

Fetal Defect Marker Proficiency Test Mailout February, 2009

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from January 27, 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 = 31	Sample #	MS 231	MS 232	MS 233	MS 234	MS 235
	Gestational Age (weeks)	19	17	19	16	18
Maternal Race	Ethnic Group	White	Hispanic	White	Black	White
Maternal Weight	Pounds (lbs)	155	150	155	135	140
Maternal Age	Years	28	25	28	30	29
Alpha-Fetoprotein (AFP)	Mean ng/ml ± Std.Dev.	157.10 ± 9.71	39.80 ± 2.90	159.30 ± 10.10	34.92 ± 1.71	17.91 ± 1.20
	MOM ± Std.Dev.	3.15 ± 0.21	1.04 ± 0.08	3.20 ± 0.24	0.87 ± 0.07	0.39 ± 0.03
Unconjugated Estriol (uE3)	Mean ng/ml ± Std.Dev.	2.07 ± 0.98	1.32 ± 0.58	2.17 ± 1.09	0.98 ± 0.37	0.73 ± 0.28
	MOM ± Std.Dev.	0.77 ± 0.18	0.72 ± 0.12	0.77 ± 0.16	0.72 ± 0.23	0.35 ± 0.11
human Chorionic Gonadotrophin (hCG)	Mean IU/ml ± Std.Dev.	19.00 ± 1.50	24.90 ± 2.21	19.10 ± 1.60	27.71 ± 2.40	35.72 ± 3.51
	MOM ± Std.Dev.	1.03 ± 0.13	1.02 ± 0.12	1.04 ± 0.11	0.83 ± 0.09	1.63 ± 0.18
Dimeric Inhibin-A (DIA)	Mean pg/ml ± Std.Dev.	193.60 ± 15.00	141.60 ± 11.21	210.50 ± 21.41	128.30 ± 8.91	263.10 ± 20.46
	MOM ± Std.Dev.	1.12 ± 0.11	0.86 ± 0.06	1.22 ± 0.15	0.71 ± 0.06	1.51 ± 0.12
Neural Tube Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Pos. (+) (100%)	Neg. (-) (100%)	Pos. (+) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Further Action G,U,A	G = 70% U = 89% A = 85%	NFA	G = 67% U = 93% A = 85%	NFA	NFA
	NTD Risk 1 in	46	8,740	37	10,000	9,000
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 = 81% U = 81% A = 75%
	Risk Est. 1 in	5,500	2,450	4,300	1,450	34
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 = 70% U = 74% A = 70%
	Risk Est. 1 in	8,100	6,000	10,000	5,000	20
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	7,225	553

*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 = 31 all-lab Consensus Values.

<u>Sample #</u>	<u>Summary Comments (Mock specimens):</u>
MS 231 Wk 19.0	This specimen was obtained from a 28 year old white woman (Gravida = 1, Parity = 0) in her 19 th week gestation with a body weight of 155 lbs. She had a personal history of pregnancy loss. Her specimen, a second pregnancy sample, was screen positive for NTD (100% consensus; MOM=3.15). 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, 70%, ultrasound, 89% and amniocentesis, 85%; The MS231 specimen had an amniotic fluid counterpart which was also elevated (MOM = 2.27).
MS 232 Wk 17.0	This specimen was procured from a 25 year old hispanic woman (Gravida = 3, parity = 2) in her 17 th week gestation with a body weight of 150 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. The labs were also in agreement that both trisomy consensus screens were negative (100%). Specimen MS232 was not paired with an amniotic fluid sample.
MS 233 Wk 19.0	This specimen was obtained from a 28 year old white woman (Gravida = 1, Parity = 0) in her 19 th week gestation with a body weight of 155 lbs. She had no personal history of pregnancy loss. Her specimen, a first pregnancy sample, was a positive screen for NTD (100% consensus; MOM=3.20). 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, 67%, ultrasound, 93% and amniocentesis, 85%. The MS233 specimen had no amniotic fluid counterpart.
MS 234 Wk 16.0	This specimen was obtained from an 30 year old black woman (Gravida = 2, parity = 1) in her 16 th week gestation with a body weight of 135 lbs. She had no family history of pregnancy complications or adverse outcomes. Her sample screened negative for NTD and her aneuploidy screen was negative for both Trisomy-21 and for Trisomy-18. No recommendation of further action was reported from participating labs. This specimen was not paired with an amniotic fluid specimen.
MS 235 Wk 18.0	This specimen was obtained from a 29 year old white woman (Gravida = 2, parity = 1) in her 18 th week gestation with a body weight of 140 lbs. She had a family history of pregnancy complications and adverse outcomes. 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, 70%; ultrasound, 74%; and amniocentesis, 70%; while the triple tests were: genetic counseling, 81%; ultrasound 81%, and amniocentesis, 75%. It was of further interest that specimen MS235 also resulted in a negative T18 screen of 100% in participating labs. This specimen was paired to an amniotic fluid specimen which also had a low AFAFP level (MOM = 0.48).

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

<u>Sample#</u>	<u>Values</u>	<u>Summary Comments:</u>
AF 231 Wk 19.0	AFP= 17.80 ± 2.50 µg/ml MOM= 2.27 ± 0.26	This sample was targeted for an elevated AFAFP value in the upper gestational age range. Eighty-one percent of the labs called AF231 an elevated specimen for NTD. This AFAFP sample was matched to a maternal serum specimen, MS231, which was also elevated (MOM = 3.15).
AF 232 Wk 21.0	AFP= 6.70 ± 0.91 µg/ml MOM= 1.23 ± 0.24	This sample was targeted for a screen negative AFAFP value in the upper gestational age range. All labs reported this specimen as a screen negative (low) AFAFP value. The AF232 specimen was not paired with a maternal serum sample.
AF 233 Wk 20.0	AFP= 6.71 ± 0.90 µg/ml MOM= 1.03 ± 0.12	This sample was targeted for a negative NTD screen AFAFP value in the upper gestational age screening range. All labs categorized this as an NTD screen negative specimen. This sample was not matched to a maternal serum specimen.
AF 234 Wk 21.0	AFP= 6.80 ± 1.11 µg/ml MOM= 1.22 ± 0.28	This sample was targeted as an NTD screen negative AFAFP value in the upper gestational age screening range. All labs categorized AF234 as a negative NTD screen specimen, and it had no maternal serum counterpart.
AF 235 Wk 18.0	AFP= 4.62 ± 0.71 µg/ml MOM= 0.48 ± 0.05	This sample was targeted for a low AFAFP value in the routine gestational age range. Most labs called AF235 a low specimen for AFAFP. This AFAFP sample was matched to a maternal serum specimen, MS235 which was low (MOM = 0.39).

