

## Fetal Defect Marker Proficiency Test Mailout March, 2010

Dear Laboratory Director,

Below you will find a summary and critique of the Proficiency Testing mail-out from January 26, 2010 for Fetal Defect Markers, which included samples for first and second trimester screening, as well as amniotic fluids. Your laboratory's results and grades are printed on a separate sheet; also included are the grades from the previous two PT events. Please review and sign your evaluation. Retain the signed packet in your files. You will need it for your next laboratory survey to demonstrate participation in the NYSPT program.

### I. Graded Results Section:

**Table 1: Second Trimester Maternal Serum: Summary of All Lab Results**

Samples *N = 30	Sample #	MS 246	MS 247	MS 248	MS 249	MS 250
	Gestational Age (weeks)	16	19	15	18	17
Maternal Race	Ethnic Group	Hispanic	White	Black	White	Asian
Maternal Weight	Pounds (lbs)	195	140	155	150	97
Maternal Age	Years	45	30	25	33	21
Alpha-Fetoprotein (AFP)	Mean	32.41	147.01	27.10	0.81	37.52
	ng/ml ± Std.Dev.	± 2.20	± 11.42	± 2.12	± 0.20	± 2.61
	MOM ± Std.Dev.	1.16 ± 0.09	2.76 ± 0.23	0.85 ± 0.08	0.02 ± 0.01	0.75 ± 0.08
Unconjugated Estriol (uE3)	Mean	1.53	2.97	1.22	1.57	2.13
	ng/ml ± Std.Dev.	± 0.72	± 1.62	± 0.61	± 0.80	± 1.09
	MOM ± Std.Dev.	1.25 ± 0.21	1.10 ± 0.17	1.18 ± 0.24	0.74 ± 0.14	1.13 ± 0.20
human Chorionic Gonadotrophin (hCG)	Mean	24.29	16.06	27.92	34.89	20.51
	IU/ml ± Std.Dev.	± 2.51	± 1.58	± 2.23	± 3.94	± 1.97
	MOM ± Std.Dev.	0.97 ± 0.12	0.83 ± 0.10	0.71 ± 0.08	1.67 ± 0.22	0.66 ± 0.08
Dimeric Inhibin-A (DIA)	Mean	137.13	206.33	131.39	141.47	153.66
	pg/ml ± Std.Dev.	± 17.34	± 20.92	± 18.02	± 17.64	± 18.56
	MOM ± Std.Dev.	0.90 ± 0.13	1.15 ± 0.15	0.73 ± 0.11	0.84 ± 0.12	0.77 ± 0.15
Neural Tube Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Pos. (+) (93%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Further Action G,U,A	NFA	G = 72% U = 82% A = 76%	NFA	NFA	NFA
	NTD Risk 1 in	5,800	110	10,000	10,000	15,000
Trisomy-21 Screen (Positive, Negative) percent 1. <u>Triple test</u>	Pos. (+) or Neg. (-)	Pos.(+) (71%)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (100%)	Neg. (-) (100%)
	Recommended Action**	G = 64% U = 64% A = 57%	NFA	NFA	G = 71% U = 76% A = 86%	NFA
	Risk Est. 1 in	118	9,550	5,850	27	5,700
2. <u>Quad Test</u>	Pos. (+) or Neg. (-)	Neg. (-) (75%)	Neg. (-) (100%)	Neg. (-) (100%)	Pos. (+) (86%)	Neg. (-) (100%)
	Recommended Action **	G = 29% U = 21% A = 25%	NFA	NFA	G = 71% U = 64% A = 71%	NFA
	Risk Est. 1 in	435	18,000	13,500	71	10,000
Trisomy-18 Screen (Positive, Negative) percent	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Recommended Action**	NFA	NFA	NFA	NFA	NFA
	Risk Est. 1 in	1,919	10,000	10,000	3310	10,000

\*N=total numbers may vary since some labs do not test all analytes. The values represent the all-lab consensus based on the arithmetic mean ± Std.Dev.; (B) = borderline positive or negative, risk reflects central tendency (Median number for 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 246 Wk 16.0	This specimen was obtained from a 45 year old hispanic woman (Gravida = 1, Parity = 0) in her 16 <sup>th</sup> week gestation with a body weight of 195 lbs. She had no personal history of pregnancy loss. Her specimen, a second pregnancy sample, was negative for NTD (100% consensus); however, a body weight correction was indicated. Her screen was negative for Trisomy 18 with all labs in agreement. However, 71% of the triple screen submissions posted a positive DS screen due to the biomarkers and to maternal age alone (1 in 22) whereas 75% of the quad screens concluded the test to be negative. Recommendations of further action were submitted for the triple test of the MS246 sample and those include genetic counseling, 64%, ultrasound, 64% and amniocentesis 57%; while quad screeners recommended genetic counseling, 29%, ultrasound, 21% and amniocentesis, 25%. This specimen had no amniotic fluid counterpart.
MS 247 Wk 19.0	This specimen was obtained from a 30 year old white woman (Gravida = 3, Parity = 2) in her 19 <sup>th</sup> week gestation with a body weight of 140 lbs. She had a family history of pregnancy complications. Her specimen, a second pregnancy sample, was a positive screen for NTD (93% consensus; MOM=2.76). 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, 72%, ultrasound, 82% and amniocentesis, 76%. The MS247 specimen had an amniotic fluid paired sample which was also elevated (MoM=2.96). The all-lab median risk for NTD of MS247 was 1 in 110.
MS 248 Wk 15.0	This specimen was procured from a 25 year old, black woman (Gravida = 2, parity = 1) in her 15 <sup>th</sup> week gestation with a body weight of 155 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 (100% consensus) with a race correction indicated. The labs were also in agreement that both trisomy consensus screens were negative. Specimen MS248 was not paired with an amniotic fluid sample.
MS 249 Wk 18.0	This specimen was obtained from a 33 year old white woman (Gravida = 2, parity = 1) in her 18 <sup>th</sup> week gestation with a body weight of 150 lbs. She had a family (sibling) history of pregnancy complications. Her sample screened negative for NTD; however, her all lab aneuploidy screen was positive for Trisomy-21 (triple, 100%; quad, 86%). Her MSAFP sample was extremely low or absent. Recommendations of further action from labs performing the T21 quad screen were: genetic counseling, 71%; ultrasound, 64%; and amniocentesis, 71%; while the triple tests were: genetic counseling, 71%; ultrasound 76%, and amniocentesis, 86%. The triple DS risk was 1 in 27, while the quad risk was 1 in 71. Specimen MS249 resulted in a negative T18 screen in 100% of the participating labs. This sample was paired to an amniotic fluid specimen which also had a very low AFAFP level (MOM = 0.01).
MS 250 Wk 17.0	This specimen was obtained from a 21 year old asian woman (Gravida = 1, parity = 0) in her 17 <sup>th</sup> week gestation with a body weight of 97 lbs. She had no family history of pregnancy complications or adverse outcomes, and her aneuploidy screen was negative for both Trisomy-21 and for Trisomy-18. A body weight correction (low side) was indicated. No recommendation of further action for the NTD and T21 screen was reported from the participating labs. This specimen was not paired with an amniotic fluid specimen.

