In Vitro Method for Detection of the Presence or Absence of Developing Malignant Tumors

White Paper

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Table of Contents

Executive Summary

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012. The most common causes of cancer death[1] are cancers of the lung (1.59 million), liver (745,000), stomach (723,000), colorectum (694,000), breast (521,000) and esophagus (400,000).

In 2016, an estimated 1,685,210 new cases of cancer will be diagnosed in the United States, and 595,690 people will die from this disease. The number of people living beyond a cancer diagnosis reached nearly 14.5 million in 2014 and is expected to rise to almost 19 million by 2024. Approximately 39.6% of men and women will be diagnosed with cancer at some point during their lifetimes. National expenditures for cancer care in the United States[2] totaled nearly $125 billion in 2010 and could reach $156 billion by 2020.

One of the major handicaps in treating this condition is the lack of a fast, effective test to identify people with abnormalities suggestive of cancer that refer them promptly for precise diagnosis. Current test methods provide diagnosis of only a few types of cancer and have downsides. Studies performed over the last decade have strongly suggested that cancer screening with current methods has the risks of overdiagnosis and overtreatment of cancers that would not threaten life or cause symptoms. Based on this, a key need is the development of biomarkers that distinguish aggressive, life-threatening cancers from non-life-threatening tumors.

Albutran has developed an in vitro blood test that can be performed in 30 minutes; it detects the onset of malignant tumor in a patient and has a sensitivity of 90% and a specificity of 90%. The test detects a cancer-specific change to the molecular conformation of serum albumin that occurs in a patient with an active malignant tumor.

Albutran's analyzer, reagents, and method are certified and approved for use in Belarus and Russia. The analyzer also has received the EU CE mark.

Since 2013, the test has been used in the Consulting and Diagnostic Center of Minsk (Belarus), in the Republican Scientific and Practical Center for Organ and Tissue Transplantation of Belarus, in the Russian Oncological Scientific Center named after N.N. Blokhin (Moscow, Russia), and other healthcare organizations. During the development of the technology involving multiple studies and clinical trials, hundreds of patients have been tested, and thousands of tests have been run.

Albutran is actively soliciting cooperation with US partners for clinical studies needed for FDA approval of the test, as well as for the development of indications for use.

Introduction

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012. The most common causes of cancer death[1] are cancers of the lung (1.59 million), liver (745,000), stomach (723,000), colorectum (694,000), breast (521,000) and esophagus (400,000).

In 2016, an estimated 1,685,210 new cases of cancer will be diagnosed in the United States, and 595,690 people will die from the disease. The number of people living beyond a cancer diagnosis reached nearly 14.5 million in 2014 and is expected to rise to almost 19 million by 2024. Approximately 39.6%of men and women will be diagnosed with cancer at some point during their lifetimes. National expenditures for cancer care in the United States[2] totaled nearly $125 billion in 2010 and could reach $156 billion by 2020.

Cancer mortality can be reduced if cases are detected and treated early. There are two components of early detection efforts: screening and early diagnosis. Screening is a means to identify asymptomatic people with abnormalities suggestive of cancer or pre-cancer and refer them promptly for diagnosis. Diagnosis is confirmation of disease by biopsy, tissue examination, or other relevant procedures in the workup following positive screening tests[3].

There have been some important successes in screening and early detection. Deaths from cervical, colorectal and breast cancer in the United States declined substantially after screening for them became common practice[4].

However, studies performed over the last decade have strongly suggested that, in addition to benefits, screening has downsides. In particular, there is the risk of overdiagnosis and overtreatment (the diagnosis and treatment of tumors that would not threaten life or cause symptoms), as well as the psychological stresses associated with a cancer diagnosis[4].

This understanding has led to intensive study of ways to identify and distinguish those screen-detected tumors that are truly life-threatening and require immediate treatment from those for which treatment is unnecessary or can be safely delayed.

