



► Immunoblot Software version 2.0.

User manual

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IMMUNOBLOT SOFTWARE - USER MANUAL

Immunoblot Software is a software tool for evaluation of BLOT, BLOT-LINE and BlueBLOT-LINE tests provided by TestLine Clinical Diagnostics, s.r.o.

▶ SYSTEM REQUIREMENTS

Minimum system requirements:

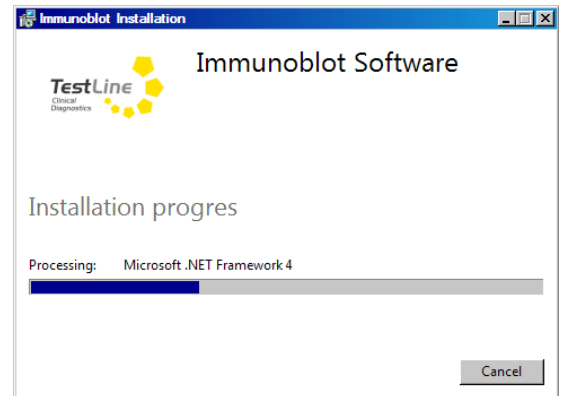
- ▶ Microsoft Windows XP SP3 or higher
- ▶ NET Framework version 4 or higher

Any scanner can be used as an input device. The scanner must be correctly installed and must be detectable by the operation system. Any printer can be used as an output device. The printer must be correctly installed and must be detectable by the operation system.

INSTALLATION

The Immunoblot Software is delivered as an *immunoblot_bundle.exe* distribution packet, which is configured to be installed automatically without a user's intervention. Administrator rights are required for the installation. You must accept the terms of license agreement to install the software correctly.

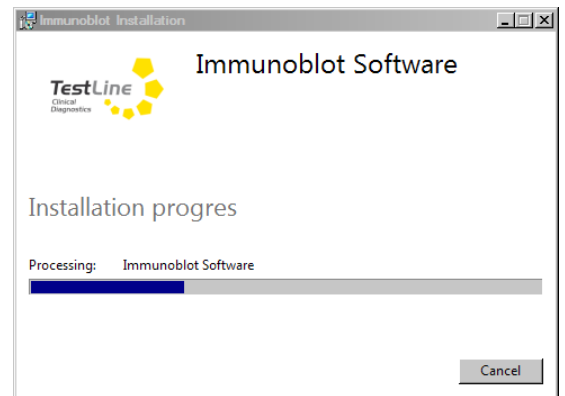
All prerequisites (such as .NET framework version 4 and previous) are already included in the installation package. The installation program automatically detects the presence of prerequisites on the computer, and if it does not find any prerequisites, it automatically installs them.



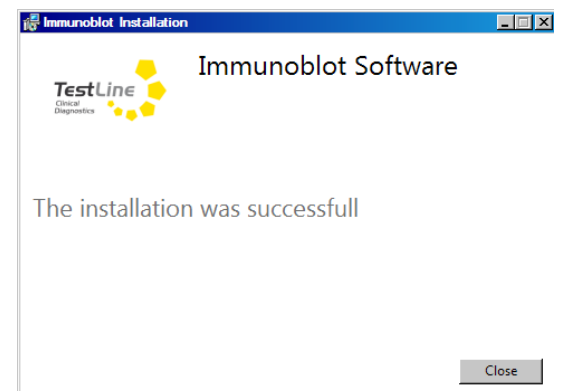
Then select a type of the installation:

- ▶ **Typical:** The Immunoblot Software without the user manual and kit definitions is installed into the **Program Files** folder.
- ▶ **Custom:** Select the installation path to the program. Kit definitions and the user manual can be installed as an option.
- ▶ **Full:** The Immunoblot Software, the user manual and kit definitions are installed into the **Program Files** folder.

The installation program also creates an icon on the **Desktop** and a shortcut in the **Start** menu.



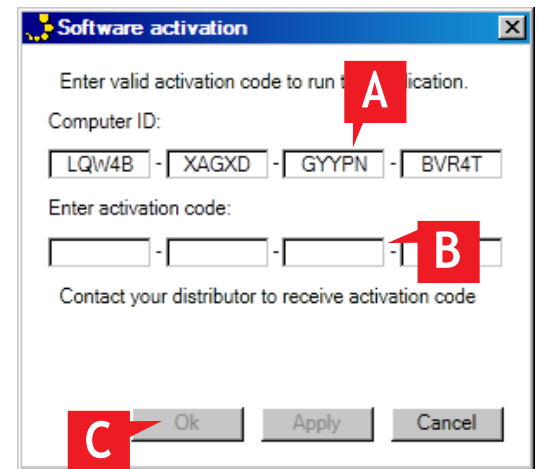
At the end you are informed that the installation was successful.



INITIALIZATION

When you first start the program, it is necessary to input the activation code. You cannot operate the program without it. If the program is installed by a service technician, he performs activation simultaneously with the installation. Otherwise proceed as follows:

- Make sure you are logged into Windows as a user with ADMINISTRATOR rights – then entered activation code is valid for all users of the computer.
- Start Immunoblot software.
- Contact TestLine service (email, phone) and give him twenty-character ID code of the computer (A).
- Service technician generates a twenty-character activation code and sends/dictates it to you. Put this code into activation code field (B).
- Click OK (C) and Immunoblot software starts.



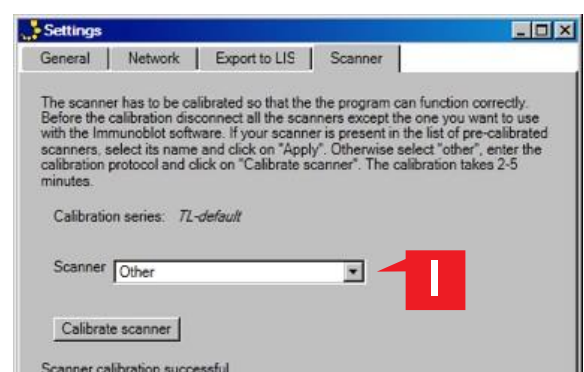
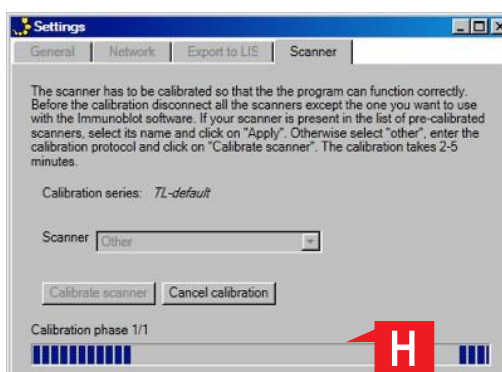
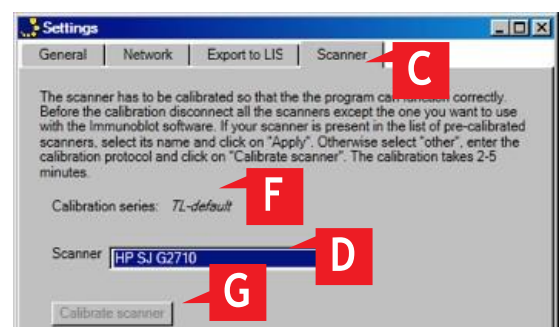
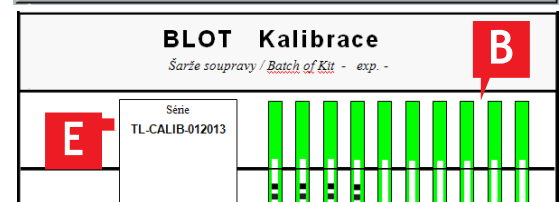
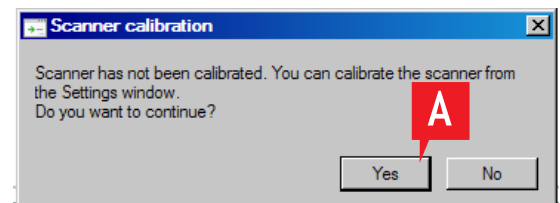
SCANNER CALIBRATION