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

#### Instructional Note:

This notice regards the demographic data provided for the mock patients in the FEDM program. For the sake of this program, it will be understood that gravida indicates the pregnant status of a woman and parity is the state of having given birth to a completed term infant or infants. Thus, a gravida = n, indicates number (n) of times pregnant including the present one; a gravida = 2 indicates that the women was pregnant once before in addition to her present pregnancy. Parity = 1 indicates the patient already has one child; however, multiple birth is also considered as a single parity.

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

## 2) AMNIOTIC FLUID AFP (NTD-analysis):

N=31; all-lab Consensus Values

Sample#	Values	Summary Comments:
AF 246 Wk 19.0	AFP= 6.80 ± 1.00 µg/ml MOM= 0.86 ± 0.09	The AF246 sample was targeted for normal AFAFP value in the upper gestational age range. All labs called AF246 a non-elevated specimen for NTD. This AFAFP sample was not matched to a maternal serum specimen.
AF 247 Wk 19.0	AFP= 23.30 ± 3.61 µg/ml MOM= 2.96 ± 0.34	The AF247 sample was targeted for a screen positive AFAFP value in the upper gestational age range. All labs reported this specimen as a screen positive AFAFP value. The AF247 specimen was paired with maternal serum sample MS247 (MoM= 2.76) which was also elevated.
AF 248 Wk 20.0	AFP= 5.21 ± 0.51 µg/ml MOM= 0.80 ± 0.08	The AF248 sample was targeted for a negative NTD screen for AFAFP in the upper gestational age screening range. All labs categorized this as an NTD screen non-elevated specimen. This sample was not coupled to a maternal serum specimen.
AF 249 Wk 18.0	AFP= 0.20 ± 0.30 µg/ml MOM= 0.01 ± 0.01	The AF249 sample was targeted as an NTD negative screen in the routine gestational age screening range. Some labs either categorized AF249 as a negative NTD screen specimen or deemed it not interpretable due to the low or nonexistent AFP level (see critique). This specimen had a maternal serum counterpart, MS249 which showed very low levels of AFP (MoM= 0.02).
AF 250 Wk 19.0	AFP= 6.41 ± 0.70 µg/ml MOM= 0.81 ± 0.08	The AF250 sample was targeted for a non-elevated AFAFP value in the upper gestational age range. Most labs called AF250 a normal MOM AFAFP specimen. This AFAFP sample was not matched to a maternal serum specimen.

## II. Non-Graded Results Section:

**Table 2: First Trimester Maternal Serum all-lab Results**

Samples *N = 16	Sample #	FT 246	FT 247	FT 248	FT 249	FT 250
	Gestational Age (weeks)	13.0	12.5	11.9	11.5	11.1
Maternal Race	Ethnic Group	Black	White	Asian	Hispanic	White
Maternal Weight	Pounds (lbs)	130	140	120	125	130
Maternal Age	Years	21	25	30	28	35
Nuchal Translucency (NT)-Associated Measurements	Crown Rump Length (mm)	67	61	53	48	44
	NT Thickness (mm)	1.50	2.74	1.24	1.22	1.19
	NT - MOM	0.91 ± 0.08	1.78 ± 0.15	0.90 ± 0.08	0.97 ± 0.09	1.03 ± 0.10
Human Chorionic Gonadotrophin (hCG) Total	Mean IU/mL ± Std. Dev.	65.82 ± 14.72	147.73 ± 42.67	76.47 ± 14.87	84.15 ± 18.27	79.76 ± 16.68
	MOM ± Std. Dev.	0.93 ± 0.12	2.05 ± 0.31	0.93 ± 0.13	0.99 ± 0.14	0.91 ± 0.11
Pregnancy-Associated Plasma Protein-A (PAPP-A)	Mean mIU/mL ± Std. Dev.	4.59 ± 2.87	2.45 ± 1.43	4.30 ± 2.62	4.06 ± 2.51	3.41 ± 2.07
	MOM ± Std. Dev.	1.72 ± 0.90	1.21 ± 0.56	2.37 ± 1.13	2.71 ± 1.32	2.72 ± 1.41
Trisomy-21 Screen (Positive/Negative) percent	Pos. (+) or Neg. (-)	Neg. (-) (100%)	Pos. (+) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Recommended Action NFA or G = % U = % A = % C = %	NFA	G = 87% U = 33% A = 27% C = 73%	NFA	NFA	NFA
	Risk Estimate 1 in	10,000	110	10,000	10,000	10,000
Trisomy-18 Screen (Positive, Negative) Percent	Pos (+) or Neg. (-)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)	Neg. (-) (100%)
	Recommended Action	NFA	NFA	NFA	NFA	NFA
	Risk Estimate	10,000	9,865	10,000	10,000	10,000

\*N=total numbers may vary since some labs do not test all analytes. (B) = borderline negative or positive; NFA = no further action; G = genetic counseling; U = ultrasound; C = chorionic villus sampling; N = number of labs participating; FT = First Trimester

**1) First Trimester Maternal Sera Only:**

**B. Narrative Evaluation of First Trimester Screening Results:**

N = 16 all-lab Consensus Values.

<u>Sample#</u>	<u>Summary Comments:</u>
FT 246 Wk 13.0	This specimen was obtained from a 21 year old Black woman of average body weight (130 lbs.). Her gestational age at time of screening was 13.0 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs were in agreement. The FT246 risk estimate for Trisomy-21 was 1 in 10,000, while the all-lab Trisomy-18 risk was also 1 in 10,000 (negative screen). All labs were in agreement that FT246 was a negative screen for both Trisomy-21 and T18.
FT 247 Wk 12.5	This specimen was procured from a 25 year old White woman of average body weight (140 lbs.). Her gestational age at time of screening was 12.5 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen positive for T21 and all testing Labs were in agreement (see Critique). The FT247 risk estimate for Trisomy-21 was 1 in 110, while the Trisomy-18 risk was 1 in 10,000.
FT 248 Wk 11.9	This specimen was obtained from a 30 year old Asian woman of average body weight (120 lbs.). Her gestational age at time of screening was 11.9 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs were in agreement. The FT248 risk estimate for Trisomy-21 was 1 in 10,000, while the all-lab Trisomy-18 risk was also 1 in 10,000 (negative screen). All labs were in agreement that FT248 was a negative screen for Trisomy-21 and Trisomy-18.
FT 249 Wk 11.5	This specimen was obtained from a 28 year old Hispanic woman of medium body weight (125 lbs). Her gestational age at time of screening was 11.5 weeks. She had no prior history of pregnancy complications and/or adverse outcomes. The FT specimen was screen negative and all testing Labs were in agreement. The FT249 risk estimate for Trisomy-21 was 1 in 10,000 while the Trisomy-18 risk was also 1 in 10,000.
FT 250 Wk 11.1	This specimen was procured from a 35 year old White woman with a body weight of 130 lbs. Her gestational age at time of screening was 11.1 weeks. She reported no prior family history of pregnancy complications. This FT specimen was screen negative for T21 and T18. The risk estimate for FT250 was 1 in 10,000, and the T18 risk was 1 in 10,000. All labs were in agreement with both aneuploid screen assessments.

