Code No. 999-60601 CE

Wako

μTASWako AFP-L3

Symbols in Product Labeling

REF	Catalog number		Consult instructions for use
IVD	In vitro diagnostics medical device		Use by (last day of the month)
CONT.	Contents of kit	Warning	Caution
AC.	Accessory		Manufacturer
Adaptor	Adaptor		Temperature limitation
Holder	Holder		
LOT	Batch code		
EC REP	Authorized Representative in the European Community		
BL	Blank		
CAL	Calibrator		
CONTROL	Control		
CONC.	Assigned value		

Intended use

The μTASWako AFP-L3 Immunological Test System is an *in vitro* device that consists of reagents used with the μTASWako i30 Immunoanalyzer to quantitatively measure, by immunochemical techniques, AFP-L3% in human serum. The device is intended for *in vitro* diagnostic use as an aid in the risk assessment of patients with chronic liver disease for development of hepatocellular carcinoma (HCC) in conjunction with other laboratory findings, imaging studies and clinical assessment. Patients with elevated AFP-L3% values (≥10%) have been shown to be associated with an increase in the risk of developing HCC within the next 21 months and should be more intensely evaluated for evidence of HCC according to the existing HCC practice guidelines in oncology.

Summary and explanation of the test

α-Fetoprotein (AFP) is a fetal serum protein[1, 2]. The presence of serum AFP in patients with hepatocellular carcinoma[3] and yolk sac tumor[4] was demonstrated in 1960s. After that, a number of reports have shown that abnormally elevated serum AFP concentrations are observed in several malignant diseases such as hepatocellular carcinoma or nonseminomatous testicular cancer. It is now clinically accepted that slight but significant increases in serum AFP occur in patients with chronic hepatitis (CH) and liver cirrhosis (LC), which are known as pre-malignant lesions[5-11]. Because AFP is increased in chronic liver diseases and HCC, it is difficult to distinguish between HCC and such benign diseases using AFP.

Human AFP, a glycoprotein with a molecular weight of approximately 70,000 daltons, has a single asparagine-linked carbohydrate chain and its heterogeneity was analyzed using various lectins with different affinities[12-16]. *Lens culinaris* agglutinin (LCA) has an affinity to the carbohydrate chain that has an additional α1-6 fucose residue bound to N-acetylglucosamine at the reducing end. Using LCA, AFP is classified into 3 microheterogeneity forms, L1, L2 and L3[14]. AFP-L1 and L3 are major components of AFP in serum of HCC patients. This addition of fucose residue is mainly observed when there is a structural change in carbohydrate chain caused by HCC.

AFP-L3% is the ratio of fucosylated AFP to total AFP. AFP-L3% is highly specific to HCC, and it is reported that elevated AFP-L3% often appears earlier than diagnosis of HCC made by imaging modalities[17-19].

The μTASWako AFP-L3 is a method for the quantitative determination of AFP-L3% based on a liquid-phase binding reaction between antigen and antibody and separation of bound and free forms by electrophoresis[20]. The μTASWako AFP-L3 assay is performed on the ''μTASWako i30'' instrument which is a fully automated immunoassay analyzer.

Principle of the method

The μTASWako AFP-L3 assay is an easy-to use system with all reagents in a single cartridge and each assay performed in a single, disposable ''chip'' using microfluidic electrophoretic separation[20]. After placing sample, reagent cartridge, wash solution and chip cassette on the instrument, the buffers, antibody solutions and sample are automatically dispensed into appropriate chip wells. The sample and Dye-Fab' solution are dispensed and form the primary immunocomplex (Dye-Fab' − AFP) in the well. Each solution is loaded into the microfluidic channel by vacuum.

Voltage is applied to the chip and DNA-Fab' moves to anode and is concentrated. The concentrated DNA-Fab' reacts with the primary immunocomplex and forms the secondary immunocomplex (Dye-Fab' − AFP − DNA-Fab'). The secondary immunocomplex is further concentrated during isotachophoresis to the anode and is thereby separated from unbound Dye-Fab'.

The concentrated secondary immunocomplexes of L3 and L1 are separated from unbound Dye-Fab' and from each other by electrophoresis into a matrix containing LCA. The LCA effects the separation of L1 and L3 by binding to L3 and altering its mobility. The two populations of Dye-Fab'-labeled AFP are detected by laser-induced fluorescence. The concentration of L1 and L3 is proportional to the fluorescence. All reactions, separations, and detection occur on a microfluidic chip.

Reagents

The μTASWako AFP-L3 kit contains a reagent cartridge and an adapter. The reagent cartridge contains the following buffers and antibodies for conducting 100 tests.

