Albumin Transport Capacity Test

A Rapid, Accurate Blood Test for

Early Identification of Sepsis Onset

White Paper

Vladimir Muravsky, PhD.

ALBUTRAN USA, LLC

Katy, Texas 77450

info@albutran.com

www.albutran.com

Table of Contents

Executive Summary

Sepsis (a.k.a. septicemia) is a severe, fast-moving infection of the blood triggering inflammation that can lead to permanent damage to organs and tissues, to organ failure, and to death. Worldwide, there are more than 30 million cases each year, leading to 8 million deaths. In the US, there are more than 1.7 million cases, resulting in 258,000 deaths yearly. In 2013, $23.7 billion was spent treating sepsis.

One of the major handicaps in treating this condition is the lack of a fast, effective test. Current test methods take up to 2 days to confirm a diagnosis of sepsis, and this time delay leads to increased costs and deaths.

Albutran has developed an in vitro blood test that can be performed in 30 minutes, which detects the onset of sepsis in a sepsis-risk patient one to two days earlier than current tests, and which has a Sensitivity of 80% and a Specificity of 80%. The test detects changes to the transport function of serum albumin under developing toxicity conditions in the body which when combined with other medical indications identifies patients at risk of developing sepsis. 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.

The test has been used in the Consulting and Diagnostic Centre 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 since 2013. 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's initial pre-submission to the FDA for the test was reviewed and a recommendation to reduce the overall original scope of the application was made. Our ongoing activity is preparing the recommended pre-submission of the test, focusing solely on the diagnosis of sepsis risk in post-surgery patients during the first 2 hours after the operation.

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 new indications for use.

Introduction

Sepsis (a.k.a. septicemia) is a severe infection of the blood that triggers inflammation, which can ultimately impair blood flow and deprive the body's organs of nutrients and oxygen. This often causes permanent damage, and in severe cases causes multiple organs to fail and death[1]. There are about 30 million cases leading to 8 million deaths worldwide each year, with numbers on the rise[2]. In the U.S., there were 1.67 million cases of sepsis, causing about 258,000 deaths in 2009[3]. Patients who do survive are more likely to have physical disabilities, cognitive impairment, and permanent organ damage[4]. Furthermore, septicemia disproportionally affects the elderly, with about two-thirds of all cases impacting people 65 and older[4].

Of the cases of sepsis in the U.S. in 2009, more than 150,000 (about 60%) of the infections were contracted as a result of complications with a device, implant or graft, or due to complications of surgical procedures or medical care[3]. Septic complications in surgery clearly continue to be a serious problem, with reports of post-operative complications ranging from 0.29 to 30%[5-10]. These post-operative and post-traumatic septic complications can not only quickly become life-threatening, but they can also prolong the hospital stay by 75%, increasing costs of treatment and often becoming the cause of death and disability in patients[3,4]. In terms of medical costs, aggregate hospital costs for septicemia were $15.4 billion in 2009, not including indirect costs[3]. For patients with septicemia as a secondary diagnosis—due to post-operative complications, for example—the average hospital stay is 15.8 days, costing $33,900[3].

According to the Agency for Healthcare Research and Quality, septicemia is the most expensive condition treated in U.S. hospitals, accounting for $23.7 billion in 2013, or 6.2 percent of the aggregate costs for all hospitalizations[11].

According to a study presented at the American Thoracic Society's annual conference, sepsis contributes to up to half of all hospital deaths in the U.S.[12]

The low success rates of sepsis treatments are largely due to lack of an effective diagnostic test for patients with high risk of sepsis or early sepsis that can provide results quickly and with high sensitivity and specificity. Indeed, the US Centers for Disease Control and Prevention has just recently begun highlighting the need for an early sepsis diagnostic.[13,14] Sepsis is typically diagnosed when a patient has a suspected or confirmed blood infection in conjunction with at least two of the following symptoms:

- Body temperature above 101°F (38.3°C) or below 96.8°F (36°C);
- Heart rate higher than 90 beats a minute;
- Respiratory rate higher than 20 breaths a minute[15].
- High white blood cell count

Those symptoms reflect a severe endogenous toxicity and appear in the patients who have already developed sepsis, and they do not help in making a well-timed, intensive interventional treatment. Unfortunately, these and other routine tests lack diagnostic accuracy and thus can be misleading[16]. Further, the only way to definitively confirm sepsis is by performing a culture of blood, urine, bronchial fluid or cerebrospinal fluid (CSF), which takes 24-48 hours[16]. Not only does this time delay put patients in additional harm's way, but clinical symptoms of sepsis are often present without a positive culture. Other methods being studied are not useful for routine diagnosis because of unknown sensitivity and specificity, high cost, duration of study and

complexity[16,17]. Given the shaky success rates of these current methods, there is clearly an unfulfilled, critical need for a quick, unequivocal test to identify patients at high risk of developing sepsis or are in the early stages of sepsis.

