

## Fetal Defect Marker Proficiency Test Mail-out from September 11, 2007 October, 2007

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from September 11, 2007 for Fetal Defect Markers, including AFP, uE3, hCG, and dimeric inhibin-A. 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.

### Maternal Serum: Summary of Sample Results

Samples *N = 31	Sample #	MS 211	MS 212	MS 213	MS 214	MS 215
	Gestational Age (weeks)	20.0	18.0	19.0	15.0	17.0
Maternal Race	Ethnic Group	White	Black	White	Asian‡	Hispanic
Maternal Weight	Pounds (lbs)	150	185	160	110	155
Maternal Age	Years	26	20	40	28	35
Alpha-Fetoprotein (AFP)	Mean ng/mL	199.59 ± 16.36	47.78 ± 3.15	31.11 ± 3.26	32.45 ± 2.60	34.93 ± 3.01
	MOM	3.41 ± 0.28	1.14 ± 0.12	0.64 ± 0.06	1.10 ± 0.11	0.93 ± 0.07
Unconjugated Estriol (uE3)	Mean ng/mL	2.51 ± 1.11	1.68 ± 0.73	0.90 ± 0.34	0.88 ± 0.31	1.01 ± 0.36
	MOM	0.74 ± 0.21	0.81 ± 0.22	0.35 ± 0.11	0.76 ± 0.24	0.59 ± 0.19
human Chorionic Gonadotrophin (hCG)	Mean IU/mL	21.35 ± 2.22	24.55 ± 2.43	40.75 ± 4.66	37.39 ± 4.08	38.89 ± 3.51
	MOM	1.20 ± 0.21	1.22 ± 0.22	2.21 ± 0.38	0.80 ± 0.16	1.58 ± 0.29
Dimeric Inhibin-A (DIA)	Mean pg/mL	241.81 ± 21.91	144.73 ± 14.93	405.82 ± 31.16	127.23 ± 13.05	229.11 ± 16.40
	MOM	1.27 ± 0.20	0.93 ± 0.14	2.37 ± 0.29	0.61 ± 0.11	1.38 ± 0.19
Neural Tube Screen (Positive, Negative)	Pos (+) or Neg. (-)	Pos. (100%)	Neg. (100%)	Neg. (100%)	Neg. (100%)	Neg. (100%)
	Further Action R,U,A	R = 14% U = 83% A = 60%	NFA	NFA	NFA	NFA
	NTD Risk	26	9,700	7,197	2,495	10,000
Trisomy-21 Screen (Positive, Negative)	Pos (+); Neg. (-)	Neg. (100%)	Neg. (100%)	Pos. (100%)	Neg. (100%)	Pos. (B) (69%)
	Recommended Action	NFA	NFA	U = 78% A = 72%	NFA	U = 61% A = 50%
	Risk Est.	6,850	2,462	10	3,000	118
1. <u>Triple test</u>	Pos (+); Neg. (-)	Neg. (100%)	Neg. (100%)	Pos. (100%)	Neg. (100%)	Pos. (B) (69%)
	Recommended Action **	NFA	NFA	U = 70% A = 65%	NFA	U = 42% A = 38%
	Risk Est.	10,000	8,267	6.	10,370	140
Trisomy-18 Screen (Positive, Negative)	Pos (+)/Neg. (-)	Neg. (-)	Neg. (-)	Neg. (B)	Neg. (-)	Neg. (-)
	Risk Est.	10,000	12,425	147	8,240	3,065

\*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 ± SD; (B) = borderline positive or negative, risk reflects central tendency (Modal number for Down positive/borderline screen). NFA = no further action; FA = further action; R = repeat; U = ultrasound, and A = amniocentesis. \*\*This percentage is normalized to labs requesting further action. ‡ Insulin Dependent Diabetic pregnancy.

**Maternal Serum Analytes: Summary of Test Results**

**A. Second Trimester Screening**

N = 31 All-lab Consensus Values.

Sample #                      Summary Comments (Mock specimens):

MS 211                      This specimen was obtained from a 26 year old Caucasian woman (gravida = 1, parity = 0) with a  
Wk 20.0                      body weight of 150 lbs. She had no family history of pregnancy complications or adverse outcomes. However, her sample screened positive for NTD; her aneuploidy screens were negative for both Trisomy 21 and 18. Recommendations of further action from labs performing the NTD screen were: repeat, 14%; ultrasound, 83%; and amniocentesis, 60%. All labs agreed that both trisomy screens were negative and the All-lab consensus for the positive NTD screen was 100%. This specimen was paired to amniotic fluid specimen AF-211, which produced a highly elevated AFAFP value (MOM=3.70).

