

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

k041847

**B. Purpose for Submission:**

New device

**C. Analyte:**

AFP-L3%

**D. Type of Test:**

Quantitative, fluorescence enzyme immunoassay

**E. Applicant:**

Wako Chemicals USA, Inc.

**F. Proprietary and Established Names:**

Wako LBA AFP-L3

Wako AFP-L3 Calibrator Set

Wako AFP-L3 Control Set

Wako LiBASys

**G. Regulatory Information:**

1. Regulation section:

21 CFR§866.6030, AFP-L3% immunological test system

21 CFR§862.1150, Calibrator

21 CFR§862.1660, Quality control material (assayed and unassayed)

2. Classification:

AFP-L3% immunological test system – Class II

Calibrator – Class II

Quality control material (assayed and unassayed) – Class I

3. Product Code:

NSF, Test, Alpha Fetoprotein L3 subfraction (AFP-L3%) for hepatocellular carcinoma risk assessment

JIT, Calibrator, Secondary

JJX, Single (specified) analyte controls (assayed and unassayed)

4. Panel:

Immunology (82), Chemistry (75)

**H. Intended Use:**

1. Intended Use:

The Wako AFP-L3% assay is intended as a risk assessment test for the development of hepatocellular carcinoma (HCC) in patients with chronic liver diseases (CLD). Elevated AFPL3% values ( $\geq 10\%$ ) have been shown to be associated with a seven-fold increase in the risk of developing HCC within the next 21 months. Patients with elevated serum AFPL3% should be more intensely evaluated for evidence of HCC according to the existing HCC practice guidelines in oncology.

2. Indication(s) for use:

Same as intended use.

3. Special condition for use statement(s):

For prescription use only.

4. Special instrument Requirements:

Use with automated analyzer LiBASys.

**I. Device Description:**

The Wako AFP-L3% device consists of reagent 1 (LCA and anion 1-conjugated anti-AFP mouse monoclonal antibody), reagent 2 (horseradish peroxidase (POD)-labeled anti-AFP mouse monoclonal antibody and anion 2 conjugated anti-AFP mouse monoclonal antibody, substrate 1 (4 acetamidophenol in 2-propanol) and substrate 2 (hydrogen peroxide) and a column. Reagent 1, reagent 2 and the column are ready-to-use. Elution buffers A to C, sample cups, inside and outside cuvettes are sold separately from kit.

The Wako AFP-L3 Calibrator set and Control set are sold separately. The calibrator set consisted of Calibrator 1 and 2. Calibrator 1 contains human AFP – L1 fraction and Calibrator 2 has human AFP-L3 fraction. The control set consisted of Control 1 and 2, each containing different concentrations of human AFP-L1 and L-3.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
No predicate
2. Predicate K number(s):  
Not applicable
3. Comparison with predicate:  
Not applicable

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

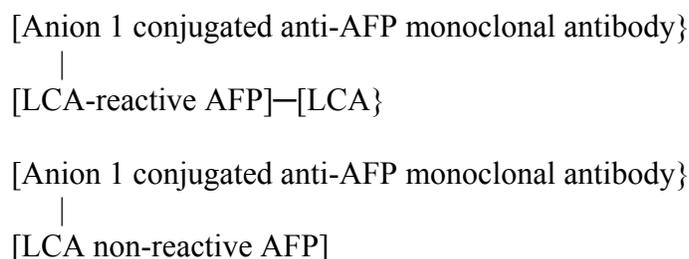
CLSI Guidelines EP5-A, EP7-A and EP6-A.