To assign correct intensity values to each line, you must calibrate the scanner, preferably immediately after the installation of the software. If a scanner you are trying to use has not been calibrated, you will be informed about it (A). If you confirm it by clicking on Yes, the program will execute the scanning and evaluation, but the results of the analysis may not be correct.

Scanner calibration does not apply to BlueBLOT-LINE kits where the calibration scale is already part of the supplied scanner.

The calibration is done using a special calibration protocol supplied with the program. The calibration procedure goes as follows:

- Insert the calibration protocol (B) into the scanner.
- Open the **Scanner (C)** in the **Settings**.
- If your scanner is present in the list of pre-calibrated scanners (D), select its name and click on *Apply*. Otherwise select other, enter the calibration protocol and click on *Calibrate scanner*.
- Check whether the calibration series is marked the same in the program (E) as in the calibration protocol (F). If the marks differ, contact your supplier to receive a new calibration protocol.
- Make sure only the scanner you want to calibrate is connected to the computer and click on the *Calibrate scanner* button (G).
- The calibration can take several minutes depending on a type of the scanner (H). After finishing the calibration the program informs you about the result (I). If the calibration fails, the scanner you used is not fit for evaluating BLOT protocols. In such case, contact your supplier to recommend/provide another scanner.

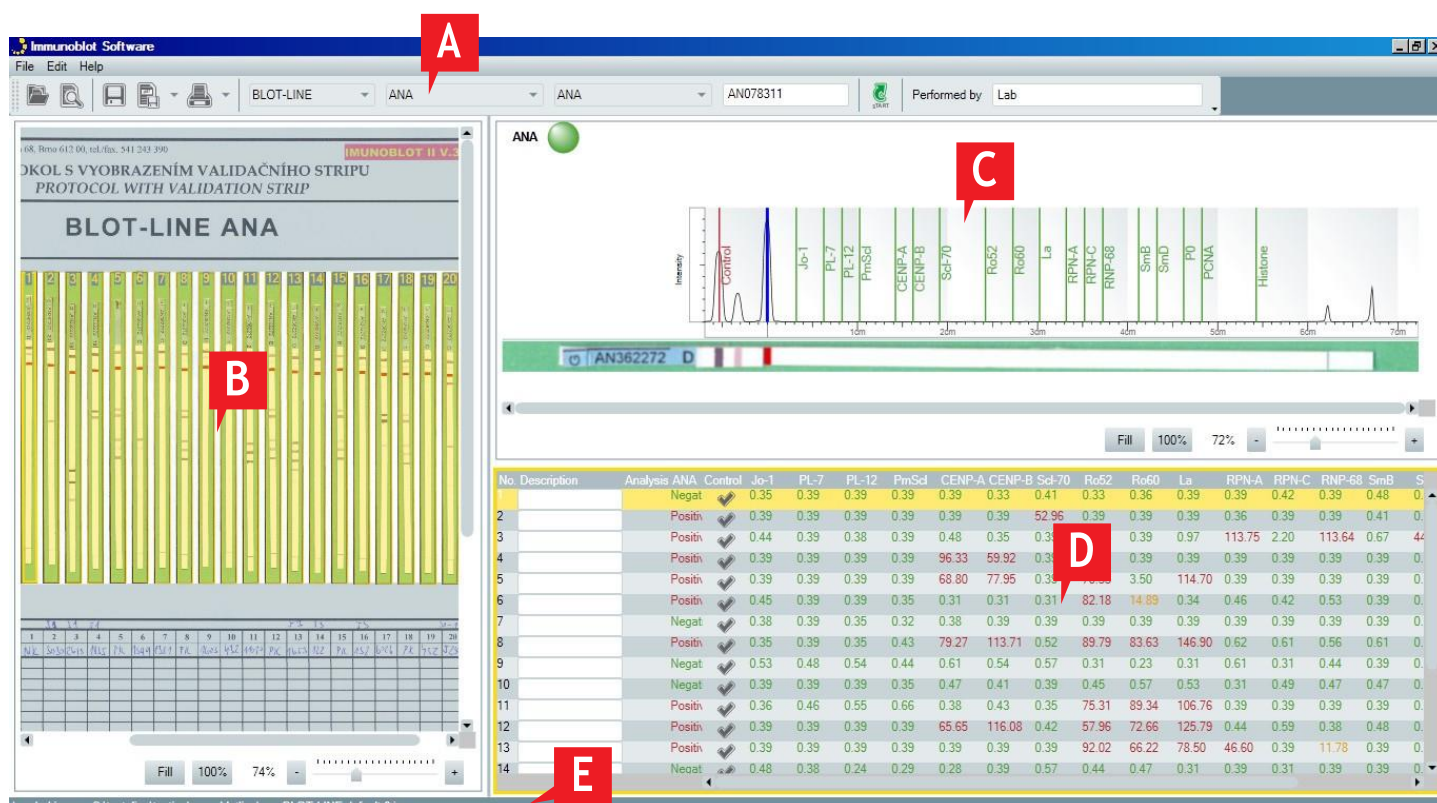


IMMUNOBLOT SOFTWARE INTERFACE

The Immunoblot Software interface consists of a single window divided into five fields:

- A) Tool bar and Menu
- B) Scan field
- C) Analysis field
- D) Evaluation table
- E) Low bar

Each field in the image is assigned a description label:



TOOL BAR AND MENU

Tool bar and Menu are used to manage following steps of the evaluation process:

1. Opening the image/Scanning the protocol/the clamp
2. Saving the scan
3. Selecting a type of the protocol, the test and the lot
4. Running the analysis
5. Printing the Results document and saving it as PDF

SCAN FIELD

It shows an opened or scanned protocol. You can use it for visual check-up of the protocol as well as for selecting particular strips after completion of the analysis. The field also allows you to adjust the zoom level.

ANALYSIS FIELD

It shows details of individual strips and a colour intensity curve of specific antigenic bands. It allows you to handle the **Start line** of the strip, or to manually exclude antigenic bands from the evaluation (see **Band evaluation**). You can adjust the zoom level in this field in the same way as in the **Scan field**.