MS 212                      This specimen was procured from a 20 year old Afro-American woman (gravida = 2, parity = 1) with a  
Wk 18.0                      body weight of 185 lbs. She had reported a family history of pregnancy loss. To date, her pregnancy appeared to follow an uncomplicated course of gestation and her sample resulted in a negative screen (100% consensus) for NTD. In comparison, all labs were also in agreement that her trisomy screens were negative. Note that this patient's sample required two algorithm adjustments, race and body weight, for her screen assessment. Specimen MS212 was not paired with an amniotic fluid sample.

MS 213                      Specimen MS213 was obtained from a 40 year old Caucasian woman (gravida = 2, parity = 0) with a  
Wk 19.0                      body weight of 160 lbs. She had a personal history of pregnancy loss. Her specimen, a second pregnancy sample was screen negative for NTD; however, her specimen was screen positive for Trisomy 21 with all labs in agreement. Recommendations of further action from labs performing the triple screen were: ultrasound, 78% and amniocentesis, 72%; correspondingly, the quad screen was ultrasound 70% and amniocentesis 65%. Interestingly, the Trisomy-18 (T18) screen for this patient was borderline negative (Risk = 147) presumably due to both low MSUE3 and MSAFP MOM results. This serum specimen did have an amniotic fluid counterpart, AF213, which produced a correspondingly low AFAFP value of 3.8 ug/ml (MOM=0.48).

MS 214                      The MS214 specimen originated from a 28 year old Asian woman (gravida = 3, parity = 2) with a body  
Wk 15.0                      weight of 110 lbs. Her family history was devoid of pregnancy complications. The sample was classified as screen negative for both NTDs and Trisomies. All labs were in agreement with both screen assessments. A race and diabetes algorithm and possibly body weight truncation adjustment was indicated. The MS214 sample was not matched to an amniotic fluid specimen.

MS 215                      Specimen MS215 represented a sample obtained from a 35 year old Hispanic woman  
Wk 17.0                      (gravida = 1, parity = 0) with a body weight of 155 lbs. She had reported a family history of adverse pregnancy outcomes in near relatives. Her MS sample was deemed screen negative (100%) for NTD with no further action recommended. Her MS specimen screened borderline positive for trisomy-21. Although, all labs were in agreement with the NTD screen assessment, her trisomy screen was only 69% positive lacking an All-lab consensus. This specimen was not matched to an amniotic fluid sample.

**B. First Trimester (FT) Mock Screening Specimens (All-lab Result):**

<u>Sample#</u>	<u>All-lab Summary Analyte Measurement (see also Figures 12-15):</u>			
FT 211	A)	Gestational	B) <u>hCG (total)</u>	C) <u>PAPP-A</u>
Wk = 11.0		Age (wks)	Mean = <u>87.89 IU/mL</u>	Mean = <u>2.52 mIU/mL</u>
(N = 12)		Mean = <u>11.0</u>	Std. Dev. (SD) = <u>7.85</u>	Std. Dev. (SD) = <u>1.22</u>
		Std. Dev = <u>0.09</u>	MOM = <u>1.00</u>	MOM = <u>2.11</u>
		% CV = <u>0.8</u>	Std. Dev. = <u>0.18</u>	Std. Dev. (SD) = <u>1.67</u>
			*BCU % CV = <u>8.0</u>	*DPC % CV = <u>6.3</u>
			*DPC % CV = <u>11.8</u>	*DSL % CV = <u>20.3</u>

This specimen was from a 25.0 year old Caucasian woman of average body weight (140 lbs). Her gestational age of pregnancy was 11.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all 12 Labs were in agreement. (\*see Critique, Pages 5-6)

<u>Sample#</u>	<u>All-lab Summary Analyte Measurement (see also Figures 12-15):</u>			
FT 212	A)	Gestational	B) <u>hCG (total)</u>	C) <u>PAPP-A</u>
Wk = 12.0		Age (wks)	Mean = <u>136.29 IU/mL</u>	Mean = <u>1.51 mIU/mL</u>
(N = 12)		Mean = <u>12.0</u>	Std. Dev. (SD) = <u>15.64</u>	Std. Dev. (SD) = <u>0.77</u>
		Std. Dev = <u>0.03</u>	MOM = <u>1.82</u>	MOM = <u>0.88</u>
		% CV = 0.2	Std. Dev. (SD) = <u>0.35</u>	Std. Dev. (SD) = <u>0.62</u>
			*BCU % CV = <u>10.2</u>	*DPC % CV = <u>7.0</u>
			*DPC % CV = <u>15.2</u>	*DSL % CV = <u>26.6</u>

This specimen was from a 22.0 year old Caucasian woman of average body weight (150 lbs.). Her gestational age of pregnancy was 12.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen positive and all 12 Labs were in agreement. (\*see Critique, Pages 5-6)

**Notice of Gravida/Parity Clarification for Present and Future Mail-outs:**

This notice regards the demographic data provided for the mock patients in the FEDM program. For the intent 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.