(1) Electrophoresis Buffer (R1) 5.6 mL
75 mmol/L Tris buffer, pH 8.0

(2) Electrophoresis Buffer (R2) 4.5 mL
75 mmol/L Tris buffer, pH 7.6
4 mg/mL *Lens culinaris* agglutinin (LCA)

(3) Electrophoresis Buffer (R3) 2.6 mL
75 mmol/L Tris buffer, pH 7.5

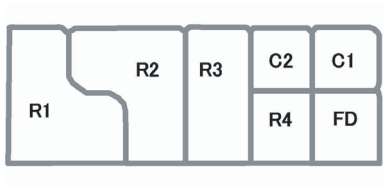
(4) Electrophoresis Buffer (R4) 1.4 mL
75 mmol/L Tris buffer, pH 7.6

(5) Labeled Antibody Solution (C1) 0.77 mL
75 mmol/L Good's buffer, pH 6.0
200 nmol/L Anion-conjugated anti human AFP antibody (mouse monoclonal antibody (DNA-Fab' (AFP))

(6) Labeled Antibody Solution (C2) 0.82 mL
50 mmol/L Phosphate buffer, pH 5.5
1000 nmol/L Fluorescent dye labeled anti human AFP antibody (mouse monoclonal antibody (Dye-Fab' (AFP))

(7) Fluorescent Dye Solution (FD) 1.4 mL
50 mmol/L Good's buffer, pH 6.0

Store the reagent cartridge at 2－10℃ (Do not freeze).



Reagent allocation in the reagent cartridge

Accessory

Adapter (Reagent cartridge opener) 1 piece

Warning and precautions

Precautions for assay

(1) For *in vitro* diagnostic use.
(2) Not to be used internally in humans or animals.
(3) Store the reagents under the specified conditions. Do not use reagents past the expiration date stated on the reagent cartridge label or more than 45 days past the opening of the cartridge.
(4) The presence of precipitates in the reagents or values of control sera outside the manufacturer's acceptable range may be an indication of reagent degradation.
(5) Do not use the reagents described above for any purpose other than described herein.
(6) Do not use the reagents described above in any procedures other than those described herein. Performance cannot be guaranteed if the reagents are used in other procedures.
(7) Do not use reagents that were frozen. Such reagents may give false results.
(8) After opening reagents, place the reagents onto the instrument immediately. Once the reagents are opened, they must be stored on the μTASWako i30.
(9) Do not use the cartridge, the adapter and other materials in the kit for any purpose other than those described herein.
(10) Operate the instruments according to the Instruction Manual.
(11) Do not reuse chips or sample cups.
(12) Calibration material is sold separately. For the usage of calibration material, refer to its package insert.
(13) It is recommended that specimen collection be carried out in accordance with CLSI Document M29-A3 and other national safety regulations. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
(14) FDA UDI (Unique Device Identification) is on the box. Keep box until reagent is finished.

Precautions for protection from hazards

(1) If the reagents come in contact with mouth, eye, or skin, wash the exposed area immediately with plenty of water. Consult a physician if necessary.
(2) μTASWako Wash Solution is 0.5 mol/L NaOH, pH 11 or higher. If the reagent comes in contact with the mouth, eye, or skin, wash off immediately with plenty of water. Consult a physician if necessary.
(3) All serum samples, and apparatuses that may be contaminated with serum, should be treated with caution to avoid infection.

This product contains components classified as follows according to the European Regulation :

Hazard designation of product

Warning

Mixture containing :

5-Chloro-2-methyl-2H-isothiazol-3-one [EC No 247-500-7] and 2-Methyl-2H-isothiazol-3-one [EC No 220-239-6] (3:1)

Information pertaining to particular dangers for man and environment

Hazard statement

May cause an allergic skin reaction.

Precautionary statement

Avoid breathing vapours/spray.
Contaminated work clothing should not be allowed out of the workplace.
Wear protective gloves/ protective clothing/ eye protection.
If on skin: Wash with plenty of soap and water.
If skin irritation or rash occurs : Get medical advice/attention.
Wash contaminated clothing before reuse.
Dispose of contents or container in accordance with local/regional/national/international regulation.

Precautions for disposal

(1) Dispose of reagents according to your local or national regulations.
(2) Wear lab protective gear when disposing of the waste liquid, used chips and sample cups to avoid infection.

Limitations of the procedure

(1) Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. The μTASWako AFP-L3 has been formulated to minimize the risk of the interference ; however, potential interactions between rare sera and ingredients can occur. For diagnostic purposes, the results obtained from this assay should always be used and interpreted in conjunction with the clinical examination, patient medical history, and other findings.
(2) Pregnancy can cause high values of AFP-L3% and AFP.
(3) AFP producing tumors other than HCC can show high values of AFP-L3% and AFP.
(4) Samples from acute hepatitis and fulminant hepatitis patients can show high values of AFP-L3% and AFP.
(5) It is recommended that this assay be used in conjunction with imaging studies for clinical diagnosis.
(6) Liver disease caused by other etiologies such as alcohol liver disease, hemachromatosis, Wilson's disease, autoimmune hepatitis and steatohepatitis have not been studied with this assay. The results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.
(7) AFP-L2 fraction, which is found in some HCC cases by using the conventional lectin-affinity electrophoresis method[21], is recognized as a part of AFP-L3 fraction in this assay system.

Instruments

The μTASWako AFP-L3 kit is designed to be used with the automated analyzer-" μTASWako i30". Refer to the Instruction Manual for a description of instrument operation and specifications.

Specimen collection and preparation

(1) Use serum as a specimen.
(2) If immediate analysis is not possible, store specimen at －80℃. AFP-L3% and AFP concentration in serum are stable for 2 years frozen at －80℃. AFP-L3% and AFP in serum are stable up to 5 days at 2－8℃. AFP-L3% and AFP in serum are stable up to 5 cycles of freeze-thaw.