EVALUATION TABLE

It shows previews of analysed strips and their description, the intensity (%) and the evaluation of each antigenic band, and also a general diagnostic classification of tested samples. The appearance and function of the evaluation table varies in each software mode. In case of the **BlueDiver** mode, it also includes the method **Planner (F)**.

The screenshot displays the 'Immunoblot Software' interface. On the left, a vertical strip of 22 immunoblots is shown. The main area features an intensity curve graph with various bands labeled: reference, control, igm, vise_bg, p83_ba, p41_bs, p39_ba, ospc_bs, ospc_bsp, ospc_bg, dbpa_p17_bg, anaplasma_p44, anaplasma_ompa, ebv_p18, and cutoff. Below the graph is a red 'F' icon. At the bottom, an evaluation table is visible.

No	Description	Analysis validity	conjugatecontrol	visebg	p83ba	p41bs	p39ba	ospcbs	ospcba	ospcbsp	ospcbg	dbpap17_bg	anap
3		↘	↘	2.75	0.78	1.96	1.57	0.39	0.78	3.14	5.49	1.18	1.18
7		↘	↘	3.53	1.18	1.57	1.57	1.57	1.57	2.35	3.92	1.57	3.14
8		↘	↘	2.35	2.75	1.57	1.57	0.78	2.35	1.18	1.96	2.35	0.78
9		↘	↘	3.92	0.78	1.57	1.57	1.18	1.18	2.35	0.39	0.78	2.35
10		↘	↘	2.75	1.96	2.75	3.53	1.57	0.78	1.57	1.18	1.96	1.96
11		↘	↘	4.71	1.57	1.18	1.57	2.35	1.96	1.57	0.78	1.18	1.18
12		↘	↘	1.96	1.57	1.18	2.35	0.78	1.18	0.78	1.57	2.75	1.18
13		↘	↘	4.71	2.75	1.96	2.75	0.78	1.18	3.14	7.06	2.75	5.49
14		↘	↘	4.31	1.96	1.18	2.35	2.35	1.18	2.35	3.53	1.18	4.31
15		↘	↘	5.88	1.18	0.78	1.96	0.39	0.78	1.18	4.31	0.78	1.57
16		↘	↘	2.35	1.18	3.14	2.35	1.96	2.35	1.57	1.18	2.75	1.96
17		↘	↘	3.14	1.18	1.57	1.18	2.35	4.31	4.31	6.67	1.57	1.96
19		↘	↘	3.14	2.35	0.78	0.78	0.78	0.78	0.78	0.39	0.39	1.96
20		↘	↘	2.75	0.78	0.39	1.57	0.39	0.39	1.18	1.57	1.18	1.18
21		↘	↘	2.35	0.78	1.96	2.75	1.18	1.18	1.57	3.53	1.57	2.35

LOW BAR

It shows a path to the file with the analysed scan. If the image has not been saved yet, the following caution is displayed: „Scan has not been saved“.

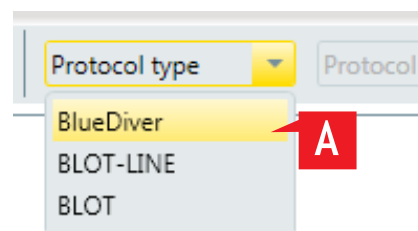
PROTOCOL PROCESSING STEP BY STEP

The chapter describes individual steps of processing the scanned protocol with strips, starting the analysis, saving and printing the **Results** document.

MODE SELECTION

The first step in processing a protocol is the mode selection. The following modes are available:

- ▶ **BLOT-LINE** – used to process strips type BLOT-LINE glued to the relevant TestLine protocols and scanned. Immunoblot BLOT-LINE mode allows you to select and evaluate one method on a protocol.
- ▶ **BLOT** – used to process strips type Western blot glued to the relevant TestLine protocols and scanned. The Immunoblot BLOT mode allows you to select and evaluate one method on a protocol.
- ▶ **BlueDiver** – used to process strips type BlueBLOT-LINE which do not require gluing and are directly inserted into the scanner along with a clamp. In the BlueDiver mode, multiple kits can be selected and simultaneously evaluated.



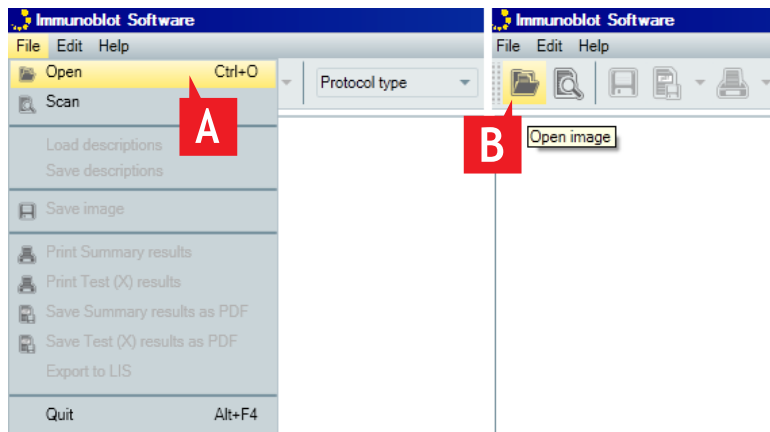
Mode selection is performed from the **Tool bar (A)**. This option affects the behavior of the program and therefore must be done at the very beginning of the evaluation process.

INPUT

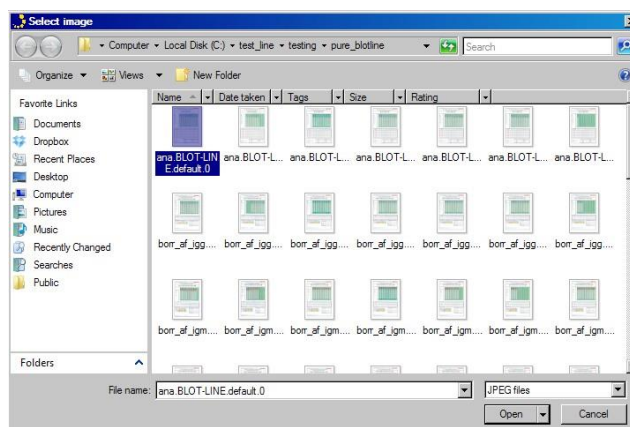
The input for the analysis is an image of a scanned protocol or a clamp. Both can be loaded from a file or directly scanned.