II. Non-Graded Results Section:

Table 2: First Trimester Maternal Serum all-lab Results

Samples N = 16	Sample #	FT 231	FT 232	FT 233	FT234	FT235
	Gestational Age (weeks)	12.0	11.0	12.0	13.1	11.5
Maternal Race	Ethnic Group	White	Hispanic	White	Black	Asian
Maternal Weight	Pounds (lbs)	150	140	150	160	125
Maternal Age	Years	25	28	25	29	21
Nuchal Translucency (NT)-Associated	Crown Rump Length (mm)	53	42	53	67	47
	NT Thickness (mm)	2.90	1.10	2.90	1.05	1.09
	NT - MOM	2.27	1.05	2.27	0.69	0.95
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	160.00 ± 22.40	81.90 ± 11.50	155.40 ± 21.70	63.00 ± 7.91	75.62 ± 8.90
	MOM ± Std. Dev.	2.18 ± 0.32	0.96 ± 0.15	2.12 ± 0.26	1.01 ± 0.13	0.91 ± 0.12
Pregnancy-Associated Plasma Protein-A (PAPP-A)	Mean mIU/mL ± Std. Dev.	1.07 ± 0.50	1.89 ± 0.85	0.91 ± 0.46	3.15 ± 1.47	2.13 ± 1.02
	MOM ± Std. Dev.	0.77 ± 0.34	1.86 ± 0.83	0.65 ± 0.32	1.44 ± 0.68	1.55 ± 0.70
Trisomy-21 Screen (Positive/Negative) percent	Pos (+) or Neg. (-)	Pos. (100%)	Neg. (100%)	Pos. (100%)	Neg. (100%)	Neg. (100%)
	Recommended Action	G = 93% U = 40% C = 47%	NFA	G = 93% U = 40% C = 47%	NFA	NFA
	Risk Estimate	18	10,000	13	10,000	9,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	Neg. (93%)	Neg. (100%)	Neg. (80%)	Neg. (100%)	Neg. (100%)
	Recommended Action	NFA	NFA	NFA	NFA	NFA
	Risk Estimate	756	12,000	297	10,000	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 231 Wk 12.0	This specimen was obtained from a 25 year old white woman of average body weight (150 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 and all testing Labs were in agreement (see Critique). The FT231 risk estimate for Trisomy-21 was 1 in 18; while the all-lab Trisomy-18 risk was 1 in 756 (93% T18 negative screen). All labs were in agreement that FT231 was a high risk Trisomy-21 pregnancy.
FT 232 Wk 11.0	This specimen was obtained from a 28 year old hispanic woman of medium body weight (140 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 FT232 risk estimate for Trisomy-21 was 1 in 10,000 while the Trisomy-18 risk was 1 in 10,000.
FT 233 Wk 12.0	This specimen was obtained from a 25 year old white woman of average body weight (150 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 and all testing Labs were in agreement (see Critique). The FT233 risk estimate for Trisomy-21 was 1 in 13; while the all-lab Trisomy-18 risk was 1 in 297 (80% T18 negative screen). All labs were in agreement that FT233 was a high risk Trisomy-21 pregnancy.
FT 234 Wk 13.1	This specimen was procured from a 29 year old black woman of average body weight (160 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 (see Critique). The FT234 risk estimate for Trisomy-21 was 1 in 10,000, while the Trisomy-18 risk was 1 in 10,000.
FT 235 Wk 11.5	This specimen was procured from a 21 year old asian woman with a body weight of 125 lbs. Her gestational age at time of screening was 11.5 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative for T21 and T18. The risk estimate for FT235 was 1 in 9,000, and the T18 risk was 1 in 10,000. All labs were in agreement with the FT235 negative screen assessments.

III. Critique and Commentary:

A) Fetal Defect Proficiency Test Mail out 1/27/09 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 attained the goals of our projected target values, risks, and outcomes. As displayed in the second trimester tables, maternal serum sample MS231 was targeted as an elevated specimen for NTD (Figs. 1 and 3), together with an elevated AF-AFP. Thus, specimen MS231 was determined to be screen positive for NTD, but negative for Trisomy-21 (T21), and Trisomy-18 (T18). For the MS231 specimen, the NTD screen resulted in a 1 in 46 all-lab risk for open neural tube defects (ONTD) and achieved 100% screen consensus. The NTD-related recommended action for the MS231 specimen was as follows: genetic counseling, 70%; ultrasound; 89%; and amniocentesis, 85%. Sample MS235, a T21 screen positive specimen, was obtained from a white woman with a prior family history of pregnancy problems. The T21 MOM results for specimen MS235 (MSAFP-MOM = 0.39, MSuE3-MOM = 0.35, MShCG-MOM = 2.10, DIA-MOM = 2.00) were all in accordance with a T21 positive screen; hence, all labs classified this specimen as T21 positive with all recommending further action. The MS235 sample from a near middle-age woman age (29 yrs.) produced a risk from the quad test (1:20) which was greater than that expected from the maternal age alone (1 in 800). Samples MS232 and MS234 produced negative screens for NTD, Trisomy-21, and Trisomy 18. Finally, the MS233 specimen was obtained from a woman of Caucasian descent and demonstrated an elevated MSAFP which served to produce a positive NTD screen in 100% of the participating labs.

Specimen MS231, together with a matched AFAFP sample, deserves further comment in that the MSAFP specimen was NTD screen positive (100%; MOM = 3.15) and the paired AFAFP sample was also elevated (MOM = 2.27) (Figs. 1 & 2). This mock patient had a family 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 the elevated AFAFP MOM, it was consistent with an NTD positive screen. No demographic correction factors were indicated for the MS231 screen assessment. An all-lab NTD risk assessment for MS231 was 1 in 46 for her screen. A subsequent high definition Stage-II ultrasound of this mock patient together with an Ache analysis later confirmed the presence of an open NTD lesion in the spinal cord of the fetus.

The MS235 specimen (gravida = 2, parity = 1; maternal age = 29) produced a positive Trisomy-21 (T21) result with both the triple and quad testing platforms. All labs reporting either triple or quad testing concluded that sample MS235 was T21 screen positive (100% all-lab consensus). Further action recommended for the T21 screen was determined as genetic counseling, 81%, 81% ultrasound (US), and 75% amniocentesis (AM) for labs using the triple screen; while genetic counseling 70%, 74% US and 70% AM was recorded for labs employing the quad screen. The recommended action on MS235 was consistent with the severity of the similar risk ratio assessments of 1 in 34 from the triple test versus a 1 in 20 risk from the quad test. Note from the point distribution graphs comparing the triple with the quad test (Figs. 5 and 6) that the MS235 point cluster in the quad assay was nearly the same but slightly lower and tighter (note scale difference) than the MS235 cluster in the triple test. As with previous specimens, the quad test signaled a slightly higher risk (difference) for Down syndrome than the triple test; hence, 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 also low (MOM = 0.48). The amniotic fluid cells of this mock patient were subjected to subsequent genotyping and indeed indicated the presence of a Trisomy-21. The MS235 specimen produced a negative screen for Trisomy-18. The age-specific risk of the MS235 sample for T21 was 1 in 800.