**AMNIOTIC FLUID AFP (NTD-analysis):**

N=31; All-lab Consensus Values

<u>Sample</u>	<u>Values</u>	<u>Summary Comments:</u>
AF 211 Wk 20.0	AFP= 24.6 ± 4.2 µg/ml MOM= 3.70 ± 0.51	This sample was targeted for a screen positive AF AFP value in the upper gestational age range. All labs reported this specimen as a screen positive elevated AF AFP value. The AF211 specimen was paired with specimen MS211 which was also elevated (MOM = 3.41).
AF 212 Wk 19.0	AFP= 7.0 ± 1.0 µg/ml MOM= 0.88 ± 0.12	This sample was targeted for a non-elevated AF AFP value in the upper gestational age group. All labs called it a non-elevated specimen for NTD. This AF AFP sample was not matched to an amniotic fluid specimen.
AF 213 Wk 19.0	AFP= 3.8 ± 0.4 µg/ml MOM= 0.48 ± 0.06	This sample was targeted for a screen negative AF AFP value in the upper gestational age screening range. All labs categorized this as a screen negative specimen. AF213 was matched to MS213 which also screened negative for NTD, but displayed a low MSAFP value of 31.11 (MOM = 0.64) consistent with a trisomy positive screen.
AF 214 Wk 16.0	AFP= 9.1 ± 1.7 µg/ml MOM= 0.62 ± 0.11	This sample was targeted as a screen negative AF AFP value in the lower routine gestational screening range. All labs categorized this as a negative AF AFP specimen; it had no maternal serum counterpart.
AF 215 Wk 17.0	AFP= 11.9 ± 1.8 µg/ml MOM= 0.99 ± 0.12	This sample was targeted for a screen negative AF AFP value in the middle gestational age range. All labs classified this as a negative (non-elevated) specimen. This AF AFP sample was not matched to an MS sample.

### **Fetal Defect Proficiency Test Mail-out 9/11/07 Critique of Maternal Serum and Amniotic Fluid Values:**

The All-lab results of the targeted values for the NTD and the Trisomy Screen attained the expectations of our projected target values, risks, and outcomes. As displayed in the above tables, maternal serum MS211 was targeted as an elevated specimen for NTD, (Fig. 1 and 3). Specimen MS213 was from a patient with a prior pregnancy loss and presently screened negative for NTD, but positive for Trisomy-21 (T21). For specimen MS211, which screened NTD positive, the All-lab recommended actions (see below) were as expected; hence, the NTD screen for MS211 resulted in a 1 in 26 risk for open neural tube defects (ONTD) and the positive screen achieved a 100% All-lab consensus. The recommended action for the MS211 specimen was the following: sample repeat, 14%; ultrasound; 83%; and amniocentesis, 60%. Sample MS215, a borderline T21 screen positive result, was obtained from a Hispanic woman with a prior family history of adverse pregnancy outcomes. The borderline positive T21 screen for sample MS215, in the face of an MS-MOM of 0.93, did not achieve the cutoff values held by all participating laboratories, hence only 69% of the labs recommended further action falling short of an All-lab consensus (80%). Specimens MS212 to MS215 were all targeted and achieved negative screens for NTD, with all labs recommending no further action. Of these samples, MS213 produced a positive screen for Trisomy-21 which is discussed below. Finally, the MS214 specimen was obtained from a woman of Asian descent and indicated both race and body weight adjustments (lower truncation) as well as diabetes in her risk assessment calculations.

Specimen MS211, together with a matched AFAFP value, proved a noteworthy case in that the MSAFP sample was screen positive (MOM = 3.4) and her paired AFAFP sample was also clearly elevated (MOM = 3.7) irregardless of the individual lab cutoff values (Fig. 1 & 2). This mock patient had a family history of adverse outcomes; thus, a paired maternal serum sample was obtained at time of amniocentesis. Her elevated MSAFP MOM of 3.41, clearly a NTD positive screen, was accompanied by an AFAFP outcome which correlated with her NTD positive MS screen. A high definition Stage-II ultrasound together with an Ache analysis later confirmed the presence of an open NTD lesion in the spinal cord from the fetus of this mock patient. An All-lab NTD risk assessment for MS211 was calculated as 1 in 26 for maternal serum alone.

The maternal serum screen for MS213 (gravida = 2, parity = 0) produced a definitive negative screen for NTD and a positive screen for Trisomy-21 (MSAFP MOM = 0.64). Moreover, the sample was accompanied by an amniotic fluid (AF) specimen which also proved to be a low AFP concentration. The AF specimen had been obtained at time of amniocentesis due to maternal age, a family history of fetal defects in sibling-related pregnancies, and to reduce anxiety in the patient. The maternal serum was procured immediately prior to the amniocentesis and the procedure produced no indication of a fetal bleed. Note also that MS213 (Fig. 4) produced a borderline negative risk for Trisomy-18 with a risk of 1 in 147 (cutoff = 1 in 100); thus, 17% of the labs called for further action.

