

LiBASys™

Performance Characteristics of AFP-L3%

INTRODUCTION

Over the last several years the biomarker AFP has been used as a tool in the diagnosis of HCC. However, AFP results are often not definitive until a tumor has reached a large size or an advanced state. Recently, a related biomarker called AFP-L3 has been shown to help rule out HCC in cases where AFP values alone are ambiguous. This data-sheet describes AFP-L3% and its use in the field of liver cancer.

AFP and AFP-L3%

AFP (α-fetoprotein) is a glycoprotein with a molecular weight of about 70,000 Daltons and has a single asparagine-linked carbohydrate chain. In some cases, blood levels of AFP are known to be elevated in the presence of liver cancer as well as in benign liver diseases, such as chronic hepatitis and cirrhosis. Heterogeneity of the carbohydrate moiety of AFP results in the formation of three isoforms called L1, L2 and L3. The carbohydrate chain of AFP-L3 is fucosylated as shown in Figure 1, and can be distinguished from the other isoforms of AFP based on its differential affinity to the lectin *Lens culinaris* agglutinin (LCA). AFP-L3% (AFP-L3 concentration/total AFP concentration) is used as an indicator of the presence of HCC.

AFP-L3% is highly specific to HCC (see Table 1). It often shows positive results (>10%) before diagnosis of HCC can be made by imaging modalities, or before greatly elevated (>200 ng/mL) levels of AFP. The mean lead time of an elevated AFP-L3% result was 205 days

before diagnosis by imaging in a North American study (manuscript in preparation). AFP-L3% can also be useful in the prognosis of HCC after therapy, and its utility in revealing malignancies has been described in several published manuscripts.^{1,2}

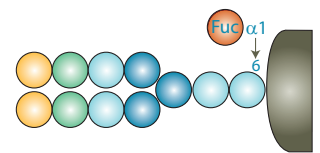


Figure 1:
Sugar chain structure of AFP-L3%

Table 1: AFP-L3% is a superior indicator of relative risk

	AFP-L3%	AFP
Cutoff value	10%	20 ng/mL
Sensitivity	51.3%	59.0%
Specificity	92.5%	75.3%
Relative risk (95% C.I.)	8.2 (4.7-14.3)	3.7 (2.1-6.8)
Positive predictive value	39.2%	18.9%
Negative predictive value	95.1%	95.0%

LIBASYS: PRINCIPLE OF OPERATION

LiBASys, seen in Figure 2, is an automated instrument used for the determination of both AFP and AFP-L3 concentrations in blood samples.³ LiBASys is an acronym that stands for Liquid phase binding assay system. In this system the antigen-antibody reaction is first performed in the liquid phase. The next step is the separation of AFP into its isoforms by anionic exchange chromatography that is assisted by the differential binding of the isoforms to LCA. Signal is amplified by antibody-conjugated peroxidase generating a fluorescent product. Fluorescence detection is used to measure activity of the analytes. A batch of up to 47 samples can be analyzed in 4 hours.

AFP-L3% test is FDA approved.



Figure 2: LiBASys

Performance Characteristics for the analysis of AFP-L3%

The within-run precision and total precision for AFP-L3% determinations are shown in Tables 2 and 3, and demonstrate that the assay is both rugged and reliable. Linearity is also very good for both AFP (Figure 3) as well as AFP-L3% (Figure 4) determinations. All data were collected in accordance with NCCLS protocol. The expected range for AFP-L3% has been reported to be less than 10% in healthy individuals.⁴ The recovery of AFP and AFP-L3% was 95.1-108.4% and 89.8-121.2%, in the concentration ranges of 15.4, 44.2, 148.6 ng/mL and 2.7, 66.6, 5.2 % respectively.

Table 2: Within-run precision

No.	Replicate	Mean		SD		CV (%)	
		AFP (ng/mL)	AFP-L3% (ng/mL)	AFP (ng/mL)	AFP-L3% (ng/mL)	AFP (ng/mL)	AFP-L3% (ng/mL)
1	21	32.5	24.8	0.48	0.82	1.5	3.3
2	21	501.9	23.1	7.31	0.70	1.5	3.0
3	21	174.2	71.5	2.39	0.53	1.4	0.7

Table 3: Total precision

	Sample 1		Sample 2		Sample 3	
	AFP	AFP-L3%	AFP	AFP-L3%	AFP	AFP-L3%
Mean value	33.1	25.4	506.5	23.3	175.3	71.8
Within run SD (Swr)	0.50	1.01	8.89	1.28	2.90	0.74
Within run CV	1.5%	4.0%	1.8%	5.5%	1.7%	1.0%
Day to Day SD (Sdd)	1.15	0.55	12.72	0.53	2.87	0.70
Day to Day CV	3.5%	2.2%	2.5%	2.3%	1.6%	1.0%
Run to Run SD (Srr)	1.41	1.14	10.76	0.67	5.46	1.64
Run to Run CV	4.3%	4.5%	2.1%	2.9%	3.1%	2.3%
Total SD (ST)	1.89	1.62	18.88	1.54	6.82	1.93
Total CV	5.7%	6.4%	3.7%	6.6%	3.9%	2.7%

Figure 3: AFP Linearity

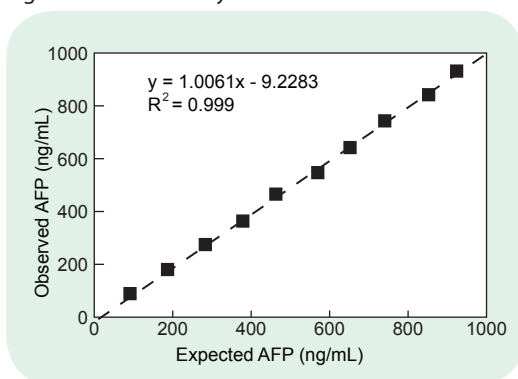
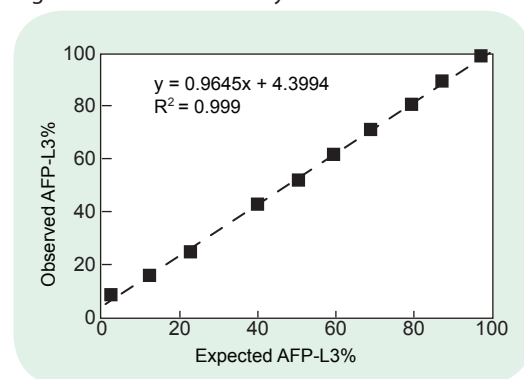


Figure 4: AFP-L3% Linearity



References

1. Leerapun, A. et al. The Utility of AFP-L3% in the diagnosis of hepatocellular carcinoma: Evaluation in a U.S. referral population. *Gastroenterology*, April 2006; 130, S2:774
2. Yamashita, F. et al. Prognostic significance of *Lens culinaris* agglutinin A-reactive α -fetoprotein in small hepatocellular carcinomas. *Gastroenterology* 1996;111:996-1001
3. Yamagata, Y. et al. Simultaneous determination of percentage of *Lens culinaris* agglutinin-reactive α -fetoprotein and α -fetoprotein concentration using the LiBASys clinical auto-analyzer. *Clin. Chim. Acta* 2003; 327:59-67.
4. Oka, H. et al., Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of *Lens culinaris* agglutinin reactive α -fetoprotein. *Journal of Gastroenterology and Hepatology* 2001; 16:1378-1383.