Procedure for μTASWako i30

Materials supplied

Refer to the section entitled "Reagents".

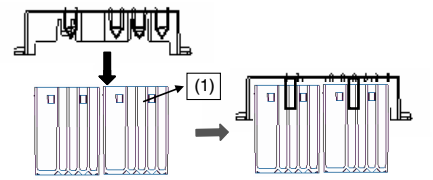
Materials required but not supplied (purchased separately)

μTASWako i30
μTASWako AFP-L3 Calibrator Set
μTASWako AFP-L3 Control L
μTASWako AFP-L3 Control H
μTASWako AFP-L3 Sample Dilution Buffer
μTASWako Wash Solution
μTASWako Chip Cassette
Sample Cup S
Pure Water

Reagent preparation

Reagents : Use as supplied. Unopened reagents are stable until expiration date printed on the label when stored at 2－10℃. Opened reagents can be used for 45 days on the μTASWako i30.

The unopened reagent cartridge is sealed with aluminum film. At the time of use, the adapter is placed on top of the reagent cartridge with the needle side downward then the aluminum seal is punctured by pressing the adapter through the cartridge completely. Lay the reagent cartridge on a flat surface when you open the reagent cartridge. Access holes are made by the needles and are used as passages for the pipetting probe. Seven access holes are observed on the reagent cartridge when above procedure is properly conducted. Do not remove the adapter after seal has been broken. Place the reagent cartridge onto the μTASWako i30 instrument according to the μTASWako i30 Instruction Manual. Store the reagent cartridge in the instrument.



Set an adapter on a reagent cartridge and fit into socket (1).

Test procedure

Refer to '' μTASWako i30 Instruction Manual'' for the details of assay procedure.

Reaction Outline

Setting of reagent cartridge, Chip Cassette and samples
↓
Dispense reagents and sample to chip
↓
Reaction with Dye-Fab'
↓
Isotachophoresis, Reaction with DNA-Fab'
↓
Capillary gel electrophoresis
↓
Fluorescent detection
↓
Peak analysis and Calculation

Calibration

Calibration is required when reagent cartridge is opened. Calibration curve is automatically produced in the μTASWako i30 by plotting fluorescence intensity of the immunocomplex peak area vs. AFP concentrations of the calibrators. The calibration curve is stable for 45 days. For a detailed description on the calibration, refer to Section 4.2 ''Calibration Operation'' of the Instruction Manual.

Quality control

A quality control program is recommended for all clinical laboratories. Daily analysis using Wako's μTASWako AFP-L3 Control L and H is recommended for monitoring the performance of the procedure. The values obtained for the controls should fall within 15% from the assigned values.

Results

The final results are automatically calculated and printed out or sent out to the host computer. The results are reported as a ratio of AFP-L3 to total AFP. Total AFP concentration is also shown in ng/mL. Refer to Section 4.11 ''Checking Test Results'' of the Instruction Manual for the information on the calculation procedure and printout format.

Reportable range

The reportable range of AFP concentration is 0.3－1000 ng/mL. The reportable range of AFP-L3% is 0.5－99.5%. AFP-L3% is calculated as follows : AFP-L3% = AFP-L3 concentration/ (AFP-L1 concentration + AFP-L3 concentration) × 100. When total AFP, AFP-L1 or AFP-L3 are under 0.3 ng/mL, AFP-L3% is not indicated. When the value exceeds 1000 ng/mL, marks H, H! or HH! appears on the screen of the μTASWako i30 and on the paper printout. When H appears, AFP and AFP-L3% value are indicated but AFP concentration exceeds the range of linearity. Dilute the sample with Sample Dilution Buffer to 1000 ng/mL or less, re-assay and multiply an obtained result by dilution factor. The dilution factors are approximately 2 to 10-fold for H, 10-fold for H! and 100-fold for HH!. Refer to Section 4.11.3 ''How to Check Printed Test Results'' of the Instruction Manual for detailed information on the marks.

Expected values

The expected values for AFP-L3% are reported to be less than 10%.[22]

Performance characteristics

Dilution Recovery

The accuracy was demonstrated by the recovery study. The results of recovery (%) ranged from 97.8% to 104.9% for AFP and ranged from 98.1% to 100.9% for AFP-L3%.