**L. Test Principle:**

Human AFP is a 70 kD glycoprotein with a single asparagine-linked carbohydrate chain. Using Lens culinaris agglutinin (LCA) which has an affinity for the carbohydrate chain that has an additional  $\alpha$  1-6 fucose residue bound to N-acetylglucosamine at the reducing end, AFP can be subdivided into 3 microheterogeneity forms, L1, L2 and L3. AFP L1 and L3 are major components of AFP in serum of HCC patients. AFP-L3% is the ratio of AFP with  $\alpha$  1-6 fucose residue to total AFP. The concentrations of LCA-reactive and LCA-nonreactive AFP are measured separately using three anti-AFP monoclonal antibodies that detect different epitope, LCA and a fluorophotometric substrate. When a patient serum sample is mixed with LCA and anion 1 (sulfated tyrosine pentamer)-conjugated anti-AFP monoclonal antibody, LCA reacts with the sugar chain of LCA-reactive AFP while anion 1 conjugated anti-AFP monoclonal antibody binds to all AFP molecules forming immune complexes. When reagent 2 is added, immune complexes react with horseradish peroxidase (POD) labeled anti-AFP monoclonal antibody and anion 2 (sulfated tyrosine pentamer)-conjugated anti-AFP monoclonal antibody. Anion 2- conjugated anti-AFP monoclonal antibody recognizes the base area of the sugar chain of AFP and competes with previously reacted LCA on LCA-reactive AFP but binds to LCA non-reactive AFP without competitive reaction. The POD labeled anti-AFP

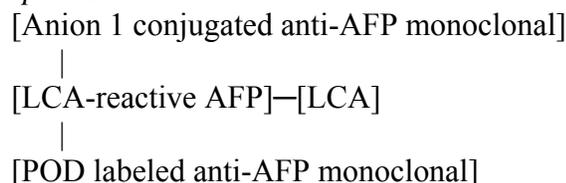
monoclonal antibody binds to all the AFP molecules in the sample. Therefore, two types of immune complexes with different anionic charges are formed. Complex 1 has 5 sulfate residues and complex 2 with 13 sulfate residues. The reaction mixture is introduced to an anion-exchange column and by means of stepwise gradient; immune complex fractions are separately eluted into reaction cups. The POD activity of each complex is measured and is determined as the increase of fluorescence intensity of 5,5'-diacetoamide-2,2'-bisphenol formed by the reaction of hydrogen peroxide and 4-acetamidophenol. The total fluorescence intensity of complex 1 and 2 represents AFP concentration and AFP-L3% is complex 1/complex 1 + complex 2. AFP concentration and AFP-L3% are obtained by comparing to standards with known AFP concentration and AFP-L3%. (see illustration below)

Step 1: Addition of LCA and anion 1-conjugated anti-AFP monoclonal antibody

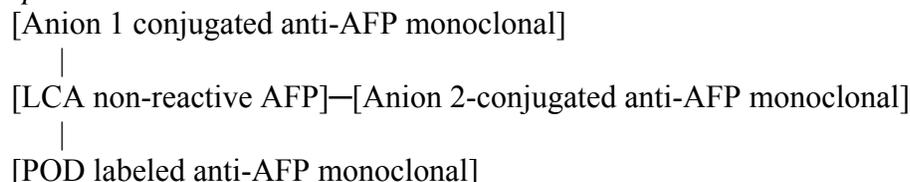


Step 2: Addition POD labeled anti-AFP monoclonal antibody and anion 2-conjugated anti-AFP monoclonal antibody

*Complex 1*



*Complex 2*



Step 3: Column separation

Step 4: Elution of immune complexes

Step 5: Addition of substrates

Step 6: Fluorescence detection

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:a. *Precision/Reproducibility:*

Within-run precision – Three control sera with low, medium and high concentration of total AFP were assayed 21 times. Control serum #1 was prepared by spiking purified AFP-L1 and L-3 into pool serum. Results are shown in the following table.

	Control 1			Control 2			Control 3		
	AFP (ng/mL)	AFP-L3 (ng/mL)	AFP-L3%	AFP (ng/mL)	AFP-L3 (ng/mL)	AFP-L3%	AFP (ng/mL)	AFP-L3 (ng/mL)	AFP-L3%
Mean	32.5	8.1	24.8	501.9	116.0	23.1	174.2	124.6	71.5
SD	0.48	0.29	0.82	7.31	4.30	0.70	2.39	2.01	0.53
CV	1.5	3.6	3.3	1.5	3.7	3.0	1.4	1.6	0.7

Total precision – Study was performed according to CLSI Guideline EP5-A. Three control sera were assayed in duplicates, two runs per day for 21 days. Results were summarized below.

