SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Alpha-fetoprotein (AFP)

Device Trade Name: ARCHITECT AFP Assay
ARCHITECT AFP Calibrators
ARCHITECT AFP Controls

Device Procode: LOK

Applicant's Name and Address: Abbott Laboratories
Abbott Diagnostics Division
100 Abbott Park Road
Abbott Park, IL 60064-3500

Date of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P120008

Date of FDA Notice of Approval: November 28, 2012

Expedited: No

II. INDICATIONS FOR USE

Reagent kit

The ARCHITECT AFP Assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of alpha-fetoprotein (AFP) in:

1. Human serum or plasma to aid in monitoring disease progression during the course of disease and treatment of patients with nonseminomatous testicular cancer.

2. Human serum, plasma, and amniotic fluid at 15 to 21 weeks gestation to aid in the detection of fetal open neural tube defects (NTD). Test results when used in conjunction with ultrasonography or amniography are a safe and effective aid in the detection of fetal open NTD.

Calibrators

The ARCHITECT AFP Calibrators are for the calibration of the ARCHITECT *i* System when used for the quantitative determination of alpha-fetoprotein (AFP) in human serum,

plasma, and amniotic fluid. The performance of the ARCHITECT AFP Calibrators has not been established with any other AFP assays.

Controls

The ARCHITECT AFP Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT *i* System when used for the quantitative determination of alpha-fetoprotein (AFP) in human serum, plasma, and amniotic fluid. The performance of the ARCHITECT AFP controls has not been established with any other AFP assays.

III. CONTRAINDICATIONS

None.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the ARCHITECT AFP Reagent Kit labeling.

V. DEVICE DESCRIPTIONS

The ARCHITECT AFP Assay is a two-step immunoassay for the quantitative measurement of AFP in human serum, plasma, and amniotic fluid using CMIA technology, with flexible assay protocols, referred to as Chemiflex.

In the first step, sample and anti-AFP coated paramagnetic microparticles are combined. AFP present in the sample binds to the anti-AFP coated microparticles. After washing, anti-AFP acridinium-labeled conjugate is added to create a reaction mixture in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of AFP in the sample and the RLUs detected by the ARCHITECT i System optics.

Kit Configurations and Components

The ARCHITECT AFP Reagent Kit is comprised of the following components:

1. ARCHITECT AFP Microparticles: 1 or 4 bottle(s) (6.6 mL per 100-test bottle/27.0 mL per 500-test bottle) of Anti-AFP (mouse, monoclonal) coated microparticles in MES buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solids. Preservative: ProClin 300.

2. ARCHITECT AFP Conjugate: 1 or 4 bottle(s) (5.9 mL per 100-test bottle/26.3 mL per 500-test bottle) of Anti-AFP (mouse, monoclonal) acridinium-labeled conjugate in MES buffer with protein (bovine) stabilizer. Minimum concentration: 400 ng/mL.

Preservatives: antimicrobial agents and sodium azide.

The ARCHITECT AFP Calibrators contain 6 Bottles (4.0 mL each) of ARCHITECT AFP Calibrators A-F. Calibrator A contains buffer solution with protein (bovine) stabilizer. Calibrators B-F contain purified AFP (from human cord serum) prepared in buffer solution with protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

The ARCHITECT AFP Controls contain 3 Bottles (8.0 mL each) of ARCHITECT AFP Controls: Low, Medium, and High. ARCHITECT AFP Controls contain purified AFP (from human cord serum) prepared in buffer solution with protein (bovine) stabilizer. Preservatives: ProClin 300 and ProClin 950.

In addition, the following components are required for the ARCHITECT AFP Reagent Kit:

1. ARCHITECT *i* System is an analyzer designed to perform fully-automated immunoassay tests based on the use of CMIA detection technology.
2. ARCHITECT *i* Pre-Trigger Solution contains 1.32% (w/v) hydrogen peroxide.
3. ARCHITECT *i* Trigger Solution contains 0.35 N sodium hydroxide.
4. ARCHITECT *i* Wash Buffer contains phosphate buffered saline solution. Preservatives: antimicrobial agents.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

For monitoring nonseminomatous testicular cancer, alternative and additional practices include physical evaluation, chest radiography, ultrasound, abdominal and pelvic computed tomography (CT) scanning, contralateral testis examination, and other immunological devices for the quantification of other analytes in human serum and plasma.

The detection of neural tube defects (NTD) can be achieved by using the following alternative practices or procedures:

a. Ultrasonography
b. Aminography
c. Amniotic fluid acetylcholinesterase (AFAChE) testing
d. The use of other devices for which there are approved premarket applications.

VII. MARKETING HISTORY

The ARCHITECT AFP Assay (LN 3P36) has been marketed outside the United States since February 2012 as a CE-marked assay available in European Union countries. Registration in countries outside the European Union is in process. This device has not been withdrawn from marketing for any reason related to its safety or effectiveness.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Patients undergoing treatment for nonseminomatous testicular cancer should not experience

any adverse effects if test results from these in vitro devices are used as an aid in managing the cancer in conjunction with other routine medical practices and procedures and all available clinical information. A false positive result would indicate that a person may be incorrectly diagnosed as having testicular cancer whereas a false negative result would indicate no change in the patient's clinical status.

The extensive follow-up testing of AFP in maternal serum and amniotic fluid reduces to a very low likelihood, but does not completely eliminate the possibility that a healthy fetus may be incorrectly diagnosed as having an NTD. Special consideration needs to be given when interpreting AFP results from pregnancies where one twin appears by ultrasound to have suffered fetal demise or an open NTD while the other appears unaffected in order to avoid a misdiagnosis of the unaffected twin. In addition, an amniocentesis procedure that may be performed as a follow-up to an elevated maternal serum AFP result carries a small miscarriage risk. The physician should discuss the risk of miscarriage with the patient.

IX. SUMMARY OF NONECLINICAL STUDIES

A. Laboratory Studies

Nonclinical studies were performed at Abbott Laboratories to evaluate the performance characteristics of the ARCHITECT AFP Assay. The studies are described below.

1. Within-Laboratory Precision (20-Day)

A 20-day precision study was performed to evaluate the precision performance of the ARCHITECT AFP Assay based on the Clinical and Laboratory Standards Institute (CLSI) Guideline EP5-A2 (Evaluation of Precision Performance of Clinical Chemistry Devices). Testing was performed using 3 lots each of ARCHITECT AFP Reagents and Calibrators, 1 lot of ARCHITECT AFP Controls, and 4 instruments (2 each of *i*2000 and *i*2000$_{SR}$). Each reagent lot was matched with a different lot of calibrator. A single calibration per reagent lot was performed on each instrument by testing the calibrators in replicates of 2. The calibration generated for each reagent lot was stored on each instrument for the duration of the study. The controls (low, medium and high) and serum-based panels were tested with a minimum of 2 replicates 2 times per day (separated by a minimum of 2 hours) for a total of 20 testing days.

The results are summarized in the following table.

Table 1. ARCHITECT AFP Within-Laboratory Precision (20-Day)

Instrument	Reagent Lot	Sample	N	Mean (ng/mL)	Within-Run		Within-Laboratory Precision (Total)[a]	
					SD	%CV	SD	%CV
*i*2000$_{SR}$ (1)	1	Low Control	120	19.81	0.317	1.6	0.327	1.6
		Medium Control	120	199.11	3.165	1.6	3.263	1.6
		High Control	120	950.53	16.41	1.7	17.20	1.8
	2	Low Control	120	20.02	0.349	1.7	0.349	1.7
		Medium Control	120	195.29	2.725	1.4	3.043	1.6
		High Control	120	928.69	16.34	1.8	17.62	1.9
	3	Low Control	120	20.23	0.248	1.2	0.286	1.4
		Medium Control	120	198.45	2.743	1.4	3.058	1.5
		High Control	120	955.96	17.38	1.8	17.38	1.8
*i*2000$_{SR}$ (2)	1	Panel 1	120	3.01	0.070	2.3	0.082	2.7
		Panel 2	120	9.54	0.191	2.0	0.201	2.1
		Panel 3	120	577.58	13.13	2.3	13.97	2.4
		Panel 4	120	1514.74	41.43	2.7	47.76	3.2
		Panel 5	120	1763.53	43.35	2.5	51.11	2.9
	2	Panel 1	120	3.10	0.060	1.9	0.065	2.1
		Panel 2	120	9.67	0.188	1.9	0.202	2.1
		Panel 3	120	564.10	13.44	2.4	14.35	2.5
		Panel 4	120	1489.93	43.56	2.9	44.07	3.0
		Panel 5	120	1729.19	50.29	2.9	54.34	3.1
	3	Panel 1	120	3.15	0.061	1.9	0.068	2.2
		Panel 2	120	9.73	0.190	2.0	0.197	2.0
		Panel 3	120	559.72	12.05	2.2	12.05	2.2
		Panel 4	120	1490.94	43.96	2.9	45.61	3.1
		Panel 5	120	1743.06	53.14	3.0	55.15	3.2
i 2000 (1)	1	Low Control	120	19.53	0.403	2.1	0.419	2.1
		Medium Control	120	192.55	3.896	2.0	4.161	2.2
		High Control	120	925.64	20.13	2.2	22.57	2.4
	2	Low Control	120	19.60	0.460	2.3	0.476	2.4
		Medium Control	120	192.92	4.090	2.1	4.233	2.2
		High Control	120	917.39	25.48	2.8	26.69	2.9
	3	Low Control	120	19.46	0.412	2.1	0.438	2.2