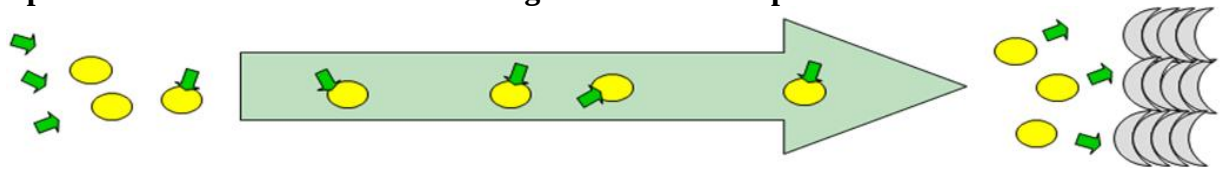
Role of serum albumin in the detoxification process

Serum albumin is the main carrier protein in the circulatory system of the body. Albumin provides the transport of cellular metabolism products, mediators, and other hydrophobic compounds, the totality of which reflects the nature and intensity of physiological and pathological processes in the body.

Serum albumin also is the main protein providing binding of the toxic molecules of hydrophobic origin and their uptake to hepatocytes in the circulatory system. Albumin also takes part in binding of reaction-active hydrophilic toxins.

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

Operations of albumin molecule during metabolite transport:

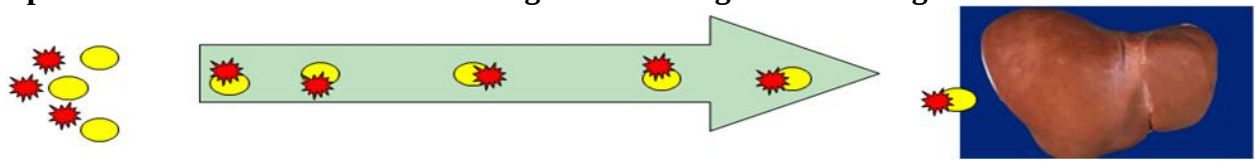


(1) Binding *(2) Transport in the circulatory system* *(3) Transfer to target cells*

In a healthy person, the concentration of toxins carried by albumin molecules is relatively low and most of the albumin molecules retain a high binding capacity. Permanent synthesis and filtration of albumin molecules in the liver maintains the functional activity of albumin in the body.

In a patient developing septicemia, there are three main processes which determine the initiation and course of the disease; (1) the increasing infusing of toxins in the circulatory system from the nidus of infection (source) or inflammation process, (2) the binding of toxins by albumin molecules and delivery of them to the detoxifying organs such as the liver and kidneys, and (3) the removal of the toxins from the body by the detoxifying organs.

Operations of albumin molecule during toxin binding and removing



(1) Binding *(2) Transport in the circulatory system* *(3) Filtration of the albumin in hepatocytes*

When the production of toxins is too high, or the capacity of the detoxifying organs is not great enough to remove the increased toxins, the toxin concentration in the circulatory system is increased. With such a condition, the albumin molecules can be overloaded with the toxins. This results in escalating toxicity of the blood, initiation of the inflammatory cascade and the development of disseminated intravascular coagulation and side effects such as multi-organ failure. It is important to note, that the critical ratio of the toxin production and removing capacity of detoxifying organs depends on the individual characteristics of the patient and the disease.

Pathogenesis of sepsis

Sepsis can be simply defined as a spectrum of clinical conditions caused by the immune response of a patient to an infection that is characterized by systemic inflammation and coagulation. It includes the full range of response, from systemic inflammatory response (SIRS), to organ dysfunction, to multiple organ failures and ultimately to death.

The sequence of events in the patient developing sepsis is:

(1) The systemic inflammatory cascade is initiated by an elevated concentration in the blood of various bacterial products, most of which are toxic themselves and are able to activate macrophages to produce pro-inflammatory cytokines; i.e., **sepsis is initiated by elevated endogenous toxicity in the blood.**

(2) The pro-inflammatory cytokines directly and indirectly (through secondary mediators) cause the activation of the coagulation cascade leading to fibrin deposition and disseminated intravascular coagulation, the complement cascade and the production of prostaglandins and leukotrienes. Clots lodge in the blood vessels which lower perfusion of the organs and can lead to endothelial cell damage and multiple organ system failures.

(3) The body then regulates this response by producing anti-inflammatory cytokines which are manifested in the patient by a period of immunodepression.

The cumulative effect of this cascade is an unbalanced state of the patient, with inflammation dominant over anti-inflammation, and coagulation dominant over fibrinolysis. Microvascular thrombosis, hypoperfusion, ischemia, and tissue injury result. Severe sepsis, shock, and multiple organ dysfunctions may occur, leading to severe illness and death.

Important aspects are:

- The systemic inflammatory cascade can be activated in a patient not only by septicemia (presence and multiplication of bacteria in the blood) but also by high levels of harmful substances released by injured (necrotic) tissue;
- The bacteria in the blood which are triggering the disease can be transient and may not be detected at the time when the patient shows symptoms. Most patients are classified as septic-based on a clinical suspicion of infection (a positive blood culture is detected in only about 30% of cases);
- A positive blood culture can be observed also in patients with bacteremia, who however do not develop septicemia.