You can open the image from a file using one of the following options:

- A) Select **File – Open** in the **Menu**
- B) Click on the **Folder icon (open image)** in the **Tool bar**

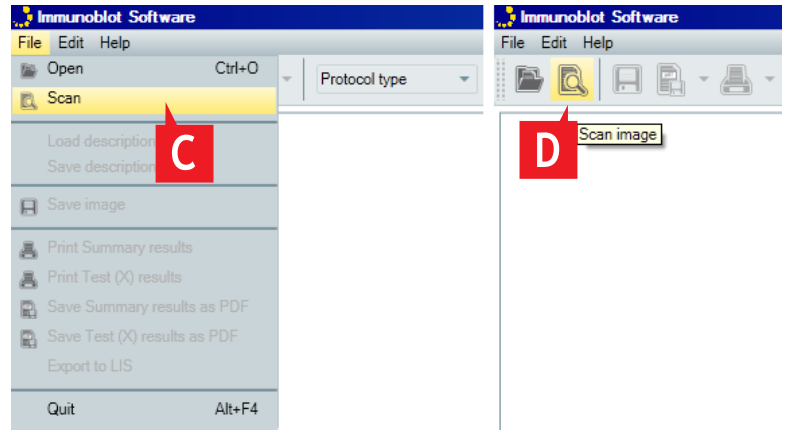


A dialog box allows you to specify a path to the file with the scanned protocol/clamp:

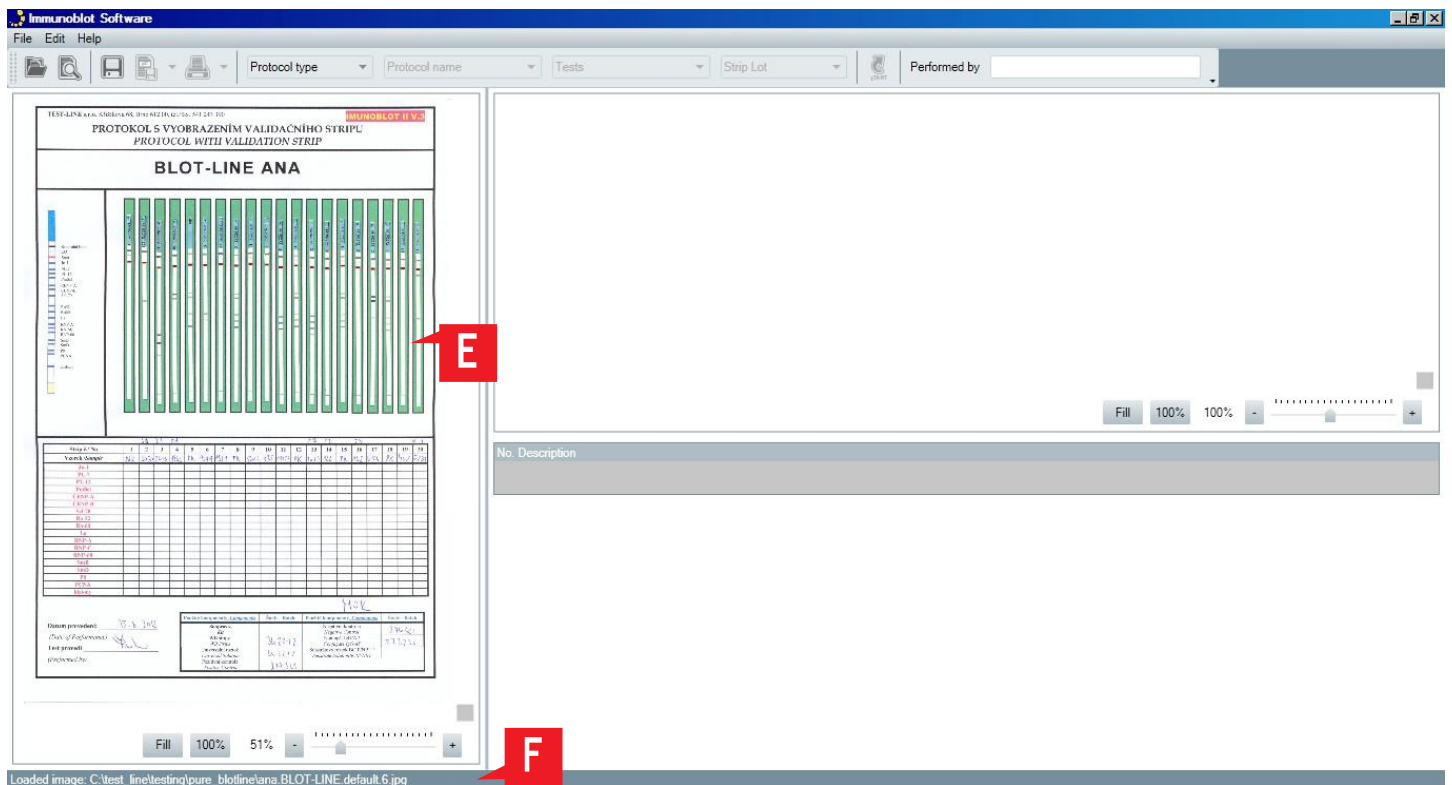


You can use one of the following options to direct scan:

- C) Select *File – Scan* in the **Menu**
- D) Click on the *Scan icon (scan image)* in the **Tool bar**

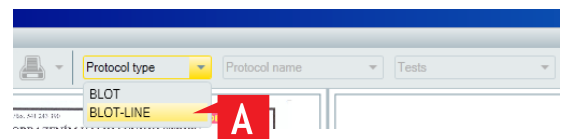


If the protocol/clamp has been scanned successfully, the image is displayed in the **Scan field (E)** along with the text “Scan has not been saved” in the **Low bar (F)**. When opening the image from a file, a path to the file is displayed in the **Low bar (F)**.

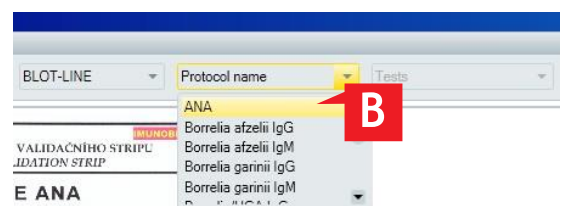


IMMUNOBLOT BLOT/BLOT-LINE MODE - SELECTING A TYPE OF THE PROTOCOL, THE TEST AND THE LOT

The program in the **Immunoblot mode** allows you to process two types of protocols. First, you need to specify whether you evaluate a protocol type BLOT or BLOT-LINE which allows drop-down menu **Protocol type (A)** in the **Toolbar**.

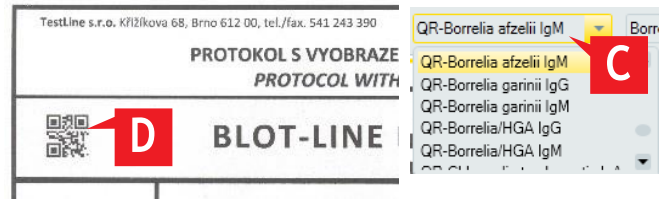


After selecting a protocol type you can select a kit type. This can be done in the drop-down menu **Protocol name (B)** in the **Tool bar**. You cannot select the kit type without first selecting the protocol type.



Since version 1.3 of the Immunoblot software, there are now available QR variants of protocols **(C)** in addition to classic BLOT-LINE protocols known from previous versions. Differences between classical and QR variant are as follows:

- 1) The performance of the **Conjugate control** is defined for QR variants of protocols (see chapter Details of the analysis – Conjugate control).
- 2) QR variant of protocol contains QR code (**D**) in the upper part of a protocol, which allows automatic kit detection.