	Sample 1			Sample 2			Sample 3		
	AFP (ng/mL)	AFP-L3 (ng/mL)	AFP-L3%	AFP (ng/mL)	AFP-L3 (ng/mL)	AFP-L3%	AFP (ng/mL)	AFP-L3 (ng/mL)	AFP-L3%
Total Mean	33.1	8.4	25.4	506.5	117.9	23.3	175.3	126	71.8
Within-run SD	0.50	0.38	1.01	8.89	7.98	1.29	2.90	2.7	0.73
Within-run CV (%)	1.5	4.5	4.0	1.8	6.8	5.5	1.7	2.1	1.0
Day- to-Day SD	1.14	0.29	0.55	12.72	3.44	0.53	2.86	2.67	0.70
Day-to-Day CV (%)	3.4	3.5	2.2	2.5	2.9	2.3	1.6	2.1	1.0
Run-to-Run SD	1.42	0.66	1.15	10.76	3.69	0.67	5.46	6.36	1.62
Run-to-Run CV (%)	4.3	7.9	4.5	2.1	3.1	2.9	3.1	5.0	2.3
Total SD	1.89	0.81	1.63	18.88	9.44	1.55	6.81	7.41	1.91
Total CV (%)	5.7	9.6	6.4	3.7	8.0	6.7	3.9	5.9	2.7

Reproducibility – Within-run precision studies were performed by 3 clinical laboratories using two samples with different concentrations of AFP and AFP-L3. The samples were assayed in 21 replicates each run for two runs. Percent CV for AFP and AFP-L3 ranged from 1.0% to 3.9% and 1.5% to 6.9% respectively.

Recovery – Five different concentrations of AFP [(48.6, 118.8, 288.4, 436.1 and 699.0 ng/mL) containing AFP-L3 concentrations of 9.9, 90.5, 42.4, 137.4 and 290.8 ng/mL and calculated AFP-L3% of 20.3, 76.2, 14.7, 31.5 and 41.6%] were spiked into aliquots of 3 serum samples with endogenous AFP concentrations of 15.4, 44.2 and 148.6 ng/mL [containing AFP-L3 concentrations of 0.4, 29.4 and 7.7 ng/mL]. Percent recovery for AFP and AFP-L3 ranged from 95.1% to 108.4% and 95.3% to 118.2% respectively.

b. *Linearity/assay reportable range:*

The assay measuring range is 0.8 ng/mL to 1000 ng/mL

Linearity was evaluated according to CLSI Guideline EP6-A. For AFP, a pool serum sample was spiked with AFP-L1 and AFP-L3

to achieve two samples, one with approximately 25% and the other 80% AFP-L3. These two samples were serially diluted with saline. Each of the seven dilutions was assayed in five replicates in two runs. For AFP-L3, three samples (50 ng/mL, 200 ng/mL and 900 ng/mL AFP-L1 and AFP-L3) were mixed in various proportions with three low AFP-L3 samples. Each sample was assayed in five replicates in two runs. The assay is linear up to 1000 ng/mL AFP.

Linearity studies were also performed in 3 clinical sites. Thirteen samples with same AFP-L3% but total AFP concentrations covering the assay linear range (0-1000 ng/mL) were analyzed in quadruplicates and for two runs. Eleven samples with same total AFP concentration but AFP-L3% covering the assay linear range (0-100%) were similarly assayed. Results for all three sites are summarized below.