**III. Critique and Commentary:**

**A) Fetal Defect Proficiency Test Mail out 1/26/10 of Second Trimester Maternal Serum and Amniotic Fluid Values:**

In general, the all-lab results of the targeted values for the NTD and the Trisomy Screen were consistent with the goals of our projected target values, risks, and outcomes. As displayed in the second trimester tables, maternal serum sample MS247 was targeted as an elevated specimen for NTD (Figs. 1 and 3) and the AF-247 sample was matched to that specimen. Thus, specimen MS247 was screen positive for NTD, but negative for Trisomy-21 (T21), and Trisomy-18 (T18). For the MS247 specimen, the NTD screen resulted in a 1 in 110 all-lab risk for open neural tube defects (ONTD), and it achieved a 100% NTD screen consensus. The NTD-related recommended action for specimen MS247 was genetic counseling, 72%; ultrasound; 82%; and amniocentesis, 76%. Sample MS249, a T21 screen positive specimen, was obtained from a white woman with a prior (sibling) history of pregnancy complications. The T21 MOM results for specimen MS249 (MSAFP-MOM = 0.02, MSuE3-MOM = 0.74, MShCG-MOM = 1.67, DIA-MOM = 0.84) were all in accordance with a T21 positive screen; labs using the quad test classified this specimen as a T21 positive screen (86%) and most recommended further action (see below). The MS249 sample was from a 33 year old woman and produced a risk from the quad test of 1 in 71 and a triple test risk of 1 in 27, which were greater than that expected from the maternal age alone (1 in 450). Finally, samples MS246 and MS248, and MS250 produced negative screens for NTD, Trisomy-21, and Trisomy 18; however, corrections for body weight were indicated for both MS246 and MS250.

Specimen MS246 produced an interesting case in the DS risk assessment using the triple versus the quad testing platforms. This sample triggered a Down syndrome (DS) risk in 71% of the laboratories using the triple test platform (N = 14), while the quad test users produced a DS risk in only 25% of the labs (N = 28). While less than 4% of the labs in this PT program employ the triple test and not the quad test, this sample represents a call for caution in the triple test only users. However, most labs in this PT program use the quad test in clinical practice, but report the results of both test platforms in the NYS PT survey.

The ACOG Practice Bulletins of May 2001 (Clinical Management Guidelines for Obstetricians and Gynecologist, #27) and of January 2007 (#77) recommend the use of the quad over that of the triple test platform.

Specimen MS249 is of special interest in that the sample was a T21 positive screen with a virtual absence of AFP (MSAFP MOM=0.02). Moreover, MS249 showed normal levels of both HCG (MOM=1.67) and DIA (MOM=0.84), and slightly low uE3 (MOM=0.74). Low levels of AFP and uE3 screening values, by themselves are not indicative of a Down syndrome; however, the combination of the two low analytes in the triple and quad tests resulted in a pregnancy at risk for a T21 positive screen in this particular situation. Interestingly, the triple test DS risk was (1 in 27) greater than the quad risk (1 in 71). This indicated that Inhibin A has a modulating effect on the screen outcome. This mock patient had a prior history of pregnancy complications, thus, paired maternal serum and amniotic fluid samples had been obtained for analysis at time of specimen collection. Subsequent Stage-II ultrasound, Ache, AFAP, and amniocentesis karyotyping were found to be unremarkable in this mock patient. Eventually, this mock patient was deemed a false positive screen for T21 even though both the triple and quad tests had generated high DS risks. However, specimen MS249 displayed one unusual biomarker in that MSAFP was virtually absent. All participating labs had suggested some further actions (see above) for both triple and quad testing. As discussed below, very low or absent AFP levels have been associated with a very rare benign condition termed congenital deficiency of AFP (CD of AFP). However, only three labs classified MS249 as a CD of AFP.

The CD of AFP should be considered in cases of very low or absent maternal serum AFP in the second trimester screening for Down syndrome. However, this condition can only be considered after a normal karyotype result is obtained following amniocentesis. Though rare, the CD of AFP has now been published in multiple case reports throughout the world including 15 in France, 4 in Israel, 2 in the United States, and one in Algeria (see refs 31-35). Congenital deficiency of AFP was first described by Greenberg et al in 1992 in the United States following routine prenatal screens (35). Greenberg reported two infants which demonstrated low or absent AFP in maternal serum, amniotic fluid, cord blood, and in newborn dried blood spots. In both cases, healthy normal infants were delivered with good Apgar scores and normal karyotypes. At 1.0 year of age, both infants were doing well with no physical or developmental problems. After that time, both patients were lost to follow-up and the cause of the AFP deficiencies was not known.

In 1997, Sher and Shohat published a Down syndrome (DS) screening case study which produced a borderline DS screen involving the total absence of MSAFP (33). The DS screening profile produced a MSAFP MoM = 0.00; uE3 MoM = 0.63; and a hCG MoM=1.63 with a borderline DS negative risk of 1 in 290. Ultrasound revealed a fluid-filled sac in juxtaposition to the stomach, and the genotype was 47 XX + 21 confirmatory for Down syndrome. The authors stated that despite publication of previous cases showing that CD of AFP is a benign condition, amniocentesis should be recommended for extremely low AFP screening results. In a later case study, two patients with congenital absence of AFP in the course of first cousin pregnancies were reported by Sharony et al (32). In this latter report, the patients' samples were studied by radioimmunoassay (RIA), immunohistochemical staining, comparative genomic hybridization (CGH), and fluorescence *in situ* hybridization (FISH) analysis. The first patient had inflammatory infiltrate sites in the placenta (deciduitis) accompanied by a placental monosomy-16, but had a normal fetus confirmed by CGH and FISH analysis. Histochemically-localized AFP was present in both fetal and maternal tissues. In the second patient described by Sharony, the triple screen profile demonstrated a AFP MoM = 0.00, hCG MoM= 0.42, and uE3 MoM= 0.97 with a cord AFP level = 0.00; however, a normal karyotype was present. Again, this patient's results were accompanied by inflammatory sites in the placenta (chorioamnionitis) and a placental monosomy-16. Interestingly, both families were unrelated according to family history and both resulted in normal, healthy newborn outcomes.

