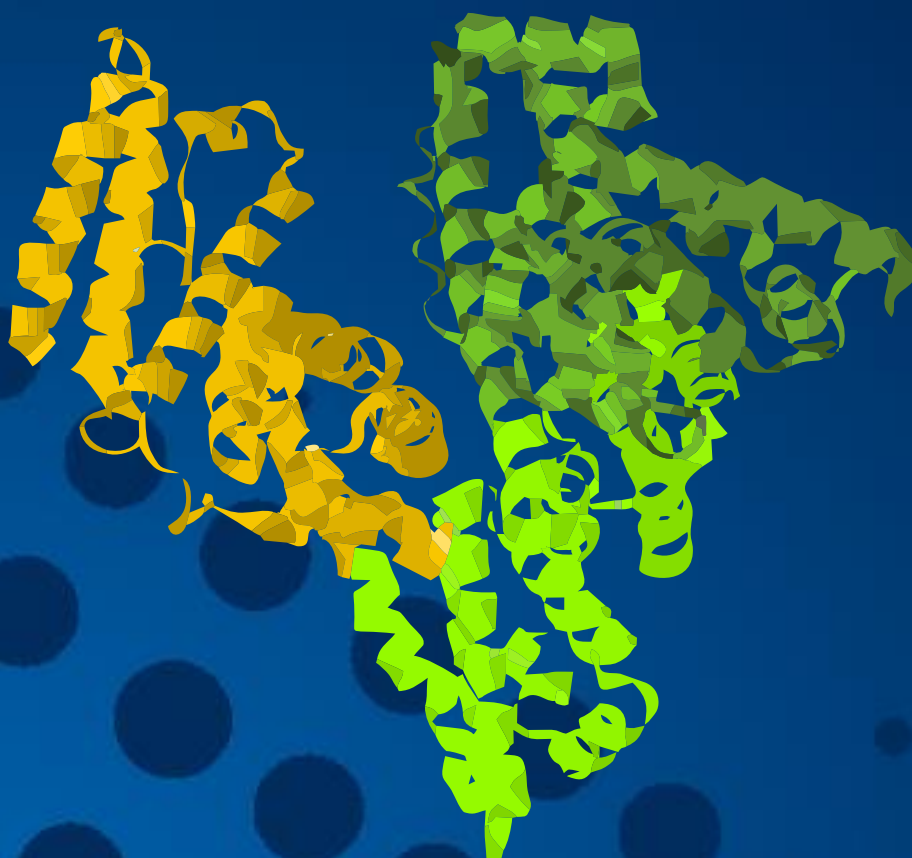




MedInnovation
Advanced Albumin Research



**Analysis of functional characteristics of
serum albumin for cancer diagnostic**

Principles - Albumin

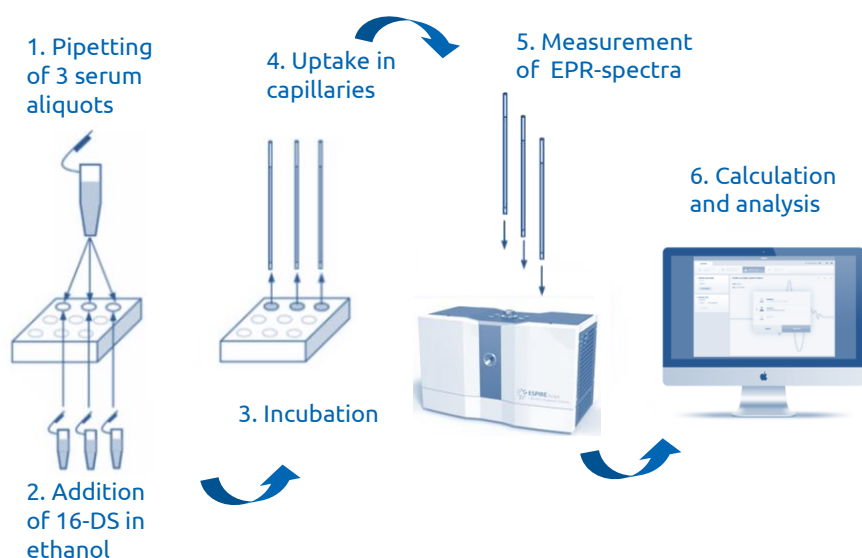
Albumin is the most abundant protein in human blood serum. It is produced in the liver and has a serum half-life of approximately 19 days.

Transporting a large variety of hydrophobic substances like fatty acids, drugs and metabolites is one of its main physiological functions [1].