Regarding the Trisomy screen, the MS213 specimen (gravida = 2, parity = 0; maternal age = 40) was intended to produce a positive Trisomy-21 (T21) screen with both the triple and quad testing platforms, which indeed was the case. The labs reporting either triple or quad testing concluded that sample MS213 was T21 screen positive (100% all lab consensus). Further action recommended for the T21 screen was determined as 78% ultrasound (US) and 72% amniocentesis (AM) for labs using the triple screen, and 70% US and 65% AM for labs employing the quad screen. Some labs also recommended genetic counseling (26%). The recommended action on MS213 reflected the severity of the risk ratio assessment of 1 in 10 from the triple test versus a 1 in 6 from the quad test, regardless of the software program employed. Note from the point distribution graphs comparing the triple with the quad test (Figs. 5 and 6) that the MS213 point cluster in the quad assay was just slightly lower than the MS213 cluster in the triple test. As consistently seen before, the quad test signaled a slightly higher risk for Down syndrome while the triple test also yielded a significant risk; overall both screens signaled a very high risk for Down Syndrome. Both mid-term risks for DS were greater than that expected from the maternal age alone (1 in 607).

The specimen MS215 provided an interesting borderline positive screen result for Down Syndrome (T21). The MSAFP was slightly low (MOM = 0.93) while the uE3 was definitely low (MOM = 0.59); however, both the MShCG and DIA were slightly elevated, MOM = 1.58 and MOM = 1.38, respectively. Nonetheless, 59% of the participating laboratories called MS215 a positive screen for T21 which did not achieve an All-lab consensus (80%). It is germane to this discussion that the All-lab T21 risk for MS215 was 1 in 118 for the triple test and 1 in 140 for the quad test. Thus, the quad test indicated a slightly less risk for T21 than did the triple test. The quad test also presented with less further actions indicated.

The performances of the various kits for maternal serum analytes (AFP, uE3, hCG, Inhibin A) are presented in a bar graph format (Figs 7-10) for each of the five MS samples. As shown in the MS-AFP graph, AFP mass measurements among the individual kits largely agreed, although Siemens/Bayer-Centaur was somewhat higher, and Siemens/DPC Immulite and Beckman Unicel or Access were marginally lower for some samples. For uE3, the All-lab mean was higher than 1.0 (see Figure 8) due to the labs employing Siemens/DPC Immulite and Siemens /DPC Immulite 2000 which yielded values averaging 1.5 to 1.8 times higher than the

median (see dotted line). In contrast, Diagnostic Systems Lab RIA/EIA results were at the mean level while Beckman Unicel or Access measured uE3 values 25 to 30% lower than the median. These results continue to demonstrate some inherent differences as to how these assays recognize the uE3 in our mock sample preparations. Regarding the hCG kits, laboratories employing Abbott AxSYM values were not displayed due to kit recall and technical difficulties, while Siemens/Bayer-Centaur, Siemens/DPC Immulite, and Beckman Unicel or Access all yielded equivalent mean (1.0) hCG values. In order to enhance uniformity among the various kits employed to measure hCG, we incorporate an intact (total) hCG recombinant analyte into our PT specimens. Labs lacking peer group companions and in-house assays will continue to be deemed non-gradable (NG) for hCG as well as other analyte groups as the situation dictates. Finally, the method comparison of Inhibin-A is displayed in Figure 10 for the Beckman Unicel or Access versus the Diagnostic System Labs (DSL) assay platforms. Other than Beckman being slightly higher than DSL, the kit performances for Inhibin-A were essentially equal.

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 approached that observed with the maternal serum samples. While results from Siemens/Bayer-Centaur, and Abbott-AxSYM Kits kits were higher, Beckman Unicel or Access and Siemens/DPC Immulite were about 10% lower than the All-lab mean as seen in previous mail-outs. Finally, please be advised that these amniotic fluid specimens are derived from actual AF samples, and therefore these results are directly relevant to patient screening.

For informational purposes, it was deemed of interest to provide the software package usage by our participating laboratories. The alpha and Benetech software packages are each used by 20 and 23%, of the labs respectively; RMA software is employed by 30%; while in-house software comprised 17%, and 10% of labs use programs classified as "other" which are proprietary software packages.

