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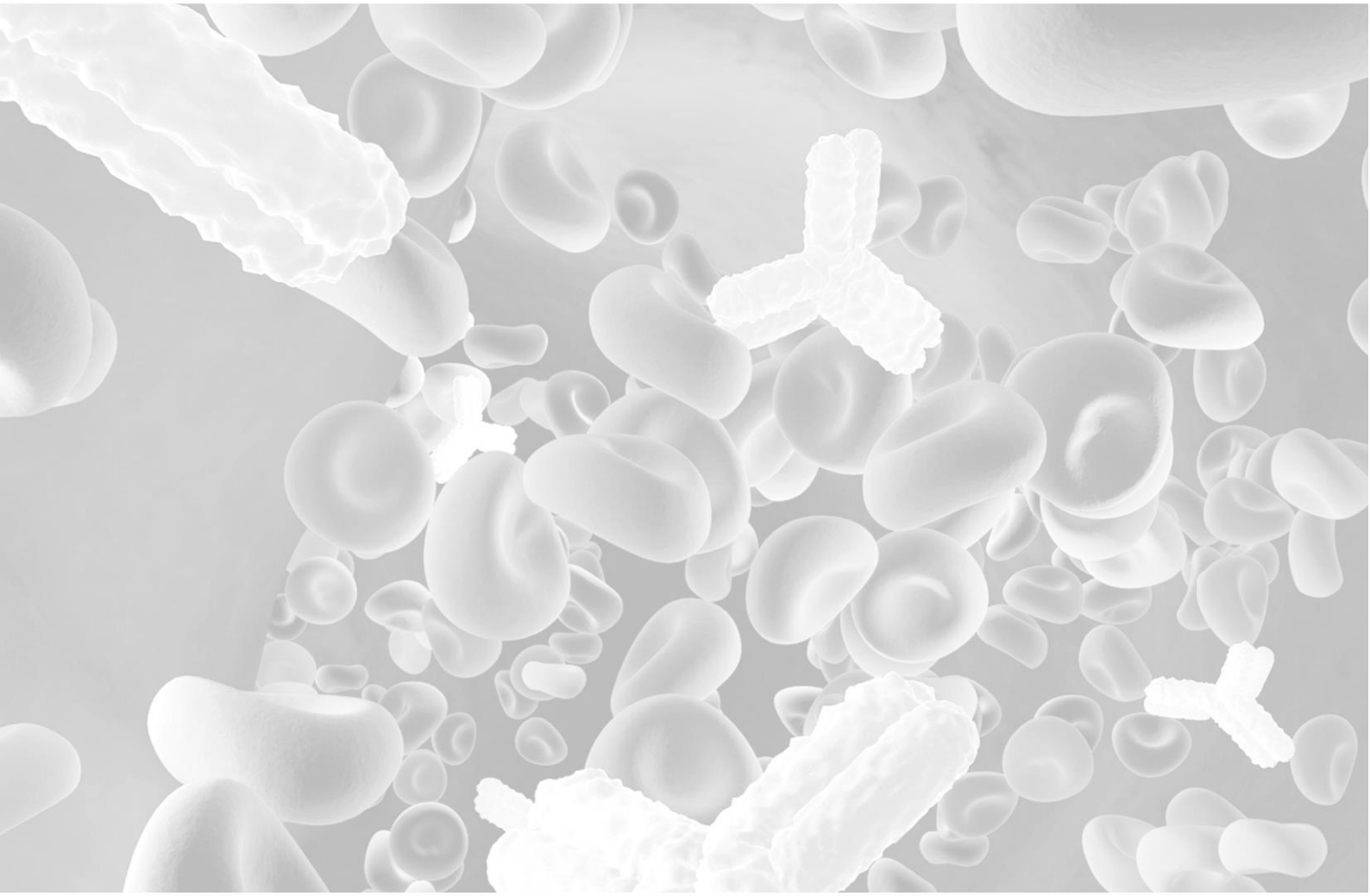


Antibody Index Software

User manual version 2.9

Cat.No.: SwAI01

TestLine
Clinical
Diagnostics

CONTENT

Antibody index - software

1 Introduction

Antibody index – a software intended for evaluation of specific antibody responses in the neuroborreliosis patient samples in the EIA Borrelia garinii VlsE IgG, EIA Borrelia garinii IgM and EIA Borrelia recombinant IgG, IgM kits of the TestLine Clinical Diagnostics s.r.o. company. The software has been developed by FASK, spol. s r.o. [1] Based on the inputs and requirements of TestLine Clinical Diagnostics s. r.o.

2 Installation

The software is installed from the installation medium (folder) by running the setup.exe file. The installation requires administrator rights. The installation requires the Microsoft NET Framework 4.0 package. If this interface has not been installed the automatic installation is started from the supplied medium prior to the installation of the Antibody index software (AI). The software installation itself does not require any configuration. The software is installed by default in the "C:\Program Files\FASK\Antibody index\" folder. The folder may be changed during the installation.