A second study emerged from Sharony et al in 2004 in which they addressed the genetic nature of CD of AFP (34). This study was a follow-up report of the two previous patients demonstrating an absence of MSAFP (see above); this study involved searching for mutations in the AFP gene itself. The group identified a mutation in which both patients' genes showed a frameshift after codon 294 (Thr 294) that led to a stop codon at AFP amino acid sequence # 318; this truncation resulted in the absence of the entire 3<sup>rd</sup> domain and a portion of domain-2 of AFP. Both of the affected infants were found to be homozygous for the mutation, presented a history of normal development, and were asymptomatic. Thus, the elimination of 48% of the amino acids from the AFP molecule resulted in normal fetal development that was compatible with postnatal life and exhibited reproductive capability in the males (the fathers had the mutation). Although CD of AFP is a very rare condition, this genetic condition should be considered in adults being monitored for post-operative serum AFP, such as in cases of hepatomas and germ cell tumors.

A recent genetic study of CD of AFP was reported by Petit et al in which a new mutation in the AFP gene was described following a Down syndrome positive screen derived from a mother having an artificial insemination pregnancy (35). These French investigators reported a mutation in exon-5 of the AFP gene which had led to a total absence of AFP both in maternal serum and in amniotic fluid. At 14 weeks gestation, the AFP MoM was 0.06, hCG MoM was found elevated at 4.74 MoM, amniotic fluid AFP was undetectable, but a normal karyotype was present (46XY). Ultrasound examinations at 14, 22, and 32 weeks proved to be normal and a healthy infant was delivered at 37 weeks. After PCR-amplification, the whole AFP gene was sequenced and a new mutation was found and determined to be a guanine to adenine transition in position 543 which created a premature stop codon in amino acid position tryptophan-181. Thus, the AFP molecule consisted only of 181 amino acids which constituted nearly 90% the first domain of AFP, but eliminating domains two and three. Despite these findings, the infant experienced both normal fetal development and an unremarkable birth. As discussed in the above reports, CD of AFP is a very rare, benign trait generally diagnosed at time of prenatal screening for Down syndrome. Low or undetectable level of AFP during gestation has an estimated frequency of 1 in 105,000 (37). In screening situations, the difficulty lies in distinguishing really low AFP values in patients at high risk for chromosomal abnormalities from that of CD of AFP. Fortunately, many clinical manifestations can be excluded by cytogenetic and biochemical investigations in conjunction with ultrasound examinations.

However, CD of AFP is not detectable until the second trimester since AFP levels cannot be measured in the first trimester. It is of interest that CD of AFP can be compared to An-Albuminemia (AALB), a very rare metabolic abnormality considered to be benign in infancy but problematic during pregnancy (36). Moreover, AALB is thought to be responsible for severe disorders of later pregnancy such as intrauterine growth retardation and intrauterine death. It has been further speculated that albumin deficiency might be responsible for precocious fetal loss in unexplained cases of complicated pregnancies.

Due to the high level of expression during embryo/fetal development, it was previously assumed that AFP was essential during the course of mammalian development. However, the above studies would argue that AFP is not necessary for the completion of pregnancy and for the birth of a viable normal newborn. Interestingly, the development of an AFP gene knockout mouse model is consistent with the present clinical observations in that AFP does not appear essential for development during pregnancy (37). An AFP-gene knockout study by Gabant et al (37) showed that neither the embryos/fetuses nor the maternal placental tissues were dependent on AFP for the successful completion of pregnancy and full term birth. Indeed, the mutant homozygous adult male mice were viable and fertile as were the fathers in the above human clinical studies. In contrast, the rodent AFP-null females were infertile due to a dysfunction in the hypothalamic/pituitary system, resulting in anovulation (38). However, an infertility history from the females in the affected families has not yet been determined, since many of the females have not yet reached the age of fertility to compare it to the rodent model. Unlike the hereditary persistence of AFP, which may be detrimental in some cases, CD of AFP seems to largely a benign condition.

In summary, it can be seen from the preceding paragraphs that the Down syndrome positive screen produced by specimen MS249 did potentially produce a false positive result. An actual DS screen outcome was published by Sher and Shohat; however, in their case it turned out to be a confirmed positive screen for Down syndrome (33). By comparison of the triple test result, it was evident that the MoM profiles were similar; AFP MoM= 0.0 (NYS), AFP MoM= 0.0; uE3 MoM= 0.74 (NYS); uE3 MoM= 0.63; hCG MoM= 1.17 (NYS), hCG MoM= 1.63. Unlike our intended mock specimen MS249, the Sher and Shohat patient case report was a positive Down's screen confirmed by karyotyping. The NYS mock patient was intended to mimic a true CD of AFP as reported in the other case histories stated above (see refs 35 & 36). Thus, a CD of AFP should be considered when a screen result of very low or absent AFP is encountered following triple or quad testing in Down syndrome prenatal screening programs. Unexpectedly in the present PT-mailout, the triple screen results from MS249 produced an even greater triple screen DS risk (1 in 27) than the quad screen (1 in 71) demonstrating the influence of Inhibin A on the risk outcome.

Comments from the PT laboratories regarding the CD of AFP in Amniotic fluid is worthy of further discussion. Approximately 50% of participating labs did not classify AF249 as a negative screen, but rather requested further action or provided additional recommendations. The various responses were as follows: 1) the sample may have been urine; 2) AFP gene mutation present, ultrasound suggested; 3) not consistent with an ongoing pregnancy or with an actual amniotic fluid sample; 4) Fern test recommended (see below); 5) CD of AFP present; and 6) could not provide a MoM or an interpretation. The verification that the sample was indeed an amniotic fluid sample instead of urine can be ascertained by performing a total protein quantitation on the sample. Amniotic fluid has a total protein value (Bradford test) of 5-8 mg/ml, while urine contains little or no protein unless proteinuria is present (detectable with a dipstick bromophenol-blue test). Some labs recommended a Fern test be employed. The Fern test can be used in two fashions; first, it can be used to detect leakage of amniotic fluid from the placental membranes; and second, it is a test of elevated estrogenic activity in cervical mucus smears to indicate that ovulation has occurred. Finally, some labs elected not to provide an AF MoM or its interpretation. The labs suggesting that the congenital absence of AFP was due to an AFP gene mutation were mostly correct in their assessment of this screening anomaly.

