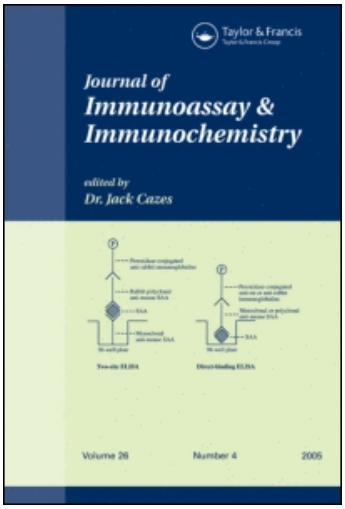

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Clinical Performance of the AMDL DR-70™ Immunoassay Kit for Cancer Detection

Dongfang Wu[a]; Xin Zhou[a]; Guoliang Yang[b]; Yuntao Xie[b]; Mingbai Hu[b]; Zhangqi Wu[c]; Gang Yang[a]; Minxiang Lu[a]

[a] 2nd Affiliated Hospital, Hubei Medical University, Department of Clinical Laboratory, Wuchang, Wuhan, China [b] 2nd Affiliated Hospital, Hubei Medical University, Oncology Institute, Wuhan, China [c] Chinese Academy of Science, Wuhan Institute of Virology, Wuhan, China

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CLINICAL PERFORMANCE OF THE AMDL DR-70™ IMMUNOASSAY KIT FOR CANCER DETECTION

Dongfang Wu[1], Xin Zhou[1], Guoliang Yang[2], Yuntao Xie[2], Mingbai Hu[2], Zhangqi Wu[3], Gang Yang[1], Minxiang Lu[1]

[1]Department of Clinical Laboratory, 2nd Affiliated Hospital, Hubei Medical University, 29 Donghu Road, Wuchang, Wuhan, China
[2] Oncology Institute, 2nd Affiliated Hospital, Hubei Medical University, Wuhan, China
[3]Wuhan Institute of Virology, Chinese Academy of Science, Wuhan, China

ABSTRACT

A clinical study using DR-70™ immunoassay for the detection of 13 different cancers have been conducted with 277 healthy subjects and 136 cancer patients. The test results showed that the DR-70™ immunoassay kit was capable of detecting cancers with high degree of specificity and sensitivity. At 95% specificity level, the sensitivity of the assay was 87.8%, 92.6%, 65.2% and 66.7%, respectively for lung, stomach, breast and rectum cancers. Furthermore the test kits were shown to be stable and performed reproducibly.

INTRODUCTION

A clinical trial using the DR-70™ immunoassay for the detection of lung cancer was conducted at the Cross Cancer Institute in Edmonton, Alberta, Canada in 1993-95 (1). The trial results, based on 237 cancer patients and 244 normal controls, showed that the DR-70™ immunoassay detected both small cell and non small cell lung cancer with an overall sensitivity of 66% at 92% specificity. In addition to the detection of lung cancer, the DR-70™ immunoassay was also shown to detect a number of other cancers. In an attempt to assess the potential of using this assay as a cancer screening tool, we have conducted a clinical study utilizing the DR-70™ assay for the detection of cancer of the lung, stomach, breast, rectum, colon, liver, ovary, esophagus, uterus, etc. The present study involves 277 healthy individuals and 136 cancer patients. The results of the study, which are encouraging, are the subject of this communication.

CLINICAL SPECIMENS

CONTROLS

The control sera were drawn from healthy individuals who came to our clinic for routine check-ups. There were 163 males and 114 females with ages ranging from 18 years to 60 years. All the control subjects gave negative test results for hepatitis surface antigen A, B & C and for syphilis. They all had normally functioning livers, kidneys and lungs.

CANCER PATIENTS

Of the 136 cancer patients, 74 were male and 62 were female. They were all patients of our clinic who sought treatment here. The presence of cancer in these patients was confirmed by X-ray, ultra-sound, CT, biopsy and/or surgical procedures. The patients range in age from 21 years to 80 years. The composition of the cancer patients is: 41 lung, 27 stomach, 23 breast, 15 rectum, 6 colon, 5 liver, 6 ovary, 5 esophagus, 3 cervical, 2 trophoblast, 1 thyroid, 1 malignant lymphoma and 1 pancreas.