Beside this it maintains the oncotic pressure and buffers the pH of the blood.

For long chain fatty acids seven binding sites are known [2], three of them with high and four with lower affinity [3]. The binding sites with high affinity are described as long and narrow pockets, whereas those with low affinity are short and wide [2].

During the last years low molecular weight biomarkers bound to serum carrier proteins like albumin were intensively investigated, assuming they might have a potential for early disease detection [4, 5, 6].

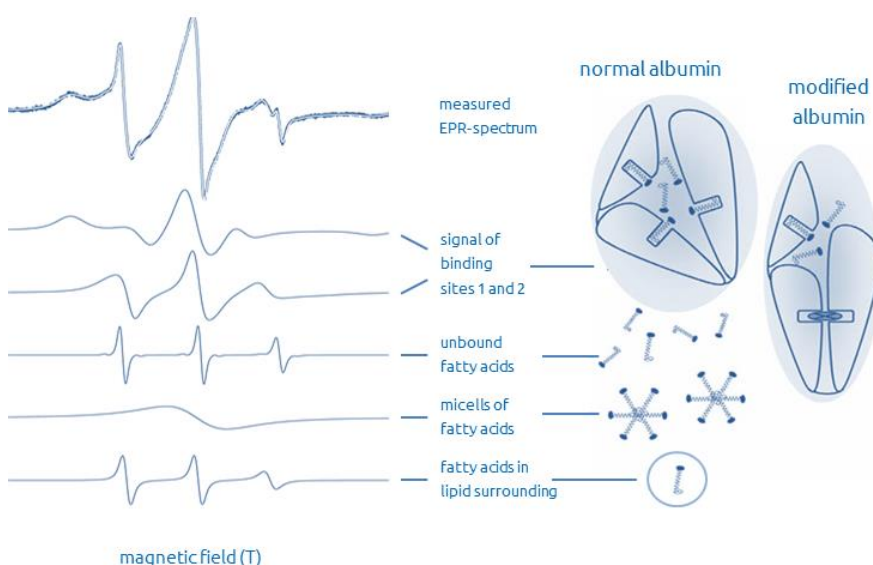


EPR technology

The albumin-functionality-test uses electron paramagnetic resonance spectroscopy (EPR) to estimate the functionality of albumin in human serum. This platform solution consists of different modules for different diseases.

It is based on a comparison of three different albumin-ethanol-fatty acid solutions, which simulate binding, transport and release conditions in vitro [7, 8]. By adding a spin-labelled fatty acid, the binding sites of albumin can be investigated.

Binding constants, binding capacities and biophysical parameters of both binding site types in the three different serum-ethanol-fatty acid solutions can be estimated by simulating the EPR spectra and hence the transport parameters (BE = binding efficiency, RTQ = real transport quality, DTE = detoxification efficiency) can be calculated.



Equipment



- Applicable in standard laboratory routine – easy to handle.
- Automated device – generating parameter control algorithms, automated measurement procedure, signal registration and pre-processing of spectra as an integral process.
- Provides high accuracy, stability and sensitivity – at a high throughput rate.
- Guarantees comparable results in the analysis of several aliquots of one sample.
- Especially designed for the analysis of samples of biologic materials, where molecular conformation changes depending on temperature, pH and other factors occur.
- All algorithms programmable and provide a wide range of routine as well as scientific applications.



- Accurate contact and non-contact sample preparation system for handling of chemical or biological solutions or suspensions
- Avoids human pipetting errors, shows highly reproducible results and increases productivity
- High resolution touch panel display with an intuitive user friendly interface
- High precision of pipetting with less than 2% (10-14µl)



Diagnostic Kit

- Set of solutions of 16-doxyl-stearic acid in ethanol (three different concentrations with different cap color)
- 96-well microtiter plates for sample incubation
- Lid for microtiter plate
- Glass capillaries
- Wax on undercoat for capillary sealing
- Laboratory film for microtiter plate's wells closure during incubation
- Package



Applications

- Cancer diagnosis & monitoring
- Quality control of commercial albumins
- Estimation of albumin transport and detoxification parameters in patients with several diseases

Analytical requirements

Criteria for exclusion

Acute exacerbations of chronic inflammatory diseases, such as Crohn's disease, ulcerative colitis or rheumatoid arthritis distort test results.

For regeneration of serum albumin it is necessary to keep a time lag of four weeks before blood sampling.

After surgery, chemo and/or radiation therapy, it is also necessary to keep a time lag of four weeks before blood sampling. This also applies to diseases with inflammatory processes.