Instrument	Reagent Lot	Sample	N	Mean (ng/mL)	Within-Run		Within-Laboratory Precision (Total)[a]	
					SD	%CV	SD	%CV
		Medium Control	120	194.92	3.602	1.8	3.602	1.8
		High Control	120	942.88	25.44	2.7	26.75	2.8
*i*2000 (2)	1	Panel 1	120	3.05	0.075	2.5	0.082	2.7
		Panel 2	120	9.63	0.204	2.1	0.205	2.1
		Panel 3	120	569.00	15.65	2.8	16.04	2.8
		Panel 4	120	1530.709	56.56	3.7	60.76	4.0
		Panel 5	120	1796.54	63.91	3.6	70.19	3.9
	2	Panel 1	120	3.10	0.070	2.3	0.074	2.4
		Panel 2	120	9.68	0.186	1.9	0.212	2.2
		Panel 3	120	553.43	13.33	2.4	13.97	2.5
		Panel 4	120	1454.43	46.28	3.2	49.52	3.4
		Panel 5	120	1693.81	53.64	3.2	56.31	3.3
	3	Panel 1	120	2.87	0.071	2.5	0.077	2.7
		Panel 2	120	9.31	0.237	2.6	0.262	2.8
		Panel 3	120	583.73	14.56	2.5	16.26	2.8
		Panel 4	120	1456.89	44.11	3.0	45.56	3.1
		Panel 5	120	1666.40	46.68	2.8	49.73	3.0

[a]Within-Laboratory (Total) variability contains within-run, within-day, and between-day variance components.

The total imprecision results for the ARCHITECT AFP Assay for each of the samples tested are shown below:

- 1.4 to 2.4 %CV for the low control
- 1.5 to 2.2 %CV for the medium control
- 1.8 to 2.9 %CV for the high control
- 2.1 to 2.4 %CV for panel 1
- 2.1 to 2.8%CV for panel 2
- 2.2 to 2.8 %CV for panel 3
- 3.0 to 4.0 %CV for panel 4
- 2.9 to 3.9 %CV for panel 5

2. *Limit of Blank (LOB), Limit of Detection (LOD), Limit of Quantitation (LOQ)*

A LoB, LoD and LoQ study was performed based on guidance from the CLSI document EP17-A (Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline). Four zero-level samples (Calibrator A, 0 ng/mL) were obtained for the study. Eight low-levels samples (2 samples at each of 4 unique target concentration levels of approximately 0.50, 1.00, 1.50, and 2.00 ng/mL) were prepared by gravimetrically diluting Calibrator B with Calibrator A. The samples were tested in a minimum of 3 replicates in five separate runs over a minimum of three days using 3 reagent lots and 2 instruments. The observed LoB was 0.00 ng/mL, the LoD was 0.04 ng/mL, and the LoQ was 2.0 ng/mL.

3. Spiked Recovery

A spiked recovery study was performed to evaluate the ability of the ARCHITECT AFP Assay to accurately recover known concentrations of AFP spiked into specimens. The recovery of the ARCHITECT AFP Assay was considered acceptable if the % recovery was:

a) 100±10% when analyzing serum samples spiked with known amounts of AFP (using the WHO 1st International Standard 72/225) across the measuring interval of the assay.
b) 100±10% when analyzing diluted amniotic fluid sample spiked with known amounts of AFP (using the WHO 1st International Standard 72/225) across the range of 312.5-1,250 ng/mL.

The study was performed with 16 low-level AFP serum specimens and 14 normal amniotic fluid specimens. The serum specimens were spiked with the WHO 1st International Standard 72/225 to create test samples across the measuring interval of the assay (2.0-2,000 ng/mL). The amniotic fluid specimens were spiked with the WHO 1st International Standard 72/225 and diluted 1:40 using the ARCHITECT *i* Multi-Assay Manual Diluent to create test samples with AFP concentrations within the range of 312.5 to 1250 ng/mL which would equate to 12.5 to 50 µg/mL. The samples were tested using the ARCHITECT AFP Assay on 1 instrument and the resulting percent recovery was calculated. For serum specimens, the mean percent recovery was 103.1% (range: 99.5% to 108.6%). For amniotic fluid specimens, the mean percent recovery was 101.2% (range: 95.1% to 107.3%).

4. Linearity

A linearity study was performed based on guidance from the CLSI document EP6-A (Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach). Three high AFP samples were prepared by diluting each of 3 neat AFP serum specimens with 3 low AFP serum specimens to a target concentration of 2,500 ng/mL (high samples). The three low AFP specimens had concentrations below the Limit of Quantitation (LoQ) of 2.0 ng/mL (low samples). These high AFP samples were further diluted to create three sets of 11 samples with the three low AFP specimens. Three additional samples were prepared by diluting the low

samples with ARCHITECT *i* Multi-Assay Diluent. All samples were tested as a set within a single run. The ARCHITECT AFP Assay demonstrated linearity from 0.91 ng/mL to 2000 ng/mL.

5. *Interference/Analytical Specificity*

i. Potentially Interfering Substances

To assess effects of interfering substances on the ARCHITECT AFP Assay, a variety of substances (endogenous substances, human anti-mouse antibodies (HAMA), rheumatoid factor (RF), medications/therapeutic drugs and other potentially cross-reactive substances in human serum/plasma) were evaluated based on guidance from the CLSI document EP7-A2 (Interference Testing in Clinical Chemistry – Approved Guideline, 2nd Edition).

For the endogenous substances (bilirubin, hemoglobin, total protein and triglycerides), each was spiked into a minimum of 13 replicate serum samples with 2 levels of AFP (approximately 10 and 1000 ng/mL). The spiked and unspiked samples were assayed, and the AFP concentrations of the spiked samples were compared to the samples that were not spiked with interfering substance. The % interference was calculated as [(Mean/Median Test Result) – (Mean/Median Reference Result)]/(Mean/Median Reference Result) x 100. The ARCHITECT AFP Assay was considered not susceptible to the endogenous interferents under evaluation if the % difference (bias) in AFP concentration was within or equal to ±10% of the corresponding reference sample for the low and high analyte levels (approximately 10 and 1,000 ng/mL, respectively). The results are summarized the table below.

Table 2. Potentially Interfering Endogenous Substances

Potentially Interfering Endogenous Substance	High Test Level	% Interference	
		10 ng/mL AFP	1000 ng/mL AFP
Bilirubin (Unconjugated)	20 mg/dL	-0.5	0.3
Bilirubin (Conjugated)	20 mg/dL	-0.9	-0.8
Hemoglobin	500 mg/dL	-0.2	-1.3
Total Protein	12 g/dL	2.9	-0.2
Triglycerides	3000 mg/dL	-1.0	-1.9

For HAMA and RF interferences, serum specimens from individuals with the substances were divided into 3 samples. Two of the samples were spiked to 2 levels of AFP (approximately 10 and 1000 ng/mL). The unspiked and spiked samples were assayed, and the AFP concentrations of the spiked samples were compared to the samples that were not spiked with interfering substance. The %recovery was calculated as [(Mean/Median Spiked Result) – (Mean/Median

Unspiked Result)]/(Mean/Median Amount AFP Added) x 100. The acceptance criterion was ±10%. The results are summarized in table below.

Table 3. HAMA and RF Interference

Potentially Interfering Substances	N	% Recovery	
		10 ng/mL AFP	1000 ng/mL AFP
Human Anti-Mouse Antibodies	13	104.7	105.9
Rheumatoid Factor	13	104.6	102.3

For medication/therapeutic drugs and potentially cross-reactive substances found in patient specimens, the substances were spiked into a minimum of 13 serum samples with 2 levels of AFP (approximately 10 and 1000 ng/mL). The unspiked and spiked samples were assayed, and the AFP concentrations of the spiked samples were compared to the reference samples. The %interference was determined as [(Mean/Median Test Result) – (Mean/Median Reference Result)]/(Mean/Median Reference Result) x 100. The acceptance criterion was ±10%. The results are summarized in the table below.

