510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k081709

B. Purpose for Submission:
New Device

C. Measurand:
Alpha-Fetoprotein (AFP)

D. Type of Test:
Automated chemiluminescent immunoassay

E. Applicant:
Olympus America Inc.

F. Proprietary and Established Names:
Olympus AFP Test System

G. Regulatory Information:

1. Regulation section:
   866.6010 Tumor-associated antigen immunological test system
   862.1150 Calibrator
   862.1660 Quality control material (assayed and unassayed)
2. Classification:
   Class II
3. Product code:
   LOJ Kit, Test, Alpha-fetoprotein for testicular cancer
   JIT Calibrator, secondary
   JJX Single (specified) analyte controls (assayed and unassayed)
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   The **Olympus AFP assay** is a paramagnetic particle (Dynabeads®), chemiluminescent immunoassay for the quantitative determination of alpha-fetoprotein levels in human serum/plasma using the Olympus AU3000i Immunoassay System. The Olympus AFP assay is intended for use as an aid in the management (monitoring) of patients with non-seminomatous germ cell tumors.
   The **Olympus AFP calibrator** is for calibrating the quantitative Olympus assay on the Olympus AU3000i Immunoassay.
   The **Olympus AFP control** is used for the quality control of the Olympus AFP assay on the Olympus AU3000i Immunoassay System.
2. Indication(s) for use:
   Same as above
3. Special conditions for use statement(s):
   Prescription use only
4. Special instrument requirements:
   Olympus AU3000i Immunoassay System (k062581)

I. Device Description:

The Olympus AFP Test System consists of two reagents (Reagent 1 and Reagent 2), calibrator and control material. Reagent 1 consists of paramagnetic particles coated with murine monoclonal anti-AFP antibody, Tris buffer, protein stabilizers and preservative. Reagent 2 consists of alkaline phosphatase labeled murine monoclonal anti-AFP antibody conjugate, MES buffer, protein stabilizers, detergent and preservatives. The calibrator is AFP prepared in bovine matrix with preservatives and the control is AFP prepared in human matrix with preservatives.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Roche Elecsys AFP Assay
   Roche Elecsys PreciControl Tumor Marker Control
   Roche Elecsys AFP CalSet
2. Predicate K number(s):
   k981282
   k050387
   k043095
3. Comparison with predicate:

AFP Test System

Similarities		
Item	New Device	Predicate
Intended Use	The Olympus AFP assay is intended for use as an aid in the management (monitoring) of patients with non-seminomatous germ cell tumors.	Same
Measurement	Quantitative	Same
Assay Methodology	Chemiluminescent, two-site immunoassay	Same
Capture Antibody	Murine monoclonal	Same
Solid phase	Microparticle	Same
Storage	2-8^{o}C	Same

Differences		
Item	New Device	Predicate
Instrument	Olympus AU3000i ™ Test System	Roche Elecsys 1010/2010 and Modular analytics E170 immunoassay analyzers
Matrix	Serum and plasma (Lithium heparin)	Serum and plasma (sodium heparin, EDTA, or sodium citrate)
Measuring Range	0.1 – 390 ng/mL	0.6 – 1210 ng/mL

Differences		
Item	New Device	Predicate
Conjugate	Alkaline phosphatase	Streptavidin
Sample volume	20µl	10µl
Stability	2-8°C for 28 days	2-8°C for 4 -12 weeks depending on instrument

Calibrator

Similarities		
Item	New Device	Predicate
Intended Use	For calibration	Same
Traceability	1st IRP WHO Reference Standard 72/225 for human AFP	Same
Storage	2-8°C	Same

Differences		
Item	New Device	Predicate
Instruments	Olympus AU3000i ™ Test System	Elecsys immunoassays systems
Reagent Preparation	Liquid	Lyophilized
Quantity	One	4 x 1.0 mL
Stability; Open	2-8°C for 28 days	When reconstituted: 5 hours on board 6 weeks at 2-8°C 12 weeks at -20°C
Composition	Bovine	Human
Levels	One (~390 ng/mL)	Two (~ 6.0 and 60 ng/mL)

Control

Similarities		
Item	New Device	Predicate
Intended Use	Quality control	Same
Composition	Human serum	Same
Stability; Open	2-8°C for 28 days	When reconstituted: 5 hours on the analyzer Two weeks at 2-8°C 1 month at -20°C
Storage	2-8°C	Same

Differences		
Item	New Device	Predicate
Instruments	Olympus AU3000i ™ Test System	Elecsys and cobas e immunoassay analyzers

Differences		
Item	New Device	Predicate
Reagent Preparation	Ready-to-use	Lyophilized; 4 vials that are reconstituted into 3 mL distilled water each.
Levels	One (~10.2 ng/mL)	Two

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI EP9-A2 Method Comparison and Bias Estimation Using Patient Samples
CLSI C28-A2 How to Define and Determine Reference Intervals in the Clinical Laboratory.