3 Starting the program

The program can be started from "Start/Programy/FASK/Antibody index/Antibody index.exe". When installed, the program is in a "Lite" mode when it is possible to create up to 2 calibration curves for each investigated class of antibodies.

To run the full version, first load the license file (*.ini).

The license file is sent to the users electronically based on a phone or e-mail purchase order sent to the TestLine Clinical Diagnostics s r.o.: order@testlinecd.com, phone: + 420 541 243 390, or to a responsible representative of TestLine at the following address: stoklaskova@testlinecd.com, phone: + 420 549 121 213.

4 Program license setup

Upon receiving the license file, save it in the computer, where the Antibody index program has been installed.

Start the Antibody index program.

Open the Application menu, click on License, search for the license file and confirm by clicking on Open. Now the program is fully licensed and you can create as many calibrations as required.

5 Working environment description

This paragraph describes the application main menu including the activities, which can be carried out.

Applications menu

- Database management (archiving, recovery)
- Language selection
- License management
- Closing the application

Patients menu

- Enables to enter new patient records
- Enables to display and edit the information on the patients

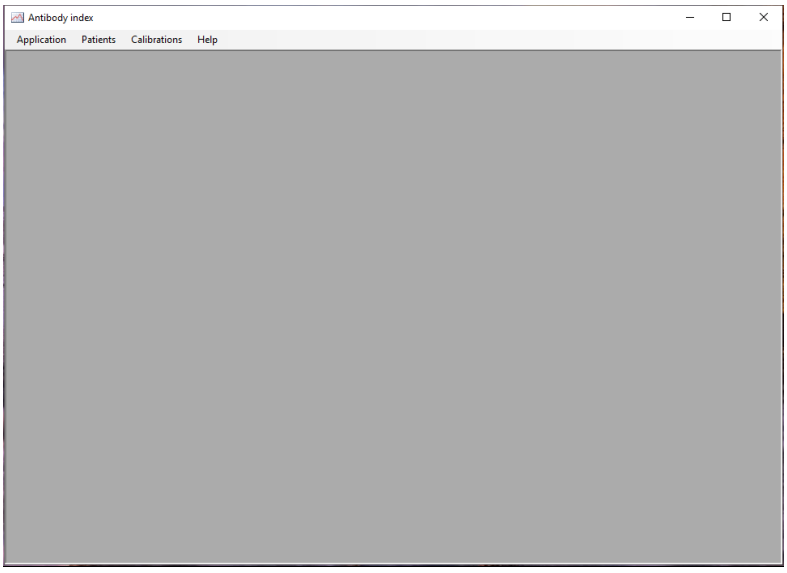
Calibrations menu

- Enables to display of the stored calibrations
- Enables to enter a new calibration

Help menu

- Help
- About the application
- Switching between the opened windows

Figure 1 AI Application



6 Material and input data needed to determine the antibody index

6.1 Calibration curve

The calibration curve is included in the SW and is available from Positive Control and CUT-OFF values provided for the EIA Borrelia garinii IgG, IgM and EIA Borrelia recombinant IgG, IgM kits.

6.2 Absorbance of the cerebrospinal fluids and sera

For the cerebrospinal fluid and the serum dilution procedure and EIA test performance, see the manuals for the EIA Borrelia garinii VlsE IgG, EIA Borrelia garinii IgM, and EIA Borrelia recombinant IgG, IgM kits.

6.3 Concentration values of albumin and total immunoglobulin (IgG and IgM) in the cerebrospinal fluid and the serum

Concentration value is collected nephelometrically within the biochemistry tests of the cerebrospinal fluid and the serum.

7 Value definition – AI calculation using Reiber's formula

The program uses the Reiber's method to calculate the antibody index. [2]

Using the concentration values of albumin and total immunoglobulin (IgG and IgM) in the cerebrospinal fluid (mg/l) and the serum (g/l), the program calculates ratios:

QAlb = concentration of albumin in the cerebrospinal fluid / concentration of albumin in the serum

QIgG = total concentration of immunoglobulin IgG in the cerebrospinal fluid / total concentration of immunoglobulin IgG in the serum

QIgM = total concentration of immunoglobulin IgM in the cerebrospinal fluid / total concentration of immunoglobulin IgM in the serum

AU - arbitrary units corresponding to the cerebrospinal fluid or serum absorbance values that are obtained from the calibration curves

QSpec (IgG) = arbitrary units in the cerebrospinal fluid of the IgG class / arbitrary units in the serum of the IgG class

QSpec (IgM) = arbitrary units in the cerebrospinal fluid of the IgM class / arbitrary units in the serum of the IgM class

The upper limit of the QAlb range is statistically defined by the equation for QLim:

$$QLim\ (IgG) = 0{,}93\ x\ \sqrt{QAlb^2 + (6\ x\ 10^{-6})}\ - 1{,}7\ x\ 10^{-3}$$

$$QLim\ (IgM) = 0{,}67\ x\ \sqrt{QAlb^2 + (120\ x\ 10^{-6})}\ - 7{,}1\ x\ 10^{-3}$$

AI – antibody index expressing the ratio of concentrations of the specific antibodies in the cerebrospinal fluid and the serum in relation to the brain capillary barrier and the total concentration of the immunoglobulin in the cerebrospinal fluid and the serum.