## **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 (except for MS249 with absence of AFP: see kit median). For uE3, the mean/all kit median for Beckman UNICEL and Access-2 hovered about 1.0 (see Fig. 8); however, labs employing DPC Immulite 2500 or Immulite 2000 yielded values achieving 2.2 to 2.5 times higher than the mean/all kit median (see dotted line). In contrast, both Beckman Access2/Unicel and DPC were nearly equivalent when the kit MOM medians were compared (Fig. 8B). Regarding the hCG kits (see Fig. 9), the Siemens/Bayer ADVIA-Centaur /ACS-180, was slightly above the 1.0 kit median, the Beckman Access2 and UNICEL DXL yielded similar mean hCG values hovering about and above the 1.0 mean/all kit median value. While DPC Immulite or Immulite 2000 kits demonstrated slightly lower values (Fig. 9). 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. Beckman kits were equal to or marginally higher and DSL was 15 to 20% lower than the 1.0 mean/all kit median value (Fig. 10). Labs lacking peer group companions and in-house assays will be deemed non-gradable (NG) for individual analyte groups as the situation dictates.

The bar graph in Figure 11 is provided to display kit performances among the amniotic fluid (AF-AFP) test samples. As shown in the amniotic fluid bar graph, overall kit performance slightly wavered about the 1.0 mean/all kit median mark except for sample AF249. The AFAFP-249 vacancy is explained by the total absence of AFP in this specimen. Overall Siemens/Bayer ADVIA-Centaur/ACS-180 and DPC immulite/2000 kits were 5 - 20% higher (except for sample AF249) Beckman Unicel and Beckman Access/2, were about 15% - 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.

### **C) Second Trimester Screening Software Utilized:**

For informational purposes, it was deemed of interest to provide the software package usage by our participating laboratories. The alpha and Benetech software packages were each used by 24% of the labs respectively; Robert Maciel (RMA) software was employed by 31%; while in-house software comprised 14% and 7% of labs used programs classified as “other” which are proprietary software packages.

### **D) First Trimester Screen:**

Five first trimester maternal serum mock samples have been provided and will be included in all future mailouts in order to survey and assess New York State licensed laboratories concerning participation and assay capabilities in first trimester Down syndrome screening. All laboratories that are **validation-approved** and presently performing first trimester Down syndrome screening are **REQUIRED** to test and report screen results; however, the laboratory results will **not** be graded at this time. Those laboratories not presently offering the test, nor planning to implement the test, can request that no further samples be sent to them. The FT sample (FT = first trimester) information provided to participating labs included maternal age, nuchal translucency (NT measurements in millimeters), last menstrual period (LMP), and draw date. Crown-rump length (CRL) measurements, race, and maternal body weight have also been included in the case histories to better evaluate all-lab participant NT information requirements.

As demonstrated in the FT table 2 (Section – II) above, the all lab measurement of the 13.0 week Black FT246 specimen for total hCG resulted in a mass mean of  $65.82 \pm 14.72$ , with a non-elevated MOM = 0.93. Furthermore, the all-lab mass mean for PAPP-A was  $4.59 \pm 2.87$  mIU/ml with a MOM = 1.72. The all-lab T21 risk assessment was 1 in 10,000 for the FT246 specimen. Even with the differences in the PAPP-A kits, all labs agreed that the FT246 sample was screen negative (See Figure 14 risk distribution) in accordance with the normal analyte MOMs. The risk cut-off level for Blacks ranges from 200 to 270 among the participating labs. Thus, the FT246 sample resulted in a 100% all lab T21 negative screen assessment. No further action was indicated. Finally, the FT246 specimen also screened negative for T18 (1 in 756) using a cutoff of 1 in 100.

As displayed in the FT table 2 (Section – II) above, the all lab measurement of the 12.5 week Caucasian FT247 specimen for total hCG resulted in a mass mean of  $147.73$  IU/ml  $\pm 42.67$ , with an elevated MOM =  $2.05 \pm 0.31$ . Furthermore, the all-lab mass mean for PAPP-A was  $2.45 \pm 1.43$  mIU/ml with a MOM =  $1.21 \pm 0.56$ . The all-lab T21 risk assessment was 1 in 110 for the FT247 specimen. Analyte MOM measurements for the first trimester Down syndrome screen detection are associated with raised NT, low PAPP-A, and high hCG MOMs. Even though the PAPP-A was 1.2 MOM, the elevated NT and hCG MoMs were sufficient to produce a positive screen, and thus the FT247 results were indeed consistent with a T21 positive screen. Further actions by the labs included genetic counseling, 87%; ultrasound, 33%; and amniocentesis/CVS = 27/73%. Finally, the FT247 specimen screened negative for T18 (1 in 297) using a cutoff of 1 in 100 with a risk of 1 in 12,000.

As shown in the above First Trimester table 2 (Section-II) for the FT248 Asian specimen, the gestational age all-lab mean was reported as 11.9 weeks. Assay measurements from FT-screening participating laboratories resulted in an all-lab total hCG mass measurement of  $76.47 \pm 14.87$  IU/ml, while the all-lab PAPP-A mass assessment was  $4.30 \pm 2.62$  mIU/ml. The first trimester all-lab Trisomy-21 screen consensus for FT248 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 FT248 achieved a MOM value of 0.93. In comparison, the all-lab MoM for PAPP-A was 2.37. All labs agreed that the FT248 sample was screen negative for Trisomy 21 (See Fig. 14 risk distribution). The FT248 specimen also resulted in a negative screen for Trisomy-18 with a risk assessment of 1 in 10,000.

In the FT249 Hispanic sample, the gestational age all-lab mean was reported as 11.5 weeks. Assay measurements for FT249 resulted in an all-lab total hCG mass measurement of  $84.15 \pm 18.27$  IU/ml, while the all-lab PAPP-A mass assessments were  $4.06 \pm 2.51$  mIU/ml (see Figs. 12 and 13). The first trimester all-lab trisomy-21 consensus for FT249 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 FT249 for total hCG resulted in a MOM value of 0.99 and the all-lab MoM mean for PAPP-A was 2.71. All labs agreed that the FT249 sample was screen negative for Trisomy 21 (See Fig. 14 risk distribution). The all-lab T18 risk assessment for FT249 was 1 in 10,000, hence, the FT249 specimen resulted in a negative screen for Trisomy-18.

As demonstrated in the FT table 2 (Section-II) above for the White FT250 specimen, the gestational age all-lab mean was reported as 11.1 weeks. Assay measurements from FT-screening participating laboratories resulted in the all-lab total hCG mass measurement of  $79.76 \pm 16.68$  IU/ml, while the all-lab PAPP-A mass assessment was  $3.41 \pm 2.07$  mIU/ml. The first trimester all-lab trisomy-21 screen consensus for the FT250 specimen was negative (100%). The all-lab FT Trisomy-21 risk assessment was 1 in 10,000. As observed in the table (Table 2, Section – II), the all lab measurement of total hCG MOM for FT250 produced a value of 0.91; in comparison, the all-lab MOM mean for PAPP-A resulted in 2.72. All labs agreed that the FT250 sample was screen negative for Trisomy 21. (See Fig. 14 risk distribution). The FT250 specimen also resulted in a negative screen for Trisomy-18 with an all-lab risk assessment of 1 in 10,000.