L. Test Principle:

The Olympus AFP assay is a two-step paramagnetic particle enzyme immunoassay. Samples are incubated with a monoclonal anti-AFP antibody bound to paramagnetic particles. The AFP reacts with the paramagnetic particles is washed and incubated with a second monoclonal anti-AFP antibody conjugated with alkaline phosphatase to form a sandwich complex. A chemiluminescent substrate is added to react with the bound phosphatase. Light generated by the reaction is measured by the luminometer. The light emission is proportional to the quantity of AFP in the sample. Results are calculated from a predefined calibration curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

   Four sites evaluated precision of 3 serum pools and 2 controls. Precision pools were prepared centrally and provided to the study sites. The low precision pool was prepared from patient serum. The medium and high precision pools were prepared from patient serum spiked with human AFP. Three different lots were evaluated at the 4 sites and testing was based on CLSI EP5-A2. Acceptance criteria %CV ≤5% was met.

			Repeatability (Within Run)		Between Run		Between Day		Within Laboratory (Total)	
Pools	Site	Mean (ng/ml)	SD (ng/ml)	CV (%)	SD (ng/ml)	CV (%)	SD (ng/ml)	CV (%)	SD (ng/ml)	CV (%)
Low	1	1.222	0.018	1.4	0.014	1.2	0.005	0.4	0.023	1.9
	2	1.231	0.016	1.3	0.017	1.3	0.012	1.0	0.026	2.1
	3	1.279	0.014	1.1	0.022	1.7	0.000	0.0	0.025	2.0
	4	1.250	0.014	1.1	0.020	1.6	0.016	1.2	0.029	2.3

Medium	1	24.654	0.420	1.7	0.557	2.3	0.000	0.0	0.697	2.8
	2	24.376	0.377	1.5	0.568	2.3	0.166	0.7	0.701	2.9
	3	25.232	0.324	1.3	0.650	2.6	0.098	0.4	0.733	2.9
	4	23.149	0.349	1.5	0.322	1.4	0.316	1.4	0.570	2.5
High	1	140.795	2.459	1.7	2.557	1.8	0.788	0.6	3.634	2.6
	2	147.815	2.688	1.8	3.614	2.4	2.516	1.7	5.160	3.5
	3	152.006	1.864	1.2	4.169	2.7	0.000	0.0	4.566	3.0
	4	141.883	2.606	1.8	3.630	2.6	2.920	2.1	5.338	3.8

The data for each site was combined. The %CV for the low, medium and high samples were determined for within-run (1.3, 1.5, and 1.7), between run (1.5, 2.2, and 2.4), respectively.

The control (~10.1 ng/mL) provided with the Olympus AFP was also evaluated and met the acceptance criteria.

Lot-to-lot: To measure the variation in concentration between multiple lots of the Olympus AFP Test system, 3 serum samples of low (~1.2 ng/mL), medium (~25ng/mL) and high (~ 145 ng/mL) were tested in parallel on 3 different lots. The %CV ≤ 3.7%.

b. *Linearity/assay reportable range:*
Linearity: To assay linearity across the measuring range of the assay, human serum pool was spiked with AFP (from human amniotic fluid) to just above the measuring range (~408 ng/mL) and then diluted with Sample Diluent (SDIL1) to create 11 concentrations that spanned the measuring range. Samples were evaluated in triplicate. The acceptance criteria was met.

Range (ng/mL)	**Slope (95% CI)**	**Intercept ng/mL (95% CI)**	**Correlation Coefficient**	**n**
0.036 to 408 ng/mL	0.9762 (0.96 – 0.99)	-0.632 (-3.06 – 1.80)	0.999	11

Dilution recovery: To demonstrate the linearity of the assay, 3 patient samples were prepared in 2-fold dilutions to a total of 4 levels. In addition a "neat" sample was run. Percent recovery is calculated by comparing the observed AFP results with the expected value. Samples concentrations prior to dilution: ~74 ng/mL, 151 ng/mL and 370 ng/mL. Mean % recoveries were between 92% and 100%. Acceptable recovery within ±10% was met.