Specimen MS233 deserves further comment in that the sample was NTD screen positive (100%; MOM=3.20). This mock patient had no prior history of pregnancy loss or complications; thus, a paired amniotic fluid was not available for analysis at time of venipuncture specimen collection. This MS specimen represented a 19 week gestational age sample, thus, an amniocentesis and ultrasound would be indicated. Although time would not permit a second sample MS repeat, an MS-specimen could be 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 adjunct to subsequent diagnostic testing to eliminate the possibility of a closed NTD. Subsequent Stage-II ultrasound and amniocentesis genotyping 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 MS233 displayed an unusual biomarker profile in its screen results. The MSAFP was elevated (MOM = 3.20) the uE3 level was low (MOM = 0.77), the MShCG was low normal (1.04 MOM) as was the DIA normal (MOM = 1.22). In view of these results, most of the participating laboratories called MS233 a positive screen NTD, and screen negative T21 and T18, and most of the labs suggested further actions. The presence of an unexplained elevated AFP in a somewhat normal maternal serum profile prompts one to consider conditions where AFP predicts poor outcomes. Adverse pregnancy outcomes relative to an elevated maternal serum AFP (MOM = >2.5) usually involve placental dysfunction and/or birth defects accompanied by sonographic adverse findings and growth retardation (see below). Interestingly, the MS233 specimen was obtained from a 28 year old Caucasian patient and adverse and complicated outcomes were not a part of her personal family history.

It has long been observed that elevated concentrations in maternal serum MSAFP alone were predictive of fetal abnormalities or placental complications at mid trimester. Hence, increased surveillance is recommended in pregnancies where the MSAFP in the second trimester is inexplicably elevated (1,2). Uterine arterial doppler and placental ultrasound have been employed to confirm predicted severe placental dysfunction and adverse outcomes by means of elevated MShCG in conjunction with MSAFP (3). Hence, isolated elevations of MSAFP alone do not confer an increased risk of fetal congenital anomalies and placental dysfunction. Elevated MSAFP levels in combination with sonographic markers have been found to be associated with a high incidence of structural anomalies and aneuploides (4). For example, fetuses with mosaic trisomy-13 may be associated with both elevated MShCG and MSAFP levels in the second trimester (5, 6). Although MSAFP levels in the second trimester are not always elevated in normotensive women who later produced a growth-retarded fetus, MSAFP levels are significantly higher in women who develop cases of severe pre-eclampsia (7). Finally, MSAFP concentrations have been reported to be significantly higher in cases of threatened miscarriage compared with gestational-age matched controls (8). See additional references below.

Elevated AFP levels had been used since 1976 as an indicator for perinatal distress conditions such as bilateral renal agenesis, fetomaternal transfusion, pre-eclampsia, intra-uterine growth retardation (IUGR), and fetal demise (3). In many instances, AFP accumulates in a biological compartment (such as amniotic fluid) by: 1) leakage from fetal serum and cerebrospinal fluid (NTD); 2) exposure of blood vessels in extruding viscera leading to transudation of AFP (exophthalmos); 3) expedited protein filtration and passage into urea (congenital nephrosis); 4) impaired fetal swallowing or digestion in amniotic fluid (GI anomaly) and; 5) altered or obstructed transplacental passage such as in placenta accreta (Table-A). The early developmental malformations reported in the literature were structural in nature and late pregnancy placental complications were directly life threatening to the fetus and oftentimes the mother. Such conditions included severe pre-eclampsia, premature labor, intrauterine and/or perinatal death, preterm birth, fetal wastage, and trophoblast abnormalities including placental previa and disruption. Non-pathological elevations of AFP in pregnancy can be the result of physiological or procedural phenomena such as twinning or multiple pregnancy, low birth weight, prematurity, or incorrect gestational age dating. Fetal defects and malformations can also be parsed by classifying them according to high or low levels of AFP in biological fluids (Table-A below). Elevated serum and amniotic fluid (AF)-AFP levels are usually indicative of the presence of an anatomical lesion such as observed in NTD, anencephaly, ventral wall defects, gastrointestinal atresia, renal anomalies, poly-and oligohyramnios, cystic hygromas (with fetal hydrops), teratomas, blastomas, and disruption of placental barriers (1-9 references below).

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

1. Thomas, R. L. and K. J. Blakemore (1990). "Evaluation of elevations in maternal serum alpha-fetoprotein: a review." Obstet Gynecol Surv 45(5): 269-83.
2. Walters, B. N., T. Lao, et al. (1985). "alpha-Fetoprotein elevation and proteinuric pre-eclampsia." Br J Obstet Gynaecol 92(4): 341-4.
3. Crandall, B. F. and C. Chua (1997). "Risks for fetal abnormalities after very and moderately elevated AF-AFPs." Prenat Diagn 17(9): 837-41.
4. Benn, P. A., A. Craffey, et al. (2000). "Elevated maternal serum alpha-fetoprotein with low unconjugated estriol and the risk for lethal perinatal outcome." J Matern Fetal Med 9(3): 165-9.
5. Mulch, A. D., S. P. Stallings, et al. (2006). "Elevated maternal serum alpha-fetoprotein, umbilical vein varix, and mesenchymal dysplasia: are they related?" Prenat Diagn 26(8): 659-61.
6. Entezami, M., S. Runkel, et al. (1997). "Diagnostic dilemma with elevated level of alpha-fetoprotein in an undiagnosed twin pregnancy with a small discordant holoacardius acephalus." Am J Obstet Gynecol 177(2): 466-8.
7. Nadel, A. S., M. E. Norton, et al. (1997). "Cost-effectiveness of strategies used in the evaluation of pregnancies complicated by elevated maternal serum alpha-fetoprotein levels." Obstet Gynecol 89(5 Pt 1): 660-5.
8. Milunsky, A., S. S. Jick, et al. (1989). "Predictive values, relative risks, and overall benefits of high and low maternal serum alpha-fetoprotein screening in singleton pregnancies: new epidemiologic data." Am J Obstet Gynecol. 161(2): 291-7.
9. Wilkins-Haug, L. (1998). "Unexplained elevated maternal serum alpha-fetoprotein: what is the appropriate follow-up?" Curr Opin Obstet Gynecol. 10(6): 469-74.

