

**SENSITIVITY & SPECIFICITY OF
DR-70™ LUNG CANCER IMMUNOASSAY**

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ABSTRACT

A multi-center study in the U.S. and China involving a total of 393 healthy subjects and 203 lung cancer patients was conducted to investigate the utility of DR-70™ immunoassay to detect lung cancer. Interference to DR-70™ test due to

different disease states and blood collection procedures were also investigated. Benign and non-cancer diseases that can give rise to elevated DR-70™ levels included pneumonia, lung infection, burns, trauma due to surgery, arthritis, renal failure, sepsis etc. The study also showed that most consistent DR-70™ test results were obtained when blood was collected in vacutainers containing SST gel and clot activator. It is important that the DR-70™ test not be performed on samples of patients suffering from the aforementioned diseases. As these non-cancer disease states and improper blood collection procedures may lead to false positive results.

INTRODUCTION

Fields et al.¹ concluded that the serum DR-70™ level is promising for use as a marker in the assessment of patients with lung cancer. The study showed that normal DR-70™ levels were similar in both male and female subjects. The DR-70™ levels of current smokers and ex-smokers were, respectively, about 15% and 4% higher than non-smokers. The levels were also higher by approximately 15% in controls 65 years of age or older, versus those less than 65 years of age¹. The mean DR-70™ level of cancer patients was 3.5 fold higher than the mean value of control non-cancer subjects. The sensitivity of the test for lung cancer was 67% and the specificity was 91%¹. Wu et al.² recently reported the successful detection 13 different cancers including lung cancer with DR-70™. The results, based on 277 healthy subjects and 136 cancer patients, showed an overall sensitivity and specificity of 84% and 95%, respectively. Contrary to the studies of Fields et al¹ and Wu et al^{1,2}, Stieber et al³ reported the lack of success in the use of DR-70™ for detecting lung cancer. In order to resolve these diametrically different conclusions on the validity of using DR-70™ as a test for lung cancer, we conducted studies in China and in the U.S. Results of the

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investigations are reported herein. Possible factors that may be contributing to the differences between results reported by Fields et al¹ and Wu et al², and those of Stieber et al³ will be discussed.

CLINICAL SPECIMENS

(a) Study in China

Control Subjects.

The sera were drawn from 335 healthy individuals who attended the Clinic of 2nd Affiliated Hospital of Hubei Medical University for routine check-ups. Sera were obtained by venipuncture and drawn into SST tubes. There were 190 males and 145 females with age range of 18 to 70 years old with an average age of 50.3 years of age. All control subjects gave negative test results for hepatitis surface antigens A, B, C and syphilis. They all have normal functioning livers, kidneys and lungs.

Cancer Patients.

Of the 83 cancer patients, 55 were male and 28 were female. They were all patients of our clinic who sought treatments here. The presence of cancer in these patients were confirmed by X-ray, ultra-sound, CT, biopsy and/or surgical procedures. The age of the patients range from 29 to 80 years old.

(b) Study in the U.S.

Control Subjects.

The sera were drawn from 58 healthy volunteers.

Cancer Patients.

Sera from 120 lung cancer subjects were obtained from Austin Medical Ventures.

Patients With Benign, Non Cancer Conditions.

Sera from patients with other "benign", non-cancerous conditions were obtained from the Department of Pathology, University of California at Irvine, after approval by the University of California, Irvine, Institutional Review Board.

MATERIALS AND METHODS

Sera

Unless otherwise stated, all sera were obtained by the following procedures:

Approximately five (5) mL venous blood was drawn into a serum separation vacutainer tube (Becton Dickinson, Franklin Lakes, NJ 07417) in the morning before the consumption of food. This tube contained SST gel and clot activator. The blood was left at room temperature for 30 min, then centrifuged at 1500 rpm for 15 min. The resulting clear serum was analyzed. For comparison, sera were obtained blood that was purposely drawn into "red top" tubes, i.e. vacutainer tubes without the SST gel and clot activator.

Plasma

Plasma from healthy volunteers was obtained by drawing the blood into vacutainer tubes containing buffered sodium citrate.

