

K990138

JUN 21 1999

510(k) SUMMARY OF SAFETY AND EFFECTIVENESS FOR THE
DSL 10-8400 ACTIVE™ AFP ELISA KIT

Name of Device: DSL 10-8400 ACTIVE™ AFP ELISA Kit
Classification Name: Enzyme Linked Immunosorbent Assay, Alpha-Fetoprotein
Analyte Code and Name: Alpha-Fetoprotein
Regulatory Class: II

Submitter: John Class
Diagnostic Systems Laboratories, Inc.
445 Medical Center Boulevard
Webster, Texas 77598 USA
Phone: 281-332-9678
E-mail: Jclass@dslabs.com

Date: January 18, 1999

DEVICE DESCRIPTION

The DSL ACTIVE™ AFP ELISA assay is intended for the quantitative determination of AFP in human serum. It is intended for *in vitro* diagnostic use to aid in the management of patients with nonseminomatous testicular cancer.

The DSL-10-8400 ACTIVE™ AFP ELISA is an enzymatically amplified "two-step" sandwich-type immunoassay. In the assay, Standards, Controls and unknown serum samples are incubated in microtitration wells which have been coated with anti-AFP antibody. After incubation and washing, the wells are treated with another anti-AFP detection antibody labelled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the substrate tetramethylbenzidine (TMB). An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 and 620 nm.

The absorbance measured is directly proportional to the concentration of AFP present. A set of AFP Standards is used to plot a standard curve of absorbance versus AFP concentration from which the AFP concentrations in the unknowns can be calculated.

Alpha-Fetoprotein (AFP) is a 68 kDa protein which is produced primarily during fetal life by the fetal liver yolk sac [1].

Elevated AFP levels are seen in patients with nonseminomatous testicular cancer. More than 95% of testicular cancers belong to a heterogeneous group called germ-cell tumors because it is widely believed that they arise in primordial germ cells [3]. Germ cell tumors (GCTs) are classified either as seminomatous or as nonseminomatous. The latter can be further classified as embryonal carcinoma, teratoma, or choriocarcinoma. The seminoma histologic

subtype can be found in 40% of all germ cell tumors while the nonseminoma histologic subtype can be found in 60% of germ cell tumors [4]. The different histologic types of germ cell tumors may occur singly or in various combinations. Elevated AFP levels have been observed in patients diagnosed as having seminomatous testicular cancer with nonseminomatous elements, but not in patients with pure seminoma [5-10].

Both AFP and hCG are measured in testicular cancer. Approximately 40% of patients with nonseminomatous germ stem cell tumors have elevation of only one marker [11]. During the clinical course of the disease, the levels of the two markers do not always parallel each other. A direct relationship has been observed between the incidence of elevated AFP levels in nonseminomatous testicular cancer, and the stage of the disease [5-7]. Elevation of AFP (> 10 IU/L or 12.1 ng/mL) occurs in 80% of metastatic and in 57% of stage 1 nonseminomatous germ cell tumors [11]. In Clinical Stage 2B or higher, AFP and/or hCG are elevated in 65-80% of the cases with increasing frequency according to the bulk of the disease [13].

The usefulness of AFP measurements in the management of nonseminomatous testicular cancer patients undergoing cancer therapy has been well established [5, 7, 14]. Current management of testicular germ cell tumors relies upon the use of serum tumor markers which can indicate the presence of small foci of active tumor that cannot be detected by currently available imaging techniques [11]. Serum markers augment and complement information obtained from radiographic and other staging procedures [15]. Also, the short half-lives of tumor markers facilitate their use in assessing tumor burden during therapy. AFP has a serum half-life of 3.5 - 6 days [16]. AFP and/or hCG levels are elevated before orchiectomy in about 60% of all Clinical Stage I patients but follow a normal decline after the testicle is removed [13].

For patients in clinical remission following treatment, AFP levels generally decrease [7]. Post-operative AFP levels which fail to return to normal strongly suggest the presence of residual tumor [5, 7, 17]. Following successful resection of primary or metastatic disease, AFP and hCG decline at a rate proportional to their respective half-lives [16]. An elevated actual half-life of serum markers following orchiectomy or retroperitoneal lymph node dissection may indicate the presence of occult, persistent disease [15].