MATERIALS AND METHODS

SERA

Two ml venous blood was drawn into a serum separation tube (Becton Dickinson) in the morning before the consumption of any food. The blood was left at room temperature for 30 minutes, then it was centrifuged at 1500 rpm for 15 minutes. The resulting clear serum was taken for analysis.

DR-70™ TEST KIT

The kits, obtained from AMDL, Inc., 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, TMB substrate solution, stop solution, wash buffer, low serum control, high serum control and 5 calibrators.

DR-70™ TEST PRINCIPLE

The DR-70™ test is an enzyme linked immunosorbent assay (ELISA) using affinity purified rabbit 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 an immuno-sandwich. The bound enzyme conjugate is quantitatively measured with TMB substrate. Immediately after stopping the enzymatic reactions, the absorbance of the solution is read at 450 nm.

DR-70™ ASSAY PROCEDURE

All sera were diluted 200 fold with the diluent solution supplied in the kit. Typically, 10 µl of serum were added to 2000 µl diluent. Upon proper mixing, the diluted serum was added to two adjacent wells of a dilution plate. Each well received 200 µl as duplicate samples. Using an 8-channel pipettor, 100 µl of the diluted serum was removed from the dilution plate and 100 µl was delivered 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 using 300 µl of wash solution for each wash. The wells were dried with a stream of air for 2 minutes. Then 100 µl of TMB substrate solution were added to each well, protected from direct light and incubated for 10 minutes for color development. Finally, 100 µl stopping solution were 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

STANDARD CURVE

The standard curve for DR-70™ was constructed based on the absorbance values obtained from the 5 calibrators supplied with the kit. The concentrations of DR-70™ in the calibrators were 0.0, 1.5, 3.0, 9.0 and 18.0 µg/ml. The resulting absorbance values were 0.020, 0.168, 0.357, 0.962 and 1.650, respectively. A typical standard curve using 5 calibrators is presented in Fig. 1 with a curve fitted to a quadratic equation with $r^2 = 0.993$.

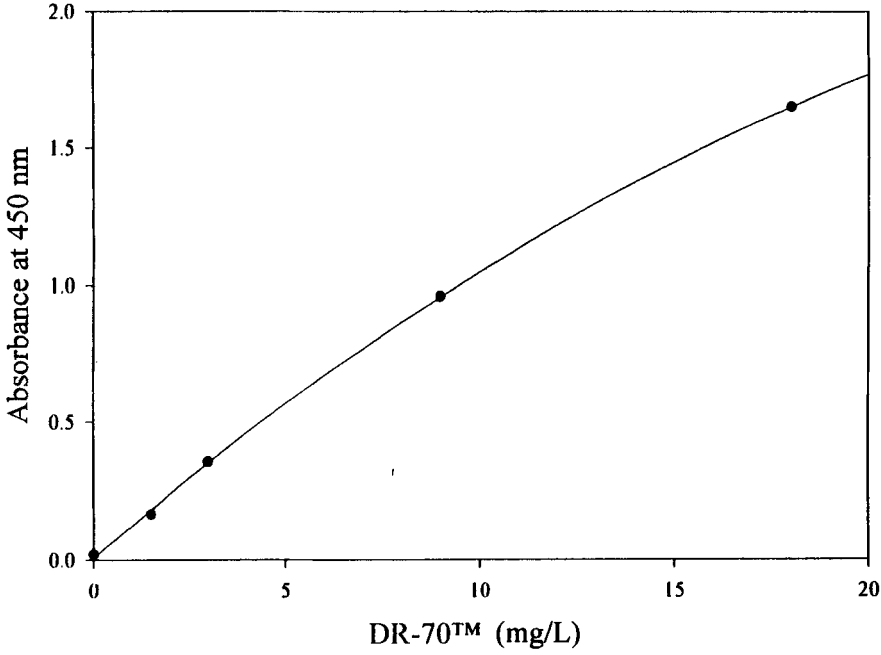

FIG. 1. Standard curve with DR-70TM concentration (mg/L) plotted against absorbance at 450 nm.