Sample	Sample Series A		Sample Series B		After Mixed Sample Series (A:B=9:1 mixed) (Expected Value)		Obtained Value		Recovery (%)	
	AFP (ng/mL)	AFP-L3% (%)	AFP (ng/mL)	AFP-L3% (%)	AFP (ng/mL)	AFP-L3% (%)	AFP (ng/mL)	AFP-L3% (%)	AFP (%)	AFP-L3% (%)
1	14.6	12.1	552.0	30.4	68.3	26.9	66.8 68.3	26.4 26.8	97.8 100.0	98.1 99.6
2	14.6	12.1	1062.0	40.4	119.3	37.3	120.6 121.2	36.9 36.8	101.1 101.6	98.9 98.7
3	14.6	12.1	2088.0	47.8	221.9	45.7	232.7 232.2	46.1 45.8	104.9 104.6	100.9 100.2
4	211.3	73.1	552.0	30.4	249.6	63.7	256.5 252.4	63.4 63.7	102.8 101.1	99.5 100.0
5	211.3	73.1	1062.0	40.4	300.6	61.5	308.6 315.0	60.9 61.2	102.7 104.8	99.0 99.5
6	211.3	73.1	2088.0	47.8	403.2	60.0	414.8 422.6	60.0 59.4	102.9 104.8	100.0 99.0
7	596.9	48.6	552.0	30.4	592.4	46.9	595.6 585.4	46.7 46.9	100.5 98.8	99.6 100.0
8	596.9	48.6	1062.0	40.4	643.4	47.2	638.5 648.6	46.9 46.7	99.2 100.8	99.4 98.9
9	596.9	48.6	2088.0	47.8	746.0	48.4	740.7 759.4	48.7 48.5	99.3 101.8	100.6 100.2

Precision

[Within-run precision]

Within-run precision studies were performed for the AFP-L3 assay over the reportable range for both AFP and AFP-L3% using 4 serum samples and 2 levels of controls. The results of CV% for each sample measured in 21 replicates ranged from 0.7% to 1.5% for AFP and ranged from 0.3% to 5.6% for AFP-L3%. This study was conducted in accordance with CLSI EP5-A2.

Sample	Number of Replicates	AFP			AFP-L3%		
		Mean (ng/mL)	SD (ng/mL)	CV (%)	Mean (%)	SD (%)	CV (%)
Serum 1	21	8.8	0.13	1.5	6.1	0.25	4.1
Serum 2	21	18.7	0.24	1.3	10.1	0.57	5.6
Serum 3	21	411.6	4.69	1.1	76.0	0.20	0.3
Serum 4	21	971.5	6.45	0.7	48.8	0.17	0.3
Control L	21	53.1	0.38	0.7	27.8	0.13	0.5
Control H	21	212.7	1.79	0.8	19.2	0.17	0.9

[Total precision]

Total precision studies were performed for the AFP-L3 assay over the reportable range for both AFP and AFP-L3 using 7 pooled human serum samples and 2 levels of controls. Three samples (5, 6 and 7) were pooled human serum samples near the clinical decision point and were prepared without spiking with analytes. The results of CV%, measured over 21 days, for all samples ranged from 1.4% to 3.1% for AFP and ranged from 0.4% to 6.3% AFP-L3%. This study was conducted in accordance with CLSI EP5-A2.

Sample	Number of test days	AFP			AFP-L3%		
		Mean (ng/mL)	ST (ng/mL)	CV (%)	Mean (%)	ST (%)	CV (%)
Serum 1	21	8.9	0.18	2.0	6.5	0.41	6.3
Serum 2	21	18.8	0.40	2.1	9.7	0.38	3.9
Serum 3	21	414.1	11.86	2.9	75.9	0.31	0.4
Serum 4	21	945.2	23.67	2.5	48.6	0.32	0.7
Serum 5	21	17.0	0.52	3.1	8.0	0.43	5.4
Serum 6	21	20.6	0.51	2.5	9.8	0.27	2.8
Serum 7	21	27.5	0.52	1.9	8.9	0.28	3.1
Control L	21	52.2	0.85	1.6	28.0	0.23	0.8
Control H	21	203.7	2.86	1.4	19.2	0.17	0.9

Linearity

The assay was verified to be linear for the reportable ranges of 0.3－1000 ng/mL of AFP and 0.5－99.5% of AFP-L3% according to CLSI EP6-A.

Limit of Detection

The limit of detection (LoD) study was carried out consistent with CLSI EP17-A ''Protocols for Determination of Limits of Detection and Limits of Quantitation ; Approved Guideline'' (Vol. 24, No. 34, 2004). From the results, the LoD for AFP-L1 and AFP-L3 was found by calculation using the equation given in CLSI EP17-A (section 4.3.2) because data distributions for both AFP-L1 and AFP-L3 were Gaussian. The LoD, the point at which the analytes are distinguished from blank, for AFP-L1 and AFP-L3 was found to be 0.030 ng/mL and 0.028 ng/mL, respectively.

Interference testing

Potential interfering substances listed below were evaluated by determining recovery in the presence of known amounts of these substances. For AFP and AFP-L3%, no significant effect from the potential interferents occurred. This study was conducted in accordance with CLSI EP7-A.