Because at the moment the albumin-functionality-test does not give an information about the location of the cancer, it is inadvisable to use it in a screening situation.

For an adequate analysis we require:

- At least 2 ml blood stored at 8°C. The whole blood should attain to MedInnovation GmbH within 24 hours after sampling.
- "Pure" (centrifuged) serum should attain to us within at least 4 days after sampling. Centrifugation should be done at 1,000 – 1,500 x g at room temperature. Serum or EDTA plasma must be transferred into a separate and labeled tube.



- Blood withdrawal systems with anticoagulant (except EDTA) or gel are not allowed and if possible please don't use vacuum collection systems.
- Storage of samples for more than 4 days must be at less than -25°C, repeated freeze-thaw cycles of serum / plasma is not permissible.
- Study sample, which will be stored for more than 3 month should be stored at temperature less than -35°C, better at -80°C
- It is allowed to have a light breakfast.
- Blood withdrawal should be done in the morning.

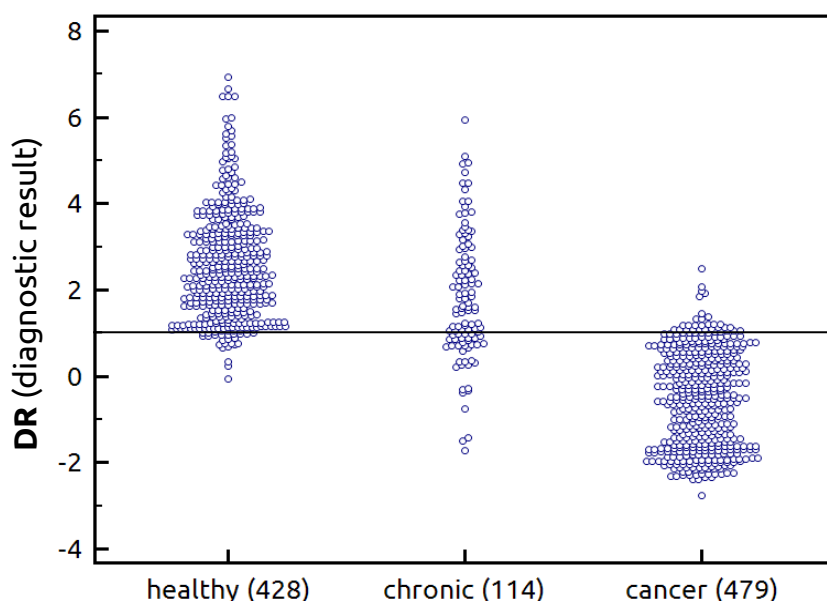
Modified binding characteristic can be detected by Electron Paramagnetic Resonance Spectroscopy (EPR).

An integral discrimination function (DR – **D**iagnostic **R**esult) can be calculated from the parameters achieved from the EPR spectra.

In case of a DR value less than 1.0 the albumin conformation has changed and there is a very high probability of an active malignant process.

In case of a DR value higher than 1.0 a high probability of no malignant disease exists. In a threshold range from 0.8 to 1.2 is still a high risk of the presence of a malignant disease.

In clinical studies the albumin-functionality-test showed – in dependence of the location – a high sensitivity (about 90%) and specificity (about 90%) [10 - 14].



Fields of application

- therapy monitoring and relapse control
- early detection of cancer in populations with elevated risk (contact with carcinogenic substances, accumulation of cancer in family history)

Albumin-functionality-test

The albumin-functionality-test offers a possibility for easy and fast detection of active tumor growth, independently from type or location.

The variety of albumin function for all transport stages is based on the high conformational flexibility of this protein.

In the case of cancer, several specific peptides and lipids are present in the blood circuit in very low concentrations [5, 9].

Some of these molecules preferentially accumulate to serum albumin and influence the albumin function by changing its conformation.