Spiked Recovery: Three serum pools across the assay linear range were spiked with three different amounts of AFP. Each spiked sample was then diluted into a low (~ 10 ng/mL), medium (~ 56 ng/mL) and high sample (~168 ng/mL). The neat spiked solution and corresponding dilutions were run in quadruplicate. Mean % recoveries ranged ~91% to 104%. Acceptance criteria ±10% were met.

c. *Traceability:*
The Olympus AFP is traceable to the 1st IRP WHO Reference Standard

72/225 for human AFP.

d. Stability:
Real-time, on-board, open vial stability were conducted for AFP test reagents, calibrator and controls. The results support the 28 day claim.

f. Detection limit:
The limit of blank was determined by running 60 replicates of the blank sample and taking the 95th percentile of the blank samples.
The limit of detection was determined using 5 serum samples tested 12 times. The limit of detection was determined to be (0.048 ng/mL).
The limit of quantification (LoQ) study was based on EP17A. The AFP LoQ pools were prepared using 3 different serums. LoQ pool levels 1-4 and level 6 were prepared by diluting a female human serum (base concentration = 0.98 ng/mL) with assay diluent. The 2 remaining levels (5 and 7) were stripped serum samples. A total of 40 replicated were measured incorporating 2 reagent lots, 2 instruments, across 4 days. The LoQ was determined to be 0.077 ng/mL which represents the lowest concentration of AFP that can be measured with a total imprecision of ≤17.2%

g. Analytical specificity:
Interference by Heterophilic antibodies and Rheumatoid Factor was determined using human serum samples spiked with AFP to the desired levels to create base pools with two levels of AFP (~5.0 ng/mL and ~47 ng/mL). Expected analyte values were compared to observed values and % recovery calculated. No significant interference was seen with 2225 IU/mL RF in samples at the designated AFP concentrations. Interference by HAMA (1825 ng/mL) was not significant; however a statement cautioning against HAMA interference is included in the package insert.

Interference and cross-reactivity: To test the susceptibility of the AFP test to common interfering substances, the following substances were tested by spiking them into a human serum sample containing AFP 8.7 ng/mL. The interference was calculated by comparing the recovery of AFP in the samples containing interferents to the control sample containing no interferents. Bilirubin is unconjugated. Interference was ≤ 5% at the concentrations indicated:

Bilirubin	≤ 3% up to 40 mg/dl Bilirubin
Haemolysate	≤ 5% up to 5 g/L Haemolysate
Intralipid™	≤ 5 % up to 10 g/L Intralipid™

The following interferent and cross-reacting substances were tested by adding the identified substances in known concentrations to a serum pool containing AFP at a concentration ~5 ng/mL. The compounds did not show interference >10% at the specific levels indicated.

Drugs	Amount added	% Recovery (spiked/control)
Acetaminophen	200 µg/mL	101%
Acetyl Cysteine	164 µg/mL	101%
Aspirine	1,032 mg/mL	104%
Ampicillin - Na	1,064 mg/mL	99%
Ascorbic acid	289,2µg/mL	104%
Bleomycin	10 mUI/mL	104%
Carboplatin	1,036 mg/mL	101%
Cefoxitin	2,44 mg/mL	101%
Cisplatin	1,980 mg/mL	95%
Cyclophosphamide	984 µg/mL	100%
Cyclosporine	5 µg/mL	103%
D-actinomycin	2,5 µg/mL	101%
Doxycycline	50 µg/mL	102%
Etoposide	100 µg/mL	101%
Ibuprofen	1,13 mg/mL	100%
Ifosfamide	4,5 mg/mL	93%
Levodopa	27,7 µg/mL	105%
Methotrexate	1,125 mg/mL	94%
Methyldopa + 1,5	21,6 µg/mL	104%
Metronidazole	232 µg/mL	102%
Naprosyn sodium	1,2 mg/mL	103%
Phenylbutazone	420,8 µg/mL	99%
Rifampicin	68,4 µg/mL	101%
Paclitaxel	10 µg/mL	100%
Theophylline	119,5 µg/mL	101%
Vinblastine	109 µg/mL	100%
Vincristine	100 µg/mL	98%

