists between the exons 7 and 8, and the novel mRNA isoform (termed AFP-V mRNA) is synthesized using the exon V. In this study, a reverse transcription-polymerase chain reaction was performed and quantitative analysis was done on the AFP mRNA to identify the exon V and the AFP-V mRNA in humans. As a result, 2 novel exons, the exons VA and VB, were identified. Furthermore, 3 novel AFP mRNAs, the AFP-V1, -V2, and -V3 mRNA, were demonstrated to be expressed through alternative splicing. Expression of the AFP-V2 mRNA isoform and the wild-type mRNA was differentially regulated, implying that the AFP-V mRNA isoforms could be used in diagnosis and classification of hepatocellular carcinoma and ovarian carcinoma.

(3) Title: Maternal plasma inhibin A at 11-13 weeks of gestation in hypertensive disorders of pregnancy.

Source: Prenat Diagn. 2009, 29:753-760

Authors: Akolekar R, Minekawa R, Veduta A, Romero XC, Nicolaidis KH

Abstract: OBJECTIVE: To investigate the potential value of maternal plasma inhibin A in first-trimester screening for preeclampsia (PE). METHOD: The concentration of inhibin A at 11-13 weeks was measured in samples from

121 pregnancies that developed PE, 87 cases of gestational hypertension (GH) and 208 normal controls. The distributions of inhibin A multiple of median (MoM) in the control and hypertensive groups were compared. Logistic regression analysis was used to derive algorithms for the prediction of hypertensive disorders. RESULTS: The maternal plasma inhibin A MoM was significantly higher in the early and late PE groups (1.55 MoM and 1.24 MoM, respectively; $p < 0.0083$), compared to the controls (0.98 MoM), but not in GH. Significant contributions for the prediction of PE were provided by maternal factors, plasma inhibin A and uterine artery pulsatility index (PI) and with combined screening the detection rates for early and late PE were 88% and 42%, respectively, for a false positive rate of 10%. CONCLUSION: The proposed combined screening test could be used to identify women at high risk for PE and intensive monitoring in such patients would lead to earlier identification of the disease which could potentially improve pregnancy outcome.

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

1) Title: A novel, label-free immunosensor for the detection of alpha-fetoprotein using functionalised gold nanoparticles.

Source: Clin Biochem 2009, 17:17

Authors: Liang W, Yi W, Li S, Yuan R, Chen A, Chen S, Xiang G, Hu C

Abstract: BACKGROUND AND OBJECTIVES: Novel immunoassay methods based on electrochemical sensors have been developed, but most of these immunosensors are unsuitable for clinical detection because their preparation requires complicated chemical procedures and because their detection sensitivity is restricted. In order to develop a highly sensitive, label-free amperometric sensor for immunoassays, we synthesised novel, functionalised gold nanoparticles (SV-GNP) by covalently capping the surface of gold nanoparticles (GNP) with 1,1'-bis-(2-mercapto)-4,4'-bipyridinium dibromide, a kind of sulfhydryl viologen (SV). DESIGN AND METHODS: We fabricated an immunosensor in a multi-step fashion, by first coating the SV-GNP onto a glassy carbon electrode surface; the resulting electrode core could then adsorb a suitable antibody in a second step to afford the desired immunosensor. alpha-fetoprotein (AFP) was used as a model analyte in this work. RESULTS: The anti-AFP/SV-GNP-modified electrode was sensitive to AFP with a linear relationship between 1.25 and 200 ng/mL and a correlation coefficient of 0.9983; the detection limit at a signal to noise ratio of 3 was 0.23 ng/mL under optimal conditions. In addition, the proposed immunosensor exhibited good sensitivity, selectivity, stability and long-term maintenance of bioactivity. CONCLUSION: The described immunosensor preparation and immunoassay methods offer promise for label-free, simple, and cost-effective analysis of biological samples.

(2) Title: Automated support-resolution strategy for a one-way chemiluminescent multiplex immunoassay.