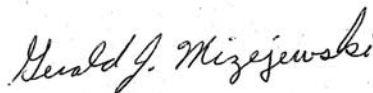
#### **D. 1.) First Trimester Assay kit Performance:**

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 the Beckman Unicel/Access kit measuring 10-20% above the kit median and DPC being about (10-15%) lower. In contrast, results from the two PAPP-A kits varied widely with the mean/all kit median values from Diagnostic Systems Lab (DSL) being less than half of those obtained with DPC Immulite or Immulite 2000 kit. However,

this difference was not equalized when the PAPP-A kit MOMs were compared, DPC Immulite being more than double that of DSL and Beckman.

**E) First Trimester Screening Software Utilized:**

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



G.J. Mizejewski, Ph.D.

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detection rate (DR), false-positive rate (FPR), and costs of contingent triple-screening were calculated and compared with routine double-screening methods. RESULTS: Six of 24 DSFN and 3 of 322 DSTN were contingent triple-screening positive. Compared with routine double-screening, DR of contingent triple-screening increased by 10% (from 50% to 60%) without a significant increase of FPR ( $p > 0.05$ ). When compared with routine triple-screening, uE3 costs in contingent triple-screening were reduced by more than 84.3%. CONCLUSIONS: Second-trimester maternal serum contingent triple-screening could be effective and suitable for prenatal care in Mainland China. Governments and Health Agencies of other developing countries may also find this strategy cost-effective.

2) Title: Maternal serum triple marker levels in immigrants to Israel from ethiopia.

Source: Fetal Diagn Ther 2009, 26:200-202.

Authors: Sharony R, Itzhaky D, Amiel A, Fejgin M, Cuckle H

Abstract: OBJECTIVES: To determine if Ethiopian immigrants have similar triple marker levels as the general Israeli population. MATERIAL AND METHODS: Second-trimester maternal serum results on 346 Ethiopians were obtained from records of 36,309 women. Two series were constructed for comparison among patients screened between 2000 and 2001 ('old group') and 2005 and 2007 ('new group'). RESULTS: The median and 95% confidence intervals (CI) were: alpha-fetoprotein (AFP) 1.080 multiples of median (MoM) (95% CI 1.03-1.13); human chorionic gonadotrophin (hCG) 0.895 MoM (95% CI 0.82-0.97), and unconjugated estriol (uE(3)) 1.050 MoM (95% CI 1.00-1.10). The differences between the AFP and hCG medians for the old and new series were not statistically significant ( $p = 0.06$  and  $0.20$ ) whilst the uE(3) difference was significant ( $p = 0.04$ ). There was a general tendency for the levels to be closer to 1 MoM over time. CONCLUSIONS: Triple marker serum levels of AFP and hCG among Ethiopian patients may need to be slightly corrected, particularly for hCG.

3) Title: Identification of second trimester screen positive pregnancies at increased risk for congenital heart defects.

Source: Prenat Diagn. 2009, 29:570-577.

Authors: Jelliffe-Pawlowski LL, Walton-Haynes L, Currier RJ

Abstract: OBJECTIVE: To examine whether second trimester biomarkers could be used to identify screen positive pregnancies at increased risk for congenital heart defects (CHDs) and measure the effect of using different biomarker cut points on the detection of CHDs and on the performance of predictive models. METHODS: Included were 19,402 pregnancies without chromosomal defects, which were screen positive for Down syndrome or other birth defects based on maternal serum measurements of alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), and unconjugated estriol (uE3). Logistic regression models were built that compared biomarkers for CHD cases compared to controls. RESULTS: CHD cases were more likely to be screen positive for trisomy-18, to have a nuchal fold (NF)  $\geq 5$  mm, and/or to have an hCG multiple of the median (MoM)  $\geq 95$ th percentile in models that considered screen positive grouping. In models that did not consider screen positive grouping, cases were more likely to have a NF  $\geq 5$  mm, an AFP MoM  $\leq 10$ th percentile, an hCG MoM  $\leq 25$ th percentile, and/or an hCG MoM  $\geq 75$ th percentile. CONCLUSION: Along with NF, second trimester maternal serum biomarkers may be useful indicators for fetal and newborn evaluation for CHDs in screen positive pregnancies without identified chromosomal defects.

#### VI. Potentially helpful website connections/locations:

- 1) [pregnancy.about.com/cs/afp/a/afptest.htm](http://pregnancy.about.com/cs/afp/a/afptest.htm)
- 2) [health.allrefer.com/health/alpha-fetoprotein-info.html](http://health.allrefer.com/health/alpha-fetoprotein-info.html)
- 3) [headtoe.apta.org/topic/medtest/hw1663/results.htm](http://headtoe.apta.org/topic/medtest/hw1663/results.htm)
- 4) [www.pregnancy-info.net/slpha\\_feto\\_protein.html](http://www.pregnancy-info.net/slpha_feto_protein.html)
- 5) [www.healthopedia.com/alpha-fetoprotein](http://www.healthopedia.com/alpha-fetoprotein)

January 2010

## **Teachings on Alpha-fetoprotein**

### **Vol. 4, Part 8**

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

#### **Fetal Erythropoiesis:**

The existence of a relationship between AFP and fetal erythropoiesis comes as no surprise, since the fetal liver and yolk sac constitute the major embryonic/fetal sites of both AFP synthesis and erythropoiesis (182). In fact, tumors of the yolk sac and the liver (hepatomas) are known to synthesize and secrete AFP (183). Patients with hepatomas are known to present with erythrocytosis and elevated erythropoietin which are associated with polycythemia in these individuals (184, 185). Levels of SAFP are also elevated in weanling rats born to mothers with anemia that was induced by serial bleeding episodes during pregnancy (174). Furthermore, elevated levels of serum SAFP persist in the nude mouse up to or beyond the 20<sup>th</sup> week following birth (186). This maintenance of high SAFP levels has been attributed to the continued presence of hematopoietic foci present in the livers of nude mice during much of their adult lifespan. Finally, high levels of SAFP are maintained in tumor-bearing mice that have verified hemolytic anemia of the patient (187). Such situations regarding AFP have been confirmed in subsequent studies of fetal aplastic crisis (189).

Meticulous clinical studies by Bartha and co-workers determined that significant negative correlations existed between maternal AFP and fetal hemoglobin levels (182, 188,). These investigators also found a negative correlation between fetal levels of SAFP and levels of fetal red blood cells, hemoglobin, hematocrit, erythropoietin, and transferrin. Concomitantly, in this study, erythropoietin levels were positively correlated with levels of fetal red blood cells, hemoglobin, and erythroblasts, and with hematocrit. This research group concluded that AFP

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must play a regulatory/modifying role in fetal erythropoiesis. Thus, we might expect a high AFP level in anemic fetuses, and a low one under conditions of hemoconcentration.