AI calculation for IgG:

If QLim(IgG) > QIgG, then AI(IgG) = QSpec(IgG) / QIgG

If QLim(IgG) < QIgG, then AI(IgG) = QSpec(IgG) / QLim(IgG)

AI calculation for IgM:

If QLim(IgM) > QIgM, then AI(IgM) = QSpec(IgM) / QIgM

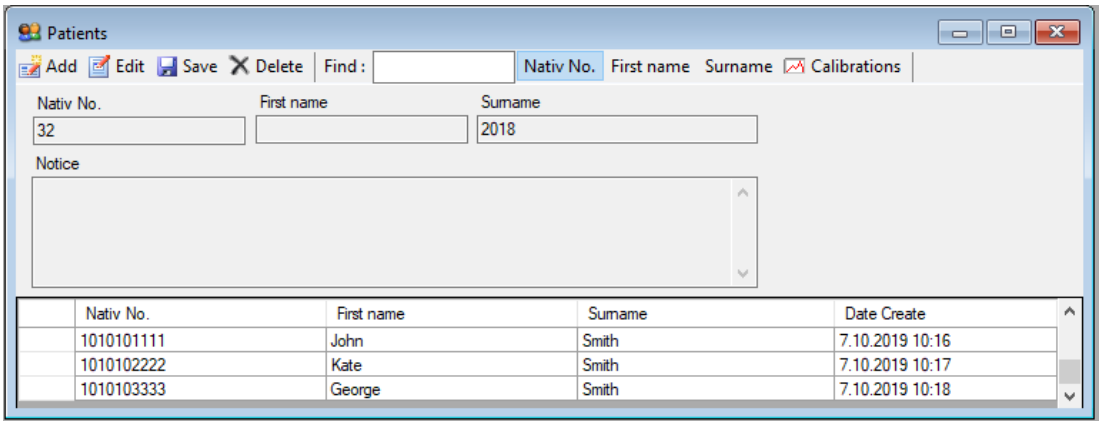
If QLim(IgM) < QIgM, then AI(IgM) = QSpec(IgM) / QLim(IgM)

8 Working procedure

8.1 Patients

Open Patients menu and click on *Show* to open the Patients window.

Figure 2 Patients window

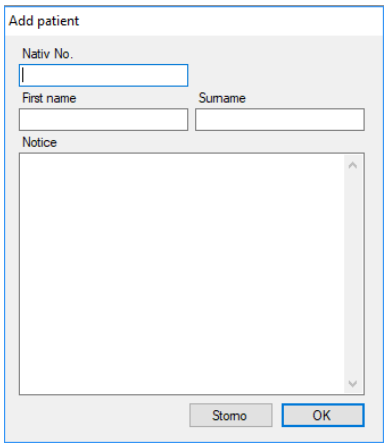


The list includes all the patients in the database.

The patients can be searched by the three criteria: the personal identification number, the name, and the surname Push the relevant button and enter the criterion in the *Find* box.

To enter the new patient data, click on the *Add* icon.

Figure 3 Adding the new patient



To modify the already entered patients, click on the *Edit* button.

The patient record may be deleted from the database by pushing the *Delete* button.

Upon modification of any inputs, the changes must be saved by pushing the *Save* button.

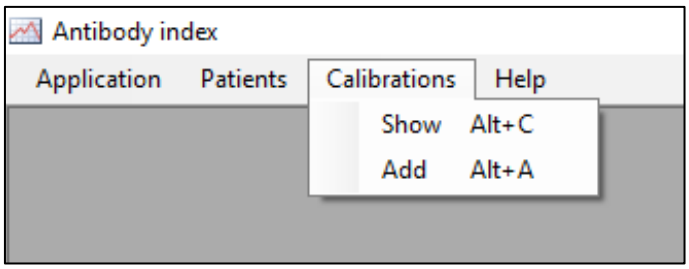
Patients can be sorted by birth number, first name, surname or date of record creation.

When you select a patient from the list, you can click on the *Calibration* button to search the calibrations that have been assigned to this patient by the date.

8.2 Calibration setup

Open the Calibration menu and select *Add*.

Figure 4 Setting up the new calibration



In the New calibration window, enter the date either using a keyboard or by clicking in the calendar. The Value deviation box allows to set an allowable deviation range in percent for individual points of the calibration (we recommend leaving 30) curve. Confirm by *OK*.