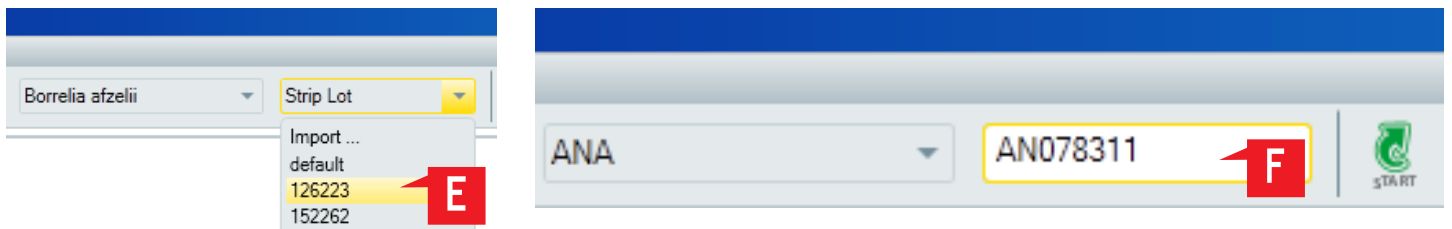
In the case of scanning / opening a protocol with a printed QR code, the program automatically chooses the **Protocol type (A)** and the **Kit type (B)**. If automatic loading for some reason (damaged QR code) fails, continue with the selection of the protocol type and the kit type as in previous versions of the program (**B**).

ATTENTION: In the case of a manual choice, after loading protocol with QR code (**D**) select always QR variant of the protocol (**C**)!!!

Strip lot selection depends on the type of a processed protocol (BLOT, BLOT-LINE).

In the case of the protocol type BLOT, the **Tool bar** displays drop-down menu **Strip lot (E)** with a selection of available lots in the system. If a strip lot is not available in the system, it can be downloaded from the website of the company TestLine or imported from CD.

As for the protocol type BLOT-LINE, the **Tool bar** displays a text box (**F**), in which it is possible to write the lot number. Lot identification is not mandatory for BLOT-LINE protocols.



BlueDiver MODE - PLANNER AND SETTING KITS

Since the **BlueDiver mode** allows the evaluation of several types of kits at one time, the upper bar with the choice of the type of kits is not active in this mode. Instead of it there is the **Planner** in the **Evaluation field**. This component allows you to assign types of kits to different groups of strips.

Planner

Position	Assay	Batch	Type	Patient ID
1				
2				
3	TA	TA150214	Sample	
4				
5				
6				
7	TA	TA150214	Sample	
8	TA	TA150214	Sample	
9	TA	TA150214	Sample	
10	TA	TA150214	Sample	
11	TA	TA150214	Sample	
12	TA	TA150214	Sample	
13	TA	TA150214	Sample	
14	TA	TA150214	Sample	

TA

TB

C

- Positive Control
- Negative Control
- Sample

Set

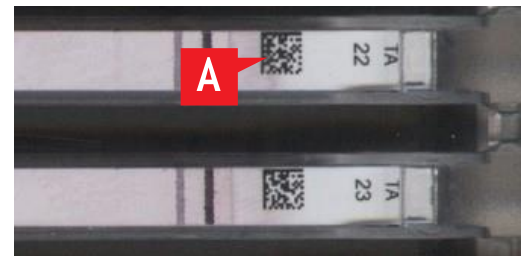
Clear

The **Planner** is divided into several fields:

- ▶ **Strip table (A)** – each row corresponds to one position in the clamp. In the table, it is possible to select one or more active rows to work with.
- ▶ **Kit selection (B)** – displays a list of available kits.
- ▶ **Type of sample selection (C)** – allows you to choose whether the strip at the appropriate position corresponds to the sample of a patient, positive or negative control.
- ▶ **Test selection (D)** – if the kit allows to perform more tests on a strip (differential diagnosis), the user can specify in this part of the **Planner**, which tests are to be analysed and which are not.

Process of planning:

The BlueBLOT-LINE strips contain **two-dimensional barcode (A)** (DataMatrix) which allows the program to automatically assign a kit to each strip. After scanning the clamp, the **Planner** will be prefilled. However, the user can change prefilled layout of strips and methods. This may be necessary, for example, when the identification code on the strip is illegible. When manually assigning a kit to a strip, proceed as follows:



Planner TA

Position	Assay	Batch	Type	Patient ID
1				
2				
3	TA	TA150214	Sample	
4				
5				
6				
7	TA	TA150214	Sample	
8	TA	TA150214	Sample	
9	TA	TA150214	Sample	
10	TA	TA150214	Sample	
11	TA	TA150214	Sample	
12	TA	TA150214	Sample	
13	TA	TA150214	Sample	
14	TA	TA150214	Sample	

TA

TB

Positive Control
 Negative Control
 Sample

Validity
 IgG Control
 Diagnose

Set

Clear

1. In the strip table, mark a strip by clicking or by dragging mark of a group (A).
2. In the list of available kits, choose a required kit (B).
3. Choose a type of a sample (C); eventually use checkboxes (D) to activate/deactivate individual tests within the chosen method.
4. Click on button **Set** (E).
5. Selection/settings can be cancelled by clicking on the **Delete** button (F).

Planner TA

Position	Assay	Batch	Type	Patient ID
1	TB		Sample	
2	TB		Sample	
3	TA	TA150214	Sample	
4				
5				
6				
7	TA	TA150214	Sample	
8	TA	TA150214	Sample	
9	TA	TA150214	Sample	
10	TA	TA150214	Sample	
11	TA	TA150214	Sample	
12	TA	TA150214	Sample	
13	TA	TA150214	Sample	
14	TA	TA150214	Sample	

TA
TB

- Positive Control
- Negative Control
- Sample

- Validity
- IgG Control
- Diagnose

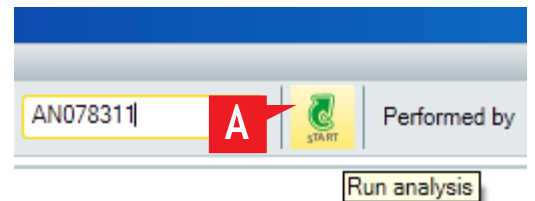
Set

Clear

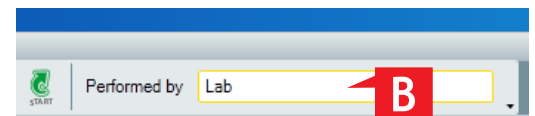
The image demonstrates a situation where kit TA was chosen for the first two strips while kit TB was chosen for the remaining strips. In case a kit is not specified for a strip, the strip will be ignored. In addition to automatic assignment of the kit type from the codes on strips, the program also recognizes a batch and automatically fills it in the **Planner (G)**.