Sepsis diagnosis

Sepsis is defined as SIRS in response to an infectious process and is typically diagnosed when a patient has a suspected or confirmed blood infection in conjunction with at least two of the following symptoms:

- Body temperature above 101°F (38.3°C) or below 96.8°F (36°C);
- Heart rate higher than 90 beats a minute;
- Respiratory rate higher than 20 breaths a minute[15].
- High white blood cell count

Those symptoms reflect a severe endogenous toxicity and appear in the patients who have already developed sepsis. However, they do not help for making an early diagnosis of the disease.

Sepsis initiation and development is a stepwise process with a cascade of clinical conditions in a patient which can be described, as follows:

(1) Contamination of the patient's blood by bacteria (by endogenous bacteria in most cases);

(2) Elevation of the blood toxicity (increasing concentration of products of the bacteria multiplying in the blood);

(3) Initiation of the systemic inflammatory cascade (the blood toxicity is increased up to some critical level);

(4) Development of SIRS (pro-inflammatory cytokines, then activation of the coagulation cascade resulting in disseminated intravascular coagulation, the endothelial cell damage, and multiple organ system failures);

(5) Period of immunodepression (the body produces anti-inflammatory cytokines).

Considering this, an efficient biomarker for prognosis and early diagnosis of sepsis must detect metabolic changes associated with developing septicemia, during the period of stages (1), (2) and (3); i.e., before or just after the initiation of SIRS.

Common laboratory tests include CBC with differential count, C-reactive protein, procalcitonin, urinalysis, coagulation profile, glucose, blood urea, nitrogen, creatinine, electrolytes, liver function tests, lactic acid level, arterial blood gas, electrocardiogram, and a chest X-ray, as well as cultures of blood, sputum, urine, and other obviously infected sites.

These tests do not help prognosis and early diagnosis of sepsis during the stages from (1) to (3), before elevating concentrations of toxic bacterial products appear in the blood and become able to initiate the systemic inflammatory cascade. The common tests are informative only at stages (4) and (5) and are used to confirm the infection causing the disease, to differentiate sepsis and SIRS, and then to manage a treatment.

Competitive sepsis diagnostic tests

Among the common laboratory tests, there are no tests providing physicians with similar information regarding escalating blood toxicity in a patient other than the Albutran Transport Capacity test.

Lactate, C-reactive protein (CRP), and procalcitonin (PCT) are commonly used for classification and management of septic patients. Lactate is used to assess tissue perfusion and is elevated with tissue hypoxia caused by hypoperfusion in severe sepsis and septic shock but not in early sepsis. C-reactive protein (CRP) and procalcitonin (PCT) are both inflammatory biomarkers, widely investigated for sepsis diagnosis. CRP is an acute-phase reactant elevated in many inflammatory conditions. PCT, the precursor of the thyroid hormone calcitonin, is also increased in the systemic inflammatory response to infection.

In sum, lactate, PCT, and CRP are helpful markers to manage patients with suspected sepsis by providing prognostic information and guiding therapy, but they have limited diagnostic utility in sepsis and no role at the early stages of sepsis.

Different research groups are studying novel biomarkers for sepsis diagnosis and management. A recent literature review identified more than 3,000 published reports of 178 sepsis biomarkers which include immune cell markers, cytokines, coagulation factors, acute-phase reactants, markers of vasculo-endothelial damage, vasodilation, and organ dysfunction[18]. These biomarkers focus on metabolic changes in a sepsis patient who has already developed SIRS. The authors of this review surmise that none of these biomarkers alone had sufficient diagnostic strength to identify septic patients.

Actually, among the common and perspective biomarkers there are none which has sufficient diagnostic strength to identify septic patients at early stages and differentiate among those patients with systemic inflammation at early stages. Considering the complex pathophysiology of sepsis, sepsis diagnosis is better attained by a combination of readouts reflecting the various aspects of the host response.

Thus, among common and prospective biomarkers, there are no biomarkers which could be direct competitors to the **Albumin Transport Capacity Test**.

The Albumin Transport Capacity Test can provide physicians with new, critical information about the elevating blood toxicity in a patient with a risk of sepsis, which in conjunction with other clinical criteria can serve as a prognosis and diagnosis of sepsis at early stages.

Albumin Transport Capacity Test: A novel approach to improve prognosis and early diagnosis of sepsis

The Albumin Transport Capacity Test is an innovative in vitro test of blood serum to indicate the ability or inability of the patient's blood transport system to carry metabolites and remove toxins.

The test can indicate the presence or absence of overload to the blood carrier protein (serum albumin) in a patient and can be used for detection of elevating blood toxicity that occurs in a patient at the conditions of pre-sepsis and early stage of sepsis.

The test can promptly provide physicians with important information that in conjunction with other clinical criteria allows them to predict and diagnose sepsis at an early stage, before the

development of severe SIRS and disseminated intravascular coagulation. The test can be quickly performed (in about 30 minutes) using a standard blood sample.