Table 4. Drug and Potentially Cross-reactive Substances

Potential Interferent	High Test Level	% Interference	
		10 ng/mL AFP	1000 ng/mL AFP
5-Fluorouracil	3 mmol/L	-0.4	0.3
Acetaminophen	6.5 mg/mL	-3.1	-3.1
Albumin	160 mg/mL	2.4	-4.1
Alpha-1-Acid Glycoprotein	2 mg/mL	0.2	-1.1
Alpha-1-Antitrypsin	5 mg/mL	8.1	0.3
Alpha-2-Macroglobulin	9 mg/mL	0.1	-0.1
Aspirin	10 mg/mL	-4.7	-4.9
Bleomycin	1000 µU/mL	-2.5	-4.3
Carboplatin	0.432 mg/mL	0.3	1.3
Ceruloplasmin	2.5 mg/mL	-0.3	-0.6
Chorionic Gonadotropin	1000 IU/mL	-1.1	-2.2
Cisplatin	1000 µg/mL	-0.6	-1.2
Cyclophosphamide	1437 µmol/L	0.3	-0.7
Etoposide	30 µg/mL	-0.8	0.3
Gamma-Globulins	30 mg/mL	-2.7	-2.4

Potential Interferent	High Test Level	% Interference	
		10 ng/mL AFP	1000 ng/mL AFP
Haptoglobin	6 mg/mL	0.7	-1.0
Ifosfamide	249 µg/mL	-3.1	-2.7
Methotrexate	2 mmol/L	-0.6	-0.5
Placental Lactogen	100 µg/mL	-3.3	-3.6
Prolactin	500 ng/mL	-4.8	-5.0
Transferrin	25 mg/mL	-1.6	-3.4
Vinblastine	500 µg/mL	-3.4	-3.5
Vincristine	1000 ng/mL	-3.1	-4.4

ii. Specimen Collection Tube Type Interference

A tube type interference study was performed to evaluate whether specific blood collection tube types are suitable for use with the ARCHITECT AFP Assay. Specimens were collected from a minimum of 40 different donors in the control (serum/plastic) tube type and the blood collection tube types selected for evaluation. The specimens were spiked with AFP positive stock to obtain AFP target concentrations spanning the measuring interval of the assay (unspiked to 1,750 ng/mL). Passing-Bablok regression plots were presented for each of the comparisons. The tube types were considered acceptable if, when compared to the control tube type, the difference in measured concentration of AFP was within or equal to ±10% across the measuring interval of the assay.

Evaluation Tube type	N	Shapire-Wilk-p-Value	Mean/Median % Difference	SD	Lower One-Sided 95% CL	Upper One-Sided 95% CL
Serum separator, plastic	46	0.0737	-0.2	5.20	-1.5	1.1
Sodium heparin	46	0.8901	-0.8	5.24	-2.1	0.5
Lithium heparin	46	0.0182	-0.4	5.43	-1.7	1.0
Dipotassium EDTA	46	0.2819	-0.2	5.23	-1.5	1.1
Sodium EDTA	46	0.0561	-0.9	4.98	-2.1	0.4

The results support the use of the following blood collection tube types with the ARCHITECT AFP Assay:

- Serum, plastic
- Serum separator, plastic

- Sodium heparin, plastic
- Lithium heparin, plastic
- Dipotassium EDTA, plastic
- Sodium EDTA, glass

6. *Within-Assay Sample Carryover*

A study was performed to evaluate the susceptibility of the ARCHITECT AFP Assay to within-assay sample carryover. Three replicates of wash buffer were tested to clear the system. A single replicate of a negative sample (*i.e.*, ARCHITECT AFP Calibrator A) was tested to serve as a sample that was not exposed to potential sample carryover (protected sample). This was followed by a single replicate of a high-level AFP serum sample (targeted to $\geq$ 1,000,000 ng/mL), then by a single replicate of the negative sample to serve as a sample exposed to potential sample carryover (unprotected sample). The sequence of wash buffer, protected negative sample, high sample, and unprotected negative sample was repeated an additional 12 times for a total of 13 iterations.

The difference between the protected negative sample and the unprotected negative sample was 0.07 ng/mL, demonstrating that the ARCHITECT AFP Assay is not susceptible to within-assay sample carryover.

7. *Verification of Dilution*

An autodilution verification study was performed to evaluate the performance of the ARCHITECT AFP automated dilution protocols versus the manual dilution methods. Twenty-one serum samples were evaluated with the 1:10 autodilution method versus a 1:10 manual dilution method. Fifteen amniotic fluid samples were evaluated with the 1:40 autodilution method versus a 1:40 manual dilution method. The performance of the ARCHITECT AFP automated dilution protocol was considered acceptable if the mean difference in concentration was within + 10% when comparing the autodiluted samples to the manually diluted samples.

For serum samples, the mean percent difference was 2.9% (range: -5.8% to 10.9%) and for amniotic fluid samples, the mean percent difference was 4.6% (range: -1.1% to 11.2%).

8. *High Dose Hook Effect*

A high dose hook study was performed to verify that the ARCHITECT AFP Assay does not exhibit a high dose hook effect when the assay is used to test specimens containing high levels of AFP. A high AFP sample was prepared with a concentration of 10,000,000 ng/mL by resuspending lyophilized AFP from purified human cord serum in normal human serum. Serial dilutions of the high AFP sample

were prepared using normal human serum according to the following table:

Dilution Factor (Dilution Level)	Volume of High AFP Sample	Volume of Normal Human Serum	Approximate AFP Concentration
Neat (Level 1)	1.0 mL	0 mL	10,000,000.0
1:8 (Level 2)	0.25 mL of Level 1	1.75 mL	1,250,000.0
1:64 (Level 3)	0.25 mL of Level 2	1.75 mL	156,250.0
1:512 (Level 4)	0.25 mL of Level 3	1.75 mL	19,531.25
1:4,096 (Level 5)	0.25 mL of Level 4	1.75 mL	2,441.41
1: 32,768 (Level 6)	0.25 mL of Level 5	1.75 mL	305.18

The mean and SD of the concentrations values were calculated for each sample that had all replicates within the measuring interval of the assay (≥ 2.0 ng/mL to ≤ 2,000 ng/mL). Each replicate of sample with a target concentration of ≥2,000 ng/mL was evaluated against the evaluation criteria. The mean and SD of the RLU values were calculated for each sample. The RLU values for Calibrator F through the highest sample concentration tested were plotted against their respective target concentrations. The upper 99% RLU tolerance limit (95% confidence) was calculated for the samples. The ARCHITECT AFP Assay was considered not susceptible to high dose hook effect if:

i. The results were reported >2,000 for samples ranging in AFP concentration from within 2,500 to 2,750 ng/mL to ≥10,000,000 ng/mL and
ii. The upper 99% RLU tolerance limit (95% confidence) of the ARCHITECT AFP Calibrator F did not overlap with the lower 99% RLU tolerance limit (95% confidence) of a sample with AFP concentration of ≥10,000,000 ng/mL.

The upper 99% RLU tolerance limit (95% confidence) of the ARCHITECT AFP Calibrator F was 2,354,106 and the lower 99& RLU tolerance limit (95% confidence) of the neat high AFP sample was 2,691,425, therefor the two values did not overlap. These results demonstrate that the ARCHITECT AFP Assay is not susceptible to high dose hook effect when testing samples with high levels of AFP.

9. Specimen Storage Stability

a. Serum and Plasma

A specimen storage study was performed to evaluate the effect of specimen handling under various conditions (room temperature storage, 2 to 8°C storage, and freeze/thaw) and time periods on serum and plasma specimens when tested with the ARCHITECT AFP Assay. A minimum of 13 specimens were collected from healthy individuals in each of the following blood collection tube types (serum, plastic or lithium heparin, plastic). AFP stock solutions were prepared

by spiking AFP, purified from human cord serum, into normal human serum to obtain target concentrations of 250 and 25,000 ng/mL.

AFP-positive stock solutions were used to supplement blood collection tubes to 2 target concentrations, 10 ng/mL and 1,000 ng/mL. The samples were supplemented on the clot or cells before the tubes were centrifuged according to the manufacturer instructions. Samples were tested at each time point for the following test conditions:

i. Condition A: (on the clot or cells)
   Time point 1 (baseline) - Samples were tested within 8 hours of draw.
   Condition B and E used Condition A as the baseline time point.
   Conditions C and D used separate baseline time points as noted below.

ii. Condition B: 2 to 8°C storage (on the clot or cells)
   Time point 2 - Samples stored at 2 to 8ºC for > 7 days
   Condition C: Approximately 22°C Storage (room temperature) (on the clot or cells)
   Time point 1 (baseline) - Samples were tested within 8 hours of draw.
   Time point 2 - Samples stored at ~22°C for > 24 hours
   Time point 3 - Samples stored at ~22°C for > 3 days

iii. Condition D: Approximately 30°C Storage (room temperature) (on the clot or cells)
   Time point 1 (baseline) - Samples were tested within 8 hours of draw.
   Time point 2 - Samples stored at ~30°C for > 24 hours
   Time point 3 - Samples stored at ~30°C for > 3 days

iv. Condition E: Freeze/Thaw Cycles# (off the clot or cells)
   Time point 2 - Samples subjected to 3 freeze/thaw cycles
   Time point 3 – Samples subjected to 5 freeze/thaw cycles

Note: The samples for Condition E were stored at 2 to 8ºC for a minimum of 7 days prior to subjecting the samples to the freeze/thaw cycles

Time point 1 served as the baseline time point for each of the test conditions.