DR-70™ LEVELS IN SERUM OF NORMAL CONTROLS AND CANCER PATIENTS

CONTROL SUBJECTS: The DR-70™ level for control subjects was established from the level of 277 controls. The average level was 1.66 mg/L. Based on a 95% confidence limit, the upper limit for the normal DR-70™ level can be set at 4.0 mg/L.

CANCER PATIENTS: A total of 136 cancer patients were enrolled in this study. The DR-70™ level of cancer patients is listed in Table 1. The average DR-70™ level of the cancer patients as a group is 13.31 mg/L. This is more than 3 times the upper limit for the control subjects.

Figure 2 showed the individual values of DR-70™ in both the normal (non-cancer) and cancer patients. The colon and rectum cancer patients were grouped in one column under C/R. Cancers

Table 1: DR-70 Level in Serums of Cancer Patients

Cancer Category	Number of Cancer Patients	Average DR-70 Conc. (±, mg/ml)	Number of Positive Patients	Sensitivity of DR-70 test
Lung	41	9.65 ± 7.47	36	87.8%
Stomach	27	11.56 ± 8.53	25	92.59%
Breast	23	5.81 ± 3.63	15	65.22%
Rectum	15	8.38 ± 5.98	10	66.66%
Colon	6	9.23 ± 4.74	5	
Liver	5	8.37 ± 4.49	4	
Ovary	6	24.19 ± 13.79	6	
Esophagus	5	10.44 ± 5.58	5	
Cervix	3	11.03 ± 8.11	3	
Trophoblast	2	5.51	2	
Thyroid	1	8	1	
Malig. Lymphoma	1	11	1	
Pancreas	1	49.94	1	

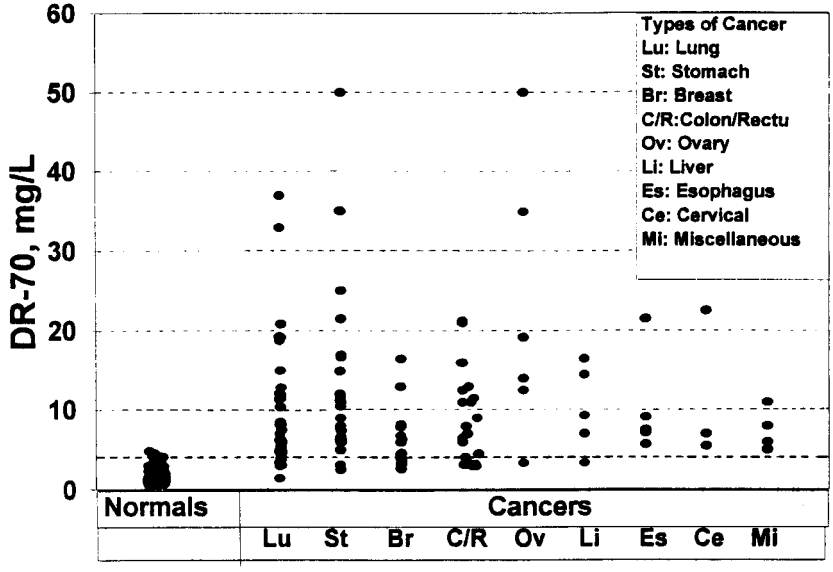

FIG. 2. Individual serum values of DR-70TM in control, non-cancer subjects and cancer patients. The heavy dashed line represents upper normal limit DR-70TM value (the cut-off value) which is set at 4 mg/L.

of the trophoblast, thyroid, lymph and pancreas were also grouped in one column under Miscellaneous (Mi). All other cancers were listed in different individual columns.