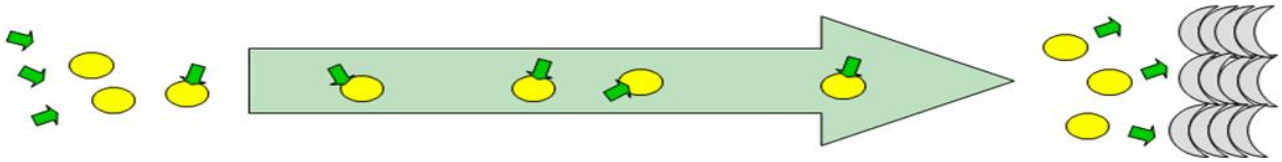
Based on this, a key need is the development of biomarkers that may distinguish aggressive, life-threatening cancers from non-life-threatening tumors[4].

Role of serum albumin in metabolite transport

Serum albumin is the main carrier protein in the circulatory system of the body. Albumin transports cellular metabolism products, mediators, and other hydrophobic compounds; all of these reflect the nature and intensity of physiological and pathological processes in the body.

During this metabolite transport, the albumin molecules perform complex operations: at the beginning (1) they efficiently **bind** metabolites; then, during the **transport** in the circulatory system (2), they strongly **retain** them in a bound state; and at the destination, (3) they provide efficient dissociation and **release** to target cells.

Operations of albumin molecule during metabolite transport:



(1) Binding *(2)* ***Transport*** *in the circulatory system* *(3)* ***Release*** *to target cells*

Variation of the albumin binding parameters during the metabolite transport is based both on the conformational flexibility of the albumin molecule and the strong allosteric interactions between its binding sites.

Binding of carried metabolites always causes conformational changes in the albumin molecules. The nature and extent of those modifications depend on the intensity of physiological and pathological processes in the body.

In a healthy person, the albumin molecules in the blood plasma are always loaded with multiple metabolites. Their molecular conformation (i.e.: "native" conformation of the albumin molecule) is significantly different than the conformation of albumin molecules which have been artificially purified by removing of bound substances. The routine, established cellular metabolism of organs and tissues, as well as the routine, permanent synthesis and filtration of albumin molecules in the liver, maintain the healthy albumin condition in the body.

In a patient who has a pathological process in the body, the levels of metabolites carried by the albumin change, depending on the type and severity of the disease. The conformation of albumin molecules may be modified as a consequence of these changing metabolite levels.

Carcinogenesis

Tumors typically progress in a stepwise fashion[5, 6]:

(1) Hyperplasia - cells divide too often but appear normal

(2) Dysplasia - the tumor cells and tissue appear abnormal. Additional genetic changes in the hyperplastic cells lead to increasingly abnormal growth. The cells and the tissue no longer look normal. The cells and the tissue may become disorganized.

(3) Carcinoma *in situ* - tumor contains primarily altered cells and is growing larger; it has not left the site of origin. Additional changes make the cells and tissues appear even more abnormal. The cells are now spread over a larger area, and the region of the tissue involved primarily contains altered cells. A key facet of *in situ* growths is that the cells are contained within the initial location and have not yet invaded other tissues. Cancers of this type are often totally curable by surgery because the abnormal cells are all in one location.

(4) Metastatic cancer - tumor has begun to invade nearby or distant tissues. These metastatic tumors are the most dangerous and account for a large percentage of cancer deaths.

During the early stages of cancer (steps 1 and 2), tumors are typically benign and remain confined within the normal boundaries of a tissue. In step 3, the tumors become cancerous and begin to grow.

In step 4, tumors become malignant, and they gain the ability to break through the cell boundaries and invade adjoining tissues[7].

Metabolic changes during cancer

For many years, cancer was fundamentally considered a disease of uncontrolled cell proliferation. Although significant metabolic changes were acknowledged to occur in cancer cells[8], this was considered a secondary phenomenon.

More recently, the metabolic changes that occur during cancer are being reconsidered as more central to the disease itself: the effects of oncogenes and tumor suppressors on proliferation are closely associated with metabolic changes which can either be a consequence or be necessary to promote cell growth or to support biomass accumulation and drive the cancer phenotype[9].

Altered metabolism is a hallmark of cancer. In almost all solid and hematological malignancies, metabolic pathways involved in energy production or the biosynthesis of carbohydrates, lipids, amino acids, or nucleotides are hijacked to meet the specific nutritional and growth requirements of the tumor. These alterations often favor the growth of rapidly dividing cancer cells and inhibit metabolic signaling that prevents tumor initiation or attenuates tumor progression and metastatic dissemination. Thus, cancer can be thought of as a metabolic disease[10].