Table A- Biological compartments in which AFP levels may be elevated during pregnancy^a

<p>I. Fetal Compartment: Serum = 3-4 mg/mL < peak at 10-13 weeks Manifestations</p> <ol style="list-style-type: none"> a. Twins/multiple gestation a. Fetal tumors b. Cystic adenomatoid growth c. Underestimated gestational age e. Renal agenesis f. Urethra obstruction g. Oligohydraminos h. Recent fetal demise 	<p>III. Placental Compartment: Abnormal Location, Placentation, Size, Anatomy=50 µg/ml at term Manifestations</p> <ol style="list-style-type: none"> a. Ectopic pregnancy b. Abdominal pregnancy c. Fetal-maternal hemorrhage d. Preeclampsia e. Poor perinatal outcome f. Placenta accreta g. Increase placental/fetal ratio h. Cystic vascular changes i. Hemangioma j. Rh-immunization/hydrops fetalis k. Triploidy l. Abruption placenta
<p>II. Amniotic Fluid Compartment: Fluid = 10:g/ml peak at 12-14 weeks Manifestations</p> <ol style="list-style-type: none"> a. Open neural tube defect b. Gastroschisis c. Omphalocele d. Congenital skin defect e. Cystic hygoma/turner syndrome f. Amniotic band syndrome g. Teratoma h. Duodenal atresia i. Esophageal atresia j. Diaphragmatic hernia k. Cystic adenomatoids (lung) l. Congenital nephrosis 	<p>IV. Maternal Compartment: Serum = 150 ng/ml = peaks at 32wks: Manifestations</p> <ol style="list-style-type: none"> a. Low body weight b. Blood pressure c. Liver cancer d. G.I. cancer e. Premature labor f. Germ cell tumor g. Hepatitis/cirrhosis h. Ataxia teleangiectasis i. Tyrosinemia j. Mosaic trisomy-8

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. 8); however, labs employing DPC Immulite 2500 or Immulite 2000 yielded values achieving 1.3 to 1.7 times higher than the mean/all kit median (see dotted line). In contrast, Beckman Access/2 and Unicel measured uE3 values 35 to 45% lower than the mean/all kit median of 1.0. 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 1% 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 Unicel versus the Diagnostic Systems Lab (DSL) assay platforms. Other than Beckman being marginally higher and DSL being slightly lower, both kit performances for Inhibin-A approximated 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. While Siemens/Bayer ADVIA-Centaur/ACS-180 and Abbott-AxSYM kits were 15-20% higher (except for sample AF230) Beckman Unicel and Beckman Access/2, were about 10% - 20% lower than the 1.0 mean/all-kit median. Finally, please be advised that these specimens are derived from actual AF samples, and therefore these results are directly relevant to patient screening. Please note: the samples AF234 and AF235 (Siemens/Bayer/ACS) exhibited higher means possibly due to the presence of a small sample size (N=3) in participating labs using that kit.

C) 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 - 28% of the labs respectively; Robert Maciel (RMA) software was employed by 34%; while in-house software comprised 11% 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 now 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 12.0 week Caucasian FT231 specimen for total hCG resulted in a mass mean of 160.0 IU/ml \pm 22.4, with an elevated MOM = 2.18. Furthermore, the all-lab mass mean for PAPP-A was 1.07 \pm 0.50 mIU/ml with a MOM = 0.77 \pm 0.34. The all-lab T21 risk assessment was 1 in 18 for the FT231 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT231 sample was screen positive (See Figure 14 risk distribution) demonstrating a high hCG MOM = 2.18, a low PAPP-A MOM = 0.77, and an increased NT MOM = 2.27. The risk cut-off level for Caucasians ranges from 200 to 270 among the participating labs. Since analyte MOM measurements for first trimester Down syndrome screen are associated with increased NT, low PAPP-A, and high hCG, the FT231 results would be consistent with a positive screen outcome. Thus, the FT231 sample resulted in a 100% all lab T21 positive screen assessment. Further actions by the labs included genetic counseling, 93%; ultrasound, 40%; and CVS = 47%. Finally, the FT231 specimen screened negative for T18 (1 in 756) using a cutoff of 1 in 100.

In the FT232 Hispanic sample, the gestational age all-lab mean was reported as 11.0 weeks. Assay measurements for FT232 resulted in an all-lab total hCG mass measurement of 81.90 \pm 11.5 based on two present methods, Beckman Access/2 or Unicel and Siemens/DPC Immulite or 2000, while the all-lab PAPP-A mass assessments were 1.89 \pm 0.85 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 FT232 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 FT232 of total hCG resulted in a MOM value of 0.96 \pm 0.15. The all-lab MoM mean for PAPP-A was 1.86 \pm 0.83. 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. 13 for mean/all kit medians). These two methods measure PAPP-A quite differently. For example, the DPC Immulite PAPP-A assay mass mean for FT232 was 3.23mIU/ml compared to Diagnostic Systems Lab 1.50 mIU/ml. Regardless of this, all the labs agreed that the FT232 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 were consistent with the screen negative outcome. The all-lab T21 risk assessment for FT232 was 1 in 10,000. The FT232 specimen also resulted in a negative screen for Trisomy-18.

As displayed in the FT table 2 (Section – II) above, the all lab measurement of the 12.0 week Caucasian FT233 specimen for total hCG resulted in a mass mean of 155.40 IU/ml \pm 21.70, with an elevated MOM = 2.12. Furthermore, the all-lab mass mean for PAPP-A was 0.91 \pm 0.46 mIU/ml with a MOM = 0.65 \pm 0.32. The all-lab T21 risk assessment was 1 in 13 for the FT233 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT233 sample was screen positive (See Figure 14 risk distribution) demonstrating a high hCG MOM = 2.12, a low PAPP-A MOM = 0.65, and an increased NT MOM = 2.27. The risk cut-off level for Caucasians ranges from 200 to 270 among the participating labs. Since analyte MOM measurements for the first trimester Down syndrome screen are associated with increased NT, low PAPP-A, and high hCG MOMs, the FT233 results were consistent with a positive screen outcome. Thus, the FT233 sample resulted in a 100% all lab T21 positive screen assessment. Further actions by the labs included genetic counseling, 93%; ultrasound, 40%; and amniocentesis/ CVS = 47%. Finally, the FT233 specimen screened negative for T18 (1 in 297) using a cutoff of 1 in 100.

As shown in the above First Trimester table 2 (Section-II) for the FT234 Afro-American specimen, the gestational age all-lab mean was reported as 13.1 weeks. Assay measurements from FT-screening participating laboratories resulted in an all-lab total hCG mass measurement of 63.00 ± 7.91 IU/ml based on the two methods above; while the all-lab PAPP-A mass assessment was 3.15 ± 1.47 mIU/ml. The first trimester all-lab Trisomy-21 screen consensus for FT234 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 FT234 achieved a MOM value of 1.01 ± 0.13 . In comparison, the all-lab MoM for PAPP-A was 1.44 ± 0.68 , 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 FT234 sample was screen negative for Trisomy 21 (See Fig. 14 risk distribution). The FT234 specimen also resulted in a negative screen for Trisomy-18 with a risk assessment of 1 in 10,000.