A relationship between of AFP level and serum folate deficiencies during pregnancy is also related to anemias. The increased demand for folic acids (FA) during pregnancy results from FA transport to the fetus, placental tissue growth elevated erythrocyte synthesis, and urinary loss replacement. It is generally accepted that a steady depletion of maternal serum folate occurs during pregnancy, and is accompanied by a mild physiologic anemia (190). The serum folate decline has been attributed to rapid plasma clearance, fetal transfer, hemodilution, and possibly hormonal effects, as seen in users of oral contraceptives. There appears to be an elevated incidence of the megaloblastic-type anemias in folate-deficient pregnant women (191). In addition, a number of complications have been associated with folate deficiency in pregnant women, although some of these associations are questionable. Among the complications are: (a) abruption placentae, (b) toxemia of pregnancy, (c) spontaneous abortion and fetal death, (d) neural tube defects, including spina bifida and anencephaly, (e) hydrocephalus, (f) prematurity - low birth weight, (g) hemolytic anemias, (h) various anatomic congenital malformations, and (i) twin pregnancies (192, 194). It is quite remarkable that AFP concentrations tend to be elevated in pregnancy complications linked to folate deficiencies. However, AFP is elevated in some instances where deficiency in FA is implicated, and in which a biochemical rather than an anatomic basis is involved.

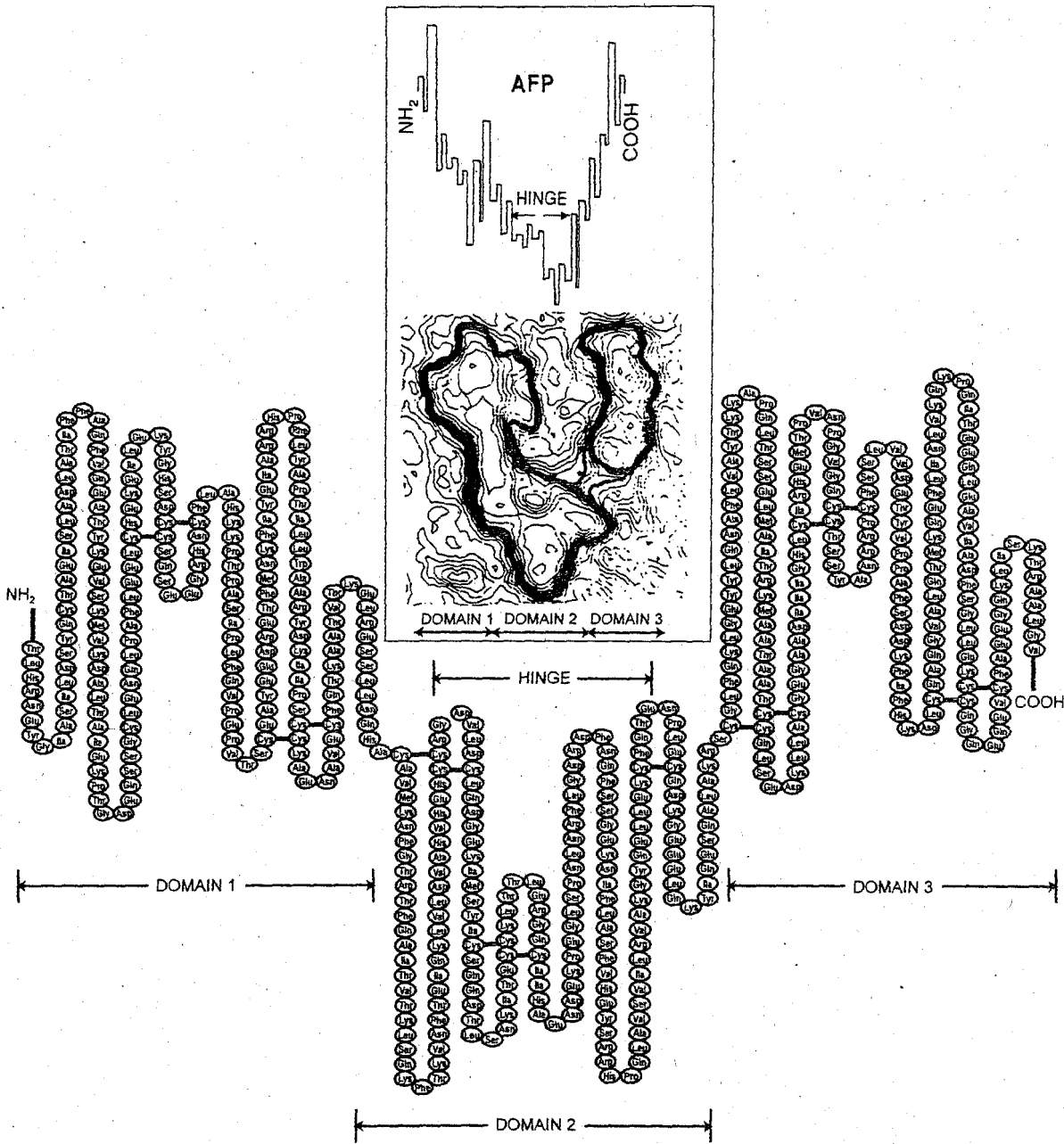
In light of the above associations, we may consider the following reported observations. First, both HAFP and ALB (193, 194) can bind FA in a low-affinity, high-capacity manner (Fig. - 2). Even though FA binding affinity is low ( $10^{-3}M^{-1}$ ), the high concentrations of AFP in early pregnancy (3 mg/ml) are sufficient to bind a considerable amount of FA. It is of interest that