The proliferation of cancer cells necessarily requires cellular building blocks such as fatty acids, nucleic acids, proteins, and lipids. Cancer cells receive these building blocks from the circulatory system. However, coincidently, they release back into the blood the waste products of their metabolism, changing the overall metabolite composition of the circulatory system.

Role of serum albumin in tumor development

With respect to a developing tumor, the role of albumin is similar to that in normal cells: albumin delivers fatty acids, which are the main nutrition for tumor cells, evacuates waste products of cellular metabolism, and transports tumor-derived mediators to organs and systems of the body[11-14].

In the earliest stages of tumor development, hyperplasia and dysplasia, when the tumor is benign and the tumor cells have an unchanged metabolism, the albumin provides transport of metabolites for the tumor cells in the same manner that it does for normal tissue, and there is no significant change to the load or role of albumin molecules.

In the early stages of malignant tumors, however, the tumor cells change their metabolism and begin uncontrolled proliferation along with the production of metabolic mediators which inhibit prevention systems of the host body[15-22]. When the tumor mass is small, the quantity of tumor-derived mediators released into the blood is minimal. Albumin molecules bind tumor-derived mediators and conceal them within the wider albumin pool that detains delivery of tumor-derived mediators and thereby suppresses their influence on the prevention systems of the body. Thus, in the early stages of malignant tumors, albumin suppresses the influence of tumor signals on the prevention systems of the host organism and proliferation of cancer cells[23-25].

After the tumor initiates steady growth that is coupled with increased tumor mass and systemic intervention in the organism, the albumin molecules become substantially loaded with tumor-

derived mediators. Under these conditions, the albumin performs an effective targeted transfer of the tumor mediators and does not prevent, but rather supports, active growth of the tumor.

In cancer patients after an effective antitumor treatment, when the tumor has been removed from the body or its growth has been depressed, the release of active tumor metabolites and their transport by albumin molecules are stopped or significantly reduced. Under such conditions, the role of albumin molecules is restored to "normal" – albumin suppresses the influence of tumor residues on the prevention systems of the host organism and thereby helps the recovery.

Thus, in a cancer patient, the role of the albumin differs, depending of the stage of cancer. In the early stages of cancer, when the tumor mass is small, the loading of albumin molecules is "normal", and the role of albumin is to suppress the influence of tumor signals on the body systems and cancer cell proliferation. In a patient with a developed cancer, who has a relatively larger and more malignant tumor, the albumin molecules are substantially loaded with tumor-related metabolites[26,27] and play a role of supporting the tumor growth.

Additionally, albumin is the main carrier protein for fatty acids, one of the important cellular building blocks required for proliferation of cancer cells. Albumin molecules directly interact with cellular membranes to deliver such metabolites to cancer cells and load the tumor-derived molecules. In a cancer patient, this loading of albumin molecules with the tumor-delivered metabolites depends on the type of tumor cell metabolism (normal or altered) and the activity of their proliferation.

Along with the tumor mass, the loading of albumin molecules and their role in tumor growth depend also of the total amount of albumin in the body: decreased serum albumin is associated with unfavorable prognosis in patients with malignant tumors[28-39].

Cancer diagnostics in vitro

There are two approaches to cancer detection in vitro; both are based on the metabolic changes occurring in cancer patients.

First, the traditional approach is the detection in the patient's fluids of specific substances, which are produced by cancer or by other cells of the body in response to cancer or certain benign (noncancerous) conditions and have much higher levels in cancerous conditions. Many different tumor markers have been characterized and are in clinical use. Some are associated with only one type of cancer, but others are associated with two or more cancer types. However, no "universal" tumor marker that can detect all types of cancer has been found. Additionally, not every patient with a particular type of cancer will have a higher level of a tumor marker associated with that cancer. Moreover, tumor markers have not been identified for every type of cancer[40].

Summarizing the results of the investigations following the traditional approach, which were performed during the last decades, no specific substance has been discovered in patient fluids that meets both of these criteria:

- has a much higher level in cancer patients than in cancer-free individuals;
- is considered a "universal" marker for malignant tumors.