#### Total AFP

	Site 1		Site 2		Site 3	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
Slope	1.0074	1.0067	1.0031	1.0144	0.9921	0.9888
Intercept	-6.2226	-3.1165	-3.5117	-2.3976	-5.6467	-2.8759
R <sup>2</sup>	0.9988	0.9997	0.9994	0.9992	0.9974	0.9997

#### AFP-L3%

	Site 1		Site 2		Site 3	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
Slope	1.0494	0.9958	0.9942	0.9933	0.9946	0.9992
Intercept	3.8505	-0.7228	0.3529	-0.3858	-1.3065	-1.1302
R <sup>2</sup>	0.9835	0.9995	0.9999	0.9996	0.998	0.9992

#### c. Traceability (controls, calibrators, or method):

The 1<sup>st</sup> International standard for AFP from WHO was used to assign values to the calibrators and controls.

The primary standards for calibrator 1 and 2 are prepared by diluting purified AFP-L1 and AFP-L3 respectively. Values are assigned using the WHO AFP standard. The primary standards are then used as calibrators to assign values to bulk preparations of AFP-L1 and AFP-L2 by assaying on four LiBASys instruments together with a previous lot of calibrators. If the bulk preparations meet the established acceptance criteria, the solutions are subdivided. One vial is selected from each of the new lot of calibrators for product testing with a previous lot of calibrators and using the primary standards for calibration. AFP values of the new lot are calculated from the mean results of both bulk and product tests. The AFP-L3% of calibrator 1 is assigned 0% and that of calibrator 2 is assigned as 100%.

The two AFP-L3 controls are prepared by mixing purified AFP-L1 and AFP-L3 and assayed on 5 LiBASys instruments using the primary standards 1 and 2 for calibration and compared to previous lot of AFP-L3 control set. Procedures for value assignment and product testing are similar to that described for calibrators.

*d. Detection limit (functional sensitivity):*

The minimal detectable concentration (MDC) for AFP was determined by assaying 21 replicates of a deionized water sample and is defined as mean concentration of AFP +2SD. Data showed the mean AFP concentration was 0.14 ng/mL with a SD of 0.06 ng/mL. The MDC was therefore 0.26 ng/mL. The analytical sensitivity claim is 0.8 ng/mL.

Functional sensitivity was determined by assaying 11 serum samples with AFP-L1 and AFP-L3 concentrations between 0 and 65 ng/mL. For each concentration, 8 replicates were run and the percents CV obtained of the replicates were plotted against the respective AFP-L1 or L3 concentrations. Functional sensitivity was established to be 10 ng/mL.

*e. Analytical specificity:*

Interference was determined by spiking different concentrations of each interfering substance into aliquots of a low and a high AFP serum sample. The AFP samples were prepared by spiking in AFP-L1 and L-3 into a pooled serum. The interfering substances tested include hemoglobin (0-1000 mg/dL), free bilirubin (0-40 mg/dL), conjugated bilirubin (0-30 mg/dL), intrafat (0-2%), rheumatoid factor (0-550 IU/mL), glucose (0-1000 mg/dL), galactose (0-200 mg/dL) and ascorbate (0-50 mg/dL). Glucose concentration >600 mg/dL can result in decreased AFP-L3%. Decreased AFP-L3% results were also observed for free and conjugated bilirubins >20 mg/dL (this information is in the package insert).

Drug interference was determined by additive studies of vitamins B1, B6 and B12, interferons (IFN)  $\alpha$ ,  $\beta$  and  $\gamma$ , ibuprofen, acetaminophen and acetylsalicylic acid. Results of AFP and AFP-L3% were not affected by Vitamins B1 (14 mg/dL), B6 (25 mg/dL), B12 (50 mg/dL), IFN- $\alpha$  (3,000 units/mL), IFN- $\beta$  (3,000 units/mL), IFN- $\gamma$  (3,000 JRU/mL), Ibuprofen (40 mg/dL), acetaminophen (20 mg/dL) and acetylsalicylic acid (50 mg/dL). Percent recoveries ranged from 95.2% to 105.6%.