Source: Anal Chem. 2009, 81:5484-5489

Authors: Yang Z, Liu H, Zong C, Yan F, Ju H

Abstract: An automated support-resolution strategy was designed to couple with a flow-through immunosensing system for performing a one-way chemiluminescent (CL) multiplex immunoassay. Different from multilabel and multichannel-based detection techniques, this immunoassay method employed a single horseradish peroxidase (HRP) label in one way. With the use of carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP) as model analytes, the capture antibodies for CEA and AFP were immobilized on the inner wall of a glass tube and the surface of paramagnetic microspheres (PMs), respectively. The on-line incubation could be performed in the glass tube after introducing the mixture of CEA, HRP-labeled anti-CEA antibody, AFP, anti-AFP immobilized PMs, and HRP-labeled anti-AFP antibody. With the use of a wash step, the formed sandwich immunocomplexes were separated automatically and the immunocomplex immobilized PMs were captured in another unmodified glass tube with a magnet. The CL signals from the two glass tubes were near-simultaneously collected with the aid of an optical shutter to perform quantitative detection. CEA and AFP could be rapidly assayed in the ranges of 1.0-60 and 1.0-80 ng/mL within 27 min. The assay results of clinical serum samples with the proposed method were in an acceptable agreement with the reference values. This system provides a promising multiplex immunoassay approach for clinical applications.

(3) Title: Immunonanogold Catalytic-Cu₂O Particle Resonance Scattering Spectral Determination of Trace alpha-Fetoprotein

Source: Chemical Journal of Chinese Universities-Chinese 2009, 30(6):1109-1115

Authors: [Jiang ZL](#). [Zhang YL](#). [Liang AH](#). [Wei LL](#). [Wang SM](#)

Abstract: 15 nm-nanogold was used to label monoclonal goat antihuman alpha-fetoprotein antibody (GAFP) to obtain immunonanogold probe(AuGAFP) for alpha-fetoprotein (AFP). Both nanogold and the probe have catalytic effect on the slow Cu₂O particle reaction between Fehling reagent and glucose that exhibit a resonance scattering peak at 620 nm. Combining AFP-AuGAFP immunoreaction with centrifugation technique, a highly sensitive immunonanogold catalytic-Cu₂O particle resonance scattering spectral assay for AFP was proposed. With addition of AFP, the AFP-AuGAFP immunocomplex increased, the excess probe in the supernatant decreased, and the resonance scattering intensity at 620 nm decreased linearly. The decreased intensity Delta I-RS was linear to AFP concentration [rho(AFP)] in the range of 0.10-16.0 ng/mL, with a regression equation of Delta I-RS = 4.27 rho(AFP) + 1.28, and a detection limit of 0.05 ng/mL. This method was applied to the detection of AFP in sera, with high sensitivity and good selectivity, in addition to low-cost reagents and easy controlling reaction.

E). Special Abstract Selection:

1) Title: The effectiveness of prenatal serum biomarker screening for neural tube defects in second trimester pregnant women: a meta-analysis

Source: Prenat Diagn 2009, 16:16

Authors: Wang ZP, Li H, Hao LZ, Zhao ZT

Abstract: BACKGROUND: Neural tube defects (NTDs) are common and serious birth defects all over the world. Prenatal screening for NTDs using maternal serum alpha-fetoprotein (MSAFP) during the second trimester of pregnancy has been widely used, but its effectiveness remains unclear. METHODS: We evaluated the studies published in the English and Chinese on MSAFP screening for NTDs. The homogeneity of the studies was evaluated by the forest graph. Meta-analysis was applied to calculate the combined effect values and their 95% confidence intervals. RESULTS: As many as 22 articles were selected according to the criteria and were included in the meta-analysis, for a total of 684 140 pregnant women screened during the second trimester. All the studies selected were homogenous according to the forest graph ($\chi^2 = 25.17, P > 0.10$). The combined relative risk estimate was 0.25 and its 95% confidence interval was 0.21 to 0.29. The combined protective rate was 75%. The sensitivity and specificity of MSAFP screening were 75.1 and 97.7%, respectively. CONCLUSION: Use of MSAFP during the second trimester in pregnant women is an effective measure for the screening of NTDs.