①Hemoglobin

Hemoglobin	(mg/dL)	0	193.3	386.6	579.9	773.2	966.5
AFP	(ng/mL)	18.5	18.6	19.2	19.0	19.0	19.4
	Recovery (%)	100.0	100.5	103.8	102.7	102.7	104.9
AFP-L3%	(%)	10.5	10.6	10.2	10.5	10.6	10.0
	Recovery (%)	100.0	101.0	97.1	100.0	101.0	95.2

②Bilirubin

Bilirubin	(mg/dL)	0	7.3	14.6	21.8	29.1	36.4	72.8
AFP	(ng/mL)	20.6	20.7	20.6	20.6	20.4	20.4	20.5
	Recovery (%)	100.0	100.5	100.0	100.0	99.0	99.0	99.5
AFP-L3%	(%)	10.0	9.7	9.6	9.8	9.6	9.4	9.2
	Recovery (%)	100.0	97.0	96.0	98.0	96.0	94.0	92.0

③Conjugated bilirubin

Conjugated bilirubin	(mg/dL)	0	8.0	16.1	24.1	32.1	40.2	80.4
AFP	(ng/mL)	20.4	20.7	20.6	20.3	20.5	20.2	20.5
	Recovery (%)	100.0	101.5	101.0	99.5	100.5	99.0	100.5
AFP-L3%	(%)	9.6	9.8	9.8	9.6	9.6	9.9	9.6
	Recovery (%)	100.0	102.1	102.1	100.0	100.0	103.1	100.0

④Triglycerides

Triglycerides	(mg/dL)	0	45.2	90.4	135.6	180.8	226.0	452.0
AFP	(ng/mL)	21.1	21.4	21.6	22.0	22.2	21.1	21.1
	Recovery (%)	100.0	101.4	102.4	104.3	105.2	100.0	100.0
AFP-L3%	(%)	9.8	10.0	9.8	9.8	9.9	10.0	10.1
	Recovery (%)	100.0	102.0	100.0	100.0	101.0	102.0	103.1

⑤Ascorbic acid

Ascorbic acid	(mg/dL)	0	10	20	30	40	50
AFP	(ng/mL)	19.7	20.8	19.9	20.7	20.8	19.6
	Recovery (%)	100.0	105.6	101.0	105.1	105.6	99.5
AFP-L3%	(%)	10.0	10.4	10.6	10.5	10.4	10.4
	Recovery (%)	100.0	104.0	106.0	105.0	104.0	104.0

⑥Glucose

Glucose	(mg/dL)	0	200	400	600	800	1000
AFP	(ng/mL)	19.5	20.0	20.3	20.0	20.3	20.1
	Recovery (%)	100.0	102.6	104.1	102.6	104.1	103.1
AFP-L3%	(%)	9.8	10.0	10.2	10.4	10.1	10.5
	Recovery (%)	100.0	102.0	104.1	106.1	103.1	107.1

⑦Galactose

Galactose	(mg/dL)	0	40	80	120	160	200
AFP	(ng/mL)	19.7	20.6	20.5	20.9	20.7	20.1
	Recovery (%)	100.0	104.6	104.1	106.1	105.1	102.0
AFP-L3%	(%)	10.2	10.0	9.8	10.6	10.3	10.0
	Recovery (%)	100.0	98.0	96.1	103.9	101.0	98.0

⑧Rheumatoid factor

Rheumatoid factor	(IU/mL)	0	100	200	300	400	500
AFP	(ng/mL)	22.3	22.8	22.5	22.4	22.7	23.3
	Recovery (%)	100.0	102.2	100.9	100.4	101.8	104.5
AFP-L3%	(%)	10.4	10.8	10.0	10.5	10.7	10.1
	Recovery (%)	100.0	103.8	96.2	101.0	102.9	97.1

⑨Vitamin B1

Vitamin B1	(mg/dL)	0	10	20	30	40	50
AFP	(ng/mL)	19.7	19.9	19.9	20.1	20.0	20.4
	Recovery (%)	100.0	101.0	101.0	102.0	101.5	103.6
AFP-L3%	(%)	10.5	10.7	9.6	10.5	9.9	10.0
	Recovery (%)	100.0	101.9	91.4	100.0	94.3	95.2

⑩Vitamin B6

Vitamin B6	(mg/dL)	0	10	20	30
AFP	(ng/mL)	20.1	20.0	20.0	20.0
	Recovery (%)	100.0	99.5	99.5	99.5
AFP-L3%	(%)	9.8	9.5	10.5	10.1
	Recovery (%)	100.0	96.9	107.1	103.1

⑪Vitamin B12

Vitamin B12	(mg/dL)	0	10	20	30	40	50
AFP	(ng/mL)	20.1	20.2	20.3	20.3	20.1	20.3
	Recovery (%)	100.0	100.5	101.0	101.0	100.0	101.0
AFP-L3%	(%)	10.0	9.6	10.2	10.3	9.8	10.3
	Recovery (%)	100.0	96.0	102.0	103.0	98.0	103.0

⑫Ibuprofen

Ibuprofen	(mg/dL)	0	10	20	30	40	50
AFP	(ng/mL)	22.4	22.2	22.6	22.6	22.4	22.0
	Recovery (%)	100.0	99.1	100.9	100.9	100.0	98.2
AFP-L3%	(%)	10.0	10.3	9.8	9.9	9.5	10.1
	Recovery (%)	100.0	103.0	98.0	99.0	95.0	101.0

⑬Acetaminophen

Acetaminophen	(mg/dL)	0	4	8	12	16	20
AFP	(ng/mL)	21.2	21.9	22.0	22.6	22.2	21.1
	Recovery (%)	100.0	103.3	103.8	106.6	104.7	99.5
AFP-L3%	(%)	9.9	10.3	10.0	9.7	10.0	10.3
	Recovery (%)	100.0	104.0	101.0	98.0	101.0	104.0