Human Proteins	Amount added	% Recovery (spiked/control)
human alpha globulin (ξ2 Macrogl)	2,5 mg/mL	95%
human alpha-1-acid glycoprotein	10 mg/mL	99%
human alpha-1-antitrypsin	20 mg/mL	99%
hCG	1000 IU/mL	98%
human Gamma Globulin	100 mg/mL	92%
human Placental Lactogen	500 µg/mL	105%
human Serum Albumin	25,1 mg/mL	107%
human Transferrin	100 mg/mL	106%

Hook Effect (Prozone): The presence of high dose effect was tested by analyzing a concentrated sample of purified AFP antigen both neat and on dilution with the measuring range of the AFP assays. No Hook Effect was demonstrated at the highest concentration evaluated (2,160,000 ng/mL).

h. Assay cut-off:
Refer to clinical studies below.

2. Comparison studies:

a. Method comparison with predicate device:
A total of 289 serum samples were analyzed using the Olympus AFP Test and compared to the predicate. Samples were run in singlicate and the data was analyzed using Deming regression. The correlation coefficient (r) was calculated using ordinary linear fit regression. The results are shown below:

Comparator	**Sample range**	**Slope (95%CI)**	**Intercept ng/mL (95%CI)**	**r**	**n**
Roche Elecsys AFP	0.89 – 349.56	1.02 (0.99 – 1.05)	-0.19 (-0.56 – 0.18)	0.996	289

b. Matrix comparison:

To demonstrate the performance of Li-heparin plasma when compared to serum, AFP concentrations were measured in 50 matched samples were tested. The slope, intercept were calculated by Deming regression. The results of the study demonstrate that results obtained using Li-heparin plasma samples are consistent with those obtained using serum across the range.

Range (ng/mL)	**Slope (95% CI)**	**Intercept ng/mL (95% CI)**	**Correlation Coefficient**	**n**
1.044 to 359.1 ng/mL	1.007 (0.988 – 1.027)	-1.065 (-1.72 – 1.51)	0.997	50

3. Clinical studies:

A clinical study was performed to assess the performance of the Olympus AFP assay to monitor patients with non-seminomatous testicular cancer. Seventy-three (73) retrospective serial serum sample sets (total of 308 evaluable samples) with clinical data from men diagnosed with testicular cancer were tested. Inclusions and exclusion criteria for the samples were provided. Samples were selected for age (range 1 to 56 years old), race/ethnicity (specimens from African America were not evaluated in this study), and stage of disease (stage I through IV).

A longitudinal analysis of serial draws from 73 patients was performed. All patients were categorized as Active/Progressing, Responding, Stable or No Evidence of Disease (NED). Disease progression was determined by the patient's physician based on physical examination, radiographic findings, and surgical procedures.

The Reference Change Value (RCV) was used to identify a significant change in AFP levels. For this calculation, the RCV was derived using the formula RCV = $2.33\ (Sw+a^2)^{1/2}$ where $Sw+a^2$ is the addition of the analytical variation based on the claimed imprecision (5%) and the biological variation (12%) squared. (Calculations taken from Trapé J. et al. Reference change value for alpha-fetoprotein and its application in early detection of hepatocellular carcinoma in patients with hepatic disease. Clin Chem 2003;49:1209-1211.) The RCV for the Olympus AFP test was calculated to be 30% and 31.6% for the predicate.

Per Visit Analysis:

Changes in AFP concentrations and in disease status were analyzed on a per visit basis. Patients were categorized as Active/Progressing, Responding, Stable or No evidence of Disease (NED) by the attending physician based on clinical information. The table below shows the distribution of results when compared to the disease status for the Olympus AFP Test:

Change in AFP	Change in Disease State				
	Responding N (% T)	Stable N (% T)	No Evidence of Disease N (% T)	Progressing N (% T)	Total N (% T)
> 30.0 % increase	6 (2.6 %)	6 (2.6 %)	9 (3.8 %)	24 (10.2 %)	**45 (19.2 %)**
No Significant Change	5 (2.1 %)	17 (7.2 %)	70 (29.8 %)	14 (6.0 %)	**107 (45.1 %)**
> 30.0 % decrease	20 (8.5 %)	22 (9.4 %)	18 (7.7 %)	24 (10.2 %)	**83 (35.8 %)**
Total	**31 (13.2 %)**	**45 (19.2 %)**	**97 (41.3 %)**	**62 (26.4 %)**	**235 (100 %)**

The following two tables show per visit clinical performance results for the Olympus AFP test and predicate device when analyzed as "Progression" and "No Progression" with "No Progression" consisting of responding, stable and NED.