As demonstrated in the FT table 2 (Section-II) above for the Asian FT235 specimen, the gestational age all-lab mean was reported as 11.5 weeks. Assay measurements from FT-screening participating laboratories resulted in the all-lab total hCG mass measurement of 75.62 ± 8.90 IU/ml based on the two methods, while the all-lab PAPP-A mass assessment was 2.13 ± 1.02 mIU/ml. The first trimester all-lab trisomy-21 screen consensus for the FT235 specimen was negative (100%). The all-lab FT Trisomy-21 risk assessment was 1 in 9,000. As observed in the table (Table 2, Section – II), the all lab measurement of total hCG MOM for FT235 produced a value of 0.91 ± 0.12 . In comparison, the all-lab MOM mean for PAPP-A resulted in 1.55 ± 0.70 , a relatively high value. 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 FT235 sample was screen negative for Trisomy 21. (See Fig. 14 risk distribution). The FT235 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 differed somewhat, with Beckman Unicel/Access kit measuring a little higher than the DPC kits and DPC being about 10% 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 about half of those obtained with DPC Immulite or Immulite 2000 kit.

G.J. Mizejewski, Ph.D.

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Abstracts

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

(1) Title: Prenatal diagnosis, fetal surgery, recurrence risk and differential diagnosis of neural tube defects.

Source: [Taiwan J Obstet Gynecol.](#) 2008 Sep;47(3):283-90.

Authors: Chen CP

Abstract: Prenatal screening with alpha-fetoprotein (AFP) and ultrasonography have allowed the prenatal diagnosis of neural tube defects (NTDs) in current obstetric care, and open spina bifida has been considered a potential candidate for in utero treatment in modern pediatric surgery. This article provides an overview of maternal serum AFP screening, amniotic fluid AFP assays, amniotic fluid acetylcholinesterase immunoassays and level II ultrasound for NTDs, prenatal repair of fetal myelomeningocele, recurrence risk of NTDs, and differential diagnosis of NTDs on prenatal ultrasound.

(2) Title: Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis.

Source: [BMC Pregnancy Childbirth.](#) 2008 Aug 4;8:33.

Authors: [Morris RK](#), [Cnossen JS](#), [Langejans M](#), [Robson SC](#), [Kleijnen J](#), [Ter Riet G](#), [Mol BW](#), [van der Post JA](#), [Khan KS](#).

Abstract: BACKGROUND: Reliable antenatal identification of pre-eclampsia and small for gestational age is crucial to judicious allocation of monitoring resources and use of preventative treatment with the prospect of improving maternal/perinatal outcome. The purpose of this systematic review was to determine the accuracy of five serum analytes used in Down's serum screening for prediction of pre-eclampsia and/or small for gestational age. METHODS: The data sources included Medline, Embase, Cochrane library, Medion (inception to February 2007), hand searching of relevant journals, reference list checking of included articles, contact with experts. Two reviewers independently selected the articles in which the accuracy of an analyte used in Down's serum screening before the 25th gestational week was associated with the occurrence of pre-eclampsia and/or small for gestational age without language restrictions. Two authors independently extracted data on study characteristics, quality and results. RESULTS: Five serum screening markers were evaluated. 44 studies, testing 169,637 pregnant women (4376 pre-eclampsia cases) and 86 studies, testing 382,005 women (20,339 fetal growth restriction cases) met the selection criteria. The results showed low predictive accuracy overall. For pre-eclampsia the best predictor was inhibin A > 2.79 MoM positive likelihood ratio 19.52 (8.33,45.79) and negative likelihood ratio 0.30 (0.13,0.68) (single study). For small for gestational age it was AFP > 2.0 MoM to predict birth weight < 10th centile with birth < 37 weeks positive likelihood ratio 27.96 (8.02,97.48) and negative likelihood ratio 0.78 (0.55,1.11) (single study). A potential clinical application using aspirin as a treatment is given as an example. There were methodological and reporting limitations in the included studies thus studies were heterogeneous giving pooled results with wide confidence intervals. CONCLUSION: Down's serum screening analytes have low predictive accuracy for pre-eclampsia and small for gestational age. They may be a useful means of risk assessment or of use in prediction when combined with other tests.

(3) Title: Multiple-marker screening for Down's syndrome: a method of assessing the statistical robustness of proposed tests.

Source: [J Med Screen.](#) 2008;15(2):55-61.

Authors: [Morris JK](#), [Bestwick J](#), [Wald NJ](#).

Abstract: OBJECTIVES: Antenatal screening for Down's syndrome relies on the use of multiple markers in combination. Markers that are highly correlated can cause statistical instability. We used the maximum variance inflation factor (VIF(max)) to determine whether a screening test using multiple markers was robust to imprecision in the estimation of the marker distribution parameters. METHODS: The VIF(max) for a specified screening test was calculated from the correlations between markers in Down's syndrome pregnancies for six tests: integrated and serum integrated tests without repeat measurements, both tests with repeat measurements across trimesters analysed in the standard way, and both tests with repeat measurements analysed as cross-trimester (CT) marker ratios. The screening performance of each test using published parameter values, in terms of the false-negative rates for a 3% false-positive rate (FN(3)), were calculated for simulated populations with medians 0.2 standard deviations (SD) higher or lower than the published values (to reflect imprecision in parameter estimation) for pregnancy-associated plasma protein A and unconjugated oestriol in affected pregnancies. For each test, the VIF(max) value was compared with the coefficient of variation of the FN(3) (FN(3) CV). An independent set of 27 Down's syndrome pregnancies was used to determine how many had meaningless low risks (<1 in 10,000) with each test. RESULTS: Tests with VIF(max) values greater than 5 had FN(3)CV values over 50%, but those with VIF(max) values less than 5 had FN(3) CV values less than 21%. The numbers of Down's syndrome pregnancies with meaningless low risk estimates in the independent set were 18 (64%) in tests with VIF(max) values > or =5 and none for those with values <5. CONCLUSION: VIF(max) values of 5 or more suggest instability. The tests using CT marker ratios were stable (VIF(max) < 3), but the tests using repeat measurements in the standard manner were not (VIF(max) > 5).

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

(1) Title: Liver tumours in patients with Fanconi anaemia: a report of three cases.

Source: [Eur J Gastroenterol Hepatol](#). 2008 Oct;20(10):1036-9.

Authors: [Ozenne V](#), [Paradis V](#), [Vullierme MP](#), [Vilgrain V](#), [Leblanc T](#), [Belghiti J](#), [Imbert A](#), [Valla DC](#), [Degos F](#).