DR-70™ Test Kits

The kits, obtained from AMDL (Tustin, Ca 92780), contained a plate of 12 x 8 well strips coated with affinity-purified rabbit anti-DR-70™ antibodies, a vial of antibody-peroxidase conjugate, one vial each of diluent, substrate solution, stop solution, wash buffer, low serum control, high serum control and 5 calibrators.

DR-70™ Test Principle

DR-70™ test is an enzyme linked immunosorbent assay (ELISA) using

affinity purified anti-DR-70™ immobilized on the bottom of the well to capture DR-70™ antigen from the diluted serum. The captured antigens, upon washing, are then complexed by peroxidase labeled anti-DR-70™ conjugate to form immuno-sandwich. The bound enzyme conjugate is quantitatively measured with TMB substrate. Upon stopping the enzymatic reactions, the absorbance of the solution is read at 450 nm.

DR-70™ Assay Procedure

All serums were diluted 200 fold with diluent solution in the kit. Typically, 10 uL of serum were added to 2000 uL diluent. Upon proper mixing, the diluted serum was added to two adjacent wells of a dilution plate. Each well received 200 uL as duplicate samples. Using an 8-channel pipettor, remove 100 uL of the diluted serums from the dilution plate and deliver 100 uL to the antibody-coated plate. The plate was sealed with a plate sealer and incubated at room temperature for 15 minutes. The plate was then washed 6 times with 300 uL wash solution in each wash. Wells were dried by blowing with a stream of air for 2 minutes. All wells were filled with 100 uL of enzyme-antibody conjugate and incubated at room temperature for 15 minutes. The plate was then washed 6 times with 300 uL wash solution in each wash. Wells were dried by blowing with a stream of air for 2 minutes. Then 100 uL of TMB substrate solution was added to each well, protected from direct light and incubated for 10 minutes for color development. Finally, 100 uL stopping solution was added to each well to stop the enzymatic reactions. The absorbance of the solution was read at 450 nm in an ELISA reader. From the absorbance of the 5 calibrators, a standard curve was constructed. The DR-70™ level of the serum was read from this standard curve.

RESULTS

Serum DR-70™ Level in Control Subjects

In the study conducted both in China and in the U.S., the normal serum DR-

70™ level in 393 healthy control subjects ranged from approximately 1 mg/L to less than 9 mg/L.

Specificity and Sensitivity of DR-70™ Test for Lung Cancer Patients

Eighty-three lung cancer patients were enrolled in the study conducted in China. Using the DR-70™ test and setting the specificity requirement at 95%, a sensitivity of 87% was obtained by using 335 control subjects and 83 lung cancers (Fig. 1, Table 1). In the U.S. study, 58 healthy controls and 120 lung cancer patients were enrolled in testing the DR-70™ assay. The results showed that at 96% specificity, a sensitivity of 86% was achieved (Figs. 2, 3, Table 2).

Effects of Vacutainer With and Without SST Gel and Clot Activator on the Values of DR-70™ Measured

The blood of 12 control subjects was collected in vacutainers with and without SST gel and clot activator, the DR-70™ values in the resulting sera were analyzed immediately and at 2 other time intervals. The overall results of this study suggested that fresh sera collected with and without SST gel and clot activator showed lower values compared to the sera collected, and under identical conditions stored 5 hours at room temperature and 53 hours at room temperature. However, sera obtained from blood collection using vacutainer with SST gel and clot activator showed significantly lower DR-70™ results than the sera obtained from blood collected in vacutainers without SST gel and clot activator (Table 3).

Conditions Causing Elevation of Serum DR-70™ Level

Certain benign, non-cancer conditions were found to cause elevation of serum DR-70™ level. They include: pneumonia, sepsis, cellulitis, acute and chronic infection, burns, trauma, surgery, renal insufficiency, jaundice and rheumatoid arthritis. The DR-70™ level in patients with examples of these conditions are listed in Table 4.