The Albumin Transport Capacity test indicates the presence or absence of overload to the blood carrier proteins in a patient and can be used in conjunction with other clinical data for diagnosis of patients with suspected or diagnosed septicemia:

- As a screening test to detect escalating toxicity in patients;
- To detect patients with a high risk of sepsis and SIRS among post-surgery patients, in the first hours immediately following the operation;
- To detect high risk of sepsis and SIRS in patients entering the emergency department;
- To determine when sepsis treatment is effective, or needs to be changed.

The Albumin Transport Capacity Test can be used also in diagnosis of patients with suspected or diagnosed blood toxicity of non-septic origin:

- To determine when patients need intensive antibiotic therapy or if it can be excessive;
- As a screening test to detect pregnant women with severe preeclampsia;
- To determine when patients with insufficient function of their liver or kidneys need hemodialysis or hemosorption;
- To detect acute resection and dysfunction of renal grafts in kidney transplant recipients;
- To determine when chemo- or radiotherapy causes severe toxicity in a cancer patient and needs to be stopped, or can be continued.

The test can help improve patient care and provide physicians with important information that allows them to treat sepsis patients at an early stage and to avoid an excessive antibiotic therapy for sepsis-free patients. The test can be used in conjunction with other laboratory tests and clinical indices that allow diagnosing sepsis patients one to two days earlier than currently used tests.

The clinical utility of the test has been confirmed through clinical studies conducted in Russia, Belarus, and Germany, and the results have been published in reputable scientific journals and presented at scientific conferences[19-51].

Science behind the test

The strength of binding and retaining the toxins (i.e. binding constant) is the parameter determining the detoxifying activity of albumin molecules. The excessive binding of toxins causes an allosteric modification of the albumin molecules that can result in a reduced binding constant for the protein. Such albumin molecules may lose or significantly reduce their ability to bind subsequent toxic molecules. 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.

The Albumin Transport Capacity Test measures this change in the carrying capacity of albumin and is covered by US patent 8632986 (also published as EP2353010), “Methods for detection of

toxemia". The high sensitivity of the albumin binding constant to toxin load is the crux of the test technology.

The test analyzes the serum albumin using Electron Paramagnetic Resonance Spectroscopy (EPR). The spin probe 16-doxyl stearate, that 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 albumin binding parameters, such as a binding constant and binding capacity, and provides an estimation of the capacity of the albumin transport system.

- Spin Probe – the molecule that contains a stable free radical and is able to bind to other molecules. In the Albumin Transport Capacity Test the spin probe (16-doxyl stearate) is able to bind to both specific and unspecific binding sites of the albumin molecule. These albumin sites are highly sensitive to allosteric interactions. The binding constant for the spin probe is very high for normal albumin but decreases significantly (drastically in the case of sepsis) when the albumin molecules are overloaded by bound compounds or affected by toxins. Under the same conditions, a change to the binding capacity in the affected albumin is less significant than to the binding constant.
- Exposure to a high magnetic field and microwaves causes a resonance of the free radical in the spin probe. The resonance response of the spin probe is measured as an EPR spectrum that reflects the structure and the properties of the protein molecule to which the spin probe is bound.
- EPR spectroscopy – the technique for the measurement and interpretation of EPR spectra.
- Initially, the method of EPR spectroscopy of serum albumin in vitro was developed in the Belorussian Research Institute of Oncology and Medical Radiology[38-49].
- Subsequently, the method was improved in cooperation with experts at the Transfusion Medicine and Medical Physics Institutes of Leipzig University[27,28,30,34,35,37], University Clinic Charité (Berlin) [29], Oregon Health & Science University (USA) [28,29,33] and others.

It has been confirmed in clinical studies, that the significant reduction of serum albumin binding parameters (the functional activity of albumin as the carrier protein) in the patients who develop severe toxicity because of sepsis, or SIRS from some other origin, is detected much earlier than the appearance of other clinical symptoms[20-23, 26, 29].

In the studies, it was confirmed that in patients developing sepsis at an early stage (before the appearance of clinical symptoms of sepsis), the transport capacity of serum albumin was significantly reduced, down to 30% or lower compared to a control group (Russian cost basis). In such patients, the conventional clinical symptoms of sepsis appear significantly later, one to two days after the dysfunction of albumin was observed, at which time the albumin transport capacity was reduced drastically, down to 2 - 9% of the original transport capacity. In a follow-up study of sepsis patients, it was confirmed that the level of albumin transport capacity correlates with the course of the disease[22, 23, 26, 29].

In summary, the Albumin Transport Capacity Test

- is a marker of toxicity in a patient
- evaluates the loading of patient's detoxifying system

Clinical Results

A number of clinical studies across a variety of patients with sepsis, SIRS, pregnant women with preeclampsia and recipients of renal grafts have shown the test to have a high specificity and high sensitivity. Sensitivity is the ability of a test to identify those with sepsis or escalating toxicity of another origin correspondingly to the studied disease (true positive rate). Specificity is the ability of the test to identify those without toxicity (true negative rate). The sensitivity and specificity of the test are much higher than other available tests detecting these diseases. The sensitivity and specificity values can be seen below in the short summaries for some clinical trials that were conducted.