Moreover, the traditional tumor markers do not distinguish between malignant tumors that are truly life-threatening and require immediate treatment (malignant cancer at stages "carcinoma *in situ*" and

"metastatic cancer") from those for which treatment is unnecessary or can be safely delayed (non-malignant tumors at the stages of "hyperplasia" and "dysplasia").

The second, more recent approach is to detect interrelated metabolic changes in the complex of multiple metabolites in blood plasma. This allows for the identification of cancer-specific metabolic signatures in a patient[41-46]. Most of these plasma metabolites are not specific for cancer, and the changes in their levels in blood plasma are relatively small.

Competitive cancer diagnosis tests

More than 20 tumor markers are currently in use[47]. The most commonly used are:

- alpha-fetoprotein (AFP),
- cancer antigen 125 (CA125),
- cancer antigen 15-3 (CA15-3),
- carbohydrate antigen 19-9 (CA19-9),
- carcinoembryonic antigen (CEA),
- human chorionic gonadotropin (HCG or b-HCG) and
- prostate-specific antigen (PSA).

Such tumor markers are very useful in determining whether a tumor is responding to treatment or assessing whether it has recurred. However, no tumor marker identified to date is sufficiently sensitive enough or specific enough to be used on its own to screen for cancer[40].

For example, the PSA test is often used to screen men for prostate cancer, but most men with an elevated PSA level do not have prostate cancer. Results from two large randomized controlled trials, the NCI-sponsored Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) and the European Randomized Study of Screening for Prostate Cancer, showed that PSA testing at best leads to only a small reduction in the number of prostate cancer deaths. Moreover, it is not clear whether the benefits of PSA screening outweigh the potential harm caused by the follow-up diagnostic tests and treatments for cancers which, in many cases, would never have threatened a man's life. Similarly, results from the PLCO trial showed that CA-125, a tumor marker that is sometimes elevated in the blood of women with ovarian cancer but can also be elevated in women with benign conditions, is not sufficiently sensitive or specific to be used to screen for ovarian cancer in women[40].

Moreover, among the currently known tumor markers, there exists no "universal" tumor marker for the proliferation of malignant cells in the body, nor any marker that could help to distinguish malignant tumors that are truly life-threatening and require immediate treatment (active malignant tumor) from those for which treatment is unnecessary or can be safely delayed (benign tumor or inactive malignant tumor).

In the last decades, cancer researchers are turning to serum/plasma proteomics (the study of protein structure, function, and patterns of expression) and metabolomics (metabolite profiling of serum/plasma) in hopes of discovering new biomarkers that can be used to identify disease in its early stages, to predict the effectiveness of treatment, or to predict the chance of cancer recurrence after treatment has ended [40, 48].

These methods have not yet been established in routine clinical practice. The main limitations are the high cost of equipment, the need for highly trained personnel, and the fact that there is still no established, adequately sensitive, and specific serum biomarker panel that could be used for diagnostic or screening purposes[49].

Another promising approach is to develop cancer tests based on the detection of interrelated metabolic changes in a complex of multiple metabolites in blood plasma, which can be a cancer-specific metabolic signature in a patient. Recent pilot studies have demonstrated that such an approach might allow development of diagnostic tests for lung, breast and pancreatic cancers, as well as for distinguishing prostate cancer from benign prostatic hyperplasia[41-46]. The major limitations, which prevent rapid integration into clinical practices of metabolic profile methods, are similar to the issues with proteomics methods: the high cost of equipment, the need for highly trained personnel, and the need to establish metabolic profiles of serum/plasma which could be used for diagnosis.

Among the foregoing commonly used and newly discovered tumor markers, none provide physicians with comprehensive overview information regarding the presence or absence of malignant cell proliferation somewhere in a patient. However, we see promise in another option, an Albumin Conformation Test, discussed next.

Albumin Conformation Test: A novel approach to improve cancer diagnosis

- We have developed an Albumin Conformation Test, a new in vitro test of blood serum that detects changes in the conformation of serum albumin molecules that relate to changes in the carried load of adherent molecules[50,51,52]. We have evidence that these changes can indicate the presence of active proliferation of malignant cells in the examined patient by detecting albumen molecular conformational changes as the adherent metabolic products shift from normal metabolism to include more abnormal load burden. These changes can be used in conjunction with other clinical data: As a screening test to detect cancer-specific metabolic alterations in patients
- To determine whether the cancer growth has stopped or the disease is progressing
- To determine when cancer treatment is effective or needs to be changed

The test can promptly provide physicians with important information that allows them to diagnose malignant tumors at an early stage. The test can be quickly performed in about 30 minutes using a standard blood sample.