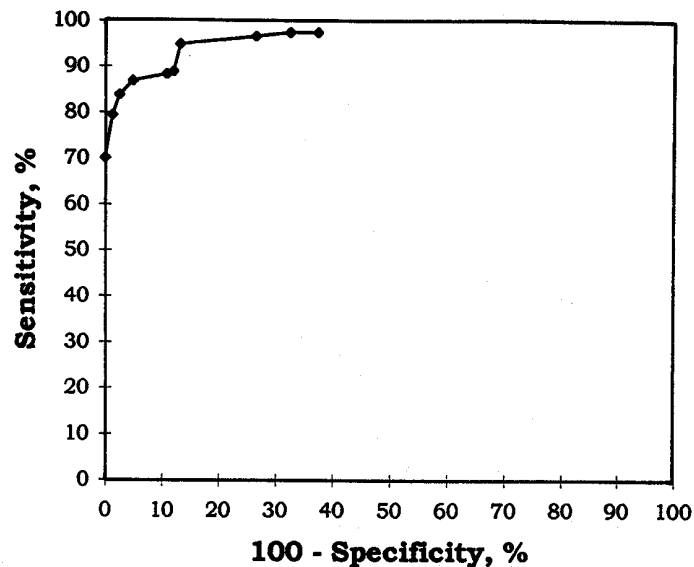


Fig. 1: Receiving Operating Characteristics (ROC) curve of DR-70™ immunoassay for lung cancer discriminating patients with lung cancer from control healthy subjects based on the study in China.

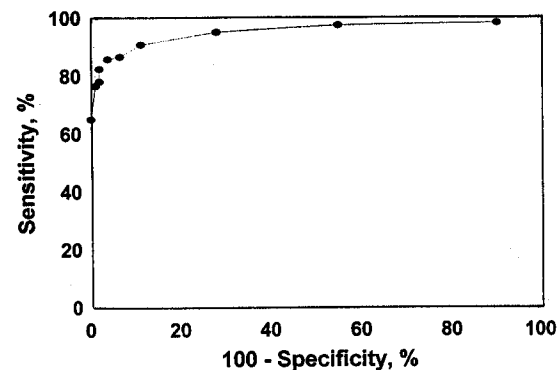


Fig. 2: Receiving Operating Characteristics (ROC) curve of DR-70™ immunoassay for lung cancer in discriminating patients with lung cancer from control healthy subjects based on the study in the U.S.

Table 1: The Specificity and Sensitivity of DR-70™ Lung Cancer Immunoassay at Different Cutoff Levels.

DR-70™ Cutoff Level (mg/L)	Specificity %	Sensitivity %
6.0	97.6	62.7
5.5	97.6	67.5
5.0	96.7	73.5
4.5	94.9	86.7
4.0	89.0	88.0
3.5	88.4	89.2
3.0	86.9	95.2
2.5	83.9	97.6

Total Number of Lung Cancer Patients: 83
 Total Number of Normals: 335

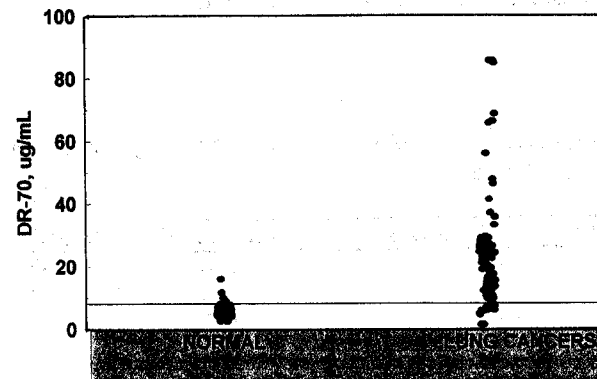


Fig. 3: Scattergram for DR-70™ values of control healthy subjects and lung cancer patients.

Table 2: The Specificity and Sensitivity of DR-70™ Lung Cancer Immunoassay at Different Cutoff Concentration.

DR-70™ Cutoff Level (mg/L)	Specificity %	Sensitivity %
16	100	65
12	99	76.6
11	98.1	78
10	98.1	82.5
9	96.2	85.8
8	93.5	86.7
7	88.8	90.8
6	72.2	95
5	45.3	97.5
4	10.1	98.3
Total Number of Lung Cancer Patients: 120		
Total Number of Normals: 108		

Table 3: Comparison of the DR-70™ values in sera from blood collected in vacutainer with SST gel and clot activator versus vacutainer without SST gel and clot activator at 2 time intervals. The number of control subjects in this study is 12.