#### First Trimester Screen:

Two first trimester maternal serum mock samples were included in the present mail-out as an investigational probe for future Down syndrome First Trimester PT mail-outs. The probe was mailed in order to survey New York State licensed laboratory clientele concerning lab participation and assay capabilities in first trimester screening for DS. The results will not be graded and the survey results are intended to serve as informational-gathering events in anticipation of a future mandated implementation. However, laboratories that are validation-approved and presently performing first trimester Down Syndrome screening are required to test and report screen results from the present mail-out. Those laboratories not presently offering the test, or not planning to implement the test, can request that no further samples be sent to them (see answer sheet).

The sample FT211 (FT = first trimester) information provided to lab participants included maternal age, nuchal translucency (NT-MOM), last menstrual period (LMR), and draw date. This time, crown-rump length (CRL) measurements were not included in the case history in order to probe laboratory nuchal translucency data requirements. Race and body weight were included in the present mail-out. The gestational age All-lab FT211 mean was reported as 11.0 weeks. Assay measurements from FT-screening participating laboratories resulted in the All-lab total hCG MOM measurement of  $1.00 \pm 0.18$  based on two methods, namely, Beckman Unicel or Access and Siemens/DPC Immulite or Immulite 2000; while the All-lab PAPP-A MOM assessments were 2.11 (see Section B, pages 2-3). The first trimester All-lab (N = 12) Trisomy-21 consensus for FT211 was screen negative. The All-lab FT trisomy-21 risk assessment was 1 in 3,220, while the Down syndrome's risk due to maternal age alone was 1 in 1,040. As observed in the First Trimester table above (Section – B, Page 3) the All-lab mass measurement for FT211 of total hCG achieved an arithmetic mean of  $87.89 \text{ IU/mL} \pm 7.85$  (MOM = 1.0). However, the hCG assay percent CV resulted in a value of 8.0% (Beckman kit) and 11.8% (Siemens/DPC kit) demonstrating a relatively high variance among the participating labs. Second trimester hCG CVs usually ranged from 5 to 9%. In comparison, the All-lab MOM mean for PAPP-A was  $2.11 \pm 1.67 \text{ mIU/mL}$ , a relatively high value. (Figures 12 to 15). The high percent CVs demonstrated by the PAPP-A mass measurements consequently yielded an All-lab % CV of 79.0% due to the differential values of the Diagnostic kit (CV = 20.3%) versus the Siemens/DPC immulite kit (CV = 6.3%) (See Figure 12 Medians). Despite the disparate variances in which the PAPP-A Siemens/DPC assay mass mean was high ( $4.12 \text{ mIU/mL}$ ) compared to Diagnostic systems ( $1.72 \text{ mIU/mL}$ ), all labs agreed that the FT sample was screen negative (See Figure 14&15 point distributions). The All-lab risk cut-off levels (N = 12) were found to range from 200 to 270. Since PAPP-A measurement for first trimester Down Syndrome are associated with low MOM values, higher MOMs would be consistent with a screen negative outcome. The FT211 specimen also resulted in a negative screen for Trisomy-18.

As shown above in the First Trimester table above (Section – B, Page 3), the All-lab mass measurement for FT212 of total hCG achieved an arithmetic mean of  $136.29 \text{ IU/mL} \pm 15.64$ , and an elevated MOM = 1.82. However, the hCG percent CV resulted in a value of 11.5% demonstrating a high variance among the participating labs. In comparison, the All-lab mass mean for PAPP-A was  $1.51 \pm 0.77 \text{ mIU/mL}$  and a low MOM = 0.88. (Figures 12 & 13) The All-lab risk assessment was 1 in 8, while the Down syndrome's risk due to maternal age alone was 1 in 1,140. The relatively high standard deviation demonstrated by the PAPP-A

measurement consequently yielded an All-lab MOM % CV of 69.7% due to the differential values of the Diagnostic Systems kit (CV = 26.6%) versus the SIEMENS/DPC immullite kit (CV = 7.0%) (see Figure 13 Means). Despite the disparate variances in which the PAPP-A SIEMENS/DPC kit mean was high (2.50mIU/mL) compared to Diagnostic systems (1.01mIU/mL), all labs agreed that the FT sample was screen positive (See Figure 14&15 point distributions). As stated above, the All-lab risk cut-off levels (N = 12) were found to range from 200 to 270. (All-lab risk = 1 in 220) Since PAPP-A measurement for first trimester Down Syndrome are associated with low MOM values, low PAPP-A and high hCG MOMs would be consistent with a screen positive outcome. Overall, both FT211 and FT212 resulted in 100% all lab screen agreements. Regarding the Trisomy-18 risk, the FT212 specimen triggered a borderline negative screen (1 in 103) with a cutoff of 1 in 100.

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**New References (Suggested reading):**

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#### Abstracts

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

(1) Title: First- and second-trimester evaluation of risk for Down syndrome.