As recently as the 1970s, nonseminomatous germ cell tumors were often fatal. Due to advances in chemotherapy, most patients are cured, even those with disseminated disease [3]. The clinical use of AFP and hCG measurements has been essential to this success. Many patients have a marker surge during the first week of chemotherapy, presumably secondary to tumor lysis. AFP may increase from 20% to 200% over pretreatment levels [15]. Chemotherapeutic responses are accompanied by a decline in marker levels. Persistent marker elevation is usually the result of residual malignancy. Rising marker values may occur before or after clinical recurrence and one marker may rise in discordance with the other [16].

Tumor recurrence is often accompanied by a rise in serum AFP values prior to clinical evidence of progressive disease [5-6].

Elevated serum levels of AFP are also associated with some non-testicular cancers. Increased serum concentrations of AFP were first observed in human subjects with primary hepatocellular carcinoma [12]. Subsequently, elevated serum AFP values have been associated with other malignant diseases such as teratocarcinoma (with yolk sac components) of the ovary, endodermal sinus tumors, certain gastrointestinal tumors (with and without liver metastasis), and tumors of other tissues [13-14, 17-21]. A study performed at the National Institutes of Health and the Mayo Clinic demonstrated elevated AFP values in patients with pancreatic, gastric, colon, and lung cancer [15]. In additional studies, AFP was elevated in 60-80% of patients with hepatocellular cancer, in 23% of

patients with gastrointestinal cancer and in 10% of patients with liver metastasis from various tumor types [13]. However, a normalization of markers may not mean that all viable tumor has been eliminated [15].

Notably however, elevated serum AFP concentrations have also been reported in patients with noncancerous diseases such as ataxia telangiectasia, heredity tyrosinemia, neonatal hyperbilirubinemia, acute viral hepatitis, chronic active hepatitis, cirrhosis, and other benign hepatic conditions [15, 17, 24-29]. AFP is modestly elevated (up to 100 ng/mL) in 20% of patients with non-malignant liver disease [13]. Due to its lack of specificity for malignant conditions, AFP testing is not recommended as a screening procedure to detect cancer in the general population.

The absorbance measured is directly proportional to the concentration of AFP present. A set of AFP Standards is used to plot a standard curve of absorbance versus AFP concentration from which the AFP concentrations in the unknowns can be calculated.

SUMMARY OF SUBSTANTIAL EQUIVALENCE STUDY

The DSL ACTIVE™ AFP ELISA is substantially equivalent to the Abbott IMx AFP Immunoassay.

In order to demonstrate substantial equivalence between the two assays, male human serum samples (n = 73) were collected and assayed using both methods. Samples were chosen based on expected AFP levels so that samples with low, intermediate and high levels would be evaluated. Linear regression analysis of the results obtained for the comparison gave the equation $Y = 1.0(X) + 5.8$ with a correlation coefficient of (r) = 0.99.

CHARACTERIZATION OF ANTIBODY

The detection and the coating antibody are highly specific for human AFP and do not cross react with human albumin. The affinity constant ranges from $3 - 4 \times 10^{10}$ L/mol.

SUMMARY OF NONCLINICAL STUDIES

PERFORMANCE CHARACTERISTICS

All performance characteristics are stated in ng/mL. To convert to nmol/L:
$$\text{ng/mL} \times 0.068 = \text{nmol/L}$$

I. Sensitivity

The theoretical sensitivity, or minimum detection limit, calculated by the interpolation of the mean plus two standard deviations of 22 replicates of the 0ng/mL AFP Standard, is 0.7 ng/mL.

II. Precision

The intra-assay precision was determined from the mean of 14 replicates each with three male human serum samples. The following results were obtained:

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	Coefficient of Variation (%)
I	49.3	3.4	6.9
II	163.9	6.6	4.0
III	260.8	12.8	4.9

The inter-assay precision was determined from the mean of 4 replicates each in 2 separate runs each day for 20 days with three human serum samples. The following results were obtained:

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	Coefficient of Variation (%)
I	16.7	1.8	10.7
II	99.5	7.6	7.6
III	290.5	12.8	5.6

III. Recovery

Three male human serum samples containing different levels of endogenous AFP were spiked with known amounts of AFP and assayed. The following results were obtained:

Sample	Engogenous (ng/ml)	Added (ng/ml)	Expected (ng/ml)	Observed (ng/ml)	Recovery (%)
I	0	25.0	25.0	28.3	113
		200.0	200.0	193.4	97
		300.0	300.0	327.4	109
II	14.3	10.0	24.3	28.3	116
		200.0	214.3	202.1	94
		300.0	314.3	361.8	115
III	23.2	50.0	73.2	79.9	109
		200.0	223.2	217.1	97
		300.0	323.2	367.7	114

IV. Linearity

Three male human serum samples were diluted with the 0 ng/mL AFP Standard and assayed. The Following results were obtained:

Sample	Dilution Factor	Expected (ng/ml)	Observed (ng/ml)	Recovery (%)
I	---	---	47.9	---
	1:2	24.0	21.5	90
	1:4	12.0	10.1	84
	1:8	6.0	4.4	73
II	---	---	229.5	---
	1:2	114.8	114.8	100
	1:4	57.4	59.4	103
	1:8	28.7	29.3	102
	1:16	14.3	14.9	104
	1:32	7.2	7.5	104
III	---	---	368.9	---
	1:2	184.4	210.8	114
	1:4	92.2	96.9	105
	1:8	46.1	46.2	100
	1:16	23.1	22.1	96
	1:32	11.5	10.3	90

V. Specificity

The following substances did not interfere with the measurement of AFP in the DSL-10-8400 ACTIVE™ AFP ELISA.

Non-interfering Compounds	Added Concentration
Prolactin	1000 µg/L
HLH	10,000 µg/L
HTSH	100 mlU/L
HCG	10,000 IU/L
Aminophylline	100 µg/ml
Atropine	100 µg/ml
Furosemide	100 µg/ml
Theobromine	100 µg/ml
Diethylsibesterol	100 µg/ml
Megesterol Acetate	100 µg/ml
4-Acetamidophenol	100 µg/ml
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
Ibuprofen	100 µg/ml
Amethopterine	100 µg/ml

SUMMARY OF CLINICAL STUDIES

To demonstrate that the DSL 10-8400 Active AFP ELISA is safe and effect as an aid in nonseminomatous testicular cancer patient management, the following clinical studies were performed.

I. EXPECTED VALUES

Each laboratory should establish its own range of expected AFP values. In a study conducted with apparently normal healthy adults, using the DSL AFP ELISA, the following values were observed:

Population	N	0 - 10 ng/mL	10 - 20 ng/mL	20 - 500 ng/mL
Males	199	195	4	0
Females	72	69	1	2

In this study 97.4% of healthy individuals had AFP values less than 10 ng/mL; 98.0% of the healthy males had AFP values less than 10 ng/mL.

II. LONGITUDINAL STUDY OF NONSEMINOMATOUS TESTICULAR CANCER PATIENTS

Three patients with diagnosed nonseminomatous testicular cancer were serially monitored over the course of their treatment. The DSL ACTIVE™ AFP ELISA was used to measure the AFP levels of these patients' serum samples.

Patient number one had a relapse of nonseminomatous testicular cancer, which did not respond to chemotherapy treatment. His serum AFP levels rose steadily, except for a small decrease at six months, to an eventual AFP concentration over 450 ng/ml (AFP) (Table 1, Graph 1).

Patient number two responded to chemotherapy treatment. His serum AFP levels were stable, with no clinical evidence of nonseminomatous testicular cancer, 18 months post treatment (Table 1, Graph 1).

Patient number three also responded to chemotherapy treatment. His serum AFP levels decreased steadily during treatment (Table 1, Graph 1).

Additionally, the serum AFP levels of fifteen male patients with nonseminomatous testicular cancer were determined using the DSL Active AFP ELISA. The results following results were obtained:

Population	N	0 – 8.9 ng/ml	> 8.9 – 100 ng/ml	> 100 – 400 ng/ml	> 400 ng/ml
Males	15	33.3%	46.7%	6.7%	13.3%

CONCLUSION OF CLINICAL AND NONCLINICAL STUDIES

The DSL Active™ AFP ELISA is a safe and effective assay to aid in the management of patients with nonseminomatous testicular cancer. The clinical, nonclinical and method comparison studies support this conclusion.