Samples were tested in a minimum of 2 replicates at baseline (Condition A or time point 1) and after each of the test conditions using one lot each of ARCHITECT AFP Reagents, Calibrators, and Controls on a minimum of 1 ARCHITECT *i*2000SR instrument. The specimen storage of serum and plasma was considered acceptable if the ARCHITECT AFP Assay had a difference in measured concentration of AFP within or equal to + 10 % when comparing baseline control specimens tested within 8 hours from draw on the cells/clot to the same samples stored at the following conditions:

i. 2 to 8°C for > 7 days

ii. room temperature for > 24 hours
iii. > 3 freeze/thaw cycles

The results showed that specimens that were:

i. stored at 2 to 8°C for > 7 days yielded a mean (or median) % difference from baseline ranging from -0.1% to 0.4%
ii. stored at approximately 22°C for > 24 hours or > 3 days yielded a mean (or median) % difference from baseline ranging from -1.3% to 0.2%
iii. stored at approximately 30°C for > 24 hours or > 3 days yielded a mean (or median) % difference from baseline ranging from -0.5% to 0.7%
iv. subjected to 3 or 5 freeze/thaw cycles yielded a mean (or median) % difference from baseline ranging from -2.2% to -0.3%.

The data demonstrate that specimens stored at 2 to 8°C for up to 7 days, specimens stored at room temperature (approximately 22 to 30°C) for up to 3 days, and specimens subjected to 5 freeze thaw cycles are acceptable for use in the ARCHITECT AFP Assay.

b. Amniotic Fluid

A specimen storage study was performed to evaluate the effect of specimen handling under various conditions (room temperature storage, 2 to 8°C storage, and freeze/thaw) and time periods on amniotic fluid specimens when tested with the ARCHITECT AFP Assay. Frozen amniotic fluid specimens representing a minimum of 13 donors were obtained. The specimens were tested using the ARCHITECT AFP Assay to determine the endogenous AFP level of each specimen. Specimens that were not within the desired AFP range of 12.5 to 50 µg/mL (*i.e.*, 12,500 to 50,000 ng/mL) were supplemented with AFP purified from human cord serum to obtain concentrations within the desired AFP range. Samples were tested at each time point for the following test conditions:

i. Condition A:
   Time point 1 (baseline)
ii. Condition B: 2 to 8°C storage
   Time point 2 - Samples stored at 2 to 8ºC for > 1 day
   Time point 3 - Samples stored at 2 to 8ºC for > 3 days
   Time point 4 - Samples stored at 2 to 8ºC for > 5 days
iii. Condition C: Approximately 22°C Storage (room temperature)
   Time point 2 - Samples stored at ~22°C for > 24 hours
   Time point 3 - Samples stored at ~22°C for > 2 days
iv. Condition D: Approximately 30°C Storage (room temperature)
   Time point 2 - Samples stored at ~30°C for > 24 hours
   Time point 3 - Samples stored at ~30°C for > 2 days
v. Condition E: Freeze/Thaw Cycles
   Time point 2 - Samples subjected to 1 freeze/thaw cycle
   Time point 3 - Samples subjected to 2 freeze/thaw cycles

Time point 4 - Samples subjected to 3 freeze/thaw cycles

Samples were tested using the 1:40 automated dilution procedure in a minimum of 2 replicates at baseline (Condition A or time point 1) and after each of the test conditions using one lot each of ARCHITECT AFP Reagents, Calibrators, and Controls on 1 ARCHITECT *i*2000SR instrument. The specimen storage of amniotic fluid was considered acceptable if the ARCHITECT AFP Assay had a difference in measured concentration of AFP within or equal to + 10 % when comparing baseline control specimens to the same samples stored at 2 to 8°C, room temperature, and exposed to freeze/thaw cycling.

The results showed that specimens that were:

i. stored at 2 to 8°C for > 1 day, > 3 days, or > 5 days yielded a mean (or median) % difference from baseline ranging from -0.5% to 0.2%
ii. stored at approximately 22°C for > 24 hours or > 2 days yielded a mean (or median) % difference from baseline ranging from -1.8% to -0.2%
iii. stored at approximately 30°C for > 24 hours or > 2 days yielded a mean (or median) % difference from baseline ranging from -2.0% to -1.7%
iv. subjected to 1, 2, or 3 freeze/thaw cycles yielded a mean (or median) % difference from baseline ranging from -1.1% to -0.6%.

The data demonstrate that specimens stored at 2 to 8°C for up to 5 days, specimens stored at room temperature (approximately 22 to 30°C) for up to 2 days, and specimens subjected to 3 freeze thaw cycles are acceptable for use in the ARCHITECT AFP Assay.

c. Sample On Board Stability

A sample on board stability study was performed to evaluate serum samples when stored on the ARCHITECT *i* System (on board storage) and tested using the ARCHITECT AFP Assay. Normal human serum was supplemented with AFP-positive stock solution to yield AFP samples at the two desired analyte levels:

i. Low AFP sample with a target concentration value of 10 ng/mL
ii. High AFP sample with a target concentration values of 1,000 ng/mL.

Both high and low samples (a minimum of 13 sample cups) were immediately tested (baseline, time point 1) or stored on board the instrument for more than 3 hours (time point 2). The same instrument, lot of reagent and calibrators/controls were used for both sets of tests. The sample on board stability was acceptable if, when comparing samples stored on board the instrument for ≥ 3 hours to samples tested immediately upon loading on the instrument, the % difference (bias) was within or equal to ±10 % for the low and

high AFP samples. The mean percent difference was 7.4% for the low AFP sample (~10ng/mL) and 1.9% for the high AFP sample (~1,000 ng/mL). The results support sample storage of up to 3 hours on board the ARCHITECT *i* System when tested using the ARCHITECT AFP Assay.

10. ARCHITECT AFP Reagent Stability

The reagent stability is an on-going study to establish the stability of the ARCHITECT AFP Reagents at the intended storage condition of 2 to 8°C and during on-board storage.

a. Reagent Real-Time Stability

Intended storage condition testing is performed on 3 lots of 100-test kit reagents and 3 lots of 500-test kit reagents on the ARCHITECT *i*2000/*i*2000SR. The testing is scheduled to continue for a maximum of 18 months. The components tested consisted of microparticles and conjugate. The reagents were stored continuously at 2 to 8ºC before testing at the designated time points. All time points were performed on the ARCHITECT *i*2000SR. Time point 0 consisted of 3 runs per reagent lot. The month 1 through month 12 time points consisted of a minimum of 1 run per reagent lot. For all time points, the on-test reagents were tested using a minimum of 10 replicates of the reference calibrators, controls, and panels. The expiration dating for the ARCHITECT AFP Reagents was established by evaluating:

i. the individual concentration (ng/mL) values or an RLU ratio against the stability action limits
ii. the percent shift calculated from the regressed mean concentration values (ng/mL) against the stability shift allowances
iii. the slope of the very high panel to Calibrator F ratio for significance.

Using the slope from the regression analysis, the percent shift from baseline was calculated at 12 months and evaluated against the maximum allowable stability shift that was determined through the specification setting process. At the time of the submission, the intended storage data meet the maximum percent stability shift allowance for the controls and serum panels for the ARCHITECT AFP Reagents over the 12-month time period tested at the intended storage condition (2 to 8°C). Therefore, the data support 12 months of expiration dating for the ARCHITECT AFP Reagents.

b. On-Board Storage Stability

On board storage condition testing is performed on 3 lots of 100-test kit reagents and 3 lots of 500-test kit reagents on the ARCHITECT *i*2000/*i*2000SR. The ARCHITECT AFP reagents were stored on board the ARCHITECT *i* System

while the instrument was in continuous running mode (on board evaluation). A minimum of 16 time points were performed over a minimum of 31 days. A single calibration was performed for each reagent lot during the first time point (baseline) by testing the calibrators in replicates of 2 with each reagent lot. Each time point consisted of a low AFR serum sample (targeted to 10 ng/mL) and the ARCHITECT AFP High Control (targeted to 1,000 ng/mL). Two instruments were used. The reagent on board evaluation for the ARCHITECT AFP Assay was considered acceptable if the shift from baseline over the course of 30 days was no more than ± 10% for the test kit. The test mean percent shifts ranged from -3.9% to -0.5% for the low AFP sample and -3.3% to -1.9% for the ARCHITECT control. The results support a 30-day storage of the ARCHITECT AFP reagent kit on board the ARCHITECT *i* System while the instrument is in continuous running mode.

c. Transport Simulation Stability

The reagent transport simulation stability is an on-going study to confirm that the ARCHITECT AFP Reagents can be shipped at ambient conditions from the Abbott Diagnostic Division (ADD) site to international customers. Reagents were tested at various monthly time points (6, 9, 10 and 12) after they have been subjected to elevated and freezing temperature stress conditions to simulate transport conditions using 1 lot of 100-test kit reagents on the ARCHITECT *i*2000/*i*2000SR. Reagent kits were subjected to the stress conditions presented in the table below and were designated as the elevated temperature on-test (stressed) reagents. The components tested included Calibrators A-F, Low Control, Medium Control, High Control, Low Panel (20 ng/mL), Medium Panel (200 ng/mL), High Panel (1,500 ng/mL) and Very High Panel (2,700 ng/mL).