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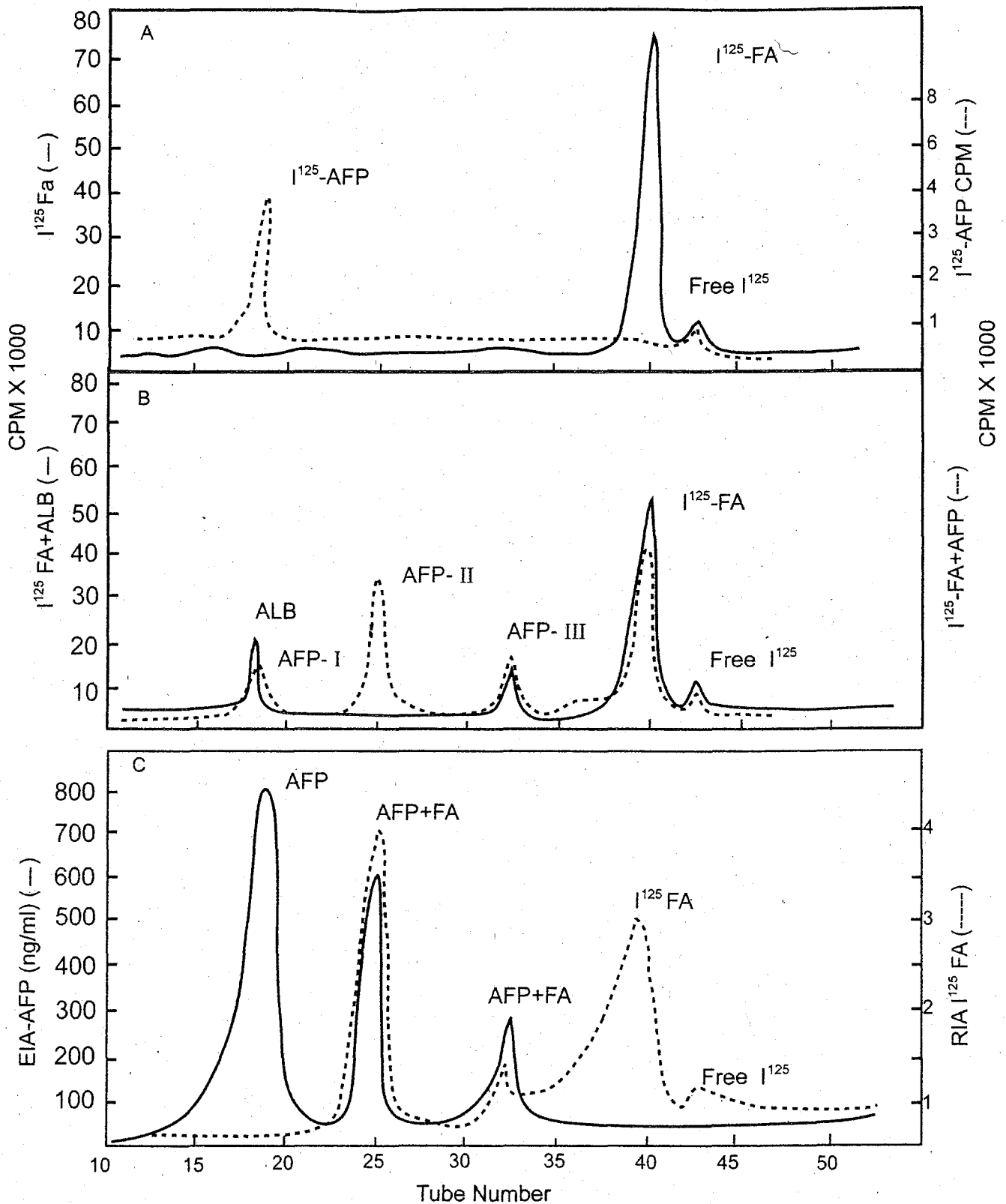
HAFP displays a Genbank amino acid sequence identity to a murine folate-binding protein at residues # 352 to 372 (50% identity) on Domain – 2 suggesting that a folic acid binding site could reside at or near this location. Second, elevated SAFP levels have been reported in patients with severe anemias (195). Third, the normal decline of maternal serum folate appears to parallel the normal fall in HAFP concentrations in both amniotic fluid (13 to 40 wks gestation) and maternal serum (30 - 40 wks gestation) (194). Fourth, in pups born to nude mice, both elevated SAFP levels and liver hematopoiesis are maintained well into adulthood (186). Thus, conditions that modulate or interfere with hematologic maturation processes in the prenatal, perinatal, or postnatal period appear to influence AFP fluid concentrations. The AFP-FA relationship is deemed important, in light of the studies linking the prevention of neural tube defects with periconceptional FA supplementation (196).

Hematopoietic stem cells are derived from the mesodermal germ layer in the embryo, and give rise to all known adult hematopoietic cell lineages (197). However, recent data suggest that bone marrow cells with a hematopoietic stem cell profile can also cross-differentiate (as totipotent cells) to endodermal cell types, such as liver cells (see below). HAFP, a differentiation marker for endodermal cells, has long been thought to be tightly regulated, in a tissue-specific manner, during development (198, 199). However, in a recent report, two new HAFP mRNA transcripts were described as AFP-variant (V) forms in hematopoietic progenitor cells that are not expressed in adult cells (199). In the AFP-V, exon 1 of AFP is replaced with one or two exons in the 5' untranslated region of the AFP gene. In cell culture lines, the AFP-V transcripts were detected in bone marrow, thymus, and brain, but were not detected in spleen, intestine, or liver, or in the hepatoma HepG2 cell line. Hematopoietic progenitor cells purified from cord blood (flow-cell sorting) also expressed the variant transcripts. The investigators asserted that

certain hematopoietic stem cells are capable of expressing fetal protein transcripts thought to be unique to the endoderm.



**Figure 1.** The primary and secondary structure amino acid sequence of human alpha-fetoprotein (AFP) is displayed. Note that the HAFP molecule is composed of three domains in a U-shaped configuration as confirmed by electron dot contour mass mapping. Human AFP belongs to the albuminoid gene family, which is characterized structurally by cysteine residues that are folded into layers that form loops dictated by disulfide bridging (15). The third domain is positioned close to a proposed "hinge" region (see arrows). The hinge concept developed from the observation that HAFP has two disulfide bridges fewer than does human albumin, providing it with a means of molecular flexibility (82). The panel in the top inset displays the two-dimensional aspect of the AFP domain structure; the panel in the bottom inset shows the electron dot contour map of the human AFP molecule (see Refs. 16, 17).



**Figure 3.** A chromatographic G-75 elution profile is shown for radiolabeled folic acid (FA) with or without complexing proteins (A). Radiolabeled  $^{125}\text{I}$ -FA was subjected to Sephadex G-75 gel filtration column chromatography using a peristaltic pump (data obtained from Refs. 193, 194). The  $^{125}\text{I}$ -FA eluted from the column in fraction tubes 38–40, while  $^{125}\text{I}$ -FA heteroprotein complex formation was detected as gel retardation in several tube locations between tubes 15 and 33, thus indicating varying degrees of dissociation (low-affinity binding) of  $^{125}\text{I}$ -FA bound to AFP. (B and C) Confirmation of the tube location for FA was made by RIA (B and C). Likewise, the fraction location of AFP shown in panel B was confirmed by

Hybritech enzyme-immunoassay in panel C. Note that human albumin (ALB; panel B) also bound to FA in a similar dissociation pattern, demonstrating a similar low affinity for FA as described in Ref. 151. The RIA for measurement of FA was obtained from Diagnostic Products Corporation as a solid-phase, no-boil assay.

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