▶ RUNNING THE ANALYSIS

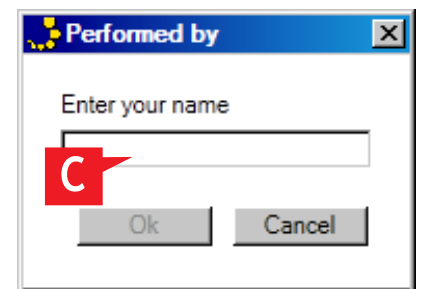
After selecting all the criteria you can start the protocol analysis by clicking on the Start icon **(A)** in the **Tool bar**. Starting the process of analysis is common for the **Immunoblot** mode and for the **BlueDiver** mode.



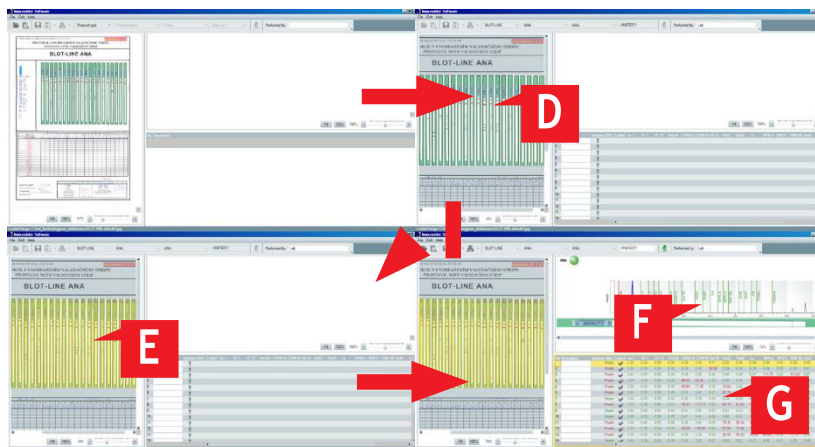
Then you must fill in the text field **Performed by (B)** in the **Tool bar**. The name you provided will also appear in the printed and saved documents.



The **Performed by** field must be filled in. The program demands you to enter your name **(C)** otherwise the analysis will not start.



The protocol analysis will take about 2-15 seconds depending on the capacity of the computer. The analysis consists of several steps which can be followed throughout the process. Firstly, a location in the protocol where the strips are fixed is set. The location is shown in sharp contours in the displayed protocol (**D**). Then individual strips are located and framed in yellow colour (**E**). Finally, the analysis is performed and the results are displayed in the **Analysis field (F)** as well as in the **Evaluation table (G)**.



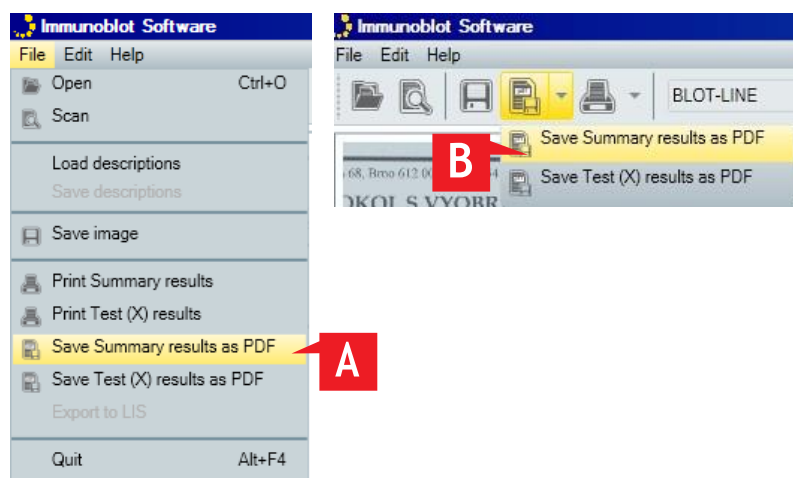
▶ PRINTING THE RESULTS AND SAVING THEM AS PDF

When the analysis is finished, you can view the results in detail (see **Details of the Analysis**).

You can choose between two types of evaluation documents. The **Summary results** protocol consists of evaluation of all antigen bands of all processed strips but it does not include some detailed information which can be found in the **Test (X) results** for each particular strip. Both types of documents can be directly printed, or they can be saved as PDF.

You can save the **Results** as PDF using one of the following options:

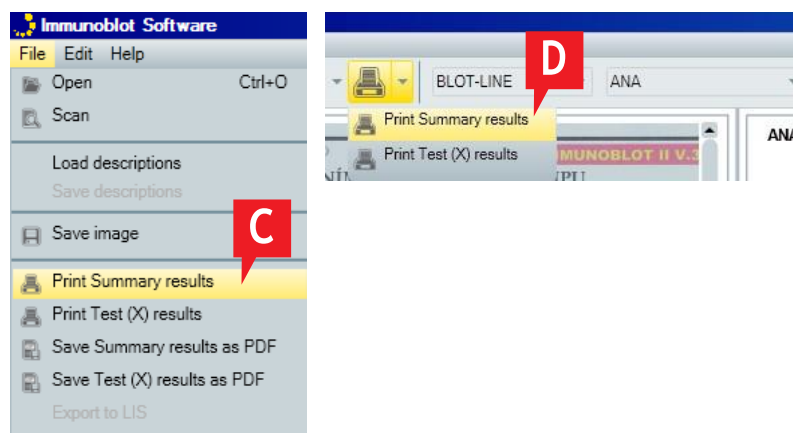
- A)** Select *File – Save Summary results as PDF / Save Test (X) results as PDF* in the **Menu**
- B)** Click on the icon *Save Results as PDF* in the **Tool bar**



A similar procedure is used for direct printing.

You can print the **Results** by selecting one of the following options:

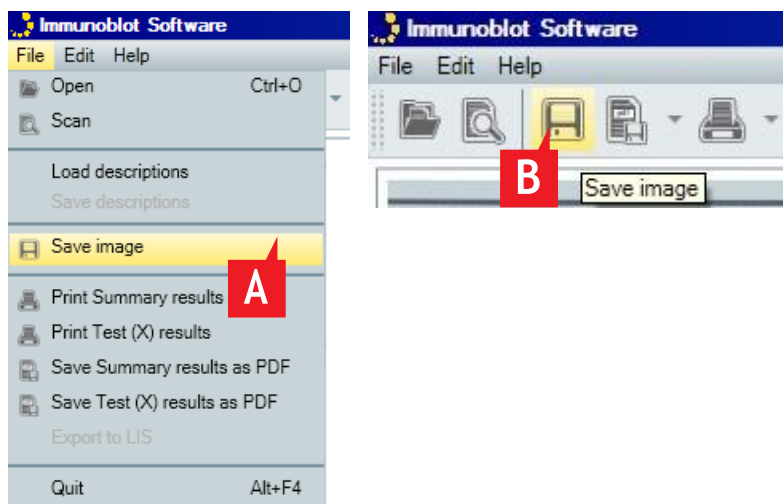
- C)** Select *File – Print Summary results / Print Test (X) Results* in the **Menu**
- D)** Click on the *Printer* icon in the **Tool bar**



SAVING THE SCANNED PROTOCOL/CLAMP

The scanned protocol/clamp can be saved for further archiving. You can do it in two ways:

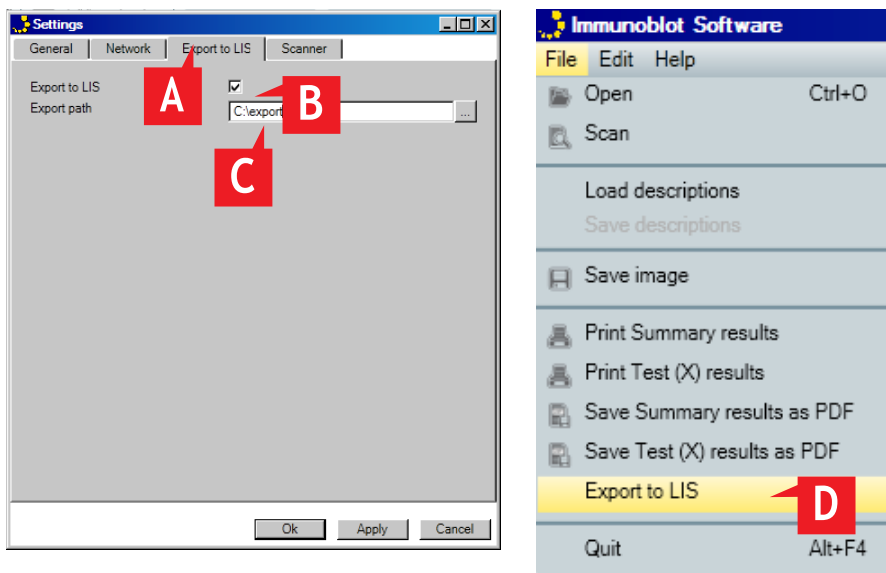
- A) Select *File/Save image* in the **Menu**
- B) Click on the *Save image* icon in the **Tool bar**



EXPORT TO THE LABORATORY INFORMATION SYSTEM

The Immunoblot software allows you to export results to the laboratory information system (LIS). After carrying out the analysis you can create an export XML file which can be imported to the LIS. To export to a XML format, you must set an export directory in the program (the LIS will then adopt the analysis results from the export directory, which must be set correctly in the LIS as well). You can set the export directory in the Immunoblot software settings using the **Export to LIS (A)**. First you must enable the export by ticking the option **(B)** and then you can specify the export directory **(C)**.

When the export directory is set, you can create an export XML file in the *File – Export to LIS* menu **(D)** after finishing the analysis.



ANALYSIS DETAILS

IMMUNOBLOT MODE - SELECTING ANALYSED STRIPS

When analyzing only some of the strips, you can select them in the **Scan field** by clicking on the yellow border of the strip (A, C). If the strip is:

1. highlighted by a full yellow frame (A) – the strip is marked and the result of its analysis is displayed in the **Evaluation table (B)**.
2. just outlined by a yellow frame (C) – the strip is not marked and the result of its analysis is not displayed in the **Evaluation table (B)**.

The unmarked strips will not be evaluated.

The screenshot shows the Immunoblot Software interface. On the left is the 'BLOT-LINE ANA' image with 20 lanes. On the right is the 'ANA' analysis field showing intensity curves for various markers. Below the analysis field is the 'Evaluation table' with columns for 'No.', 'Description', 'Analysis ANA', and various antigenic bands. A yellow border highlights the selected strip (No. 15) in the table, and a yellow frame highlights the corresponding strip in the blot image. A red callout 'E' points to the analysis field detail view for the selected strip.

No.	Description	Analysis ANA	Control	Jo-1	PL-7	PL-12	PmScd	CENP-A	CENP-B	ScI-70	Ro60	La	RPN-A	RPN-C	RNP-68	SmB	SmD	PCNA	Histone
6	Positiv	✔	0.39	0.39	0.39	0.39	68.80	77.95	0.39	0.39	0.39	114.70	0.39	0.39	0.39	0.39	0.39	0.39	0.39
7	Negativ	✔	0.45	0.39	0.39	0.35	0.31	0.31	0.31	0.31	0.39	0.39	0.34	0.46	0.42	0.53	0.39	0.39	0.35
10	Negativ	✔	0.38	0.39	0.39	0.32	0.38	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
13	Negativ	✔	0.39	0.39	0.39	0.35	0.47	0.41	0.39	0.45	0.57	0.53	0.31	0.49	0.47	0.47	0.32		
15	Positiv	✔	0.39	0.39	0.39	0.39	0.39	0.39	0.39	92.02	66.22	78.50	46.60	0.39	11.78	0.39	0.39		
16	Negativ	✔	0.39	0.39	0.39	0.39	75.08	133.41	0.39	44.19	85.81	156.34	0.39	0.39	0.39	0.39	0.39		

You can view a detail of any marked strip in the **Analysis field (E)** by clicking on the corresponding band in the table (D). Intensity curves of individual antigenic bands and their descriptions and positions are displayed for a selected strip. A strip with a currently displayed detail is highlighted in yellow colour in the **Evaluation table (D)** and its frame is shown in bold in the **Scan field (F)**. You can also change the currently displayed detail of the strip manually by using the UP (↑) and DOWN (↓) cursor keys.

IMMUNOBLOT MODE - EVALUATION TABLE

The evaluation of antigen bands is shown via numbers/symbols in the **Evaluation table** or graphically in the **Scan field**. Appearance of the **Evaluation table** in the Immunoblot mode and the BlueDiver mode is different. The BlueDiver mode will be described at the end of the chapter.

Explanation of labels, numbers and symbols in the **Evaluation table**:

No.	Description	Strip	Analysis	ANA	Control	Jo-1	PL-7	PL-12	PmScl	CENP-A	CENP
5		AN087311 16		Posit		0.06	0.32	0.09	0.09		108.95
6		AN087311 13		Positiv		1.04	0.10	0.90	2.16		0.34
10	Sample 2	AN087311 12		Positiv	✓	6.97	11.73	4.81	4.24	5.19	16.23
13	OK	AN087311 9		Positiv	✓	0.70	0.53	0.79	1.24	69.11	130.34
15		AN087311 B		Border	✓	0.38	3.12	0.60	7.77	1.50	0.09
16		AN087311 2		Positiv	✓	0.00	0.00	0.00	0.05	52.87	108.63
		AN087311 12		Positiv	✓	0.29	0.35	0.32	0.31	0.38	0.31

- A) *Number* – strips are arranged in the protocol from left to right.
- B) *Description* – a short text which can be used to mark the strip. The text will also appear in the **Results**. Descriptions of strips can be prepared in advance, they can be saved and loaded (see **Saving and loading descriptions**).
- C) *Strip* – a view of the strip for better orientation in the results. The view of the strip can be switched off (see **Program settings**).
- D) *Analysis* – can indicate possible problems in the course of the analysis.
- E) *Test name* – the interpretation of the test/test results.
- F) *Validity* – indicates whether the **Control line** has been located correctly in BLOT-LINE protocols.
- G) *Band evaluation* – percentage value of each antigenic band intensity. In some cases, this information is replaced by a symbol (see **Band evaluation**).