The Albumin Conformation Test appears to universally detect cancer of different sites and types; extensive clinical studies have shown a high specificity and sensitivity of the test to various types of cancer[50,51,53-64]. Such sensitivity is due to the fact that the Albumin Conformation Test does not diagnose a tumor itself, but rather detects the specific changes in the blood transport system of the organism arising from the influence of actively growing malignant cells.

The test can help improve patient care and provide physicians with important information that allows them to treat patients with life-threatening tumors at an early stage and to avoid overtreatment of patients with benign conditions.

Clinical studies conducted in Russia, Belarus, and Germany support its clinical utility[50,51,53-64].

Science behind the test

Serum albumin is the main carrier protein in the circulatory system of the body. Its major function is to transport fatty acids and other hydrophobic molecules. The albumin molecule globule has a flexible conformation. In the blood plasma, the albumin conformation is such that its hydrophilic sites are located on the surface of the globule and its hydrophobic sites are inside the globule; it acts as a molecular container with a hydrophilic surface and a hydrophobic interior. When it transports hydrophobic molecules in the circulatory system, the albumin hides them inside the globule[11,65]. To bind (load) or release the carried metabolites, the albumin molecule interacts directly with the cell membrane surface receptor albondin of the target cell.

The globular albumin molecule has 9 specific sites for strong binding of long-chain fatty acids, and it can also hold fatty acids in the space between the albumin domains inside the albumin globule. The hydrophobic area inside the albumin globule also serves as the path for load and release of fatty acids and is the main unspecialized binding site of the albumin for other hydrophobic molecules[11,65].

The conformation of the albumin globule is flexible and can be slightly modified, depending on bound metabolites[11]. In the blood plasma, the albumin molecules always are loaded with cellular metabolism products, mediators, and other hydrophobic compounds, all of which depend of the nature and intensity of physiological and pathological processes in the body. In healthy subjects, modifications of the albumin conformation are detectable but not significant.

However, a significant change to the albumin conformation is observed in cancer patients using fluorescent methods[66-72] and EPR spectroscopy[50,51,53-64,70]. In the clinical studies with cancer patients, it was found that, in patients with various types of active malignant tumors, a specific conformational change to the albumin conformation can be detected by the EPR method that allows early diagnosis of malignant tumors with 90% specificity and 90% sensitivity[50,51,53-64]. The presence or absence of the change to the albumin molecule conformation correlates with the activity of a malignant process and presumably reflects the loading of albumin molecules by metabolites produced by tumor cells.

The high sensitivity of the conformation of albumin molecules to tumor-derived metabolites is the crux of the test technology.

The test analyzes the serum albumin using Electron Paramagnetic Resonance Spectroscopy (EPR), a method commonly used in laboratories but not typically in health care applications. The spin probe 16-doxyl stearate, which is specific to albumin, is used to detect albumin-bound and unbound (free) portions of the spin probe in the patient's serum sample. This allows an estimation of the albumin binding parameters, such as the binding constant and binding capacity of the albumin binding sites, and provides an estimation of the conformation of the albumin molecules.

A spin probe is a molecule that contains a stable free radical and can bind to other molecules. In the Albumin Conformation Test, the spin probe (16-doxyl stearate) can bind to both the specific and unspecified binding sites of the albumin molecule[54,65]. These binding sites have a very high binding constant for the probe and are highly sensitive to allosteric interactions on the albumin molecule[73-75].

For the fatty acid spin probe, the binding constant and capacity of the unspecified binding site of the albumin molecule (the binding site formed on the hydrophobic area inside the albumin globule) depends on the pattern of the metabolites carried by the albumin. This unspecified binding site of the albumin molecule is the main "container" for transport of a variety of hydrophobic metabolites, for

which the albumin molecule has no specific binding sites[11]. Once loaded on the albumin molecule, the spin probe molecules compete with other albumin-bound metabolites for binding on the unspecified albumin site. This determines the sensitivity of the fatty acid spin probe for a load of metabolites on the albumin molecule.