⑭Acetylsalicylic acid

Acetylsalicylic acid	(mg/dL)	0	10	20	30	40	50
AFP	(ng/mL)	22.3	23.7	23.3	22.9	23.3	22.1
	Recovery (%)	100.0	106.3	104.5	102.7	104.5	99.1
AFP-L3%	(%)	10.4	10.4	10.1	10.1	10.3	9.9
	Recovery (%)	100.0	100.0	97.1	97.1	99.0	95.2

⑮IFN-α

IFN-α	(IU/mL)	0	600	1200	1800	2400	3000
AFP	(ng/mL)	22.7	22.6	22.6	23.1	22.8	23.1
	Recovery (%)	100.0	99.6	99.6	101.8	100.4	101.8
AFP-L3%	(%)	10.4	10.7	9.9	10.4	10.7	9.9
	Recovery (%)	100.0	102.9	95.2	100.0	102.9	95.2

⑯IFN-β

IFN-β	(IU/mL)	0	600	1200	1800	2400	3000
AFP	(ng/mL)	22.0	22.4	22.5	22.4	22.6	22.9
	Recovery (%)	100.0	101.8	102.3	101.8	102.7	104.1
AFP-L3%	(%)	10.3	9.8	10.9	10.7	10.7	10.9
	Recovery (%)	100.0	95.1	105.8	103.9	103.9	105.8

⑰IFN-γ

IFN-γ	(JRU/mL)	0	600	1200	1800	2400	3000
AFP	(ng/mL)	22.2	23.0	23.6	24.1	22.8	23.4
	Recovery (%)	100.0	103.6	106.3	108.6	102.7	105.4
AFP-L3%	(%)	10.4	10.5	10.3	9.7	10.6	10.8
	Recovery (%)	100.0	101.0	99.0	93.3	101.9	103.8

Interference testing with high concentration of AFP samples

Potential interfering substances listed below were evaluated by determining recovery in the presence of known amounts of these substances. For AFP and AFP-L3%, no significant effect from the potential interferents occurred. This study was conducted in accordance with CLSI EP7-A.

①Hemoglobin

Hemoglobin	(mg/dL)	0	1060
AFP	(ng/mL)	485.7	478.2
	Recovery (%)	100.0	98.5
AFP-L3%	(%)	18.3	18.2
	Recovery (%)	100.0	99.5

②Bilirubin

Bilirubin	(mg/dL)	0	75
AFP	(ng/mL)	436.9	419.7
	Recovery (%)	100.0	96.1
AFP-L3%	(%)	18.3	16.9
	Recovery (%)	100.0	92.3

③Conjugated bilirubin

Conjugated bilirubin	(mg/dL)	0	80
AFP	(ng/mL)	435.8	432.0
	Recovery (%)	100.0	99.1
AFP-L3%	(%)	18.5	17.7
	Recovery (%)	100.0	95.7

④Triglycerides

Triglycerides	(mg/dL)	0	452
AFP	(ng/mL)	522.7	522.7
	Recovery (%)	100.0	100.0
AFP-L3%	(%)	18.2	18.3
	Recovery (%)	100.0	100.5

⑤Rheumatoid factor

Rheumatoid factor	(IU/mL)	0	500
AFP	(ng/mL)	533.6	537.2
	Recovery (%)	100.0	100.7
AFP-L3%	(%)	18.3	18.3
	Recovery (%)	100.0	100.0

Correlation

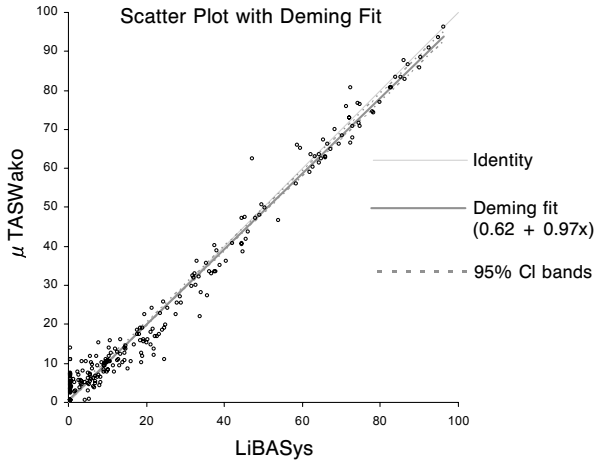
A comparison of the μTASWako AFP-L3 to a similar AFP-L3 assay (LBA AFP-L3) was performed using μTASWako i30 and LiBASys, respectively. A comparison study of 200 samples from 100 patients was conducted by both instruments. In addition, 40 serum samples spiked with AFP-L1 and AFP-L3 to cover the upper part of the reportable range were studied.

Deming analysis, excluding two outliers, shows acceptable correlation with and without spiked samples, as demonstrated in the correlation graphs given herein. Correlation #1 shows the Deming regression analysis of AFP-L3% values, with spiked samples and without outliers, run on μTASWako i30 and LiBASys. Correlation #2 shows the Deming regression analysis of AFP-L3% values, without spiked samples and without outliers.