Elevated temperatures:

Temperature Condition	Duration
15-30ºC	161.6-174.0 hours (6.8-7.25 days)
25ºC ± 2ºC	71.7-78.0 hours (3-3.25 days)
30ºC ± 2ºC	6.1-6.5 hours
37ºC ± 2ºC	72-76 hours (3 days)
40ºC ± 2ºC	1.0-1.25 hours

Freezing conditions:

Temperature Condition	Duration
15-30ºC	96-100 hours (4 Days)
-20ºC or colder	24-28 hours (1 Day)

For each time point, testing was performed using a minimum of 4 replicates each of reference calibrator, control, and panel on at least 1 ARCHITECT *i*2000SR instrument using the designated stressed reagent kits. The reagent transport simulation stability is scheduled to continue for a maximum of 18

months. To date, the above reagent transport simulation stability meet the action limits over the 12-month time period tested. Therefore, the data support the stability of the ARCHITECT AFP Reagents, with 12 months of dating, following transport at ambient temperatures from the ADD (Wiesbaden, Germany) site to international customers.

11. ARCHITECT AFP Calibrator and Control Stability

a. Calibrator and Control Real-Time Stability

The calibrator and control stability is an on-going study to establish the stability (expiration dating) of the ARCHITECT AFP Calibrators and Controls at the intended storage condition of 2 to 8°C. The testing was performed at 6, 9, 10 and 12 months. Testing was performed on 3 lots each of calibrators and controls on the ARCHITECT *i*2000/*i*2000$_{SR}$. The components tested were Calibrator A-F, Low Control and Medium Control.

The calibrators and controls were stored continuously at 2 to 8ºC before testing at the designated time points. The reference materials were all stored at or below -20 C. All time points are performed on the ARCHITECT *i*2000SR. Time point 0 consisted of 3 runs per calibrator and control lot. The Month 1 through Month 12 time points consisted of a minimum of 1 run per calibrator and control lot. For all time points, the on-test calibrators were evaluated by testing the on-test calibrators, reference calibrators, reference controls, and reference panels in a minimum of 10 replicates each using the reference reagents. For all time points, the on-test controls were evaluated by testing the on-test controls, reference calibrators and reference controls in a minimum of 10 replicates each using the reference reagents. Stability of ARCHITECT AFP Calibrators and Controls was established by evaluating:

i. the individual concentration (ng/mL) values or an RLU ratio against the stability action limits
ii. the percent shift calculated from the regressed mean concentration values (ng/mL) against the stability shift allowances
iii. the slope of the very high panel to Calibrator F ratio for significance.

The testing is scheduled to continue for a maximum of 18 months. To date, the data meet the maximum percent stability shift allowance for the ARCHITECT AFP Calibrators and Controls over the 12-month time period. Therefore, the data support 12 months of expiration dating for the ARCHITECT AFP Calibrators and Controls.

b. Calibrator and Control Transport Simulation Stability

The calibrator and control transport simulation stability is an on-going study to confirm that the ARCHITECT AFP Calibrators and Controls can be shipped at ambient conditions from the Abbott Diagnostic Division (ADD) site to international customers. Calibrators and controls were tested at various monthly time points (6, 9, 10 and 12 months) after they have been subjected to elevated and freezing temperature stress conditions to simulate transport conditions using 1 lot each of calibrators and controls on at least 1 ARCHITECT *i* 2000SR instrument. The on-test calibrators were evaluated by testing the on-test calibrators, reference controls, and reference panels in a minimum of 4 replicates each. The components tested were Calibrators A-F, Low Control and Medium Control.

Elevated temperatures:

Temperature Condition	Duration
15-30ºC	161.6-174.0 hours (6.8-7.25 days)
25ºC ± 2º C	71.7-78.0 hours (3-3.25 days)
30ºC ± 2ºC	6.1-6.5 hours
37ºC ± 2ºC	72-76 hours (3 days)
40ºC ± 2ºC	1.0-1.25 hours

Freezing conditions:

Temperature Condition	Duration
15-30ºC	96-100 hours (4 Days)
-20ºC or colder	24-28 hours (1 Day)

The reference materials were all stored at or below -20ºC. The calibrator and control transport simulation stability is scheduled to continue for a maximum of 18 months. To date, the above calibrator and control transport simulation stability meet the action limits over the 12-month time period tested. Therefore, the data support the stability of the ARCHITECT AFP Calibrators and Controls, with 12 months of dating, following transport at ambient temperatures from the ADD site to international customers.

12. Microbial Challenge Characterization

The Microbial Challenge Characterization (MCC) evaluation for the ARCHITECT AFP Reagents, Calibrators, and Controls consists of an Antimicrobial Effectiveness Testing (AET) evaluation, which establishes the level of antimicrobial protection provided by the preservative formulation, and a Microbial Interference Characterization (MIC) evaluation, which demonstrates the effect of microbial bioburden and/or its by-products on assay performance. The MCC evaluation integrates the results from both AET and MIC to determine that the product is adequately protected.

a. Antimicrobial Effectiveness Testing (AET)

An AET study was performed to establish the level of antimicrobial protection provided by the preservative formulation of the ARCHITECT AFP Reagents, Calibrators, and Controls. The on-test materials were inoculated at a concentration of 10^5 to 10^6 colony forming units per mL (CFU/mL) with each of the following microbial organisms:

Group	Organism	Organism Type
I	*Candida albicans* (*C. albicans*)	Fungal
II	*Aspergillus niger (A. niger)*[a]	Fungal
III	*Escherichia coli (E. coli)*	Bacterial
IV	*Pseudomonas aeruginosa (P. aeruginosa)*	Bacterial
VII	*Staphylococcus aureus (S. aureus)*	Bacterial

[a]*Aspergillus niger* has been renamed *Aspergillus brasiliensis*, but will be referenced as *Aspergillus niger* or *A. niger* in this study.

Control materials (uninoculated) were prepared by inoculating the material under evaluation with sterile saline. On Days 14 and 28, the uninoculated and inoculated materials were plated onto agar petri plates, incubated, and examined for growth. The number of colony forming units was counted.

With the exception of the microparticle diluent inoculated with *A. niger* at Time Point 4, the results were cidal for all microbial groups that were tested for all materials and time points. Therefore, the final AET results were *fungicidal/bactericidal* for these materials. The result from the microparticle diluent inoculated with *A. niger* at Time Point 4 was static. Therefore, the final AET results were *fungistatic/ bactericidal* for this material.

b. Microbial Interference Characterization Testing

A microbial interference characterization study was conducted to demonstrate the effect of microbial bioburden and/or its by-products on the assay performance of the ARCHITECT AFP Reagents, Calibrators, and Controls. The materials were inoculated at a concentration of 10^3 to10^4 CFU/mL with each of the microbial organisms listed below:

Group	Organism	Organism Type
I	*Candida albicans* (*C. albicans*)	Fungal
II	*Aspergillus niger (A. niger)*[a]	Fungal
III	*Escherichia coli (E. coli)*	Bacterial
IV	*Pseudomonas aeruginosa (P. aeruginosa)*	Bacterial
V	*Pseudomonas* species *(fluorescens* group*)*	Bacterial
VI	*Staphylococcus aureus (S. aureus)*	Bacterial

[a] *Aspergillus niger* has been renamed *Aspergillus brasiliensis*, but will be

referenced as *Aspergillus niger* or *A. niger* in this study.

Control materials (uninoculated) were prepared by inoculating the material under evaluation with sterile saline.

The date of inoculation was recorded as Day 0. The material was incubated at 2 to 8°C for a minimum of 35 days. Testing was initiated within seven days after Day 35. Testing was performed using one ARCHITECT *i* 2000$_{SR}$ instrument.