For measurement, a sample with the spin probe is exposed to a high magnetic field and microwaves that cause a resonance of the free radical in the spin probe. The resonance response of the spin probe is measured as a spectrum of an electron paramagnetic resonance (EPR) of the spin probe that reflects the structure and the properties of the protein molecule to which the spin probe is bound. The technique for the measurement and interpretation of EPR spectra is called EPR spectroscopy. Initially, the method of EPR spectroscopy of serum albumin in vitro was developed in the Belorussian Research Institute of Oncology and Medical Radiology[59-63,76]. Subsequently, the method has been improved in cooperation with experts at the Transfusion Medicine and Medical Physics Institutes of Leipzig University[54,55-58,64,65], University Clinic Charité (Berlin) [50], Oregon Health & Science University (USA) [50,51] and others.

Clinical studies have confirmed that a specific change in the conformation of serum albumin is detected in patients with active malignant tumors at an early stage and is not observed in cancer-free subjects, including healthy persons, patients with benign tumors, and those with non-malignant chronic diseases [50,51,53,55-64,76]. In a follow-up study of cancer patients, it was confirmed that the albumin conformation correlates with the course of the disease: the albumin conformation is restored to "normal" after an effective anti-cancer therapy; cancer-specific change to the albumin conformation is detected at an early stage of cancer relapse.

In these clinical studies, it was confirmed that, in patients with various types of malignancies, the test of albumin conformation could be used for early diagnosis of malignant tumors with 90% specificity and 90% sensitivity[50,51,53,55-64].

In summary, the Albumin Conformation Test

- is a marker for active malignant tumors in a patient
- evaluates the loading of patient's albumin by cancer-specific metabolites derived from cancer cells of a malignant tumor
- is covered by US patent 9528992 (also published as EP2817626), "Methods and Kits for Detection of Active Malignancy."

Clinical Results

A number of clinical studies examining a variety of patients with various types of cancer, healthy subjects, patients with benign tumors, and non-malignant chronic diseases have shown the test to have a high specificity and high sensitivity. Sensitivity is the ability of a test to identify those with malignant tumors (true positive rate). Specificity is the ability of the test to identify those without malignant tumors (true negative rate). Both the sensitivity and specificity of the test are about 90%. The sensitivity and specificity values can be seen below in the short summaries of some clinical trials.

The utility of the method was confirmed in the series of clinical studies carried out in clinics in Belarus, Russia, and Germany:

HUMAINE Klinikum Bad Saarow, Germany (2002-2003)

Evaluation of the utility of EPR test of serum albumin for diagnosis of chronic lymphocytic leukemia (BCLL)

Clinical study group: 9 patients with BCLL, 4 patients with benign hematological diseases, and 4 healthy persons

Confirmed diagnostic utility: sensitivity 100%, specificity 85%

Clinic for Mouth, Jaw and Facial Surgery, Johannes-Gutenberg-University Mainz, Germany (2003)

Evaluation of the utility of EPR test of serum albumin for diagnosis of malignant tumors of the maxillofacial area

Clinical study groups: Cancer patients with the following tumor sites: epithelial cancer of tongue (1), mukoepidermoid tongue cancer (1), cornified epithelial cancer of cheek (3), epithelial cancer of floor of oral cavity (1), epithelial cancer of lower jaw (1), basal cell cancer (1), basaloid epithelial cancer (1);

Control group of 10 patients with diseases of non-malign origin in regions of face, jaw, and mouth, and one patient with leukoplakia which is the disease known to be often a pre-stage of malign tumor

Confirmed diagnostic utility: sensitivity 100%, specificity 70%

Blutspendedienst Sachsen, Blutspendedienst Baden Würtenberg, Institut für Transfusionsmedizin Universitätsklinikum Leipzig, Germany 2003-2006

Evaluation of the ESR test utility for early diagnosis of cancer in the cohort of plasma and blood donors

Study design: look back samples of plasma and blood donors, which become available after the storage time required by law, were selected from blood banks and analyzed as blind samples.

Clinical study groups**:** 188 patients in total: 79 donors with a post-donation report of cancer and 99 donors who remained cancer-free after comparable time periods.