Additionally, a calculation of an estimate of agreement is given to describe how often the μTASWako AFP-L3 test agrees with the comparative test, AFP-L3 assay (LBA AFP-L3). Using the data from correlation #2 and the clinical cut-off value of 10%, a concordance rate (overall agreement) of 90.4% is found.

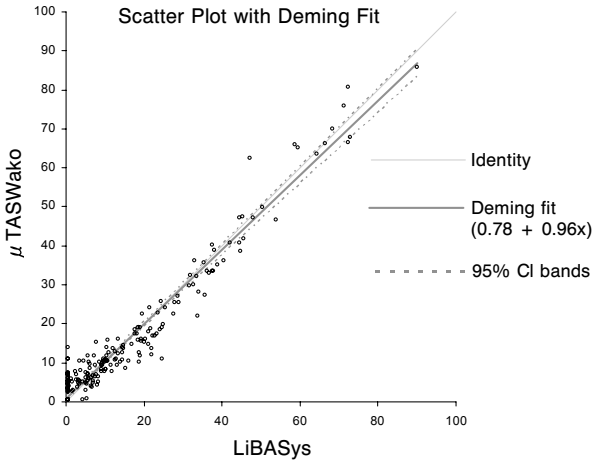
Correlation #1

Number	238	
Intercept	0.62	95% CI (−0.03 to 1.26)
Slope	0.97	95% CI (0.95 to 0.98)



Correlation #2

Number	198	
Intercept	0.78	95% CI (0.03 to 1.54)
Slope	0.96	95% CI (0.91 to 1.00)



AFP-L3% 2x2 Agreement

		LiBASys	
		≥ 10 %	< 10 %
μTASWako i30	≥ 10 %	93 (47.0%)	13 (6.6%)
	< 10 %	6 (3.0%)	86 (43.4%)

Percent positive agreement = 93.9%
Percent negative agreement = 86.9%
Overall agreement = 90.4%

Clinical Information Collected with μTASWako i30

To understand the impact of discordance in the correlation study, a secondary study was conducted to demonstrate the relative risk determinations based on the test performance for μTASWako i30 AFP-L3 assay and compared to previous results obtained on the predicate LiBASys test system. For the calculation of relative risk (RR), the original 437 samples used to determine RR in the original study were re-tested on the μTASWako i30. Specimen stability studies were conducted to demonstrate the specimens were suitable for retesting. For the definition of the Groups A, B, and C, refer to the section on ''Clinical Information Collected with LiBASys''. Of the 437 samples tested, the 54 with existing HCC diagnosis as Group D are not included in the RR calculations.

Relative Risk was calculated using Groups A and C. The following table summarizes the relative risk calculations with AFP-L3% ≥ 10 and those with AFP-L3% < 10 for the μTASWako i30 system and the previous results obtained on the predicate LiBASys test system:

Groups A and C Only	Risk (95% CI) μTASWako i30	Risk (95% CI) LiBASys
Relative Risk	10.6 (5.4 to 20.6)	7.0 (4.1 to 12.0)
Risk of HCC given AFP-L3% positive	43.3% (31.4 to 55.1)	48.8% (33.4 to 64.1)
Risk of HCC given AFP-L3% negative	4.1% (1.6 to 6.6)	7.0% (4.0 to 10.0)

CI = Confidence Interval

The patients categorized as ''Suspicious'' were treated as a separate group because no definitive diagnosis could be obtained from physicians and therefore was not included in the above risk calculation. To consider the possibility of spectrum bias by excluding this Group from the risk analysis, the following tables showed the worst case and best case scenarios to determine the effect of this group in risk estimation:

Best Case :	Risk (95% CI) μTASWako i30	Risk (95% CI) LiBASys
Relative Risk	16.9 (9.0 to 31.9)	10.4 (6.4 to 16.9)
Risk of HCC given AFP-L3% positive	57.8% (47.6 to 68.0)	60.4% (47.2 to 73.6)
Risk of HCC given AFP-L3% negative	3.4% (1.3 to 5.5)	5.8% (3.3 to 8.3)

Worst Case :	Risk (95% CI) μTASWako i30	Risk (95% CI) LiBASys
Relative Risk	1.6 (1.1 to 2.4)	1.6 (1.1 to 2.4)
Risk of HCC given AFP-L3% positive	32.2% (22.6 to 41.9)	37.7% (24.7 to 50.7)
Risk of HCC given AFP-L3% negative	19.8% (15.2 to 24.4)	23.6% (19.0 to 28.2)

Clinical Information Collected with LiBASys

Longitudinal data were collected on 437 subjects with liver disease from seven clinical sites. The study subjects consisted of 321 males and 116 females with an average age of 52.5 years and ranged from 40 to 70 years. Serum was collected on the average of every 137 days. The study subjects were categorized into three groups based on biopsy, explanted liver histology and imaging results : Group A patients developed confirmed HCC during study and with lesions of at least 0.5 cm in diameter, Group B patients were suspected of possible HCC with lesions at least 0.3 cm in diameter and high total AFP results and Group C patients did not have HCC. The risk of developing HCC among patients with an elevation of AFPL3% above 10% and among patients without such an elevation is calculated, along with their exact 95% confidence interval using AFP-L3% results from Group A and Group C. The risk of developing HCC with an elevated AFP-L3% test is 48.8%. The risk of developing HCC with a negative AFP-L3% test result is 7.0%. Their ratio is 7.0, indicating a seven-fold increase of developing HCC given an elevated AFP-L3% test result.