The ARCHITECT AFP Reagents (Microparticles and Conjugate), Calibrators, and Controls were not sensitive to the microbial organisms when inoculated at 10^3 to 10^4 CFU/mL.

iii. Microbial Challenge Characterization Conclusion

The overall microbial challenge characterization data indicate that the ARCHITECT AFP Reagents, Calibrator, and Controls are adequately protected.

13. Traceability

The ARCHITECT AFP Assay, calibrators and controls are traceable to the WHO 1st International Standard 72/225.

14. Preparation and Characterization of Anti-AFP Antibodies

This new version of ARCHITECT AFP Assay differs from the originally approved AFP assay in the anti-AFP antibodies used for coating the microparticles and the conjugate.

The ARCHITECT AFP Assay utilizes one antibody and one antibody fragment. Both are manufactured at the Dartford facility. The antibody, Anti-AFP Monoclonal Antibody (E3H219C1), is used in the ARCHITECT AFP microparticles and the other antibody, purified Anti-AFP (E3H31C2), is digested and used in the ARCHITECT AFP Conjugate.

The hybridoma cell line that produces antibodies reactive with AFP determinants was generated using a polyethylene glycol-mediated procedure which fuses spleen cells [from a BALB/c mouse that was immunized with native AFP] with the mouse myeloma cell line SP2/0. The resulting mouse monoclonal hybridoma cell line secreting anti-AFP was designated as E3H219C1 and has been characterized as described below. Mouse monoclonal AFP antibodies were obtained from the hybridoma cell line grown in hollow fiber cell culture. The antibody immunoreactivity

was confirmed by Western blot with human AFP (Scripps antigen).

The class and isotype of the antibody were determined by Roche IsoStrip. The isotype of the antibody secreted by the cells is IgG2a and the immunoglobulin light chain of this antibody is of the kappa family. Characterization testing was performed on the hybridoma cell line to demonstrate safety and efficacy, which included tests for cell bank viability and purity, as well as tests for identity, purity, stability, and suitability. The results demonstrated that the anti-AFP murine hybridoma E3H219C1 cell line and its cell banks are of acceptable quality and provide a reliable and predictable source of cells to support the manufacture of anti-APP monoclonal antibody. An anti-AFP antibody harvest is thawed, pooled, and centrifuged. The centrifuged pool is diluted with 0.14M sodium phosphate/0.1% sodium azide solution and mixed. The diluted pool is loaded onto a Protein-A column. The antibody is eluted, and the elution peak is collected. The pH of the eluted peak is measured and adjusted, if necessary. The volume and A280 of the pool are measured and the protein concentration is determined. The concentration is adjusted, if necessary, and diafiltered. At this step, the diafiltered pool is allowed to be stored up to 24 hours at 2 to 8°C until further processing. The antibody is centrifuged and filtered. The volume and A280 of the pool are measured and the final protein concentration is determined. Purified anti-AFP IgG (2a) is divided into aliquots and stored at -10°C or colder. The identity of the final product is determined by SDS-PAGE and Dot blotting and its purity is determined by GPC-HPLC and SDS-PAGE.

B. Animal Studies

None.

C. Additional Studies

1. System Reproducibility (5-Day Precision)

The ARCHITECT AFP Assay is designed to have an imprecision of ≤ 7.5% within-laboratory (Total) %CV for samples between 10 and 2000 ng/mL and an SD of ≤ 0.75 for samples less than 10 ng/mL down to the LoQ (*i.e.*, 2.0 ng/mL).

A 5-day precision study was performed for the ARCHITECT AFP Assay based on guidance from the CLSI document EP15-A2. Testing was conducted at 3 clinical sites (Milton S. Hershey Medical Center, Hershey, PA, ARUP Laboratories, Institute for Clinical and Experimental Pathology, Salt Lake City, UT and Minneapolis Medical Research Foundation, Minneapolis, MN) using 3 lots each of ARCHITECT AFP Reagents, Calibrators, and Controls and 1 ARCHITECT

*i*2000/*i*2000SR instrument per site. Three controls and 5 human serum panels were assayed in replicates of 4 at 2 separate times of day for 5 days. The results are summarized in the following table. The data are summarized in the following table.

Table 5. System Reproducibility (5-Day Precision on ARCHITECT *i* 2000/*i* 2000SR) (All Sites, All Reagent Lots)

Sample	N	Grand Mean (ng/mL)	Within-Run		Within-Day		Within-Laboratory Precision (Total) [a]		Reproducibility with Additional Component of Between-Site		Reproducibility with Additional Component of Between-Lot		Reproducibility with Additional Components of Site and Lot (Overall) [b]	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
Low	360	19.46	0.685	3.5	0.747	3.8	0.747	3.8	0.797	4.1	0.813	4.2	0.830	4.3
Medium	360	203.65	7.502	3.7	7.987	3.9	7.987	3.9	13.984	6.9	17.293	8.5	17.293	8.5
High	360	973.10	45.461	4.7	46.393	4.8	46.450	4.8	63.066	6.5	65.166	6.7	65.891	6.8
Panel 1	360	2.95	0.114	3.9	0.127	4.3	0.128	4.4	0.239	8.1	0.278	9.4	0.278	9.4
Panel 2	360	9.47	0.355	3.7	0.359	3.8	0.377	4.0	0.506	5.3	0.486	5.1	0.527	5.6
Panel 3	360	591.26	25.303	4.3	25.620	4.3	26.503	4.5	42.717	7.2	50.677	8.6	50.677	8.6
Panel 4	360	1511.80	70.406	4.7	75.992	5.0	81.050	5.4	90.629	6.0	90.629	6.0	90.629	6.0
Panel 5	360	1743.35	79.509	4.6	86.121	4.9	89.956	5.2	100.632	5.8	100.632	5.8	100.632	5.8

[a] Within-Laboratory (Total) variability contains within-run, within-day, and between-day variance components.

[b] Overall variability contains within-run, within-day, between-day, between-lot, between-site and lot-site interaction variance components.

2. Method Comparison Studies

A study was performed to compare the investigational ARCHITECT AFP to the Beckman Coulter Access AFP assay. A total of 1,472 serum and 692 amniotic fluid specimens from the clinical study were available for method comparison evaluation. All serum specimens having results within the measuring interval of the ARCHITECT AFP Assay (2.00 ng/mL to 2000.00 ng/mL) and all amniotic fluid specimens, with corresponding results on the Beckman assay, were eligible for method comparison. Each specimen, in singlicate, was tested with the ARCHITECT AFP Assay using the ARCHITECT *i*2000/*i*2000SR system and the comparator AFP assay. Each specimen was tested in replicates of 1 with one of 3 lots of ARCHITECT AFP Reagents, Calibrators, and Controls and in replicates of 2 with one of 2 lots of the comparator assay (Beckman Coulter Access DxI AFP)

reagents and calibrators. The mean result of the 2 replicates generated with the comparator assay was used in the analysis. Passing-Bablok and Deming regression were performed. The results are summarized in the following tables.

Table 6. Passing-Bablok Regression Analysis: Serum (n = 1472)

ARCHITECT AFP (ng/mL)		Comparator AFP (ng/mL)		Correlation Coefficient (r)		Intercept		Slope	
Min	**Max**	**Min**	**Max**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
2.00	1728.02	0.56	1302.56	0.985	0.983, 0.986	-0.13	-0.17, -0.08	1.06	1.05, 1.07

Table 7. Deming Regression Analysis: Serum (n = 1472)

ARCHITECT AFP (ng/mL)		Comparator AFP (ng/mL)		Correlation Coefficient (r)		Intercept		Slope	
Min	**Max**	**Min**	**Max**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
2.00	1728.0	0.56	1302.56	0.985	0.983, 0.986	-3.57	-8.00, 0.86	1.22	1.06, 1.38

Table 8. Passing-Bablok Regression Analysis: Amniotic Fluid (n = 692)

ARCHITECT AFP (ng/mL)		Comparator AFP (ng/mL)		Correlation Coefficient (r)		Intercept		Slope	
Min	**Max**	**Min**	**Max**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
1.37	52.27	1.39	39.37	0.952	0.944, 0.958	0.39	0.20, 0.55	1.08	1.05, 1.11

Table 9. Deming Regression Analysis: Amniotic Fluid (n = 692)

ARCHITECT AFP (ng/mL)		Comparator AFP (ng/mL)		Correlation Coefficient (r)		Intercept		Slope	
Min	**Max**	**Min**	**Max**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
1.37	52.27	1.39	39.37	0.952	0.944, 0.958	0.05	-0.59, 0.69	1.13	1.05, 1.21

Serial Monitoring of Nonseminomatous testicular cancer

Clinical samples from the nonseminomatous testicular cancer serial monitoring study were also analyzed on the Beckman Coulter Access AFP assay for comparison. Since the Beckman AFP test used a different criteria and not a reference change value (RCV) for analyzing their results, a RCV had to be derived for the Beckman for the purpose of this study. The RCV was derived similar to the ARCHITECT AFP test by taking into account the published biological variation for AFP and the total imprecision of the specific assay and calculated to be 39.98%. Results of the comparison study are summarized in table 10.