Confirmed diagnostic utility:

Specificity 94%;

Sensitivity, depending on the time period prior to the diagnosis of cancer:

- 0 to 3 months prior the diagnosis - 89%;
- 4 to 6 months prior the diagnosis - 84%;
- 7 to 12 months prior the diagnosis - 59%.

N. N. Blokhin Russian Cancer Research Center RAMS (2006)

Evaluation of the diagnostic utility of EPR test of serum albumin for cancer diagnosis and monitoring.

Clinical study group: 245 serum samples from 96 patients with cancer of the stomach, pancreas, esophagus, kidney, adrenal, colon, and retroperitoneal tumors, and 153 serum samples from 70 cancer-free individuals.

Confirmed diagnostic utility: sensitivity 71.4%, specificity 75.7%

A.I.Burnazian Federal Medical Biophysical Center, Moscow, Russia (2007-2010)

Evaluation of the utility of EPR test of serum albumin for cancer diagnosis.

Clinical study group: 657 serum samples, from cancer patients with various clinical conditions, and the control groups of healthy individuals and patients with chronic diseases.

Confirmed diagnostic utility: sensitivity 90.4%, specificity 100%

N. N. Blokhin Russian Cancer Research Center RAMS (2013)

Evaluation of the diagnostic utility of EPR test of serum albumin for ovarian cancer.

Clinical study group: 19 women with ovarian cancer, 5 women with benign ovarian tumors, 15 healthy women.

Confirmed diagnostic utility: sensitivity 100%, specificity 100%**Summarized data for cancer patients observed before treatment:**

Blind samples of serum and plasma EDTA from 1267 patients were studied:

- 585 healthy individuals (blood and plasma donors, volunteers);
- 128 patients with chronic non-malignant diseases;
- 554 cancer patients before treatment.

***Clinical utility of the Albumin Conformation Test**:*

Specificity:

Patient group	Number of patients	Specificity
Healthy individuals	585	***98%***
Patients with chronic non-malignant diseases	128	*73%*

Sensitivity:

Cancer patient groups	Number of patients	Sensitivity
Cancer patients, in total	554	***90%***
Gastrointestinal tumors	*84*	*93%*
Lung cancer	*26*	*96%*
Breast cancer	*31*	*87%*
Prostate cancer	*82*	*90%*
Non-Hodgkin's lymphoma	*39*	*95%*
Malignant lymphoma	*23*	*91%*
Plasmacytoma	*34*	*97%*
Leukemia	*31*	*87%*
Other cancer sites	*204*	*88%*

Approvals and Certifications

Belarus and Russia:

Analyzer, reagents and the method of investigation are certified and approved for use

- Analyzer AXM-09 and the set of reagents "ATA-test" are registered by the Ministry of Health of the Republic of Belarus (Registration Certificates No IM-7.98584 and IM-7.99443) and by Roszdravnadzor of the Russian Federation (Registration Certificates No FSZ 2012/12247 and RZN 2013/377).
- The medical technology is permitted for use by the Federal Service for Supervision of Health of the Russian Federation (Permission Certificate for the use of the new medical technology No FS 2009/315).
- Clinical guidelines "Laboratory diagnosis of a malignant proliferation by the method of EPR spectroscopy to determine changes of the transport properties of albumin in the blood serum" are approved by the Association of Professionals and Institutions of the Laboratory Services "Federation of Laboratory Medicine" of the Russian Federation[52].

The test has been used in the Consulting and Diagnostic Centre of Minsk (Belarus), in the Russian Oncological Scientific Center named after N.N. Blokhin (Moscow, Russia), and other health care organizations since 2013.

Europe:

- Analyzer AXM-09 has CE mark. Certificate of Conformity was issued by SERTIKA (Certificate Registration No. LS.08.02.2153 on June 04, 2014).

Test Procedure

The test procedure is very simple and straightforward. It consists of drawing a blood sample from the patient and analyzing it using the Analyzer AXM-09. The details of the process are:

(1) Draw venous blood from the patient,
(2) Separate serum or plasma EDTA by centrifugation of the blood sample,
(3) Mix two 50 ul aliquots of the serum sample with the reagents from the kit "ATA-test,"
(4) Incubate the probes with the shaker for 10 minutes at 37°C,
(5) Analyze the probes with the AXM-09 (duration: 8 minutes per patient sample).