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

N. N. Blokhin Russian Cancer Research Center RAMS (2013) [20, 23]

Prognosis, diagnostics and therapy monitoring of sepsis in post-surgery patients

Clinical study group: 132 oncology patients studied after scheduled operations of the thoracic and abdominal cavities, pelvic organs, kidneys, and major joints (12 of them had sepsis and 33 had SIRS).

Confirmed conclusions:

- The test has high prognostic significance for sepsis and SIRS in post-surgery patients, when assessed during the first two hours after the operation, with Sensitivity of 80% and Specificity of 80%;
- The test provides early diagnosis and monitoring of sepsis patients that allows optimizing of the treatment;
- About 25% saving of clinical expenses (Russian cost basis) for treatment and follow-up of post-surgery patients by the optimized use of antibiotics and reduced lengths of the periods of individual patient stays in the ICU.
- Other indirect inflammation markers (D-dimer, procalcitonin, fibrinogen) are less significant: in several cases, the procalcitonin level was within or slightly outside the normal range when the subject had already been diagnosed with sepsis and had a positive ATA-test.

N. N. Blokhin Russian Cancer Research Center RAMS (2006) [26]

Diagnostics and therapy monitoring of sepsis in post-surgery patients:

Clinical study groups: 40 oncology patients studied after scheduled operations of the abdominal cavities and kidneys (among them 10 had sepsis, 7 had peritonitis, 2 had pneumonia), and **30** healthy volunteers.

Confirmed conclusions:

- The test is a reliable indicator of the deterioration to transport function of serum albumin in sepsis patients;
- The test provides diagnostics of sepsis in post-surgery patients in the first and second days after the operation with Sensitivity of 80% and Specificity of 80%.

Republican Scientific and Practical Center for Organ and Tissue Transplantation (9-th Clinic of Minsk, Belarus), Belorussian State University (2013) [20, 21]

Diagnostics of acute rejection of kidney transplant, monitoring of kidney transplant functionality and efficacy of immunosuppressive medical agents

Clinical study group: 92 recipients of kidney transplants

Confirmed conclusions:

- The test is a sensitive noninvasive method that clearly demonstrated high diagnostic utility in kidney transplant recipients with acute resection and dysfunction of the kidney transplant, with Sensitivity of 73% and Specificity of 78%;
- The test is useful for monitoring of the postoperative course through the use of serial measurements starting from the early postoperative period.

Federal State Budget Institution "V.I.Kulakov Research Center for Obstetrics, Gynecology, and Perinatology" of Ministry of Healthcare of the Russian Federation (2015) [51]

Diagnostics of preeclampsia (gestosis)

Clinical study groups: 92 pregnant women with preeclampsia and 97 women with an uncomplicated pregnancy.

Confirmed conclusions:

- The test allows diagnosis of severe preeclampsia in pregnant women during the second trimester with Sensitivity of 64% and Specificity of 92%, and in the third trimester with Sensitivity of 59% and Specificity of 82%.

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” were 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 was permitted for use by the Federal Service for Supervision of Health of the Russian Federation (Permission Certificate for 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" was approved by the Association of Professionals and Institutions of the Laboratory Services "Federation of Laboratory Medicine" of the Russian Federation[50].
- Instruction for use of ATA test was registered by the Ministry of Health of the Republic of Belarus (Instructions for use No 12-00.00.000 IP, No 003-0114, 24-9802).

The test has been used in the Consulting and Diagnostic Centre 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 since 2013.

An ongoing activity is an evaluation of clinical utility for patients with different diseases and conditions in order to develop recommendations for clinical use for different groups of patients.

Europe:

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

US:

Approval by the FDA is ongoing. Pre-submission for the test (Application No Q151049/S001) was revised by the FDA and discussed in the informational meeting with the FDA.

The equipment and consumables to be supplied for the test meet the standards required for FDA approval (Appendix presents the list of international standards applied to the analyzer).

The recommendation of the FDA was to prepare individual pre-submissions for each indication for use of the test. During an approval of the test for the first indication both the analyzer and set of reagents will be approved (some technical examinations of the devices in the US could be required). For the next indications, only approval of the test (clinical utility) should be required.

An ongoing activity is to prepare the pre-submission for the first indication for use which is expected to be the test for the diagnosis of sepsis risk in post-surgery patients in the first two hours after the operation.

The cooperation of US partners is required for clinical studies expected for approval of the test by FDA, as well as for the development of new indications for use.

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) Separation of serum or plasma EDTA by centrifugation of the blood sample,

(3) Mixing of the serum sample with the reagent from the kit "ATA-test",

(4) Incubation of the probe with the shaker for 10 min at 37 °C,

(5) Investigation of the probe with the analyzer AXM-09 during 4 min.