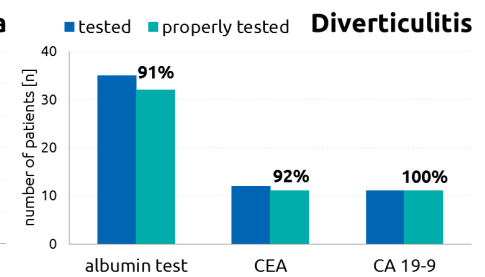
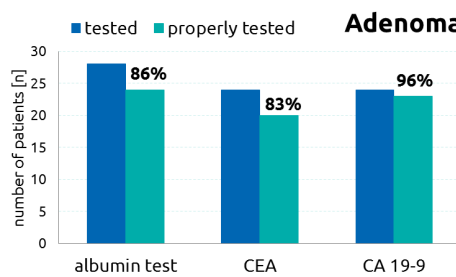
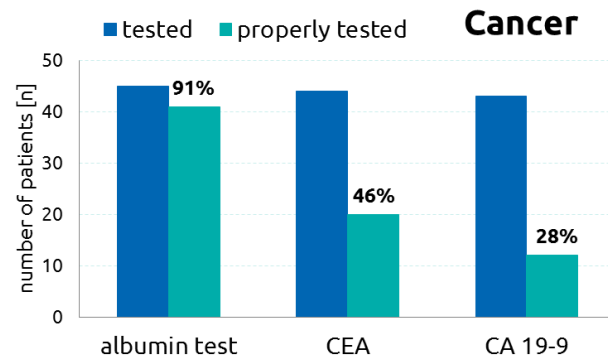
Under pathological conditions there are several modifications of conformation mobility of albumin, resulting in modified transport and altered detoxification characteristics.

A comparison of the structure of normal albumin and albumin saddled with cancer peptides or lipids reveals a dramatic extent of conformational changes.

Comparison with tumor markers

In a study investigating patients with diseases of the large bowel the albumin-functionality-test was compared to the tumor marker CEA (Carcinoembryonic antigen) and CA 19-9 [13].

In case of colorectal carcinoma the albumin-functionality-test showed clear advantage in sensitivity (91%) compared to conventional tumor markers CEA (46%) and CA 19-9 (28%).



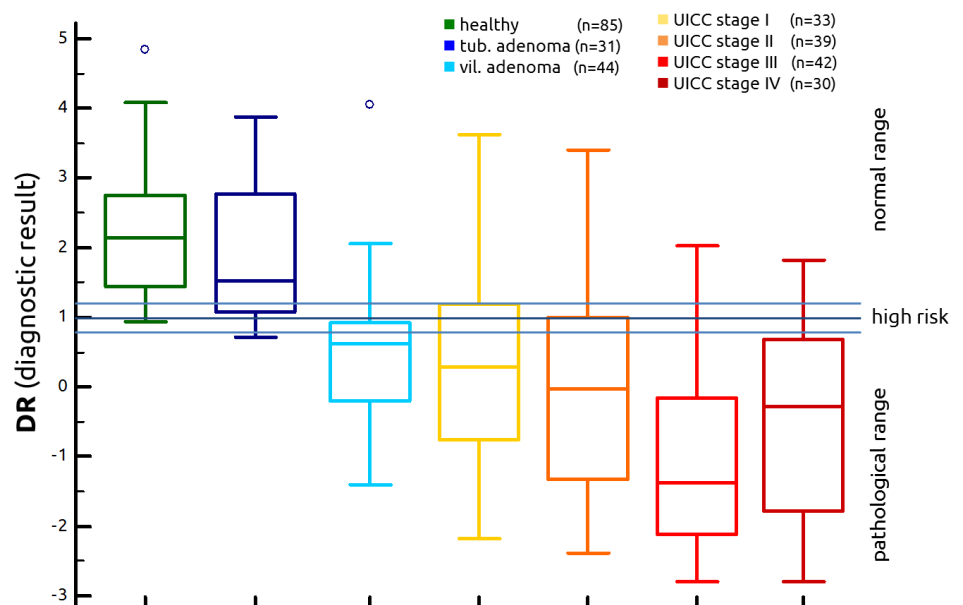
In case of adenoma and diverticulitis the albumin-functionality-test and the conventional tumor marker CEA and CA 19-9 are comparable in their specificity.

Patients with colorectal diseases

Studies with patients with malignant and benign colorectal diseases were conducted in Berlin, Bochum and Moscow.

A clear correlation between the DR value and the malignancy and the risk to malignant degeneration is recognizable.

Further investigations are on the way to enhance the number of cases to verify the first results.