- Single patient sample processing time (practical): 30 to 40 minutes, each.
- Analyzer throughput maximum (practical): 6 to 8 prepared samples per hour.

TEST SAMPLES: 100 μl of serum or EDTA plasma

Samples of serum or EDTA plasma may be stored prior to the study:

- Without freezing at a temperature of 4 to 10 degrees C for 5 days
- Frozen at minus 30 degrees C for up to 5 years

Equipment

Weight of 60 kg, dimensions 52x50x30 cm

LABORATORY ANALYZER “EPR AXM-09”

- Registration Certificate of Federal Service on Surveillance in Healthcare of Russian Federation (ROSZDARVNADZOR) no. FSZ 2012/12247 from June 01, 2012.
- Registration Certificate of Ministry of Health of the Republic of Belarus no. IM-7.98584 from September 07, 2017.
- The analyzer meets all international standards applied to in vitro devices (Appendix presents the list of applied international standards).

CONSUMABLES:

Sets of reagents for assessment of the albumin parameters in serum and plasma by the electron paramagnetic resonance method “ATA-test-C-20”, “ATA-test-C-80” (for diagnosis of growing malignant tumors)

- Registration Certificate of Federal Service on Surveillance in Healthcare of Russian Federation no. RZN 2013/377 from March 15, 2013.
- Registration Certificate of Ministry of Health of the Republic of Belarus no. IM-7.99443 from September 07, 2017.
- Sets of reagents are manufactured according to all international standards and requirements for in vitro devices.

Analyzer "AXM-09" and the reagent kits "ATA-test" are produced in the Republic of Belarus by the Research and Production Enterprise "Albutran"

MEASURED PARAMETER OF ALBUMIN:

✓ **DR** –	albumin conformation index	(For diagnosis of active malignancy)

INTERPRETATION OF “ATA-TEST” RESULTS

For cancer diagnosis and monitoring:

- Norm value: DR values greater than 1.0 indicate patients without an active malignant tumor;
- DR values equal to 1.0 correspond to the borderline (or transitional) state;
- DR values less than 1.0 indicate patients having an active malignant tumor.

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APPENDIX

International Standards Applied to the Analyzer "EPR AXM-09"

EN 55011-2012	Industrial, Scientific And Medical Equipment - Radio-Frequency Disturbance Characteristics - Limits And Methods Of Measurement
IEC 60065-2004	Audio, Video And Similar Electronic Apparatus - Safety Requirements
IEC 60601-1-2-2006	Medical electrical equipment - General requirements for basic safety and essential performance
IEC 61000-3-2-2006	Electromagnetic compatibility (EMC) - Limits - Limits for harmonic current emissions (equipment input current ≤ 16 A per phase)
IEC 61000-3-3-2005	Electromagnetic compatibility (EMC) - Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤ 16 A per phase and not subject to conditional connection.
IEC 61000-4-2-2006	Electromagnetic compatibility (EMC) - Testing and measurement techniques - Electrostatic discharge immunity test
IEC 61000-4-3-2009	Electromagnetic compatibility (EMC) - Testing and measurement techniques - Radiated, radio-frequency, electromagnetic field immunity test
IEC 61000-4-4-2006	Electromagnetic compatibility (EMC) - Testing and measurement techniques - Electrical fast transient/burst immunity test
IEC 61000-4-5-2006	Electromagnetic compatibility (EMC) - Testing and measurement techniques - Surge immunity test
IEC 61000-4-6-2011	Electromagnetic compatibility (EMC) - Testing and measurement techniques - Immunity to conducted disturbances, induced by radio-frequency fields
IEC 61000-4-8-2006	Electromagnetic compatibility (EMC) - Testing and measurement techniques - Power frequency magnetic field immunity test
IEC 61000-4-11-2006	Electromagnetic compatibility (EMC) - Testing and measurement techniques - Voltage dips, short interruptions and voltage variations immunity tests
EN 61010-1:2010	Safety requirements for electrical equipment for measurement, control, and laboratory use - General requirements
EN 61010-2-081:2002	Safety requirements for electrical equipment for measurement, control, and laboratory use - Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
IEC 61326-1:1997	Electrical equipment for measurement, control and laboratory use - EMC requirements - General requirements