RECEIVER OPERATING CHARACTERISTIC (ROC) CURVES

In addition to analytical precision and accuracy, other parameters such as sensitivity, specificity and predictive values (PV) are required to define the accuracy of a laboratory test. In the context of an immunoassay for cancer, the term "sensitivity" is used to characterize the incidence of true positive results obtained when the assay is applied to patients known to have a cancer. The term "specificity" is used to characterize the incidence of true negative results obtained when an assay is applied to subjects known to be free of cancer(2). The predictive value can be applied to either positive or negative test results. A positive predictive value indicates the frequency of cancer patients in all patients with positive test results. The negative predictive value indicates the frequency of non-cancer (control) subjects in all subjects with negative test results(3). Therefore, the sensitivity and specificity of an assay are parameters which refer to a homogeneous group of patients, whereas the terms positive or negative predictive values refer to a mixed group. Mathematically, the above parameters can be presented as follows:

$$\text{Sensitivity} = \frac{TP \times 100}{TP + FN}$$

$$\text{Specificity} = \frac{TN \times 100}{FP + TN}$$

$$\text{Positive PV} = \frac{TP \times 100}{TP + FP}$$

$$\text{Negative PV} = \frac{TN \times 100}{TN + FN}$$

TP = True Positive = number of cancer patients correctly classified by the test.

TN = True Negative = number of non-cancer patients correctly classified by the test.

FP = False Positive = number of non-cancer patients misclassified by the test.

FN = False Negative = number of cancer patients misclassified by the test.

At a specificity of 95.0%, the sensitivity of the DR-70™ assay was 87.8%, 92.6%, 65.2% and 66.7% for lung, stomach, breast and rectum cancers, respectively. The overall specificity and

Table 2: Overall Performance of DR-70, Cancer Patients vs. Control Subjects

Category	Number	Number of Positive Patients	Specificity	Sensitivity	P*
Cancer Patients	136	114		83.82 %	
Control Subjects	277	14	94.95%		<0.001

sensitivity of DR-70™ for cancers tested were respectively 95.0% and 83.8% (Table 2). The predictive values of positive and negative tests are 89.1% and 92.3%, respectively.

Depending on the level of DR-70™ used as the cut-off value, a range of specificity values and their corresponding sensitivities can be obtained for a particular cancer. The relationship between a series of specificities and sensitivities can be profiled in an ROC curve as shown in Fig. 3. The power of discrimination between the cancer patients and the control non-cancer subjects of a test can be readily observed in the ROC curve. The closer the curve is to the upper left hand corner of the plot, the greater is the discrimination power of the test.

PRECISION

The intra-day precision of the DR-70™ kit was studied by measuring high, medium and low control sera 20 times within one day. The results of the study, as presented in Table 3, indicated that the DR-70™ kit provided an acceptable precision with CV values of 4.3%, 7.6% and 11.9% for high, medium and low control sera, respectively.

DISCUSSION

Fields et al. reported in 1995 the results of a clinical trial using DR-70™ for the detection of lung cancer conducted at the Cross Cancer Institute. The trial results indicated DR-70™ detected both non-small cell lung cancer and small cell lung cancers equally well. The level of DR-70™ increases from 9.3 to 31.2 as the stage of the cancer changes from stage I to stage IV. The results also showed 83.7 % sensitivity of DR-70™ marker in stage IV patients with non-small cell lung cancer and 80.0% sensitivity in advanced patients with small cell lung cancer. The overall sensitivity of the assay was 66% at a specificity level of 92%(1). Previous results also indicated that DR-70™ was able to detect a number of cancers other than lung cancer (unpublished data from studies conducted in California, Germany and Brazil). The results from