- Single patient sample processing time (practical): 25 to 30 minutes, each.
- Analyzer throughput maximum (practical): 12 to 15 prepared samples per hour.

TEST SAMPLES: 50 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-T-20", "ATA-test-T-80" (for diagnosis of intoxication)

- 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:

✓ **DTE** – detoxifying efficiency	(For diagnosis of toxicity)

REFERENCES

1. Sepsis Fact Sheet. National Institute of General Medical Sciences, 2014. (Accessed Dec. 15, 2015, at https://www.nigms.nih.gov/Education/pages/factsheet_sepsis.aspx.)

2. GSA News and Info. Global Sepsis Alliance (GSA). (Accessed Dec. 15, 2015, at http://globalsepsisalliance.com/gsa-news-and-info/.)

3. Elixhauser A, Friedman B, Stranges E. Septicemia in U.S. Hospitals, 2009. HCUP Statistical Brief #122. Rockville, MD: Agency for Healthcare Research and Quality (AHRQ); 2011 October 2011.

4. Hall M, Williams S, DeFrances C, Golosinskiy A. Inpatient care for septicemia or sepsis: A challenge for patients and hospitals. NCHS data brief, no 62. Hyattsville, MD: National Center for Health Statistics; 2011.

5. B. A. Barnes, G.E. Behronger, F.C. Wheelock at all. Postoperative Sepsis: Trends and Factors Influencing Sepsis Over a 20-Year Period Reviewed in 20,000 Cases. /Annals of Surgery. Volume 154, Number 4, 1961. P. 585-598.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1465981/pdf/annsurg00858-0105.pdf

6. T. Jakobsona, J. Karjagina, L. Vipp at all. Postoperative complications and mortality after major gastrointestinal surgery. /Medicina 50 (2014). P. 111-117.

http://dx.doi.org/10.1016/j.medici.2014.06.002

7. B.T. Bateman, U. Schmidt, M.F. Berman at all. Temporal Trends in the Epidemiology of Severe Postoperative Sepsis after Elective Surgery: A Large, Nationwide Sample. /Anesthesiology 4 2010, Vol.112, 917-925. doi:10.1097/ALN.0b013e3181cea3d0.

http://anesthesiology.pubs.asahq.org/article.aspx?articleid=1932871

8. Vogel TR, Dombrovskiy VY, Lowry SF. Trends in postoperative sepsis: are we improving outcomes? / Surg Infect (Larchmt). 2009 Feb;10(1):71-8. doi: 10.1089/sur.2008.046.

https://www.ncbi.nlm.nih.gov/pubmed/19298170

9. T. R. Vogel, V. Y. Dombrovskiy, J. L. Carson at all. Postoperative sepsis in the United States./ Ann Surg. 2010 Dec; 252(6): 1065–1071. doi: 10.1097/SLA.0b013e3181dcf36e.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2951484/

10. Sepsis contributes to as many as half of all hospital deaths in U.S., study says. / Health System. University of Michigan. http://www.uofmhealth.org/news/archive/201405/sepsis-contributes-many-half-all-hospital-deaths-us-study

11. Celeste M. Torio, Brian J. Moore. National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2013. STATISTICAL BRIEF #204, May 2016.

https://www.hcup-us.ahrq.gov/reports/statbriefs/sb204-Most-Expensive-Hospital-Conditions.jsp?utm_source=AHRQ&utm_medium=AHRQSTAT&utm_content=Content&utm_term=HCUP&utm_campaign=AHRQ_SB_204_2016.

12. Vincent Liu, MD, MS Gabriel J. Escobar at all. Hospital Deaths in Patients with Sepsis From 2 Independent Cohorts. JAMA July 2, 2014 Volume 312, Number 1, p.90-92. http://jamanetwork.com/journals/jama/fullarticle/1873131#.U3kivi3tbAw.twitter.

13. CDC. Making Health Care Safer - Think sepsis. Time matters. CDC Vital Signs 2016.

14. Karidis A. Sepsis is a medical emergency, CDC says. It can be stopped if caught in time. The Washington Post 2016 August 23, 2016.

15. Davis CP. What are the signs or symptoms of sepsis (blood poisoning)? In: MedicineNet, Inc.; 2015.

16. Jin M, Khan AI. Procalcitonin: Uses in the Clinical Laboratory for the Diagnosis of Sepsis. Laboratory Medicine 2010;41:173-7.

17. Gelfand B, Filimonov M, Hyles T. Procalcitonin: a new laboratory marker of sepsis and septic complications in surgery. Journal of Intensive Care 2003;1.