HAMA interference – Four different concentrations of AFP [(19.4 ng/mL, 71.1 ng/mL, 238.1 ng/mL and 297.5 ng/mL) containing

AFP-L3 concentrations of 3.7, 58.3, 84.5 and 51.5 ng/mL] were spiked into aliquots of a positive HAMA serum sample. Each AFP concentration was assayed in quadruplicate and percent recoveries were determined for AFP, AFP-L3 and AFP-L3%. Percent recoveries ranged from 94.5% to 100.1% for AFP, 95% to 112.8% for AFP-L3 and 97.9% to 113.5% for AFP-L3%.

*f. Assay cut-off:*

AFP-L3% is not reported if total AFP is <10 ng/mL. Data supporting the cut-off were from published literature (Oka, H., et al. J. Gastroenterol. Hepatol. 16 (2001), 1378-1383 and Yamagata, Y., et al. Clin. Chim. Acta 327 (2003), 59-67).

2. Comparison studies:

*a. Method comparison with predicate device:*

No predicate device.

*b. Matrix comparison:*

Not applicable since only serum is the only matrix.

3. Clinical studies:

*a. Clinical sensitivity:*

Not applicable.

*b. Clinical specificity:*

Not applicable.

*c. Other clinical supportive data (when a and b are not applicable)*

Longitudinal data analysis

This is a double-blind, multi-site prospective study. Four hundred ninety six patients with liver disease were enrolled at 7 clinical sites (6 US and one Canadian). Data were collected for serum chemistries and imaging (CT, MRI, and/or ultrasound), presenting symptoms, medications, interventions, therapeutics, and other medical information required for patient management. Serum samples were collected and stored frozen. All AFP-L3% tests were performed by Wako Chemicals USA, Inc.

At the time of enrollment, subjects belonged to two categories - newly diagnosed HCC (a minimum of 50 HCC patients with tumor size <5 cm for informational purposes) or did not have HCC. The diagnosis of HCC for all patients enrolled was made by at least one or a combination of the following observations: 1) a HCC result on a liver biopsy, 2) an enlarging mass, typically greater than 2 cm by imaging (includes ultrasound, CT and MRI) with elevated serum total AFP (typically 20-200 ng/mL), 3) an enlarging mass by CT or MRI in the setting of cirrhosis, 4) a very high serum total AFP >400-500 ng/mL alone, 5) at least 3 serial blood draws that showed a rising serum AFP in the setting of a liver mass or 6) a mass on CT

scan that enhances in the arterial phase and was hypoattenuating compared to the rest of the liver in the venous phase.

The objective of the study was to observe a minimum of 30 subjects who developed verifiable HCC during the study. At the end of the study, all evaluable subjects without HCC at enrollment were categorized by the physician investigators based on biopsy, explanted liver histology, imaging results and site policies into three groups: 1) developed confirmed HCC during study and with lesions of at least 0.5 cm in diameter; 2) suspected of possibly HCC with lesions at least 0.3 cm in diameter and high total AFP results and 3) did not have HCC but had complete workup. Data were collected on study subjects for the duration of the study (approximately 3 years) or until death, developed HCC or end of study. Evaluable patients were defined as patients who had at least one valid AFP-L3% result.

In the data analysis, the number of days was calculated for each patient as the time between the first AFP-L3%  $\geq 10$  and the confirmed HCC diagnosis for group 2 patients and for the other groups, the AFP-L3 % value between study enrollment and end of study was used. The primary endpoint is the relative risk (with 95% CI) of developing HCC within the next 21 months in patients with AFP-L3%  $\geq 10\%$  as compared to the risk in patients with AFPL3%  $< 10\%$ .

The number of patients screened was 582 with 76 failed screening, 10 with no samples, 53 died during study period of other causes. Two subjects in Group 4 were less than 40 years old and were excluded from the analysis. The following table summarizes the distribution of the 494 subjects by group and clinical site. One site recruited 50% of patients. Eighty-one subjects had only one sample drawn and 415 patients had more than one sample.