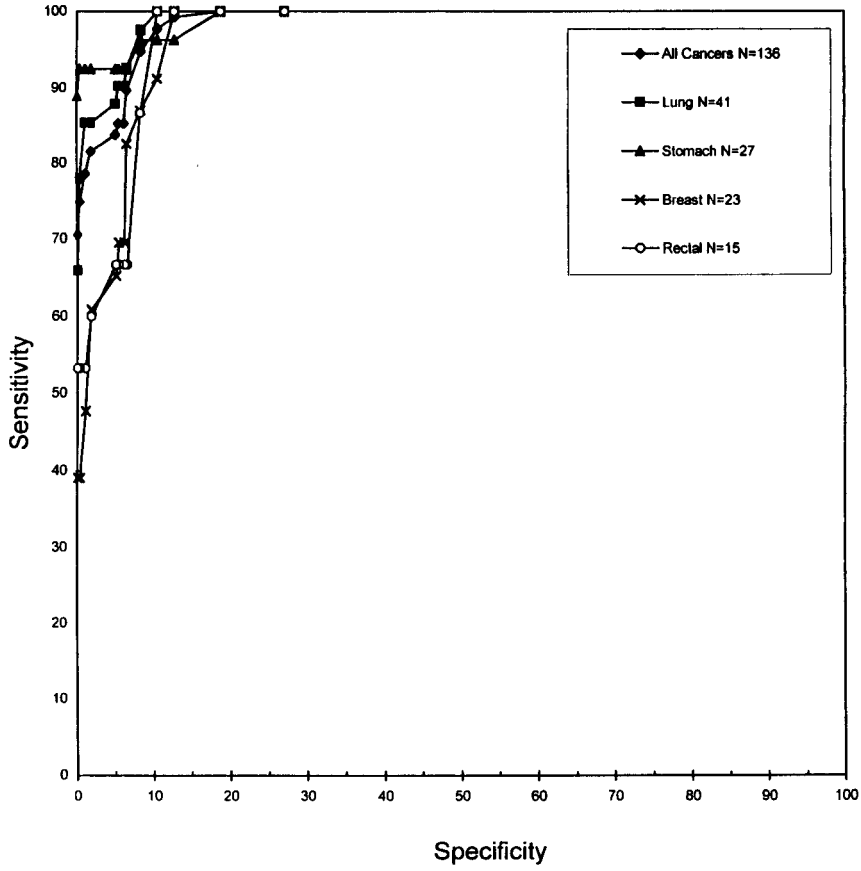

FIG. 3. Receiver Operating Characteristic (ROC) curves of DR-70TM immunoassay in discriminating patients with various cancer from control, non-cancer patients.

Table 3: Intra-day Precision Study with DR-70 Kit

Control Serum	Average Level (mg/L)	CV (%)
High	15.505	4.2753
Medium	4.98	7.5864
Low	1.613	11.9404

our study confirmed earlier observations that the DR-70™ immunoassay can indeed detect a number of cancers. In our studies, a total of 13 different cancers have been detected with high degree of sensitivity and specificity by the DR-70™ immunoassay kit (Fig.2). The application of DR-70™ immunoassay to lung cancer detection showed an overall sensitivity of 87.8% when evaluated at 95.0% specificity. A detailed ROC analysis of the data was presented in Fig. 3. The reported sensitivity of CYFRA 21-1 lung cancer immunoassay was 68% in squamous cell carcinoma of the lung when tested at 95% specificity in patients with benign lung disease (4). Our study with 27 stomach cancer patients yielded a test sensitivity of 92.6% ant a specificity of 95.0%. In comparison, the reported sensitivity of CA 72-4 for stomach cancer ranged from 27% to 84% at a specificity range of 68-93% (5,6). The results also suggest that this assay may be useful for cancer screening in, for example, an annual physical check-up.

In order to obtain reliable results with the DR-70™ assay, the assay procedure described in the product insert must be strictly adhered to (including properly washing the microwells). Patients who are suffering from infections should not take the test while the infection is still active and ongoing. Sera must be fresh and well prepared. Plasma, or serum obtained from clotted plasma, cannot be used. Hemolyzed serum, serum from incompletely clotted blood or serum from patients with SLE or suffering from infection with Histoplasma capsulatum, pneumonia, hepatitis and possibly other agents should not be used as they may give false positive test results. Highly lipidic sera may result in false negative tests. Therefore, it is best to obtain the serum sample from the patient in the morning before any meal is taken.

CONCLUSION

When the assay procedure as described in the manual is adhered to, and the patients to be tested are not suffering from acute infection, auto-immune disorders or trauma, this report clearly shows that the DR-70™ immunoassay can give reliable results that are useful in the detection, screening, and management of cancer. With lung and stomach cancers, DR-70™ provided test results with very high degrees of sensitivity and specificity. It is also demonstrated that the DR-70™ assay is capable of detecting at least 13 different cancers. This is consistent with the concept that DR-70™ can serve as a "universal tumor marker" or "pan tumor marker".

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