Figure 5 Setting up the new calibration

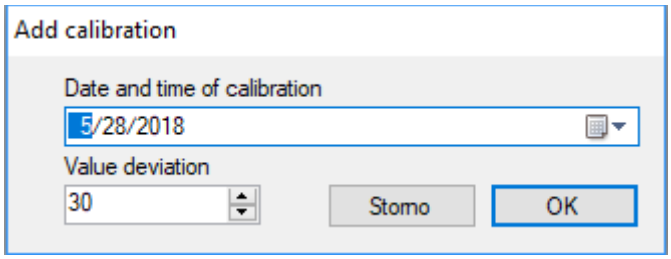
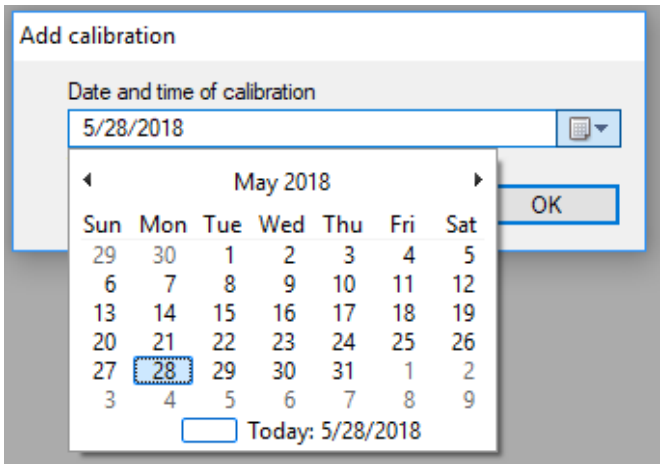


Figure 6 Selection of the calibration date



In the next window go to the IgG tab and create the calibration curve. Enter the absorbance values of relevant controls in the CUT-OFF and Positive Control fields.

Use the same procedure to enter the values to the IgM tab.

The recommended absorbance values of CUT-OFF for correct calculation of antibody index are in the range of 0.300-0.700 in the IgG class and in the range of 0.350-0.750 in the IgM class. If these values are exceeded, the Comment field will indicate that

the calibration curve values are outside the recommended validation criteria and the manufacturer recommends that the test be repeated.

Upon entering all the required data, push the *Solve* button to show the calibration curve. There is a possibility to switch between linear and logarithmic type of display (by checking off the Logarithmic box).

Figure 7 Calibration curve, empty values

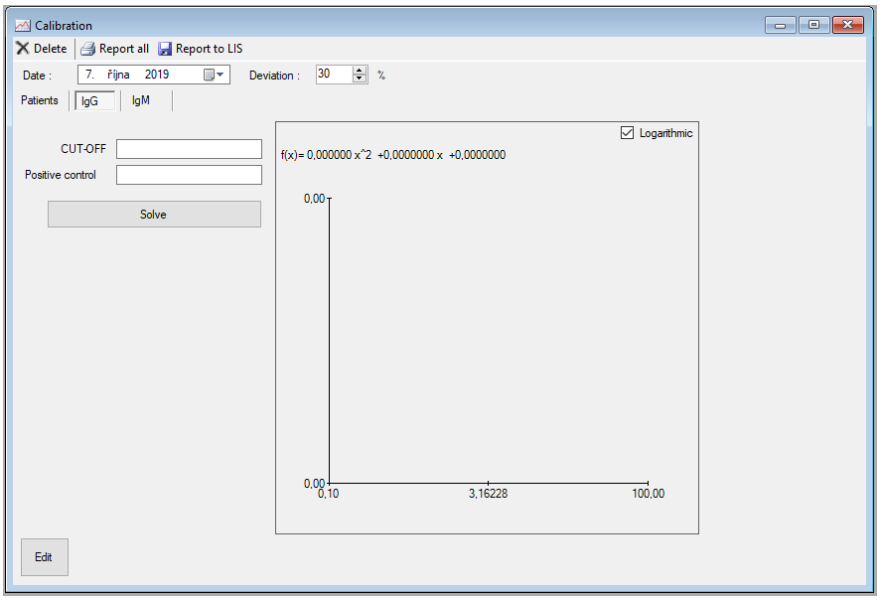
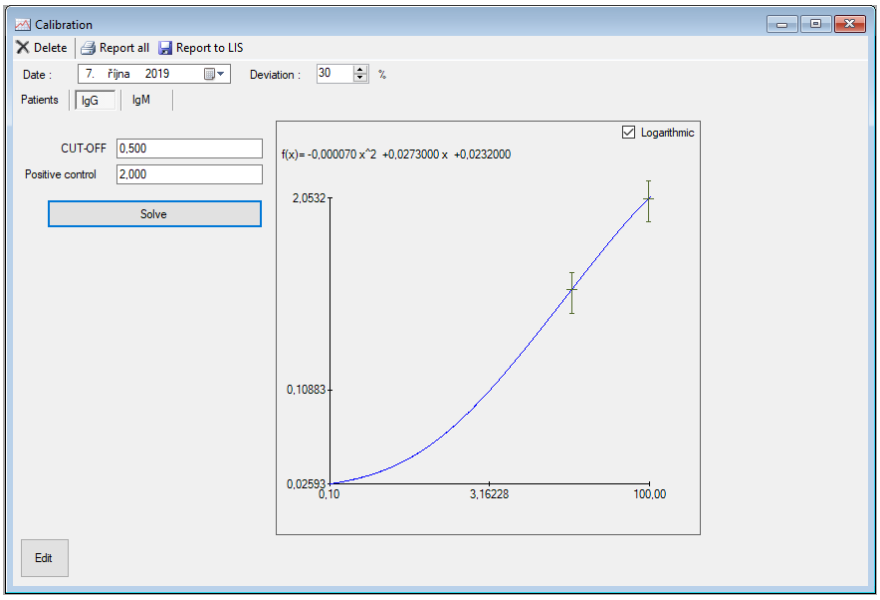