Clinical study site	Group 1	Group 2	Group 3	Group 4	Total
	HCC	HCC Dx during study	Suspicious	No HCC	
Lahey (MA)	1	3	3	43	50 (10.1%)
MCV (VA)	0	3	2	53	58 (11.7%)
Miami (FL)	7	3	2	46	58 (11.7%)
Mt. Sinai (NY)	43	27	62	118	250 (50.6%)
Toronto (Canada)	1	2	1	39	43 (8.7%)
UCSF (SF)	2	1	1	14	18 (3.6%)
UPenn (PA)	0	0	0	17	17 (3.4%)
Total	54	39	71	332	494

The following table summarizes sample collection interval by number of days for patients from Group 2, 3 and 4.

		Group 2	Group 3	Group 4
Total Number of Patients		39	71	332
Number of Samples	1	1	6	63
	2	4	7	56
	3	9	13	51
	4	5	16	48
	≥5	20	29	114
Average Number of Samples		4.5	4.3	3.6
Day Interval	Average	134.6	136.7	143.0
	Range	63.7-335.5	60.5-467	53.0-579

### Demographics

The subjects consisted of 368 males and 128 females. The gender distribution between group 2 and group 4 was similar (males: group 2 = 79.5% and group 4 = 72.3%; females: group 2 = 20.5% and group 4 = 27.7%).

		Group 1	Group 2	Group 3	Group 4	Total
		HCC	HCC Dx during study	Suspicious	No HCC	
Male	N	46	31	51	240	366
	%	85.2	79.5	71.8	72.3	74.1
Female	N	8	8	20	92	128
	%	14.8	20.5	28.2	27.7	25.8
Total	N	54	39	71	332	494

The overall mean age was 52.6 y (SD = 7.0 y) and with similar distribution between group 2 (mean age = 53 y, SD = 6 y) and group 4 (mean age = 52.1 y, SD = 7.1 y).

		Group 1	Group 2	Group 3	Group 4	Total
		HCC	HCC Dx during study	Suspicious	No HCC	
Male	Mean Age	55.1	52.6	51.8	51.1	51.8
	SD	7.6	5.4	6.1	6.1	6.3
	Range	40-70	42-70	42-69	36-70	40-70
Female	Mean Age	57.3	54.5	53.2	55.2	55.0
	SD	6.3	8.0	6.2	8.4	7.9
	Range	48-66	46-66	43-67	40-70	40-70
Total	Mean Age	55.4	53.0	52.2	52.1	52.6
	SD	7.4	6.0	6.1	7.1	7.0
	Range	40-70	42-70	42-69	36-70	36-70
	N	54	39	71	332	494

The following table summarizes the ethnic distribution of the patient groups:

		Group 1	Group 2	Group 3	Group 4	Total
		HCC	HCC Dx during study	Suspicious	No HCC	
Caucasian	N	29	29	37	231	326
	%	53.7	74.4	52.1	70.0	66.0

		Group 1	Group 2	Group 3	Group 4	Total
		HCC	HCC Dx during study	Suspicious	No HCC	
Asian	N	2	2	2	21	27
	%	3.7	5.1	2.8	6.4	5.5
Black	N	7	4	14	33	58
	%	13.0	10.3	19.7	10.0	11.7
Hispanic	N	142	2	12	39	67
	%	25.9	5.1	16.9	11.8	13.6
Other	N	2	2	6	6	16
	%	3.7	5.1	8.5	1.8	3.2
Total	N	54	39	71	330	494

The following table summarizes the history of alcoholism of the patient groups:

		Group 1	Group 2	Group 3	Group 4	Total
		HCC	HCC Dx during study	Suspicious	No HCC	
Heavy	N	4	1	7	20	326
	%	9.1	2.7	10.6	6.3	6.9
Moderate	N	9	14	12	35	70
	%	20.5	16.2	28.8	17.6	19.8
Occasional	N	11	6	19	56	92
	%	25.0	16.2	28.8	17.6	19.8
No	N	20	16	28	207	271
	%	45.5	43.2	42.4	65.1	58.3
No data	N	10	2	5	14	29
Have data	N	44	37	66	318	465
Total	N	54	39	71	332	494

Similar distribution was observed between group 2 and group 4 for HBV and HVC status. Forty-six patients were HBV positive with 10.3% in group 2 and 9.0% in group 4. Of the 331 HCV positive patients, 71.8% in group 2 and 70.5% group 4. For the 119 HBV and HCV positive patients, 17.9% in group 2 vs. 20.5% in group 4.