Source: Obstetrics & Gynecology. 110(1):10-7, 2007.

Authors: [Ball RH.](#) [Caughey AB.](#) [Malone FD.](#) [Nyberg DA.](#) [Comstock CH.](#) [Saade GR.](#) [Berkowitz RL.](#) [Gross SJ.](#) [Dugoff L.](#) [Craig SD.](#) [Timor-Tritsch IE.](#) [Carr SR.](#) [Wolfe HM.](#) [Emig D.](#) [D'Alton ME.](#) [First and Second Trimester Evaluation of Risk \(FASTER\) Research Consortium.](#)

##### Abstract:

OBJECTIVE: To investigate the differences in costs and outcomes of Down syndrome screening using data from the First and Second Trimester Evaluation of Risk (FASTER) Trial. METHODS: Seven possible screening options for Down syndrome were compared: 1) Triple Screen-maternal serum alpha fetoprotein, estriol, and hCG; 2) Quad-maternal serum alpha fetoprotein, estriol, hCG, and Inhibin A; 3) Combined First-nuchal translucency, pregnancy-associated plasma protein A (PAPP-A), free beta-hCG; 4) Integrated-nuchal translucency, PAPP-A, plus Quad; 5) Serum Integrated-PAPP-A, plus Quad; 6) Stepwise Sequential-Combined First plus Quad with results given after each test; and 7) Contingent Sequential-Combined First and only those with risk between 1:30 and 1:1,500 have Quad screen. The detection rates for each option were used given a 5% false-positive rate except for Contingent Sequential with a 4.3% false-positive rate. Outcomes included societal costs of each screening regimen (screening tests, amniocentesis, management of complications, and cost of care of Down syndrome live births), Down syndrome fetuses identified and born, the associated quality-adjusted life years, and the incremental cost-utility ratio. RESULTS: Based on the screening results derived from the 38,033 women evaluated in the FASTER trial, the Contingent Sequential screen dominated (lower costs with better outcomes) all other screens. For example, the Contingent Sequential cost 32.3 million dollars whereas the other screens ranged from 32.8 to 37.5 million dollars. The Sequential strategy led to the identification of the most Down syndrome fetuses of all of the screens, but at a

higher cost per Down syndrome case diagnosed (\$719,675 compared with \$690,427) as compared with the Contingent Sequential. Because of the lower overall false-positive rate leading to fewer procedure-related miscarriages, the Contingent Sequential resulted in the highest quality-adjusted life years as well. The Contingent Sequential remained the most cost-effective option throughout sensitivity analysis of inputs, including amniocentesis rate after positive screen, rate of therapeutic abortion after Down syndrome diagnosis, and rate of procedure-related miscarriages. CONCLUSION: Analysis of this actual data from the FASTER Trial demonstrates that the Contingent Sequential test is the most cost-effective. This information can help shape future policy regarding Down syndrome screening.

- (2) Title: Midtrimester maternal serum inhibin A levels after multifetal pregnancy reduction.  
Source: Prenatal Diagnosis. 27(5):431-4, 2007.  
Authors: [Chen HJ](#), [Huang LW](#), [Lin YH](#), [Seow KM](#), [Hsieh BC](#), [Hwang JL](#), [Tzeng CR](#).  
Abstract: OBJECTIVE: To investigate the relationship between the maternal serum inhibin A concentrations and the number of fetuses. Further, the maternal serum inhibin A levels for twin pregnancies and multiple pregnancies reduced to twins in the second trimester were compared. METHODS: Three groups of women with pregnancies following in vitro fertilization and embryo transfer were recruited for this study. Groups 1, 2 and 3 included 20 singleton pregnancies, 37 twin pregnancies, and 35 multifetal pregnancies, respectively. In group 3, multifetal reduction was performed during 10-12 weeks of gestation. Blood samples were obtained longitudinally at 10th, 12th, 15th and 18th week of gestation. RESULTS: There was a significant association between the number of fetuses and maternal plasma inhibin A prior to multifetal reduction. The inhibin A levels were not significantly different between twin and multifetal reduced twin pregnancies at 15th and 18th weeks of gestation. CONCLUSION: In multifetal reduction to twin pregnancies, the maternal serum levels of inhibin A decrease to the level of twin pregnancies during the second trimester. Therefore, inhibin A may be effectively used as a marker for Down syndrome screening in cases of twin pregnancy following multifetal reduction.