Group		A	C	Total	B
		HCC	No HCC		Suspicious*
AFP-L3	AFP-L3%≥10%	20	21	41	12
	AFP-L3%<10%	19	252	271	59
Total		39	273	312	71

Relative risk : 7.0 (95% C.I. : 4.1–12.0)
Risk of HCC given AFP-L3%≥10% : 48.8% (95% C.I. : 33.4%–64.1%)
Risk of HCC given AFP-L3%<10% : 7.0% (95% C.I. : 4.0%–10.0%)

*The patients categorized as ''Suspicious'' (far right column) were treated as a separate study group because no definitive diagnosis could be obtained from the physicians. However, for demonstrative purposes, these patients were included in the analysis to illustrate the effect of this regrouping on the relative risk calculations. The worst case and the best case scenarios are shown in the following two tables.

Best case scenario		HCC	No HCC	Total
AFP-L3	AFP-L3%≥10%	20+12=32	21	53
	AFP-L3%<10%	19	252+59=311	330
Total		51	332	383

Relative risk : 10.4 (95% C.I. : 6.4–16.9)
Risk of HCC given AFP-L3%≥10% : 60.4% (95% C.I. : 47.2%–73.6%)
Risk of HCC given AFP-L3%<10% : 5.8% (95% C.I. : 3.3%–8.3%)

Worst case scenario		HCC	No HCC	Total
AFP-L3	AFP-L3%≥10%	20	21+12=33	53
	AFP-L3%<10%	19+59=78	252	330
Total		98	285	383

Relative risk : 1.6 (95% C.I. : 1.1–2.4)
Risk of HCC given AFP-L3%≥10% : 37.7% (95% C.I. : 24.7%–50.7%)
Risk of HCC given AFP-L3%<10% : 23.6% (95% C.I. : 19.0%–28.2%)

References

(1) Bergstrand CG., Czar B., Scand. J. Clin. Lab. Invest., **8**, 174 (1956)
(2) Halbrecht I., Klibanski C., Nature, **178**, 794-795 (1956)
(3) Tatarinov YS, Vopr. Med. Khim., **10**, 90-91 (1964)
(4) Abelev GI., et al., Int. J. Cancer **2**, 551-558 (1967)
(5) Masopust J., et al., Int. J. Cancer, **3**, 364-373 (1968)
(6) Nishi S., Hirai H., Gann. Monogr. Cancer Res., **14**, 79-87 (1973)
(7) Ruoslahti E., et al., Br. Med. J., **8**, 527-529 (1974)
(8) Alpert E., Okuda K., Peters RL., eds. Hepatocellular carcinoma, New York, John Wiley & Sons, 353-367 (1976)
(9) Lehmann F-G, Wagener T., Lehmann F-G. ed. Carcinoembryonic proteins. Vol I., Amsterdam, Elsevier/North-Holland Biomedical Press, 219-231 (1979)
(10) National Institutes of Health Consensus Development Statement, ''Management of Hepatitis C : 2002'', June 10-11 (2002)
(11) National Comprehensive Cancer Network, Practice Guidelines in Oncology V.1 (2003)
(12) Smith CJ., Kelleher P.C., Biochim. Biophys. Acta, **317**, 231-235 (1973)
(13) Bϕg-Hansen T.C., et al., Scand. J. Immunol., 4(suppl), 141 – 147 (1975)
(14) Kerckaert J-P., et al., Biochim. Biophys. Acta, **576**, 99-108 (1979)
(15) Breborowicz J., et al., Scand. J. Immunol., **14**, 15-20 (1981)
(16) Miyazaki J., et al., Acta. Hepatol. Jpn., **22**, 1559-1568 (1981)
(17) Taketa K., et al., Cancer Res., **53**, 5419-5423 (1993)
(18) Sato Y., et al., N. Engl. J. Med., **328**, 1802-1806 (1993)
(19) Shiraki K., et al., Hepatology, **22**, 802-807 (1995)
(20) Kagebayashi C., et al., Anal. Biochemistry. **388**, 306-311(2009)
(21) Taketa K., et al., Electrophoresis, 10(8-9), 562-7(1989)
(22) Oka H., et. al., J. Gastroenterol. Hepatol., **16**, 1378-1383 (2001)

Ordering information

Code No.	Wako Product	Package
999-60601	μTASWako AFP-L3	100 Tests
997-60901	μTASWako AFP-L3 Calibrator Set Blank (2×2 mL) Calibrator 1 (1×2 mL) Calibrator 2 (1×2 mL)	1 Set
997-61001	μTASWako AFP-L3 Control L	4 × 2 mL
993-61101	μTASWako AFP-L3 Control H	4 × 2 mL
997-61501	μTASWako AFP-L3 Sample Dilution Buffer	3 × 10 mL
991-60801	μTASWako Wash Solution	4 × 60 mL
993-61601	μTASWako Chip Cassette	5 × 20 Pieces
452-00501	Sample Cup S	1000 Pieces

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