Abstract: Fanconi anaemia is an autosomal recessive disease, causing secondary aplastic anaemia and congenital abnormalities, associated with an increased risk of tumours. Liver cell adenoma and hepatocellular carcinoma have rarely been described. Clinical, radiological and histopathological features in three patients with Fanconi anaemia and liver tumours were analyzed. Only one patient had received androgens and none had chronic viral hepatitis. All had elevated serum ferritin with significant parenchymal iron overload. Alpha-fetoprotein levels were normal in all cases. Patient 1 had moderately differentiated hepatocellular carcinoma with venous invasion and satellite nodules. The patient underwent two consecutive resections. Patient 2 had hepatic nodules diagnosed at routine examination with radiological features of adenomas. The patient underwent resection, which showed liver cell adenoma with foci of carcinoma. Patient 3 had three nodules, with radiological and histological diagnosis of adenoma. In patients with Fanconi anaemia, androgen therapy and iron overload may contribute to the development of liver cell adenoma and hepatocellular carcinoma. Hepatocellular carcinoma may occur as a transformation of liver cell adenoma. With prolongation of survival, continued development of liver tumours can be expected. Routine detection should therefore be considered in these patients as curative resection can be performed.

(2) Title: Hereditary persistence of alpha-fetoprotein.

Source: [Pediatr Blood Cancer](#). 2009 Mar;52(3):403-5.

Authors: [Li X](#), [Alexander S](#).

Abstract: Hereditary persistence of alpha-fetoprotein (HPAFP) is a rare benign autosomal dominant disorder. Here we report a 7-year-old healthy female who was found to have elevated alpha-fetoprotein (AFP) of 55-88 ng/ml over a 2-year period. Subsequently, AFP was also determined to be elevated in another 4 out of 8 family members in three generations, consistent with an autosomal dominant inheritance pattern. Elevated AFP levels are usually related to pregnancy, congenital disorders, liver diseases, or specific malignancies. However, HPAFP should be considered in the differential diagnosis of children with unexplained elevation of AFP. This disorder can be easily confirmed by measuring AFP levels in family members or checking specific point mutations of AFP gene promoter.

(3) Title: Gastroschisis: clinical presentation and associations.

Source: [Am J Med Genet C Semin Med Genet.](#) 2008 Aug 15;148C(3):219-30.

Authors: [Hunter AG](#), [Stevenson RE](#).

Abstract: Gastroschisis is a major malformation which requires immediate surgical care to return the exposed viscera to the abdominal cavity, parenteral nutrition until bowel motility permits oral feedings, and evaluation for coexisting malformations. Almost all cases are diagnosed prenatally using midtrimester ultrasound and maternal serum alphafetoprotein measurement. This allows most infants to be delivered in a tertiary care facility where the best mode of delivery and neonatal management can be determined. About 10% of infants with gastroschisis will have other malformations. Half of these are considered related to the gastroschisis (intestinal atresia or stenosis, malrotation, cryptorchidism, amyoplasia, urinary tract obstruction). Other associated malformations occur which are not recognized to be secondary to the gastroschisis. Prominent among these are cardiac and limb defects. Fetal and neonatal mortality are increased, but neither appear related to lethal malformations.

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

(1) Title: Maternal and biochemical predictors of antepartum stillbirth among nulliparous women in relation to gestational age of fetal death.

Source: [BJOG.](#) 2007 Jun;114(6):705-14.

Authors: [Smith GC](#), [Shah I](#), [White IR](#), [Pell JP](#), [Crossley JA](#), [Dobbie R](#).

Abstract: OBJECTIVE: To determine whether maternal serum levels of alphafetoprotein (alpha-FP) and human chorionic gonadotrophin (hCG) at 15-21 weeks provided clinically useful prediction of stillbirth in first pregnancies. DESIGN: Retrospective study of record linkage of a regional serum screening laboratory to national registries of pregnancy outcome and perinatal death. SETTING: West of Scotland, 1992-2001. POPULATION: A total of 84,769 eligible primigravid women delivering an infant at or beyond 24 weeks of gestation. METHODS: The risk of stillbirth between 24 and 43 weeks was assessed using the Cox proportional hazards model. Logistic regression models within gestational windows were then used to estimate predicted probability. Screening performance was assessed as area under the receiver operating characteristic (ROC) curve. MAIN OUTCOME MEASURE: Antepartum stillbirth unrelated to congenital abnormality. RESULTS: The odds ratio (95% CI) for stillbirth at 24-28 weeks for women in the top 1% were 11.97 (5.34-26.83) for alpha-FP and 5.80 (2.19-15.40) for hCG. The corresponding odds ratios for stillbirth at or after 37 weeks were 2.44 (0.74-8.10) and 0.79 (0.11-5.86), respectively. Adding biochemical to maternal data increased the area under the ROC curve from 0.66 to 0.75 for stillbirth between 24 and 28 weeks but only increased it from 0.64 to 0.65 for stillbirth at term and post-term. Women in the top 5% of predicted risk had a positive likelihood ratio of 7.8 at 24-28 weeks, 3.7 at 29-32 weeks, 5.1 at 33-36 weeks and 3.4 at 37-43 weeks, and the corresponding positive predictive values were 0.97, 0.33, 0.47 and 0.63%, respectively. CONCLUSIONS: Maternal serum levels of alpha-FP and hCG were statistically associated with stillbirth risk. However, the predictive ability was generally poor except for losses at extreme preterm gestations, where prevention may be difficult and interventions have the potential to cause significant harm.

(2) Title: Using second trimester ultrasound and maternal serum biomarker data to help detect congenital heart defects in pregnancies with positive triple-marker screening results.

Source: [Am J Med Genet A](#). 2008 Oct 1;146A(19):2455-67.

Authors: [Jelliffe-Pawlowski LL](#), [Walton-Haynes L](#), [Currier RJ](#).

Abstract: Congenital heart defects (CHDs) are the most common of all birth defects. For many newborns with a CHD, prenatal versus postnatal detection is associated with substantially decreased morbidity and mortality risks. Although technological advances in fetal echocardiography have led to an increased capacity to detect CHDs prenatally, pregnancies without an identified risk factor are not routinely screened. With the aim of identifying pregnancies at increased risk for CHDs, this study examined the relationship between CHDs and typically collected second trimester biomarker data collected on a large population-based sample of singleton pregnancies with one or more second trimester screen positive result for Down syndrome, trisomy 18 (T-18), Smith-Lemli-Opitz syndrome (SLOS), or a neural tube defect (NTD). Where possible, logistic models for cases and controls were built and potential referral models were tested among study subsamples with information on the presence or absence of CHDs reported pre- and perinatally. When considered in combination, screen positive for T-18, screen positive for SLOS, nuchal fold measurement ≥ 5 mm, and/or having an adjusted hCG multiple of the median \geq the 95th centile detected 42.7% of all pregnancies with a CHD in the combined subsample (where co-occurrence with chromosomal defects was not considered) and detected 29.7% of all pregnancies with a CHD in the no-chromosomal defect subsample. A nuchal fold measurement ≥ 5 mm detected 18.2% of those with a CHD in the Down syndrome subsample and an adjusted hCG multiple of the median (MoM) \leq 5th centile detected 92.9% of those with a CHD in the T-18 subsample.