Figure 8 Calibration curve, filled-in and calculated values

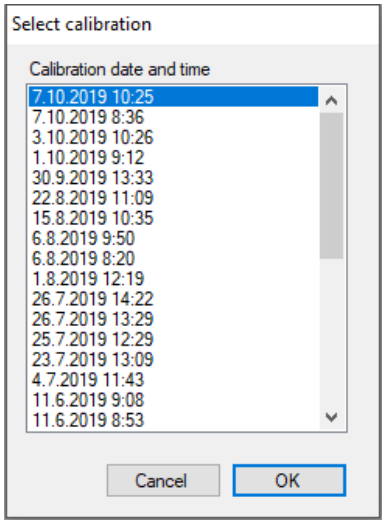


The calculated calibration curve graph includes the defined tolerance deviations from the specified values.

Note:

The program allows evaluation of the antibody index in both classes or just in one class of antibodies.

Figure 9 List of existing calibrations



Calibration → Show menu allows to select from the already created calibrations arranged by the date and time.

8.3 Inserting values and calculation

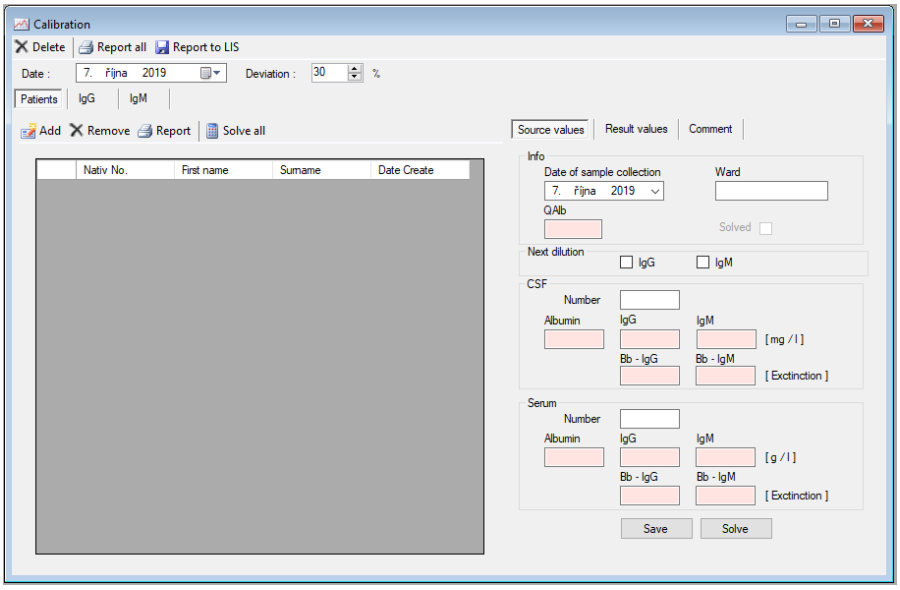
The Patients tab is used to calculate the output values of tested patients.

On the left, the list of tested patients is displayed, whereas two tabs are displayed on the right: *Source values* and *Result values*.

The input values are the measured values of the serum and the cerebrospinal fluid absorbance obtained by testing with the TestLine kits.

The program calculates the results automatically after saving the source values and clicking on the *Solve* button.

Figure 10 Calibration window



One calibration may include only one patient one time.

A new patient may be added to the calibration by clicking on the *Add* icon, which opens the Patients menu. Multiple patients can be added to a single calibration. The list includes all the patients in the database. The patients can be searched by the three criteria: the personal identification number, the name, and the surname Push the relevant button and enter the criterion in the *Find* box.

Select the required patient or patients from the list and confirm by clicking on the *OK* button.

Enter the input values in the Info, the Cerebrospinal fluid (CSF), and the Serum sections. You can switch from one section to another using the Tab key.

Enter the identifications or the values:

Date of sample collection – fill in or mark in the calendar.

Ward – identification of the department which is sending the sample.