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

- (1) Title: Malignant transformation of a well-organized sacrococcygeal fetiform teratoma in a newborn male.  
Source: Journal of the Formosan Medical Association. 106(5):400-2, 2007.  
Authors: [Chen YH](#), [Chang CH](#), [Chen KC](#), [Diau GY](#), [Loh IW](#), [Chu CC](#).  
Abstract: We report herein a case of a male newborn with a sacrococcygeal fetiform teratoma (FT). The baby presented with a large coccygeal teratoma. The preoperative diagnosis of FT was made by plain radiography, ultrasonography and magnetic resonance imaging. The baby was successfully treated by complete excision and pelvic floor reconstruction. Postoperative follow-up was uneventful until the teratoma recurred 11 months later as a malignancy. After undergoing a second operative procedure accompanied by chemotherapy, he has been doing well for 18 months.

- (2) Title: Yolk sac tumor of the vagina.  
Source: Saudi Medical Journal. 28(7):1125-6, 2007.  
Authors: Mahzouni P, Pejhan S, Ashrafi M.  
Abstract: Malignant germ-cell tumors GCT are rare tumors of childhood accounting for less than 3% of pediatric malignancies. Endodermal sinus tumors EST form the most common histologic subtype of malignant GCT. The vagina is an extremely rare site for GCTs. An 8-month-old female was admitted with a short history of vaginal bleeding, and a mass protruding from the vagina. A mass was palpable anteriorly on rectal examination. Computed tomography showed a tumor mass posterior to the bladder. A biopsy revealed a vaginal EST. The serum alpha-fetoprotein was elevated. Vaginohysterectomy was carried out. She was subsequently referred to the oncologist for further management.

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

- (1) Title: Comparative study of three amniotic fluid markers in premature rupture of membranes: prolactin, beta subunit of human chorionic gonadotropin, and alpha-fetoprotein.

Source: Gynecologic & Obstetric Investigation. 63(4):195-9, 2007.  
Authors: [Shahin M](#), [Raslan H](#).  
Abstract: OBJECTIVE: To evaluate whether prolactin, alpha-fetoprotein (AFP) or B-human chorionic gonadotropin (BHCG) is the most effective marker in vaginal fluid for diagnosing prelabor rupture of membranes (PROM). These proteins

are present in amniotic and vaginal fluid and have been reported to be potent markers of PROM, but have not been used clinically nor compared to each other. STUDY DESIGN: A total of 100 pregnant women between 28 and 37 weeks of gestation were recruited for the study. Patients were divided into 2 groups. The first group consisted of 50 pregnant women diagnosed with ruptured membranes. The second group consisted of 50 normal pregnant women seen during routine antenatal clinic visit (control) group. All women underwent speculum examination aiming to sample prolactin, BHCG and AFP in the vaginal fluid. Ultrasonographic examination for gestational age and amniotic fluid index measurement was performed. The electrochemoluminescence (ECLIA) method was used for quantitative measurement of the three proteins (the total duration of the assay was 18 min). RESULTS: Vaginal fluid concentrations of the three markers were significantly higher in the PROM group than in the control group ( $p < 0.001$ ). Receiver operator curve analysis indicated that AFP had 94% specificity, sensitivity, positive and negative predictive values, and efficiency. The other two markers have lower specificity, sensitivity, positive and negative predictive values, and efficiency: 70, 76, 71.7, 74.5 and 73% for prolactin and 72, 84, 75, 81.8 and 78% for BHCG, respectively. CONCLUSION: This work demonstrates that of the three markers investigated AFP has the highest diagnostic performance. Using the ECLIA method it can be an ideal marker for diagnosing PROM particularly in equivocal cases. The technique could be introduced into laboratory tests to meet clinical needs.

Title: Major fetal abnormalities associated with positive screening tests for Smith-Lemli-Opitz syndrome (SLOS).  
Source: Prenatal Diagnosis. 27(5):409-14, 2007.

Authors: [Craig WY.](#) [Haddow JE.](#) [Palomaki GE.](#) [Roberson M.](#)

Abstract: OBJECTIVE: Determine the relationship between positive screening interpretations for Smith-Lemli-Opitz syndrome (SLOS) and other fetal abnormalities, to aid counseling and diagnostic activities. METHODS: An SLOS screening algorithm was incorporated into California's second-trimester screening program for Down syndrome and open neural tube defects (ONTDs). Between 2002 and 2004, 777 088 pregnant women were given an SLOS risk interpretation, using alpha-fetoprotein (AFP), unconjugated estriol (uE3), and human chorionic gonadotrophin (hCG) measurements. Outcomes were obtained in 98.8% of screen-positive pregnancies. RESULTS: SLOS screen positives, alone or in combination with screen positives for other fetal disorders (Down syndrome, trisomy 18, ONTD), were associated with a high risk for fetal pathology. Type and frequency of chromosomal or anatomic abnormalities (or fetal death) varied according to screen-positive combination. Among 2018 screen-positive pregnancies, 644 fetal deaths were identified. Among the 1374 viable pregnancies, 519 were screen positive for SLOS alone; two SLOS cases and 51 other serious abnormalities were identified (14 aneuploidies; 37 anatomic). The remaining 855 were also screen positive for at least one other disorder; two SLOS cases and 327 other abnormalities were identified (180 aneuploidies; 157 anatomic). CONCLUSION: For screening programs implementing the SLOS algorithm, the present data may be useful for counseling and to guide diagnostic studies.