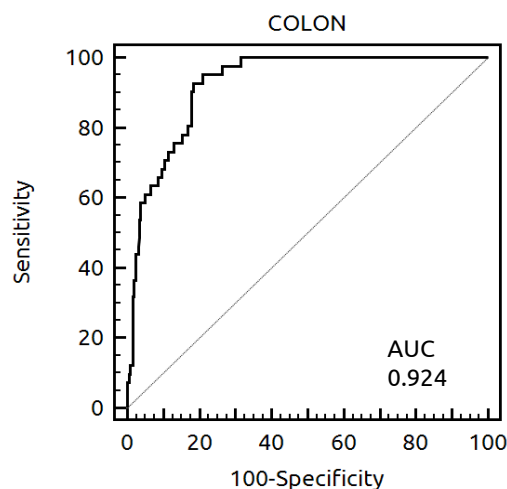
After a validation study regarding benign and malignant colorectal diseases, the albumin-functionality-test could be used for early detection of colorectal cancer in risk groups and for prolongation of colonoscopy intervals. [DRKS-ID of recent study – DRKS00025249]

Experimental results - cancer

First results for determination of cancer location

In a proof of concept project we have developed algorithms for four cancer types (colon, pancreas, prostate and mamma carcinoma).

For the classification of colon cancer the ROC curve is shown, consisting of 41 colon cancer cases and 322 patients with either pancreas, prostate or mamma carcinoma, as well as patients with benign colorectal diseases and healthy controls.



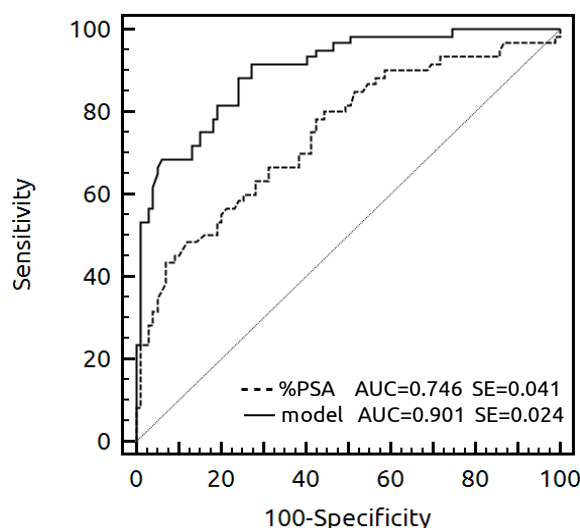
First analyses of biophysical parameters achieved from EPR spectra have shown that a discrimination of different tumor localizations is possible.

Further investigations with larger groups and more cancer localizations are on the way to verify these first results.

[DRKS-ID of recent study – DRKS00025249]

Albumin-functionality-test improves clinical diagnosis of prostate cancer

A comparison of ROC analysis of free-to-total PSA ratio (%PSA) and a parameter combination of %PSA, total PSA, albumin concentration and DR (model) of 60 prostate cancer patients and 99 age-matched healthy controls is shown.



The Study was conducted with the Department of Oncology and Cancer Epidemiology University Hospital Lund, Sweden (unpublished data).

It is possible to increase the efficiency of prostate carcinoma diagnosis without invasive method by a parameter combination of PSA related values and the albumin-functionality-test.

All values could be ascertained from the same blood sample.

Rationale of recent study (DRKS-ID – DRKS00025249)

The results presented above are validated in a current, extensive study.

The group of cancer patients is included in order to determine the sensitivity and specificity of the Albumin-functionality-test as function of cancer localization and to optimize the algorithms for the localization.

The correlation of the DR value with the tumor stage or tumor size is analyzed and compared with established tumor markers.

The additional use of the transport parameters to optimize the diagnostic performance is analyzed.

The group of benign diseases and cancer-healthy probands makes it possible, among other things, to check the model for the detection of prostate cancer.

In this multicenter, prospective study (in the area of Berlin-Brandenburg) 1500 test persons are examined with the albumin-functionality-test. The study population is divided into three different groups.

The group of patients with benign diseases should reflect the clinical application situation in which medical professionals need to differentiate between benign and malignant.

The group of healthy people, with the same age and gender structure as the other two groups, is required to determine the specificity, which is also necessary in the situation of relapse control in asymptomatic patients.

Healthy group (probands without cancer)

- 300 healthy probands
- Without any inflammatory diseases in the past four weeks
- Without cancer or cancer therapy in the last two years
- With the same age and gender distribution as in the other two groups

Cancer group

- 500 patients with therapy naive cancer
- without any cancer therapy/invasive diagnostic or inflammatory diseases in the past four weeks
- with one of the following cancer localization: breast, prostate, lung, colon, uterus, stomach, urinary bladder, kidney, pancreas or Non-Hodgkin-lymphoma (NHL)
- each cancer localization should include approximately 50 patients with the same distribution of staging

Group with benign diseases

- 700 patients with benign diseases of same organs as the cancer patients, reflecting the clinical situation in which the test is to be used (suspect for malignancy)
- At least 50 patients should be included for each organ

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