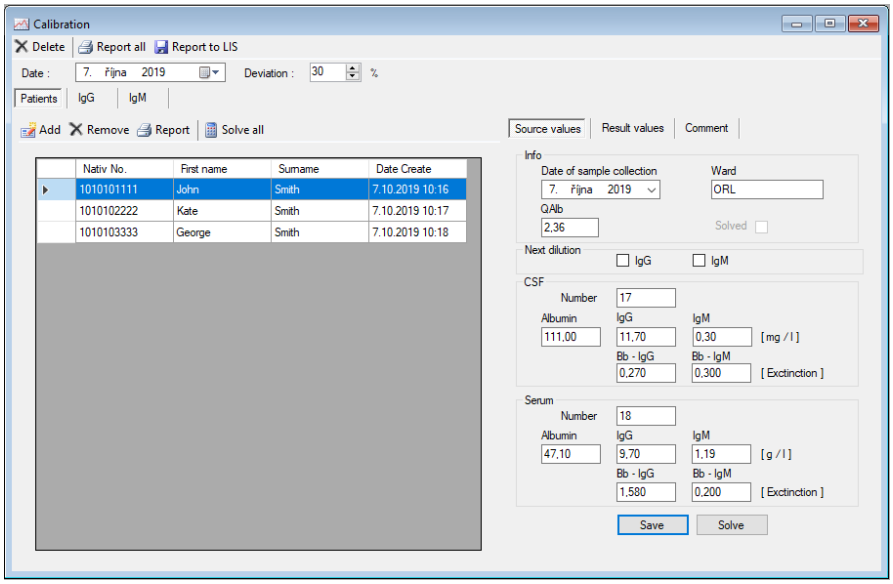
QAlb – enter the value if obtained from an external location. This box is not mandatory for the AI calculation. When you enter the value in the box, it is compared to the Qalb value calculated by the program (see below).

Number – sample identification (cerebrospinal fluid, serum).

Albumin, IgG, IgM – concentration values of albumin and total immunoglobulin (IgG and IgM) in the cerebrospinal fluid (mg/l) and the serum (g/l) (be aware of different concentration units for the cerebrospinal fluid and the serum).

Measured Extinction in the class of the IgG and IgM antibodies in the cerebrospinal fluid and serum samples.

Figure 11 Calibration window with patients



Upon entering the values, confirm their correctness using the *Save* button. Now the entered values are stored in the database and it is possible to enter values of another patient.

Upon clicking on the *Solve* button, a calculation of the results is carried out. The determination results including a literal assessment of the intrathecal synthesis are displayed in the *Result values*.

Partial calculations to determine an intrathecal synthesis of Qalb, AU, QIgG, QIgM, QSpec (IgG), QSpec (IgM), QLim (IgG), QLim (IgM) are specified in the chapter 7*. Value definition - calculation using Reiber's formula*.

The QAlb ratio value determines the brain capillary barrier. Verbal assessment (normal - minor disorder - moderate disorder - severe disorder may differ slightly from the assessment by the biochemical laboratory, since the criteria for each blood-brain barrier condition depend on the patient's age, among other things.

Table 1 Evaluation of the AI results for the IgG and IgM class antibodies

Sample	Value	Interpretation of the results
Sample X	AI < 1.4	negative AI, absence of intrathecal synthesis
Sample Y	1.4 ≤ AI < 1.5	borderline AI, the result should be evaluated in correlation with the clinical symptoms and antibody response in the serum
Sample Z	1.5 ≤ AI	positive AI, proven intrathecal synthesis

If the text "**negative result (AI cannot be calculated)**" is displayed in the result interpretation box then:

- the cerebrospinal fluid and/or serum absorbance of the samples is lower than the lowest point of the calibration curve, or
- the concentration value of total immunoglobulins IgG in the cerebrospinal fluid is lower than 9.26, or
- the concentration value of total immunoglobulins IgM in the cerebrospinal fluid is lower than 0.36.

In any case, the result of the intrathecal synthesis is considered negative.

If the text "**not determination (not tested)**" is displayed in the result interpretation box then the program could not determine the relevant value since the required input data was not supplied (e.g. Immunoglobulin concentrations, cerebrospinal fluid or serum absorbance values).