		Group 1	Group 2	Group 3	Group 4	Total
		HCC	HCC Dx during study	Suspicious	No HCC	
Only HBV positive	N	10	4	2	29	45
	%	18.5	10.3	2.8	8.8	9.1
Only HCV positive	N	26	28	43	233	330
	%	48.1	71.8	60.6	70.6	66.8
HBV and HCV positive	N	18	7	26	68	119
	%	33.3	17.9	36.6	20.5	24.0
HBV and HCV negative	N	0	0	0	0	0
	%	0	0	0	0	0
Total	N	54	39	71	332	494

Disease stage according to Child’s Classification was available for 85.6% (424/496) of patients with cirrhosis. Group 2 had more Grade C whereas group 4 had more Grade A. The number of patients with Grade B was similar (see below)

		Group 1	Group 2	Group 3	Group 4	Total
		HCC	HCC Dx during study	Suspicious	No HCC	
Grade A	N	11	8	12	118	149
	%	28.9	20.5	17.6	42.6	65.4
Grade B	N	18	19	43	113	193
	%	47.4	48.7	63.2	40.8	45.7
Grade C	N	9	12	13	46	80
	%	23.7	30.8	19.1	16.6	19.0
Total	N	38	39	71	277	422

Comparison of AFP-L3% between the 4 cohorts by Mann-Whitney Test is shown below.

	Group 1 (Max AFP-L3% before treatment)	Group 2 (AFP-L3% at start of study)	Group 2 (Max AFP-L3% before treatment)	Group 3 (within ± 3 months of enrollment)	Group 4 (within ± 3 months of enrollment)
Total # patients	54	39	39	71	332
Average AFP-L3%	11.1	10.19	17.3	6.0	3.1
AFP-L3% SD	17.7	15.99	19.0	12.8	11.2
P value vs. Group 4	<0.0001	<0.0001	<0.0001	<0.0001	

Relative risk:

Relative risk was calculated using group 2 and 4. Due to the change in the intended use claim from 12 months to 21 months, 57 patients from Group 4 who had less than 21 months follow-up (total or after a positive AFP-L3% ≥ 10) were excluded from the calculation. The following table summarizes the distribution of patients with AFP-L3% ≥10% and those with AFP-L3% <10% in these groups.

	HCC	No HCC	Total
AFP-L3% ≥10%	20	21	41
AFP-L3% <10%	19	252	271
Total	39	273	312

Risk of HCC for AFP-L3% ≥10% = 48.8% (95% CI: 33.5%-64.1%)  
 Risk of HCC for AFP-L3% <10% = 7.0% (95%CI: 4.0%-10.1%)  
 Relative Risk = 7.0 (48.8/7.0) (95% CI: 4.1 to 11.9)

Based on information available to the physicians, definitive diagnosis could not be made for patients in Group 3 at the time of the study and therefore were not included in the above risk calculation. Since this group accounted for 16% of the data, one has to consider the possibility of spectrum bias by excluding Group 3 in the risk analysis. The following tables showed the worst case and best possible case scenarios to determine the effect of this group in the risk estimation,

## (a) Worst case scenario

	HCC	No HCC	Total
AFP-L3% $\geq$ 10%	20	21+12 = 33	53
AFP-L3% <10%	19+59 = 78	252	330
Total	98	285	383

Risk of HCC for AFP-L3%  $\geq$ 10% = 37.7% (20/53)

Risk of HCC for AFP-L3% <10% = 23.6% (78/330)