REFERENCES

1. Seppälä M: Fetal pathophysiology of human -fetoprotein. *Ann NY Sci* 259:59-73, 1975
3. Lange PH. Testicular Cancer Markers. in *Human Cancer Markers*. Sell S and Wahren B 9ed.) Vlifton, Humana. 259-273, 1985.
4. Small EJ, Torti FM. Testes. in *Clinical Oncology*. New York, Churchill livingstone. 1493-1526, 1995.
5. Kohn J, Orr AH, McElwain TJ, et al. Serum Alpha-Fetoprotein in Patients with Testicular Tumours. *Lancet* 2: 433-436, 1976.
6. Scardino PT, Cox HD, Waldmann TA, et al. The Value of serum Tumor Markers in the Staging and Prognosis of Germ Cell Tumors of the Testis. *J. Urol.* 118: 994, 1977.
7. Lange PH, McIntire KR, Waldmann TA, et al. Serum Alpha-Fetoprotein and Human Chorionic Gonadotropin in the Diagnosis and Management of Nonseminomatous Germ Cell Testicular Cancer. *Medical Intelligence* 295: 1237, 1976.
8. Javadpour N, McIntire KR, Waldmann TA. Human Chorionic Gonadotropin (HCG) and Alpha-Fetoprotein (AFP) in Sera and Tumor Cells of Patients with Testicular Seminoma, A Prospective Studt. *Cancer* 42: 2768-2772, 1978.
9. Lange PH, Nochomovitz LE, Rosai J, et al. Serum Alpha-Fetoprotein and Human Chorionic Gonadotropin in Patients with Seminoma. *J. Urol.* 124: 472-478, 1980.
10. Jacobsen GK. Alpha-Fetoprotein (AFP) and Human Chorionic Gonadotropin (HCG) in Testicular Germ Cell Tumors. *Acta Path Microbiol Immunol Scand* 91: 183-190, 1983.
11. Doherty AP, Bower M, Christmas TJ. The Role of Tumour Markers in the Diagnosis and Treatment of Testicular Germ Cell Cancers. *Brit J Urol* 79: 247-252, 1997.
12. Tatarinov YS. Finding of an Embryonic Alpha Globulin in the Blood Stream in a Patient with Primary Hepatic Cancer. *Vopr Med Khim* 10: 90, 1964.
13. Klepp O. Serum Tumour Markers in Testicular and Extragonadal Germ Cell Malignancies. *Scand J Clin Lab Invest Suppl.* 51: 28-41, 1991.
14. Perlin E, Engeler JE, Edson M, et al. The Value of Serial Measurement of Both Human Chorionic Gonadotropin and Alpha-Fetoprotein for Monitoring Germinal Cell Tumors. *Cancer* 37: 215-219, 1976.
15. Bartlett NL, Freiha FF, Torti FM. Serum markers in Germ Cell Neoplasma. *Hem/Onc Clinics of N.A.* 5: 1245-1260, 1991.
16. Jacobs EL, Haskell CM. Clinical Use of tumor Markers in Oncology. in *Current Problems in Cancer*. Littleton, Mosby-Year Book. 299-359, 1991.
17. Waldmann TA, McIntire KR. The Use of a Radioimmunoassay for Alpha-Fetoprotein in the Diagnosis of Malignancy. *Cancer* 34: 1510-1515, 1974.
18. Silver HKB, Gold P, Feder S, et al. Radioimmunoassay for Human Alpha-Fetoprotein. *Proc Nat Acad Sci USA* 70: 526-530, 1973.
19. Abelev GI. Alpha-Fetoprotein in Ontogenesis and Its Association With Malignant Tumors. *Adv Cancer Res* 14: 295, 1971.
20. Maeyama M, Tayama C, Inoue S, et al. Serial Serum Determination on Alpha-Fetoprotein as a Marker of the Effect of postoperative Chemotherapy in Ovarian Endodermal Sinus Tumor. *Gynecol Oncol* 17: 104-116, 1984.
21. Yasunami R, Hashimoto Z, Ogura T, et al. Primary Lung Cancer Producing Alpha-Fetoprotein: A Case Report. *Cancer* 47: 926-929, 1981.