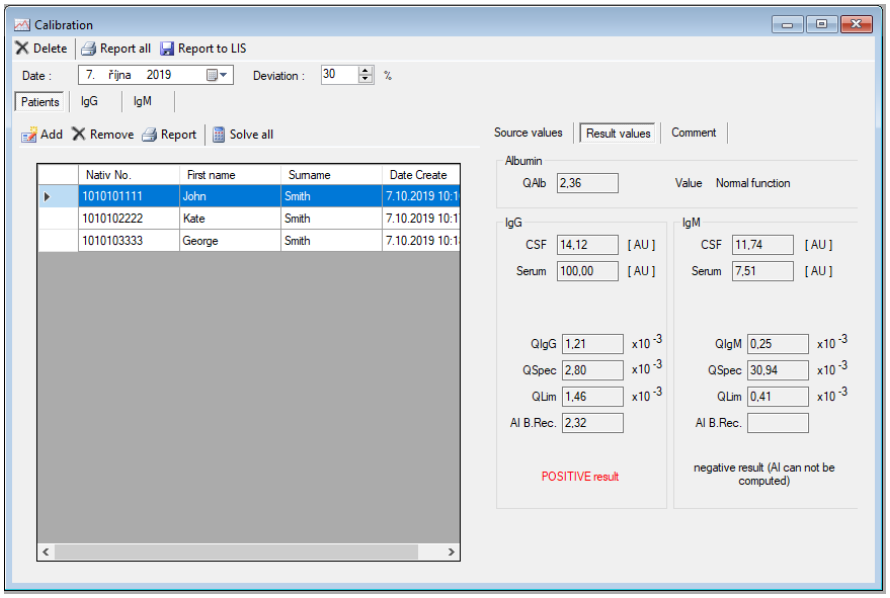
If the text "**next dilution required**" is displayed in the result interpretation box then both the cerebrospinal fluid and/or serum absorbance values exceed the absorbance of the highest point of the calibration curve whereas the AI result of this matched sample was negative. In this case, dilute the cerebrospinal fluid and serum samples using the sample diluent, see par. 14.5 in annex of the EIA kit manual and repeat the test according to the instruction 14.6 in the annex EIA kit manual. For new evaluation

of this matched sample, check the antibody class, within which the measurement is repeated in the Calibration window (“Next dilution” section). Then the program automatically recalculates the actual arbitrary units of the cerebrospinal fluid and serum samples.

Note:

The calculated QAlb value, i.e. the ratio of concentrations of albumin in the cerebrospinal fluid and the serum, is compared to the QAlb value entered in the QAlb box of the Input values window (see above). In case that the calculated and entered values differ by more than 0.1, a red exclamation mark is displayed at the result. We also recommend checking if the albumin concentration values of the cerebrospinal fluid and the serum were entered correctly.

Figure 12 Calibration window with results



If the input data need to be repaired go back to the Input values window and change the entered values.

It is possible to add or modify data of several patients at once. Gradually enter the values for individual patients. Select a new patient using the *Add* button. Before adding a new patient, always confirm the entered data by pushing the *Save* button.

Finally, confirm the calculation of all the tested patients within the current calibration by pushing the *Solve all* button.

8.4 Output reports

The program allows connect from LIS and display two output reports.

- For physicians: the output report contains detailed information on one patient and his/her results.
- For lab: the output report contains information on all the patients and their results from one calibration.

Both reports include the results and the data entered in the program:

- patient's name
- personal identification number
- identification of the department, which is sending the sample
- cerebrospinal fluid and serum sample number
- sampling date
- concentration values of albumin and immunoglobulin (IgG and IgM) in the cerebrospinal fluid and the serum
- absorbance values of the specific antibodies in the cerebrospinal fluid and the serum in both antibody classes
- calculated and arbitrary units in the cerebrospinal fluid and the serum in both antibody classes
- calculated QAlb value
- final value of the antibody index with colour coding of the positive, limit, and negative results

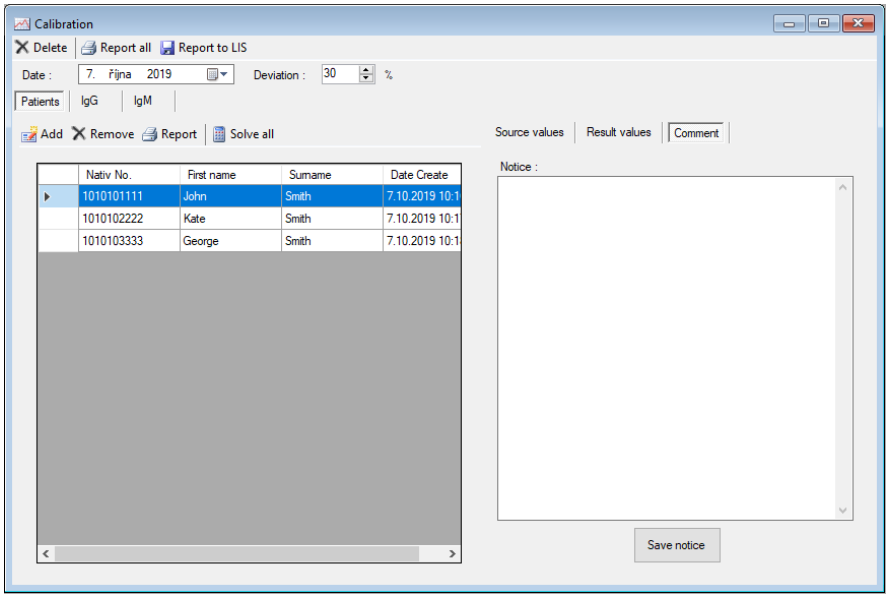
The report for the physician contains an additional literal assessment of the intrathecal synthesis

The data of the output report are exported in the HTML format and formatted for the A4 page size printing.