18. Pierrakos C, Vincent JL. Sepsis biomarkers: A review. Crit Care. 2010;14(1):R15. PMC2875530

19. T. Davydova, I. Matveeva, A. Gritsai, N. Pogosyan, L. Mamedova, V. Kuznetsov. Clinical and diagnostic value of the functional and conformational properties of serum albumin in patients with ovarian cancer (a pilot study). Women Reproductive System Tumors, 2014, 2, p.59-61 *(in Russian).*

20. Kalachik A., Ugolev I., Zabello T., Sadousky D., Oganova E., Murauski U. Electron Spin Resonance Spectroscopy Evaluation of the Serum Albumin Function Quality in Kidney Transplant Recipients. Proceedings of the National Academy of Sciences of Belarus, Series of Medical Sciences, 2014, Volume 4, p. 72-77 *(in Russian).*

21. A. Kalachyk, I. Ugolev, T. Zabello, E. Oganova, V. Muravsky. Electron Spin Resonance Spectroscopy of Serum Albumin is a Novel Test for Diagnosis of the Kidney Transplant Acute Rejection. Clin Chem Lab Med 2014; 52, Special Suppl, pp S1 – S1760, June 2014, P. 1068.

22. P. Vorobiev, L. Bezmelnitsyna, L. Krasnova, M. Holownia, I. Matveeva, T. Davydova, I. Nekhaev, A. Sytov, M. Nersesyan. Clinical and economical analysis of efficiency of ATA-test for early diagnosis of postoperative purulent-septic complications. Health care standardization problems, 2014, Volume 3-4, pp. 28-36 *(in Russian).*

23. Vorobiev P., Bezmelnitsyna L., Krasnova L., Muravsky V., Davidova T., Sytov A. Economical Justification of the Innovation Method of Laboratory Diagnostic of the Structural and Functional Changes of Serum Albumin in Septic Complications (Ata-Test). Value in Health, 2013, Volume 16, Issue 7, Page A364.

24. V. Sidorenko, V. Muravsky, E. Shimanovich, L. Zenko. Deterioration of serum albumin transport function in pregnant women with gestosis. Medical Journal (BSMU, Belarus), 2013, No 1, 149-150 *(in Russian)*

25. Liu et al. Changes in the electron paramagnetic resonance spectra of albumin-associated spin-labeled stearic acid as a diagnostic parameter of colorectal cancer. World Journal of Surgical Oncology, 2013, 11:223.

26. Davidova T., Matveeva I., Citov A., Nekhaev I., Sviridova S., Zubrikhina G., Jujginova O., Muravsky V., Gurachevskaya T. Diagnostics and monitoring of a development of purulo-septic complications in early postsurgical period in cancer patients using EPR method. International Journal of Applied and Fundamental Research. 2011, No 5, 94-95 *(in Russian).*

27. R. Jalan, K. Schnurr, R.P Mookerjee, S. Sen, L. Cheshire, S. Hodges, V. Muravsky, R. Williams, G. Matthes, N. A Davies. Alterations in the functional capacity of albumin in patients with decompensated cirrhosis is associated with increased mortality. Hepatology. 2009 Aug; 50(2); 555-64.

28. V. Muravsky, T. Gurachevskaya, S. Berezenko, K. Schnurr, A. Gurachevsky. Fatty acid binding sites of human and bovine albumins: Differences observed by spin probe ESR. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 74 (2009).

29. A. Gurachevsky, S. C. Kazmierczak, A. Jörres and V. Muravsky. Application of Spin Label EPR in the diagnosis and prognosis of cancer and sepsis. Clin Chem Lab Med 2008; 46(9); 1203-1210

30. V.Muravsky, A.Gurachevsky, G.Matthes. Disease-specific albumin patterns defined by electron spin resonance. Tumor Biol.28 (suppl. 1), 19 (2007). ISSN 1010-4283; 1423-0380

31. Gurachevsky A, Muravskaya E, Gurachevskaya T, Smirnova L, Muravsky V. Cancer-Associated Alteration in Fatty Acid Binding to Albumin Studied by Spin-Label Electron Spin Resonance. Cancer Invest. 2007. 25:378-383. ISSN: 0735-7907

32. A.Gurachevsky, E.Shimanovitch, T.Gurachevskaya, V.Muravsky. Intra-albumin migration of bound fatty acid probed by spin label ESR. Biochem Biophys Res Commun. 2007 Sep 7; 360(4):852-856

33. S. C. Kazmierczak, A. Gurachevsky, G. Matthes, and V. Muravsky. Electron Spin Resonance Spectroscopy of Serum Albumin: A Novel New Test for Cancer Diagnosis and Monitoring. Clinical Chemistry 52:11, 2129–2134 (2006)

34. P.Seidel, A.Gurachevsky, V.Muravsky, K.Schnurr, G.Seibt, G.Matthes. Recognition of malignant processes with neural nets from ESR spectra of serum albumin. Z. Med. Phys. 15 (2005) 265–272

35. K. Schnurr, V. Muravsky, G. Seibt, G. Matthes. The analysis of functional characteristics of serum albumin – a novel possibility of early cancer diagnosis. In the book "Methods, possibilities, and perspectives of pre-symptomatic tumor diagnostics", Pabst Science Publischers, 2005, p. 225-234. ISBN 1-59326-077-6 (USA)

36. Muravskaya E., Lapko A., Muravskii V. Modification of transport function of plasma albumin during atherosclerosis and diabetes mellitus. Bull Exp Biol Med. 2003 May; 135(5):433-5 *(in Russian)*