22. D'Costa M, Feld R, Laxdal V., et al. A Multicenter Evaluation of the Boehringer Mannheim ES 300 Immunoassay System. *Clin Biochem* 26: 51-57, 1993.
23. Cattini R, Cooksey M, Robinson D, et al. Measurement of Alpha-Fetoprotein, Carcinoembryonic Antigen and Prostate-Specific Antigen in Serum and Heparinised Plasma by Enzyme Immunoassay of the Fully Automated Serono SR1™ Analyzer. *Eur J Clin Chem Clin Biochem* 31: 517-524, 1993.
24. Wepsic HT. Alpha-Fetoprotein: Its Quantitation and Relationship to Neoplastic Disease. in *Alpha-Fetoprotein, Laboratory Procedures and Clinical Applications*.
25. Chen DS, Sung JL. Relationship of Hepatitis B Surface Antigen to Serum Alpha-Fetoprotein in Non-Malignant Diseases of the Liver. *Cancer* 44: 984-992, 1979.
26. Waldmann TA, McIntire KR. Serum Alpha-Fetoprotein Levels in Patients with Ataxia Telangiectasia. *Lancet* 2: 1112-1115, 1972.
27. Belanger L. Tyrosinémie Héritaire et Alpha-Foetoprotéine II. Recherche Tissulaire Comparée de L'Alpha-Foetoprotéine dans Deux Cas de Tyrosinémie Héritaire. Considérations sur L'Ontogénèse de la Foetoprotéine Humaine. *Path Biol* 21: 457-462, 1973.
28. Kew MC, Purves LR, Bersohn I. Serum Alpha-Fetoprotein Levels in Acute Viral Hepatitis. *Gut* 14: 939-942, 1973.
29. Endo Y, Kanai K, Oda T, et al. Clinical Significance of Alpha-Fetoprotein in Hepatitis and Liver Cirrhosis. *Ann NY Acad Sci* 259: 234-238, 1975.
30. Purves LR, Purves M. Serum Alpha-Fetoprotein. VI. The Radioimmunoassay Evidence for the Presence of AFP in the Serum of Normal People and During Pregnancy. *S Afr Med J* 46: 1290, 1972.
33. Primus FJ, et al. "Sandwich"-type immunoassay of carcinoembryonic antigen in patients receiving murine antibody for diagnosis and therapy. *Clin Chem* 34: 261, 1988.
34. Hansen HJ, et al. Solving the problem of antibody interference in commercial "sandwich"-type immunoassay of carcinoembryonic antigen. *Clin Chem* 35: 146, 1989.
35. Schroff RJ, et al. Human anti-murine immunoglobulin responses in patients receiving monoclonal antibody therapy. *Cancer Res* 45: 879, 1985.



DEPARTMENT OF HEALTH & HUMAN SERVICES

JUN 21 1999

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Mr. John G. Class
Manager of Regulatory Affairs
Diagnostic Systems Laboratories, Inc.
445 Medical Center Boulevard
Webster, Texas 77598

Re: K990138
Trade Name: DSL 10-8400 ACTIVE™ AFP ELISA Kit
Regulatory Class: II
Product Code: LOJ
Dated: April 20, 1999
Received: April 21, 1999

Dear Mr. Class:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the Current Good Manufacturing Practice requirements, as set forth in the Quality System Regulation (QS) for Medical Devices: General regulation (21 CFR Part 820) and that, through periodic QS inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal laws or regulations.

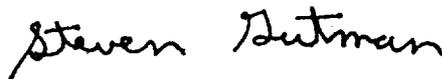
Page 2

Under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), this device may require a CLIA complexity categorization. To determine if it does, you should contact the Centers for Disease Control and Prevention (CDC) at (770) 488-7655.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification"(21 CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll-free number (800) 638-2041 or (301) 443-6597, or at its internet address "<http://www.fda.gov/cdrh/dsma/dsmamain.html>".

Sincerely yours,

A handwritten signature in black ink that reads "Steven Gutman". The signature is written in a cursive style with a large initial 'S'.

Steven I. Gutman, M.D, M.B.A.
Director
Division of Clinical
Laboratory Devices
Office of Device Evaluation
Center for Devices and
Radiological Health

Enclosure

510(k) Number (if known): K 990138

Device Name: ACTIVE™ AFP ELISA

Indications For Use:

The DSL 10-8400 AFP ELISA assay is intended for the quantitative determination of AFP in human serum. It is intended for *in vitro* diagnostic use to aid in the management of patients with nonseminomatous testicular cancer.

(PLEASE DO NOT WRITE BELOW THIS LINE. CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)



(Division Sign-Off)

Division of Clinical Laboratory Devices

510(k) Number

K990138

Prescription Use
(Per 21 CFR 801.109)

OR

Over-The-Counter Use