Sera collected in vacutainer	Average DR-70™ level (mg/L)		
	"Fresh": 30 minutes at room temperature	5 hours at room temperature	5 hours at room temperature plus 20°C for 48 hours
With SST gel and Clot Activator	5.71	6.13	6.33
Without SST gel and Clot Activator	5.64	6.53	6.92

DISCUSSION

A multi-center study to evaluate the clinical utility of DR-70™ for cancer detection has been conducted in the U.S. and China. DR-70™ immunoassay kit detects in serum specific fibrin degradation resulting from a malignancy. Also included in the study were the effects of various non-cancer diseases and

Table 4. Conditions causing elevation of serum DR-70™ level.

Patient Code	Clinical Diagnosis	DR-70™ (mg/L)
S-1	Infection	10.2
S-19	Neurogenic bladder + chronic infection	11.2
S-14	Pneumonia & Fasciitis (Necrotizing) Klebsiella	16.6
S-42	Acute Pneumonia	20.8
S-17	50% Body burns, 2 nd & 3 rd degree	8.9
S-18	Chest trauma-surgery	20.6
S-20	Renal insufficiency-jaundice	19.4
S-38	Rheumatoid Arthritis	37.6
S-45	Rheumatoid Arthritis	8.8
S-46	Rheumatoid Arthritis	18.4
S-39	Cellulitis of knee	20.0
S-48	Sepsis	15.2

laboratory factors that may influence the results of DR-70™ tests and hence their interpretations.

The DR-70™ level of 393 normal, healthy subjects was measured to be in the range of 1 to 9 mg/L. The value for serum DR-70™ level can vary significantly depending on how the serum was obtained. For example, when blood was drawn into vacutainers containing neither SST gel nor clot activator, and left in the vacutainers for 5 hours, the value for the DR-70™ level in the resulting serum increased by 16% and increased further to 20% or more for samples left in the vacutainer tubes for more than 48 hours. On the other hand, under the same conditions, when blood was obtained in vacutainers with SST gel and clot activator, the DR-70™ values increase by only 7% and 11%, respectively.

The influence of a number of non-cancer disease conditions on the value of DR-70™ was also investigated. It is clear from Table 4 that non-cancer diseases such as infection, burns, trauma due to surgery, arthritis, sepsis, etc. are likely to contribute to an increase in the level of serum DR-70™. These effects can lead to false positive results. It is therefore important to note that when DR-70™

immunoassay is prescribed to detect the presence of lung cancer, the patient should be cleared of the aforementioned diseases and that proper vacutainer and blood collection procedures are used. Interference of a tumor marker test by benign, non-cancer diseases is not uncommon. For example, CYFRA 21-1 was shown to be elevated in chronic obstructive bronchopneumopathy and in chronic renal failure⁵. Similarly, CA 125 test picked up benign pelvic diseases⁶.

When an appropriate reference group, i.e. normal and healthy individuals without the above mentioned non-cancer diseases listed in Table 1 and proper serum collection procedures are used, DR-70™ lung cancer test can perform satisfactorily with good clinical sensitivity and specificity as shown in the ROC curve analysis (Figures 1 to 3). It, therefore, appears that the major factor contributing to the vast differences between our results obtained in a multicenter study and the results of Stieber³ et al is most likely due to the difference in the reference group used. Stieber³ et al. used patients with benign diseases of the lung and smokers. The consequence of using such a reference groups, when the specificity is fixed at 95%, is an increase in DR-70™ cut-off level for "normal" subjects. This, in turn, leads to an artificially low sensitivity as was indicated in Stieber's³ report.

CONCLUSION

When patients with certain benign lung conditions such as infection or trauma and other non-malignant conditions such as burns, arthritis and sepsis are excluded, and proper blood collection and serum processing procedures are used, DR-70™ lung immunoassay is able to give results with good sensitivity and specificity relative to lung cancer. These observations strongly suggest that the DR-70™ assay may be useful as a screening test for lung cancer in asymptomatic individuals.

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