2) Title: Potential function of amniotic fluid in fetal development---novel insights by comparing the composition of human amniotic fluid with umbilical cord and maternal serum at mid and late gestation.

Source: J Chin Med Assoc. 2009, 72:368-373.

Authors: Tong XL, Wang L, Gao TB, Qin YG, Qi YQ, Xu YP

Abstract: BACKGROUND: Amniotic fluid (AF) is a dynamic and complex mixture. Up to now, little is known about the physiological functions of AF in the process of fetal development. We suppose that AF carries components such as proteins or peptides, which contribute to the regulation of fetal development. METHODS: Compositions including biochemical components and tumor markers were determined in human AF, umbilical cord serum (UCS) and maternal serum (MS) from the same subject in the range of 15-42 weeks of gestation. RESULTS: (1) The levels of primary electrolytes such as sodium, chloride, anion gap and osmotic pressure in AF was almost the same as in UCS and MS. (2) The levels of organic substances, including total protein, glucose, triglycerides, cholesterol and various enzymes, were markedly lower in AF than in UCS and MS, especially for total protein, which was 8- and 12.5-fold lower in AF than in UCS and MS, respectively. (3) The levels of tumor markers, including carcinoembryonic antigen, ferritin, cancer antigen 125 and 199, and alpha-fetoprotein in AF displayed different dynamic changes compared to UCS and MS as gestation advanced. CONCLUSION: This study demonstrated that AF is not a result of simple filtration from the blood but an independent fluid. We speculate that proteins or peptides in the amniotic fluid modulate the process of fetus development since they possess potent bioactivity on cellular growth and proliferation. AF provides a pathway to transport these "regulators" to the fetus and thus plays a pivotal role in fetal development.

3) Title: The impact of fetal gender on first trimester nuchal translucency and maternal serum free beta-hCG and PAPP-A MoM in normal and trisomy 21 pregnancies

Source: Prenat Diagn. 2009, 29:578-581.

Authors: Cowans NJ, Stamatopoulou A, Maiz N, Spencer K, Nicolaides KH

Abstract: OBJECTIVE: To investigate if fetal sex has an impact on 1st trimester combined screening for aenuploidy. METHODS: We studied the first trimester PAPP-A, free beta-human chorionic gonadatropin (beta-hCG) and nuchal translucency levels in 56,024 normal, singleton pregnancies with known fetal sex at birth. We also examined the distributions in 722 pregnancies with trisomy 21 of known fetal sex. RESULTS: We have found a 14.74% increase in first trimester maternal serum (MS) median free beta-hCG MoM, 6.25% increase of PAPP-A and a 9.41% decrease in delta NT, when the fetus was female. Analysis of data has shown that women carrying a female fetus were 1.084 times more likely to be in the 'at risk' group than those carrying a male fetus. In examining data from 722 pregnancies in which the fetus was affected by trisomy 21, we observed a similar 20.8% increase in free beta-hCG MoM, 5.7% increase in PAPP-A and a 12% decrease in delta NT when the fetus was female. Amongst the trisomy 21 cases, 88.8% of male trisomy 21 cases were detected compared with 91.2% in female cases, this difference was not statistically significant. Correcting for fetal sex redressed the balance in screen-positive rate between the sexes and had a minimal impact on detection rate. CONCLUSION: Correcting for fetal sex may be a worthwhile consideration. A cost-benefit analysis would be required to determine if it is feasible to introduce fetal gender assignment into the routine first trimester scan for the purpose of marker correction and whether this would have any significant impact.

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

Source: Ultrasound Obstet Gynecol. 2009, 33:506-511

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

Abstract: 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.

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