Table 10. Agreement for ARCHITECT AFP and Comparator AFP

ARCHITECT AFP	Comparator AFP		
	>39.98% Increase	≤39.98% increase	Total
> 39.22% increase	18 (A)	9 (B)	27 (A+B)
≤ 39.22% increase	12 (C)	166 (D)	178 (C+D)
Total	30 (A+C)	175 (B+D)	205 (A+B+C+D)

Overall Agreement = 89.76%; 95% CI = 84.77% to 93.55%
Positive Agreement = 60.00%; 95% CI = 40.60% to 77.34%
Negative Agreement = 94.86%; 95% CI = 90.46% to 97.62%.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

A. Study Design

The clinical investigation was conducted from September 2011 to April 2012. The clinical investigation was conducted in compliance with the design validation protocol, the clinical brochure, Good Clinical Practice (GCP), and applicable regulatory requirements. No adverse device events occurred during the clinical investigation.

The study was conducted at the following three clinical testing sites: Milton S. Hershey Medical Center, Hershey, PA (Clinical Testing Site 1); ARUP Laboratories, Institute for Clinical and Experimental Pathology, Salt Lake City, UT (Clinical Testing Site 2); and Minneapolis Medical Research Foundation, Minneapolis, MN (Clinical Testing Site 3).

The objective of this study was to evaluate the performance and intended use of the ARCHITECT AFP Assay in a clinical laboratory setting. The specific study objectives were:

1. To determine the regressed medians and the multiples of the regressed medians (MoM) for maternal serum and amniotic fluid specimens for gestational weeks 15 through 21, and the specificity and sensitivity at the cutoffs of 2.0, 2.5 and 3.0 MoM,
2. To determine expected values for apparently healthy individuals and to show the distribution of AFP values from individuals with nonmalignant and malignant diseases,
3. To determine the reference change value (RCV) and compare results between the ARCHITECT AFP and the comparator assay for specimens collected serially from subjects with nonseminomatous testicular cancer.

B. Specimens Used for the Clinical Studies

The 2,537 specimens tested and analyzed in the ARCHITECT AFP clinical investigation were collected from specimen collection sites as well as purchased from specimen vendors.

The 2,537 specimens were from the following categories:

- 400 serum specimens from apparently healthy individuals
- 152 serum specimens from individuals with malignant disease
- 238 serum specimens from individuals with nonmalignant disease
- 279 serial serum specimens from 72 individuals with nonseminomatous testicular cancer (post-treatment)
- 758 maternal serum specimens from singleton pregnant females at gestational weeks 15 through 21. Of the 758 maternal serum specimens,
  - 682 specimens were from pregnant females with unaffected birth outcomes,
  - 55 specimens were from pregnant females at low risk for open NTD, and
  - 21 specimens were from pregnant females with affected birth outcomes.
- 710 amniotic fluid specimens from singleton pregnant females at gestational weeks 15 through 21, with the exception of 1 specimen in week 22 and 1 specimen in week 24. Of the 710 amniotic fluid specimens,
  - 222 specimens were from pregnant females with unaffected birth outcomes,
  - 467 specimens were from pregnant females at low risk for open NTD, and
  - 21 specimens were from pregnant females with affected birth outcomes.

C. Safety and Effectiveness Results

Prenatal Testing Studies

Objectives of the ARCHITECT AFP prenatal testing studies were to determine:

1. The regressed medians and the multiples of the regressed medians (MoM) for maternal serum and amniotic fluid specimens for gestational weeks 15 through 21;
2. The specificity and sensitivity at the cutoffs of 2.0, 2.5 and 3.0 MoM.

A total of 758 maternal serum and 708 amniotic fluid specimens that were tested across the 3 clinical testing sites were included in the analysis.

Of the 758 maternal serum specimens for analysis,

1. The medians and MoM were determined using 685 specimens, which included 630 specimens with unaffected birth outcome and 55 specimens at low risk for open NTD.
2. The specificity at the cutoffs of 2.0, 2.5 and 3.0 MoM was determined using the same 630 specimens with unaffected birth outcome used in the MoM calculations and an additional 52 serum specimens with unaffected birth outcome.
3. The sensitivity was determined using 21 specimens with an affected birth outcome (i.e., open neural tube defect confirmed by subject's medical record.

Of the 708 amniotic fluid specimens for analysis,

1. The medians and MoM were determined using 687 specimens, which included 220 specimens with affected birth outcome and 467 at low risk for open NTD.
2. The specificity at the cutoffs of 2.0, 2.5 and 3.0 MoM was determined using the same 220 specimens with affected birth outcome used in the MoM calculation and an additional 2 specimens with unaffected birth outcome.
3. The sensitivity was determined using 19 specimens with an affected birth outcome (i.e., open neural tube defect confirmed by subject's medical record or by autopsy results along with the presence of acetylcholinesterase).

The regressed medians and multiples of regressed medians (MoM) of the AFP values for maternal serum and amniotic fluid specimens from unaffected or low-risk, singleton pregnancies were determined for each gestational week (*i.e.*,15 to 21) using the single replicate result of the ARCHITECT AFP Assay. The data are summarized in table 11 and 12.

Table 11. Maternal Serum AFP Regressed Medians

Gestational Week	Number of Specimens	Regressed Medians (ng/mL)	Multiples of Regressed Medians (ng/mL)		
			2.0	2.5	3.0
15	101	32.17	64.35	80.44	96.52
16	95	36.86	73.73	92.16	110.59
17	102	42.24	84.48	105.60	126.72
18	103	48.40	96.79	120.99	145.19
19	101	55.45	110.90	138.63	166.35
20	106	63.53	127.07	158.84	190.60
21	77	72.80	145.59	181.99	218.39

Table 12. Amniotic Fluid AFP Regressed Medians

Gestational Week	Number of Specimens	Regressed Medians (ug/mL)	Multiples of Regressed Medians (ug/mL)		
			2.0	2.5	3.0
15	104	16.41	32.82	41.02	49.22
16	108	13.38	26.76	33.45	40.14
17	105	10.91	21.82	27.27	32.72
18	109	8.89	17.79	22.23	26.68
19	102	7.25	14.50	18.13	21.75
20	97	5.91	11.83	14.78	17.74
21	62	4.82	9.64	12.05	14.46

Specificity and sensitivity were calculated using specimens with confirmed birth outcomes. The specificity and 95% confidence interval were estimated separately using cutoffs of 2.0, 2.5 and 3.0 MoM for maternal serum and amniotic fluid from singleton pregnant females in the gestational weeks 15 to 21 having unaffected birth outcome. The sensitivity and 95% confidence interval was estimated separately using the cutoffs of 2.0, 2.5 and 3.0 MoM for maternal serum and amniotic fluid specimens from singleton pregnant females in the gestational weeks 15 to 21 having affected (open NTD) birth outcome. The data are summarized in tables 13 and 14.

Table 13. ARCHITECT AFP Specificity: Specimens from Unaffected Singleton Pregnancies from 15–21 Weeks of Gestation

Specimen Type	Number of Specimens	Specificity (95% CI)		
		Multiples of the Median (MoM)		
		2.0	2.5	3.0
Amniotic Fluid	222	98.65% (96.10%, 99.72%)	99.10% (96.78%, 99.89%)	99.55% (97.52%, 99.99%)
Maternal Serum	682	95.45% (93.61%, 96.89%)	98.24% (96.95%, 99.09%)	99.71% (98.94%, 99.96%)

Table 14. ARCHITECT AFP Sensitivity: Specimens from Affected Singleton Pregnancies from 15–21 Weeks of Gestation

Specimen Type	Number of Specimens	Sensitivity (95% CI)		
		Multiples of the Median (MoM)		
		2.0	2.5	3.0
Amniotic Fluid	19	100.00% (82.35%, 100.00%)	94.74% (73.97%, 99.87%)	94.74% (73.97%, 99.87%)
Maternal Serum	21	95.24% (76.18%, 99.88%)	80.95% (58.09%, 94.55%)	71.43% (47.82%, 88.72%)

Nonseminomatous Testicular Cancer Monitoring Studies

1. *Expected Values*

   An objective of the ARCHITECT AFP clinical investigation was to determine expected values for apparently healthy individuals and to show the distribution of AFP values for individuals with nonmalignant and malignant diseases.

   A total of 862 serum specimens from the following categories were tested across the 3 clinical testing sites:

- 400 apparently healthy individuals (200 males and 200 females).
- 224 individuals with malignant disease (gastrointestinal, hepatocellular, pancreatic, seminoma testicular, and nonseminoma testicular). The 224 individuals were comprised of 152 individuals with malignant disease and the first available specimen of serially collected specimens from 72 individuals diagnosed with nonseminomatous testicular cancer (post-treatment).
- 238 individuals with nonmalignant disease (cirrhosis, hepatitis, pancreatitis, and genitourinary).