(3) Title: First trimester screening for Down's syndrome after assisted reproductive technology: non-male factor infertility is associated with elevated free beta-human chorionic gonadotropin levels at 10-14 weeks of gestation.

Source: [Fertil Steril](#). 2008 Oct;90(4):1206-10.

Authors: [Anckaert E](#), [Schiettecatte J](#), [Sleurs E](#), [Devroey P](#), [Smitz J](#).

Abstract: We retrospectively compared the first trimester Down's syndrome serum screening markers free beta-hCG (fbetaHCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11-14 weeks of gestation in 4,088 women with naturally conceived pregnancies and in women pregnant after ICSI (n = 163), IVF (n = 59) and frozen-thawed embryo transfer (n = 31), and we searched for a potential relationship between infertility cause and marker levels. We found lower serum PAPP-A levels in pregnancies after IVF and ICSI compared with spontaneously conceived pregnancies and non-male factor infertility was associated with elevated serum fbetaHCG levels at 11-14 weeks of gestation.

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

1) Title: Development of high-performance magnetic chemiluminescence enzyme immunoassay for alpha-fetoprotein (AFP) in human serum.

Source: [Clin Chim Acta](#). 2008 Jul 17;393(2):90-4.

Authors: [Wang X](#), [Zhang QY](#), [Li ZJ](#), [Ying XT](#), [Lin JM](#).

Abstract: BACKGROUND: A high-performance chemiluminescence enzyme immunoassay (CLEIA) for alpha-fetoprotein (AFP), a tumor marker for the diagnosis of hepatocellular carcinoma (HCC), was constructed by using magnetic particles (MPs) as both the immobilization matrix and separation tools. METHODS: A double sandwiched immunocomplex was formed through the reaction among anti-fluorescein isothiocyanate (FITC) antibody coated MPs, FITC-labeled anti-AFP antibody, AFP antigen, and alkaline phosphatase (ALP)-labeled anti-AFP antibody. The subsequent chemiluminescence reaction of ALP with 4-methoxy-4-(3-phosphate-phenyl)-spiro-(1,2-dioxetane-3,2'-adamantane) (AMPPD) gave light intensity that was directly proportional to the amount of analyte present in the samples. The effects of several physicochemical parameters, including the concentration of FITC-labeled anti-AFP antibody, the dilution ratio of ALP-labeled anti-AFP antibody, the volume of MPs and substrate, the immunoreaction time and other relevant variables upon the immunoassay were studied and optimized. RIA and microplate CLEIA were used as comparison methods. RESULTS: The

proposed method had a sensitivity of 3.0 ng/ml, low cross reactivities, and an assay time of 1 h. The linear range was 0-1200 ng/ml through using MPs and is useful for samples with extremely high AFP concentrations without dilution while avoiding the hook effect. The intra- and inter-assay precision was <3% and <5%. The present method has been successfully applied to the detection of AFP human serum with recoveries from 90 to 108%, and showed a good correlation with the commercially available AFP RIA kit. CONCLUSIONS: This proposed assay provided apparent advantages over microplate CLEIA and RIA, and facilitated the development of high-throughput screening and automated operation systems in the clinical practice.

(2) Title: Label-free electrochemical immunosensor for the determination of fetoprotein based on core-shell-shell nanocomposite particles.

Source: [Protein Pept Lett.](#) 2008;15(8):782-8.

Authors: [Sun AL](#), [Qi QA](#), [Dong ZL](#).

Abstract: A new approach toward the development of advanced immunosensors based on chemically functionalized core-shell-shell magnetic nanocomposite particles, and the preparation, characteristics, and measurement of relevant properties of the immunosensor useful for the detection of alpha-1-fetoprotein (AFP) in clinical immunoassays. The core-shell NiFe₂O₄/3-aminopropyltriethoxysilane (APTES) (NiFe₂O₄@APTES) was initially prepared by covalent conjugation, then gold nanoparticles were adsorbed onto the surface of NiFe₂O₄@APTES, and then anti-AFP molecules were conjugated on the gold nanoparticles. The core-shell-shell nanocomposite particles not only had the properties of magnetic nanoparticles, but also provided a good biocompatibility for the immobilization of biomolecules. The core-shell-shell nanostructure present good magnetic properties to facilitate and modulate the way it was integrated into a carbon paste. The analytical performance of the immunosensor was investigated by using an electrochemical method. Under optimal conditions, the resulting composite presents good electrochemical response for the detection of AFP, and exhibits wide linear range from 0.9 to 110 ng/mL AFP with a detection limit of 0.5 ng/mL. Moreover, the proposed immunosensors were used to analyze AFP in human serum specimens. Analytical results, obtained for the clinical serum specimen by the developed immunosensor, were in accordance with those assayed by the standard ELISA. Importantly, the proposed immunoassay system could be further developed for the immobilization of other antigens or biocompounds.

(3) Title: Evaluation of alpha-fetoprotein (AFP) in human serum by chemiluminescence enzyme immunoassay with magnetic particles and coated tubes as solid phases.

Source: [Anal Chim Acta.](#) 2009 Jan 12;631(2):212-7.

Authors: [Zhang Q](#), [Wang X](#), [Li Z](#), [Lin JM](#).

Abstract: In this work, the monoclonal antibodies (McAbs) to alpha-fetoprotein (AFP) were immobilized on two different solid phases, i.e., magnetic particles (MP) and coated tubes (CT). Based on this, a MP based chemiluminescence enzyme immunoassay (MP-CLEIA) and a CT based CLEIA (CT-CLEIA) were proposed for the evaluation of AFP in human serum and their analytical merits were studied and compared. By detailed discussion of several performance variants, including the concentration of immobilized McAb, dilution ratio of horseradish peroxidase (HRP) labeled McAb (HRP-McAb), total assay time, substrate volume, chemiluminescent kinetics, and hook effect concentration, the advantages of MP-CLEIA became conspicuously apparent. Moreover, in the presence of MP, the catalytic activity of labeled enzyme was kept to high extent and the stability of immunoreagents was satisfied. Finally, 59 human serum samples were detected by the MP-CLEIA and a good correlation was obtained when comparing the results with that from a commercial electrochemiluminescence immunoassay kit.

E). Special Abstract Selection:

1) Title: Obstetrical complications associated with abnormal maternal serum markers analytes.

Source: [J Obstet Gynaecol Can.](#) 2008 Oct;30(10):918-49.