Relative Risk = 1.6 (37.7/23.6) (95% CI: 1.1 to 2.4)

## (b) Best case scenario

	HCC	No HCC	Total
AFP-L3% $\geq$ 10%	20+12 = 32	21	53
AFP-L3% <10%	19	252+59 = 311	330
Total	51	332	383

Risk of HCC for AFP-L3%  $\geq$ 10% = 60.4% (32/53)

Risk of HCC for AFP-L3% <10% = 5.8% (19/330)

Relative Risk = 10.5 (60.4/5.8) (95% CI: 6.4 to 17.1)

Relative risks for patients positive for HBV or HCV infection were also analyzed. The following table shows the relative risk calculated for HBV positive patients in Group 2 was compared to those in Group 4.

	HCC	No HCC	Total
AFP-L3% $\geq$ 10%	1	2	3
AFP-L3% <10%	3	28	31
Total	4	30	34

Risk of HCC for AFP-L3%  $\geq$ 10% = 33.3%

Risk of HCC for AFP-L3% <10% = 9.7%

Relative Risk = 3.4 (95% CI: 0.5 to 23.7)

Relative risk for HCV positive patients in Group 2 compared group 4 (see below).

	HCC	No HCC	Total
AFP-L3% $\geq$ 10%	14	13	27
AFP-L3% <10%	14	221	235
Total	28	234	262

Risk of HCC for AFP-L3%  $\geq$ 10% = 51.9%

Risk of HCC for AFP-L3% <10% = 6.0%

Relative Risk = 8.7 (95% CI: 4.7 to 16.3)

Logistic regression models were run to determine the interaction effects of hepatitis virus group (HBV, HCV and both HBV and HCV), number of days and AFP-L3% values on development of HCC. The odds ratios between AFP-L3% and development of HCC were not statistically different ( $p=0.52$ ) among the hepatitis virus groups. Excluding the interaction term between AFP-L3% and hepatitis virus group, the odds ratio between AFP-L3% and development of HCC, adjusted for days and hepatitis virus group was 21.8 (95% CI 8.9, 53.4) which is greater than the odds ratio adjusted for days alone (20.9, 95% CI 8.6, 50.3).

4. Clinical cut-off:  
AFP-L3%  $\geq$ 10% considered positive for HCC.
5. Expected values/Reference range:  
Expected value range for AFP in healthy adults is 0.1 to 5.8 ng/mL according to published literature (Masseyeff R., et al. N. Engl. J. Med. 291 (1974), 532). Normal reference value for AFP-L3% is not detectable.

**N. Instrument Name:**

Wako LiBASys

**O. System Descriptions:**

1. Device Description:  
The LiBASys consists of a main analyzer, a bar code reader and a drain port. The main analyzer contains the following units: analysis, reaction, solution feed, column setup, photometric, display operation, elution accommodation, tank accommodation and power.
2. Principles of Operation:  
See Test Principles
3. Modes of Operation:  
Automated fluorescence analyzer
4. Software:  
FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types: Yes
5. Specimen Identification:  
Bar-coding of the serum samples is done before the samples are loaded into the instrument

6. Specimen Sampling and Handling:  
Serum samples are to be assayed immediately after blood collection. If not, store samples at -80°C. Patient serum samples are put into sample cups which are loaded into the sample disk housed in the analysis unit with the reaction disk.
7. Assay Types:  
Liquid binding assay using anion column separation of immune complexes.
8. Reaction Types:  
Rate assay.
9. Calibration:  
A two-point linear calibration is required every assay run.
10. Quality Control:  
Two level controls are used. Values should be within  $\pm 15\%$  of assigned values.

**P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:**

None.

**Q. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**R. Conclusion:**

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.6030 with special controls. The special control guidance document "AFP-L3% Immunological Test System" is available at [WWW.fda.gov/cdrh/oivd/guidance/1570.pdf](http://www.fda.gov/cdrh/oivd/guidance/1570.pdf)