Adding notes

A note for the physician may be attached in the Comment window of the output report. The note is saved by pushing the *Save notice* button. The text is displayed in the output report for physician**.** If the values of controls are outside the recommended validation criteria, the recommendation for retesting will also be displayed in the output for the physician.

Figure 13 Calibration window with a note



Output report for physicians

This report shows a comprehensive record for physicians.

The output report can be displayed by selecting the required patients in the list and clicking on the *Report* button.

The output report is displayed in the default HTML browser.

The text entered in the *Comment* window is shown in the note under the table.

Then the page can be saved or printed.

Figure 14: Example – Report for physicians – the patient sample suitable for print

Intrathecal synthesis - Borrelia IgG & IgM (Antibody index)

Probe

Patient	Smith George	Serum No.	20
Native No.	1010103333	CSF No.	19
Ward	KICH	Sample collection	7.10.2019

Clinical chemistry

	Albumin	IgG	IgM	Unit
Serum	54,70	12,45	1,61	g/l
CSF	394,00	45,90	1,75	mg/l

Blood-brain barrier	
QAlb	7,20

QAlb Value	Blood-brain barrier classification
< 5	Normal function
5 - 10	Mild disfunction
10 - 15	Moderate disfunction
> 15	Severe disfunction

Specific antibodies

	Extinction		Arbitrary units	
	IgG	IgM	IgG	IgM
Serum	0,750	0,330	43,58	13,03
CSF	0,320	0,100	17,00	3,40

Results

			Intrathecal synthesis
Antibody index	AI (IgG)	2,095	POSITIVE result
	AI (IgM)	4,756	POSITIVE result

Notes

Figure 145 Example – Report all – all the patient samples tested in one calibration

Results AI - Borrelia IgG a IgM (7.10.2019)

Patient	Name	Native No.	Ward	CSF No.	Serum No.	Sample collection	mg/l			g/l			Extinction				Arbitrary units				QAlb * 10^{-3}	AI (IgG)	AI (IgM)
							CSF			Serum			CSF		Serum		CSF		Serum				
							Albumin	IgG	IgM	Albumin	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM			
Smith	George	1010103333	KICH	19	20	7.10.2019	394,00	45,90	1,75	54,70	12,45	1,61	0,320	0,100	0,750	0,330	17,00	3,40	43,58	13,03	7,20	2,09	4,76
Smith	John	1010101111	ORL	17	18	7.10.2019	111,00	11,70	0,30	47,10	9,70	1,19	0,270	0,300	1,580	0,200	14,12	11,74	100,00	7,51	2,36	2,32	-
Smith	Kate	1010102222	KDIN	15	16	7.10.2019	893,00	248,00	35,80	50,90	15,90	1,89	2,557	0,577	1,624	1,797	100,00	24,14	100,00	100,00	17,54	1,34	0,71

8.5 Working with a database

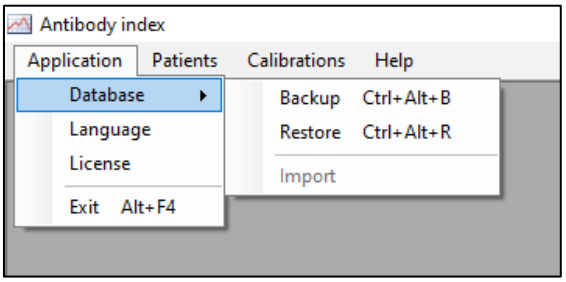
The program uses the MS Access database to store all the data created during the work with the application. The calibration curve values, the patient information, the measured values of the patients in the calibrations and their calculated results are stored.

To avoid data loss within a defined period, the application enables archiving this data and restoring the archived data.

Therefore it is necessary to backup (archive) data on a regular basis so that the data can be restored from the backup file whenever necessary.

The application does not backup the data automatically. This must be ensured by the user (PC administrator).

Figure 156 Application menu



8.5.1 Working database backup

The data backup can be selected in the main window of the program. Open the *Application* and *Database* menu.

Click on *Backup* to create a backup copy of the working database. Enter the file name and the location where the working database backup shall be stored. Click on the *OK* button to copy the database.

8.5.2 Restoring the working database from the backup file

The working database can be restored from the backup file.

Before starting the restoration, we recommend backing up the current working database (see the previous chapter “Working database backup”).

To restore the data, click on the *Restore* in the *Application, Database* menu and search for the stored backup file After the selected backup file is confirmed, all the windows opened in the application are closed and the working database is restored from the backup file. From that moment, the application works with the data restored from the backup file.

8.6 Troubleshooting

In case of any issues related to working with the program, contact the responsible person in the TestLine company at stoklaskova@testlinecd.com, phone: + 420 549 121 213 and provide a short description of the issue.

9 Literature

1. FASK, spol. s r.o., Brno, e-mail: info@fask.cz, www.fask.cz
2. TUMANI, H., NÖLKER, G., REIBER, H. Relevance of cerebrospinal fluid variables for early diagnosis of neuroborreliosis. Neurology 1995;**45**:1663-1670

CONTACT

Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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