Olympus AFP Value vs. Disease Progression

	Change in Disease State		
Change in AFP	Progression	No Progression	Total
> 30.0% increase	24	21	45
≤ 30.0% increase	38	152	190
Total	62	173	235

	Estimate	95% Confidence Interval
Sensitivity	38.7%	(26.6% - 51.9%)
Specificity	87.9%	(82.0% to 92.3%)

The table below shows the distribution of results when compared to the disease status for the Predicate Test:

Change in AFP	Change in Disease State				
	Responding N (% T)	Stable N (% T)	No Evidence of Disease N (% T)	Progressing N (% T)	Total N (% T)
> 31.6 % increase	6 (2.6 %)	7 (3.0 %)	11 (4.7 %)	26 (11.1 %)	**50 (21.3 %)**
No Significant Change	5 (2.1 %)	15 (6.4 %)	65 (27.7 %)	10 (4.3 %)	**95 (40.4 %)**
> 31.6 % decrease	20 (8.5 %)	23 (9.8 %)	21 (8.9 %)	26 (11.1 %)	**90 (38.3 %)**
Total	**31 (13.2 %)**	**45 (19.1 %)**	**97 (41.3 %)**	**62 (26.4 %)**	**235 (100 %)**

Predicate Device AFP Value vs. Disease Progression

	Change in Disease State		
Change in AFP	Progression	No Progression	Total
>31.6% increase	26	24	50
≤ 31.6% increase	36	149	185
Total	62	173	235

	Estimate	95% Confidence Interval
Sensitivity	41.9%	(29.5% - 55.2%)
Specificity	86.1%	(80.1% - 90.9%)

Olympus AU3000i AFP Concordance to Comparative Method (on a per visit basis)

	> 31.6 % increase	≤ 31.6 % increase	Total
> 30.0 % increase	**42**	3	45
≤ 30.0 % increase	8	**182**	190
Total	50	185	235

		95% Confidence Interval
% Overall agreement	95.3%	(91.8% – 97.6%)
% Positive agreement	84.0%	(70.9% – 92.8%)
% Negative agreement	98.4 %	(95.3% – 99.7%)

4. Clinical cut-off**:**
   Not applicable.
5. Expected values/Reference range:
   The distribution of AFP values in normal individuals, patients with benign conditions and malignant conditions was established. In this study, 97.5% of healthy males had AFP levels less than 7.14 ng/mL.

	Distribution of AFP values n(%)					
	N	**0 – 5 IU/mL** [0 – 6 ng/mL]	**5 – 10 IU/mL** [6 - 12 ng/mL]	**10 – 100 IU/mL** [12 – 120 ng/mL]	**101 – 325 IU/mL** [121 – 390 ng/mL]	**>325 IU/mL** [>390 ng/mL]
Apparently Healthy						
Male	206	197 (95.6%)	9 (4.4%)	-	-	-
Benign Conditions						
Prostate	108	101 (93.5%)	7 (6.5%)	-	-	-
GI/Lung	109	103 (94.5%)	5 (4.6%)	1 (0.9%)	-	-
Diabetes	106	97 (91.5%)	8 (7.5%)	-	-	1 (0.9%)
Heart/Liver	108	102 (94.4%)	6 (5.6%)	-	-	-
Malignant Conditions (treated)						
Liver	18	6 (33.3%)	3 (16.7%)	3 (16.7%)	1 (5.6%)	5 (27.8%)
Lung	83	74 (89.2%)	8 (9.6%)	1 (1.2%)	-	-
Upper GI	43	40 (93.0%)	2 (4.7%)	1 (2.3%)	-	-
Prostate/Testicular/ Bladder	228	212 (93.0%)	9 (3.9%)	5 (2.2%)	1 (0.4%)	1 (0.4%)
Colorectal	61	50 (82.0%)	8 (13.1%)	3 (4.9%)	-	-

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted information in this premarket notification is complete and supports a substantial equivalence decision.