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

(1) Title: Enhanced immunoresponse of antibody/mixed-PEG co-immobilized surface construction of high-performance immunomagnetic ELISA system.

Source: Journal of Colloid & Interface Science. 309(2):524-30, 2007.

Authors: [Nagasaki Y.](#) [Kobayashi H.](#) [Katsuyama Y.](#) [Jomura T.](#) [Sakura T.](#)

Abstract: Poly(ethylene glycol) possessing pentaethylenehexamine at one end (N6-PEG) was prepared via a reductive amination reaction of aldehyde-ended PEG with pentaethylenehexamine. Using N6-PEG, an antibody/PEG co-immobilized surface was constructed on magnetic particles via an active ester reaction method. After immobilization of the antibody on the active ester surface, N6-PEG was reacted on the magnetic beads. A sandwich enzyme-linked immunosorbent assay (ELISA) system was newly constructed using PEG/antibody co-immobilized magnetic beads combined with an alkaline phosphatase (ALP)-assisted fluorescent detection system using alpha-fetoprotein (AFP) as a model antigen. The co-immobilization of both antibody and PEG on the magnetic bead surfaces reduced the nonspecific adsorption of proteins from cell lysates. Especially, when the magnetic particle surface was modified by N6-PEG mixtures with different molecular weights of 6000 and 2500 (6 kDa:2.5 kDa=9:1 w/w), the nonspecific adsorption of proteins was strongly suppressed. It is rather surprising for us that the sensitivity of the antibody on the surface was enhanced significantly when the PEG tethered chain was constructed in between the surface antibodies. Consequently, the mixed N6-PEG treatment showed a much higher S/N ratio than for the corresponding beads treated with bovine serum albumin (BSA), a conventional blocking reagent. Actually, when alpha-fetoprotein was analyzed by the magnetic bead-assisted ELISA thus constructed, the S/N ratio was about 20-fold higher for the mixed coating with PEG (6 kDa):PEG (2.5 kDa)=9:1, compared to the conventional BSA.

(2) Title: Label-free immunosensing for alpha-fetoprotein in human plasma using surface plasmon resonance.  
Source: Analytical Biochemistry. 365(2):201-7, 2007 Jun 15.  
Authors: [Teramura Y.](#) [Iwata H.](#)  
Abstract: In this study, we attempted to develop a surface plasmon resonance (SPR)-based immunoassay sensor to detect alpha-fetoprotein (AFP) in human plasma at the nanogram level, as is required for clinical diagnosis of hepatocellular tumors. A self-assembled monolayer (SAM) surface of tri(ethylene glycol) (TEG) and carboxyl group-terminated hexa(ethylene glycol) (HEG) was employed to suppress the nonspecific adsorption of plasma components onto the sensor surface. AFP was detected by a sandwich-type immunoassay using two kinds of antibodies, primary and secondary, in this system. The SPR signal shift was further enhanced by applying an antibody (polyclonal) against the second antibody. With this method, the SPR signals were highly intensified, and so nanogram levels (ng/ml) of AFP could be easily detected with a high signal/noise ratio, as is necessary for clinical diagnosis. It is expected that our SPR-based immunoassay method can also be applicable to the detection of several other tumor markers that are present in low concentrations in human blood.

E). Review Article “Pick of the Month”

(1) Title: Placenta accreta: a review of current advances in prenatal diagnosis.  
Source: Placenta. 28(7):599-603, 2007.  
Authors: [Mazouni C.](#) [Gorincour G.](#) [Juhan V.](#) [Bretelle F.](#)  
Abstract: Placenta accreta is a life-threatening obstetrical condition requiring a multidisciplinary approach. Despite identified obstetrical risk factors, the diagnosis is often made at the time of delivery. Recent advances in biology could allow a prenatal screening of placenta accreta with the identification of biological markers in maternal blood including cell-free fetal DNA, placental mRNA, and DNA microarray. These promising technologies can detect the presence of anomalies and should play a future role in developing a better understanding of placental invasion. Ultrasound imaging is popular due to its low cost and accessibility and widely used for the screening of placenta location and potential abnormal development. This exam is associated with high sensitivity and specificity for diagnosis of placenta accreta when specific defined criteria are used for the diagnosis. A placental MRI provides a morphological description, as well as recently demonstrated topographical information that optimizes diagnosis and surgical management. The screening of placenta accreta should be improved with the use of a combination of these diagnostic techniques and benefit high-risk populations with a reduction in morbidity.