Authors: [Gagnon A](#), [Wilson RD](#), [Audibert F](#), [Allen VM](#), [Blight C](#), [Brock JA](#), [Désilets VA](#), [Johnson JA](#), [Langlois S](#), [Summers A](#), [Wyatt P](#).

Abstract: OBJECTIVE: To review the obstetrical outcomes associated with abnormally elevated or decreased level of one or more of the most frequently measured maternal serum marker analytes used in screening for aneuploidy. To

provide guidance to facilitate the management of pregnancies that have abnormal levels of one or more markers and to assess the usefulness of these markers as a screening test. **OPTIONS:** Perinatal outcomes associated with abnormal levels of maternal serum markers analytes are compared with the outcomes of pregnancies with normal levels of the same analytes or the general population. **EVIDENCE:** The Cochrane Library and Medline were searched for English-language articles published from 1966 to February 2007, relating to maternal serum markers and perinatal outcomes. Search terms included PAPP-A (pregnancy associated plasma protein A), AFP (alpha-fetoprotein), hCG (human chorionic gonadotropin), estriol, unconjugated estriol, inhibin, inhibin-A, maternal serum screen, triple marker screen, quadruple screen, integrated prenatal screen, first trimester screen, and combined prenatal screen. All study types were reviewed. Randomized controlled trials were considered evidence of the highest quality, followed by cohort studies. Key individual studies on which the recommendations are based are referenced. Supporting data for each recommendation are summarized with evaluative comments and references. The evidence was evaluated using the guidelines developed by the Canadian Task Force on Preventive Health Care. **VALUES:** The evidence collected was reviewed by the Genetics Committee of the Society of Obstetricians and Gynaecologists of Canada. **BENEFITS, HARMS, AND COSTS:** The benefit expected from this guideline is to facilitate early detection of potential adverse pregnancy outcomes when risks are identified at the time of a maternal serum screen. It will help further stratification of risk and provide options for pregnancy management to minimize the impact of pregnancy complications. The potential harms resulting from such practice are associated with the so called false positive (i.e., uncomplicated pregnancies labelled at increased risk for adverse perinatal outcomes), the potential stress associated with such a label, and the investigations performed for surveillance in this situation. No cost-benefit analysis is available to assess costs and savings associated with this guideline. **SUMMARY STATEMENTS:** 1. An unexplained level of a maternal serum marker analyte is defined as an abnormal level after confirmation of gestational age by ultrasound and exclusion of maternal, fetal, or placental causes for the abnormal level. (III) 2. Abnormally elevated levels of serum markers are associated with adverse pregnancy outcomes in twin pregnancies, after correction for the number of fetuses. Spontaneous or planned multifetal reductions may result in abnormal elevations of serum markers. (II-2) **RECOMMENDATIONS:** 1. In the first trimester, an unexplained low PAPP-A (< 0.4 MoM) and/or a low hCG (< 0.5 MoM) are associated with an increased frequency of adverse obstetrical outcomes, and, at present, no specific protocol for treatment is available. (II-2A) In the second trimester, an unexplained elevation of maternal serum AFP (> 2.5 MoM), hCG (> 3.0 MoM), and/or inhibin-A ($> = 2.0$ MoM) or a decreased level of maternal serum AFP (< 0.25 MoM) and/or unconjugated estriol (< 0.5 MoM) are associated with an increased frequency of adverse obstetrical outcomes, and, at present, no specific protocol for treatment is available. (II-2A) 2. Pregnant woman with an unexplained elevated PAPP-A or hCG in the first trimester and an unexplained low hCG or inhibin-A and an unexplained elevated unconjugated estriol in the second trimester should receive normal antenatal care, as this pattern of analytes is not associated with adverse perinatal outcomes. (II-2A) 3. The combination of second or third trimester placenta previa and an unexplained elevated maternal serum AFP should increase the index of suspicion for placenta accreta, increta, or percreta. (II-2B) An assessment (ultrasound, MRI) of the placental-uterine interface should be performed. Abnormal invasion should be strongly suspected, and the planning of delivery location and technique should be done accordingly. (III-C) 4. A prenatal consultation with the medical genetics department is recommended for low unconjugated estriol levels (< 0.3 MoM), as this analyte pattern can be associated with genetic conditions. (II-2B) 5. The clinical management protocol for identification of potential adverse obstetrical outcomes should be guided by one or more abnormal maternal serum marker analyte value rather than the false positive screening results for the trisomy 21 and/or the trisomy 18 screen. (II-2B) 6. Pregnant woman who are undergoing renal dialysis or who have had a renal transplant should be offered maternal serum screening, but interpretation of the result is difficult as the level of serum hCG is not reliable. (II-2A) 7. Abnormal maternal uterine artery Doppler in association with elevated maternal serum AFP, hCG, or inhibin-A or decreased PAPP-A identifies a group of women at greater risk of IUGR and gestational hypertension with proteinuria. Uterine artery Doppler measurements may be used in the evaluation of an unexplained abnormal level of either of these markers. (II-2B) 8. Further research is recommended to identify the best protocol for pregnancy management and surveillance in women identified at increased risk of adverse pregnancy outcomes based on an abnormality of a maternal serum screening analyte. (III-A) 9. In the absence of evidence supporting any specific surveillance protocol, an obstetrician should be consulted in order to establish a fetal surveillance plan specific to the increased obstetrical risks (maternal and fetal) identified. This plan may include enhanced patient education on signs and symptoms of the most common complications, increased frequency of antenatal visits, increased ultrasound (fetal growth, amniotic fluid levels), and fetal surveillance (biophysical profile, arterial and venous Doppler), and cervical length assessment. (III-A) 10. Limited information suggests that, in women with elevated hCG in the second trimester and/or abnormal uterine artery Doppler (at 22-24 weeks), low-dose aspirin (60-81 mg daily) is associated with higher birthweight and lower incidence of gestational hypertension with proteinuria. This therapy may be used in women who are at risk. (II-2B) 11. Further studies are recommended in order to assess the benefits of low-dose aspirin, low molecular weight heparin, or other therapeutic options in pregnancies determined to be at increased risk on the basis of an abnormal maternal serum screening analyte. (III-A) 12. Multiple maternal serum markers screening should not be used at present as a population-based

screening method for adverse pregnancy outcomes (such as preeclampsia, placental abruption, and stillbirth) outside an established research protocol, as sensitivity is low, false positive rates are high, and no management protocol has been shown to clearly improve outcomes. (II-2D) When maternal serum screening is performed for the usual clinical indication (fetal aneuploidy and/or neural tube defect), abnormal analyte results can be utilized for the identification of pregnancies at risk and to direct their clinical management. (II-2B) Further studies are recommended to determine the optimal screening method for poor maternal and/or perinatal outcomes. (III-A).

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