The distribution of AFP values (ng/mL) was determined for the apparently healthy individuals and for individuals with nonmalignant and malignant diseases. The data are summarized in the following tables.

Table 15. Distribution of ARCHITECT AFP Values in Apparently Healthy Individuals

Group/Category	n	Distribution of Values (%) by AFP Concentration						
		0 - 8.78	>8.78 - 15.00	>15 - 200	>200 - 500	>500 - 1000	>1000 - 2000	>2000
Apparently Healthy	400	97.5	2.0	0.5[a]	0.0	0.0	0.0	0.0

[a] These 2 samples had AFP concentrations of 25.16 and 27.81 ng/mL.

The observed nonparametric central 95% derived from 2.5th percentile to 97.5th percentile was calculated for the apparently healthy individuals by gender and combined. The observed nonparametric central 95% ranged from 0.89 to 8.78 ng/mL for males and females combined.

Table 16. Distribution of ARCHITECT AFP Values in Individuals with Nonmalignant and Malignant Diseases

Group/Category	n	Distribution of Values (%) by AFP Concentration Range						
		0 - 8.78	>8.78 - 15.00	>15 - 200	>200 - 500	>500 - 1000	>1000 - 2000	>2000
Nonmalignant Disease								
Cirrhosis	49	98.0	2.0	0.0	0.0	0.0	0.0	0.0
Genitourinary	26	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Hepatitis	14	90.6	6.7	2.0	0.0	0.0	0.0	0.7
Pancreatitis	14	92.9	7.1	0.0	0.0	0.0	0.0	0.0
Malignant Disease[a]								
Gastrointestinal	64	98.4	1.6	0.0	0.0	0.0	0.0	0.0
Hepatocellular	29	69.0	0.0	17.2	0.0	0.0	0.0	13.8
Pancreatic	34	85.3	0.0	5.9	2.9	0.0	2.9	2.9

Group/Category	n	Distribution of Values (%) by AFP Concentration Range						
		0 - 8.78	>8.78 - 15.00	>15 - 200	>200 - 500	>500 - 1000	>1000 - 2000	>2000
Nonseminoma	72	87.5	1.4	9.7	1.4	0.0	0.0	0.0
Seminoma	25	100.0	0.0	0.0	0.0	0.0	0.0	0.0

[a] The nonseminoma testicular samples were from treated patients. The disease status was unknown for specimens from the other malignant diseases.

2. *Change in ARCHITECT AFP vs. Change in Disease Status*

An objective of the ARCHITECT AFP clinical investigation was to compare results between the ARCHITECT AFP and the comparator assay for specimens collected serially from subjects with nonseminomatous testicular cancer.

The reference change value (RCV) was used to determine if a significant change in AFP occurred. For this calculation, the RCV for each assay (ARCHITECT AFP and the comparator) was derived by taking into account the published biological variation for AFP and the total imprecision of the specific assay. The RCV for the ARCHITECT AFP method was calculated to be 39.22% and that of the comparator to be 39.98%. A minimum of 3 serial samples were obtained from each of 72 subjects and were analyzed to determine the change in AFP concentration per sequential pair (n=207).

In addition, samples were analyzed on a per subject basis. The sensitivity and specificity of the ARCHITECT AFP test for monitoring testicular cancer was determined to be 88.95% (95% CI = 84.35% to 93.55%) and 22.86% (95% CI = 9.38% to 40.00%) respectively.

The data are summarized in tables 17 and 18.

Table 17. ARCHITECT AFP Change in AFP vs. Change in Disease Status

% Change in AFP	Change in Disease Status				
	Responding n (%)	Stable n (%)	No Evidence of Disease n (%)	Progressing n (%)	Total n (%)
> RCV increase	7 (3.38)	3 (1.45)	9 (4.35)	8 (3.86)	27 (13.04)
No Significant	20 (9.66)	38 (18.36)	70 (33.82)	18 (8.70)	146
> RCV decrease	8 (3.86)	12 (5.80)	5 (2.42)	9 (4.35)	34 (16.43)
Total	35 (16.91)	53 (25.60)	84 (40.58)	35 (16.91)	207

Table 18. ARCHITECT AFP Change in AFP vs. Change in Disease Status (Progression, No Progression)

% Change in AFP	Change in Disease Status		
	Progression	No Progression	Total
> 39.22% increase	8	19	27
≤ 39.22% increase	27	153	180
Total	35	172	207

Specificity = 88.95%; 95% CI = 84.35% to 93.55%
Sensitivity = 22.86%; 95% CI = 9.38% to 40.00%

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The data in this submission indicate the safety and effectiveness of the ARCHITECT AFP Assay to confirm the presence of alpha-fetoprotein (AFP) in human serum, plasma, and amniotic fluid. The data support the use of human serum or plasma to aid in the monitoring patients with nonseminomatous testicular cancer and the use of human serum, plasma, and amniotic fluid at 15 to 21 weeks gestation to aid in the detection of fetal open neural tube defects (NTD). Test results when used in conjunction with ultrasonography or amniography are a safe and effective aid in the detection of fetal open NTD.

The data from the nonclinical studies demonstrated acceptable precision, recovery, linearity, sensitivity, and stability for the ARCHITECT AFP Assay when used according to the instructions for use as stated in the labeling, the warnings and precautions, and the Specimen Collection and Preparation for Analysis and Limitations sections of the labeling. The data obtained from a method comparison to another approved device indicated that the ARCHITECT AFP Assay was comparable in performance.

The clinical studies in this application indicate that the ARCHITECT AFP Assay is safe and effective when used according to the directions for use in the labeling.

B. Safety Conclusions

Amniocentesis procedure performed as a follow-up to an elevated maternal serum AFP result carries various risks, including: miscarriage. Second-trimester amniocentesis carries a slight risk of miscarriage that is between 1 in 300 and 1 in 500. During amniocentesis the baby might move an arm or leg into the path of the needle. Serious needle injuries are rare. Rarely, amniotic fluid leaks through the vagina after amniocentesis. If the leak seals, the pregnancy is likely to proceed normally. It is possible, however, for chronic leakage to lead to orthopedic problems for the baby. Rarely, amniocentesis might cause the baby's blood cells to enter the mother's bloodstream. Rarely, amniocentesis might trigger a uterine infection.

For venipuncture, there is a risk of hematoma.

C. Benefit-Risk Conclusions

The probable benefits of the device are also based on data collected in clinical studies conducted to support PMA approval as described above.

The use of AFP values as a tumor marker for nonseminomatous testicular cancer and fetal neural tube defect (NTD) marker is well-established.

Prenatal testing for NTDs by measurement of AFP identifies pregnancies that are at sufficient risk for NTD to warrant genetic counseling and the offer of additional testing such as ultrasound and amniocentesis. In addition, it enables pregnant women to make informed decisions regarding the pregnancy and be better prepared in the event of the birth of an affected infant.

Abnormal test results of AFP may indicate the need for additional testing. Usually an ultrasound is performed to confirm the dates of the pregnancy and to examine the fetal spine and other body parts for defects. An amniocentesis may be needed for accurate diagnosis. Amniocentesis has a small risk of miscarriage, needle injury to fetus, amniotic fluid leaks and uterine infection.

A false positive result in maternal serum can be mitigated by follow-up testing of ultrasonography. If ultrasonography result is inconclusive, amniocentesis will be performed and amniotic fluid will be tested for AFP. In the case of a false positive AFP result in an amniotic fluid sample, the fetus would be incorrectly diagnosed as having NTD, consequently may lead to termination of pregnancy. In the case of a false negative result, risk can be mitigated by ultrasonography or amniography.

In the setting of management of patients with nonseminomatous testicular cancer, the consequences of a false negative result are substantially mitigated by clinical recommendations that at least two serial measurements are obtained before discontinuing or reducing therapy and that all patients are monitored with physical examination, tumor marker determinations including hCG and CT scan. The effects of a false positive result are likewise substantially mitigated by other diagnostic radiological examination such as CT scan.

In conclusion, given the available information above, the data support that for the ARCHITECT AFP Assay to be used with human serum or plasma to aid in monitoring disease progression during the course of disease and treatment of patients with nonseminomatous testicular cancer or with human serum, plasma, and amniotic fluid at 15 to 21 weeks gestation to aid in the detection of fetal open neural tube defects (NTD) in conjunction with ultrasonography or amniography, the probable benefit outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Based on the clinical study results and previous experience with previously approved products with similar indication for use and comparable studies, the probable benefits outweigh the probable risks.

XIV. CDRH DECISION

CDRH issued an approval order on November 28, 2012. The final conditions of approval cited in the approval order are described below.

The applicant's manufacturing facilities were inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.