37. G.Matthes, G.Seibt, V.Muravsky, G.Hersmann, G.Dornheim. Albumin transport analysis of different collected and processed plasma products, by electron spin resonance spectroscopy. Transfus Apheresis Science, 2002, 27, p. 129-135

38. E.Melnichenko, A.Kuschner, A.Milutin, A.Maschevsky, V.Muravsky. Evaluation by the enamel of temporary teeth of the ionizing radiation doses adsorbed by the children population of Republic of Belarus. Stomatologiia (Moskow) 2002, 4, p. 54-56 *(in Russian)*

39. A.Maschevsky, V.Prokchorova, V.Muravsky, S.Linev, I.Ugolev. Technology of the tooth enamel ESR-dosimetry for the individual adsorbed dose estimation. Ukrainian Radiological Journal, 1999, № 1, p. 49-50 *(in Ukrainian)*

40. V.Prokchorova, A.Maschevsky, T.Zirus, S.Lappo, L.Paschko, R.Smoliakova, L.Derjavez, N.Jurevich, V.Muravsky. The use of laboratory testing methods for breast cancer diagnostics. In the book "Actual problems of oncology and clinical radiology". Research Institute of oncology and clinical radiology, Minsk, 1998, p. 77-81 *(in Russian)*

41. R.Smoliakova, A.Maschevsky, V.Prokchorova, T.Zirus, L.Paschko, A.Milutin, V.Muravsky. Investigation of structural and functional status of albumin in cancer patients by means of ESR-spectroscopy method. Advances of medical science in Belarus, 1997, 2, p. 19-20 *(in Russian)*

42. A.Maschevsky, V.Muravsky, E.Korotkevich, S.Linev, Y.Darvin, I.Ugolev, E.Javrid, V.Prokchorova, J.Zmuschko. Methodical and metrological aspects of gamma-irradiation adsorbed dose-measuring by tooth enamel. Medical physics, 1996, № 3, p. 46-50 *(in Russian)*

43. V.Prokchorova, A.Maschevsky, V.Muravsky, A.Milutin, T.Zirus, S.Linev, R.Smoliakova, L.Paschko. Diagnostic potential of ESR-spectrometric determination of conformational-dynamical characteristics of albumin for breast cancer. In the book "Actual problems of oncology and clinical radiology". Research Institute of oncology and clinical radiology, Minsk, 1996, p. 47-55 *(in Russian)*

44. V.Muravsky A.Maschevsky, N.Krisenko, E.Javrid, S.Linev, I.Ugolev, Y.Darvin, V.Prokchorova. Parameters of the ESR spectrum spin model of tooth enamel and the use of it in ESR-dosimetry. Ukrainian Radiological Journal. 1995, № 4, p. 353-357 *(in Ukrainian)*

45. S.Linev, Y.Darvin, A.Maschevsky, V.Muravsky. Estimation of gamma-radiation absorbed doses by tooth enamel using spin model of ESR spectrum. Medical physics, 1995, №2, p. 14. *(in Russian)*

46. E.Javrid, A.Maschevsky, V.Muravsky, V.Prokchorova, E.Korotkevich, A.Milutin. A comparative study of conformation properties of human serum albumin in lung cancer by spin probe technique. Annals of Oncology, 1994, vol.5 (suppl.8), p. 9

47. A.Maschevsky, V.Muravsky, V.Prokchorova, A.Milutin. Comparative investigation of long chain fatty acid binding sites of normal serum albumin with the one from lung cancer patients carried out by the spin probe method. In the book "Blood serum albumin in clinical medicine". Serie "Fluorescence methods of investigation and clinical diagnostics", Edited by J.Grizunov and E.Dobretsov, Moskow, "IRUS", 1994, p. 176-181 *(in Russian)*

48. A.Maschevsky, V.Muravsky, A.Milutin, E.Javrid, E.Korotkevich, V.Prokchorova, T.Zirus, S.Linev. Dynamics of ESR spectra of the spin-labeled blood serum under neoplastic process. Ukrainian Radiological Journal. 1994, № 4, p. 268-270 *(in Ukrainian)*

49. A.Maschevsky, V.Muravsky. The algorithms of bio-molecules parameters estimation for the development of clinical diagnostics procedures on the base of spin probe ESR method. In the book "Actual problems of oncology and clinical radiology", Research Institute of Oncology and Clinical Radiology, Belarus Ministry of Health, Minsk, 1993. P. 31-33 *(in Russian)*

50. O. Liang, K. Schnurr, K. Waterstradt. Laboratory diagnosis of active malignant proliferation by assessment of changes to transport parameters of serum albumin using EPR spectroscopy. Laboratory service. 2015;4(3): 44-56 *(in Russian)*

51. D.Nasonova, T.Ivanetz. "Deterioration of serum albumin transport function in preeclampsia"// Reference Book of the Head of Clinical Diagnostic Laboratory. June 2015, No 6, pp. 35-40 *(in Russian)*

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