BAND EVALUATION

- A) *Negative bands* are displayed in **green** colour (percentage value of the band intensity).
- B) *Border bands* are displayed in **orange** colour (percentage value of the band intensity).
- C) *Positive bands* are displayed in **red** colour (percentage value of the band intensity).
- D) Band that does not match criteria of evaluation is marked with a **green X [X]**. It is usually a band that cannot be distinguished from background of the strip.
- E) Bands *manually excluded* from the test (e.g. dirt on the strip, scanner etc.) are assigned a **dash [-]**.
- F) Bands *considered suspicious* by the program are assigned an **exclamation mark [!]**. The program recommends you to check whether they have been correctly evaluated. If they have not been correctly evaluated, exclude them manually from the test.
- G) If the strip is mechanically damaged (it is torn or too short), all the bands are assigned a **[ø] symbol** and this strip cannot be evaluated.

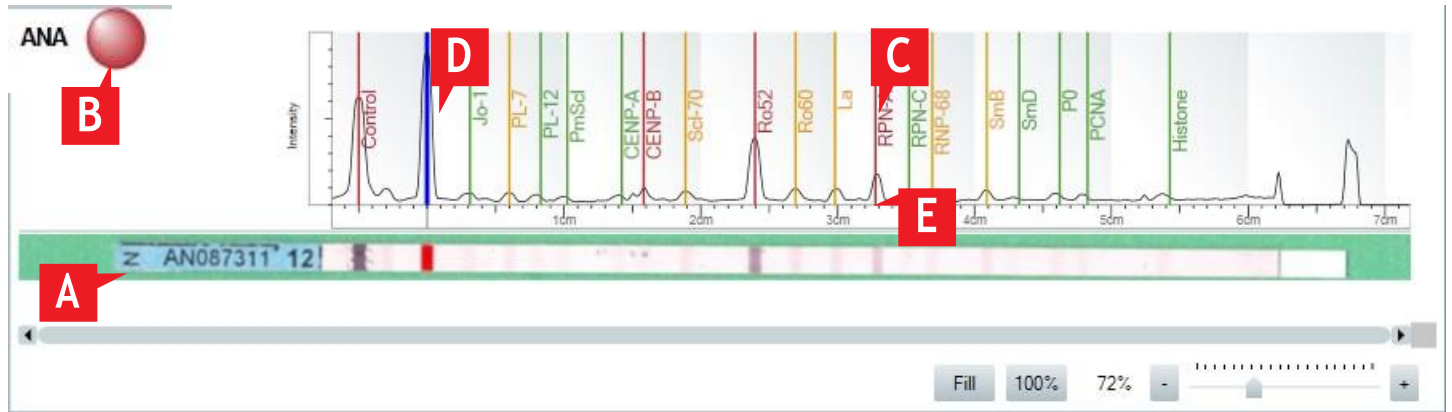
	PmScl	CENP	CENP-B	Scl-70	Ro52	Ro60
1	0.50	0.54	0.31	0.00	0.02	
0.1	56.30	109.94	0.42	5.13	109.14	
2.63	0.16	0.55	0.01	0.00	0.00	
4.49		18.31	13.30	63.57	15.70	
23.24	X	X	X	X	16.10	
1.23		0.65	1.58	0.71	1.32	
ø	ø	ø	ø	ø	ø	
0.3	0.1	0.24	0.20	0.42	0.00	
0.31	1.2	0.35	1.05	67.94	13.56	

In all cases listed above, you can view additional information concerning the evaluation by placing the mouse cursor above a given symbol. A small question mark (**H**) appears beside the cursor and the program displays a small text field near the cursor.

0.26	-	0.48	0.00	0.08	5.19	16.23	12.99	62.38	15.30
0.99	67.20	13.26	10.63	8.68	7.84	8.82	6.33	16.43	
ø	ø	ø	ø	ø	ø	ø	ø	ø	ø
69.11	130.34	2.66	105.01	111.78					

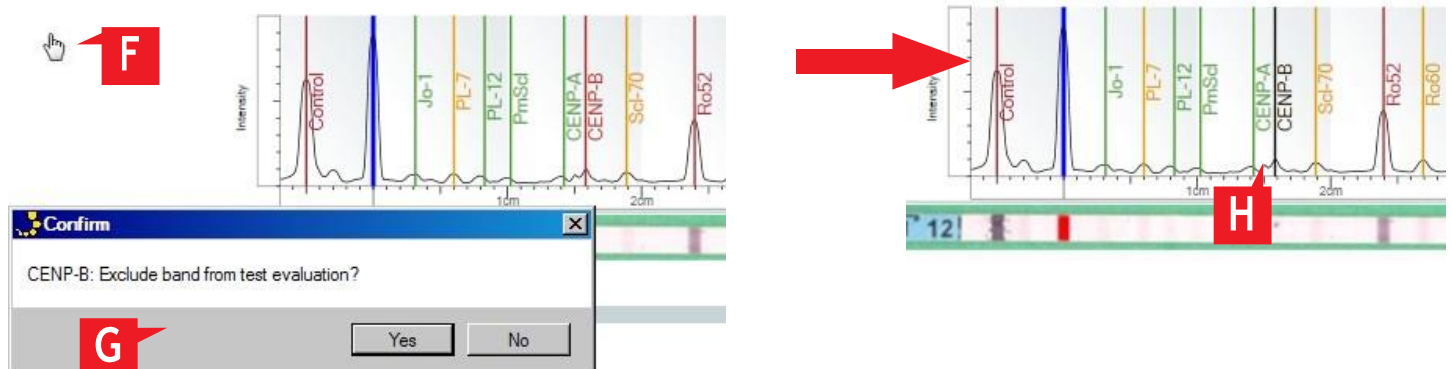
STRIP DETAIL

After finishing the analysis you can view a detail of a specific analysed strip in the **Analysis field**. The detailed view of the strip can look like this:

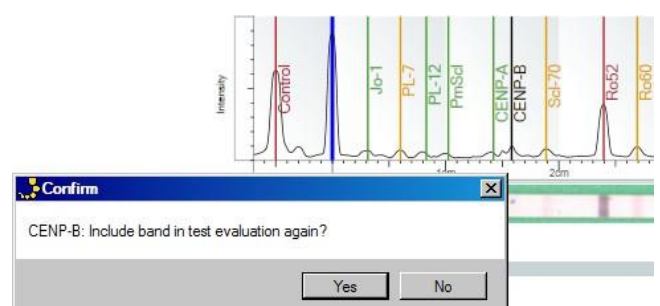


- A) *Strip image* – position on the strip corresponds to a display of individual bands in the intensity curve.
- B) *Interpretation of the test/tests results* – coloured circle/circles indicate diagnostic classification of the strip according to individual tests. **Red = positive result**, **orange = borderline result**, **green = negative result**.
- C) *Band view and evaluation* – **green = negative or unsatisfactory band**, **orange = borderline band**, **red = positive band**, **black = manually excluded band**.
- D) *Start line* – a line where an analytic area of the strip begins. Positions of all antigenic bands are related to this specific line.
- E) *Scale* – shows a position of each band in relation to the **Start line**.

The detailed view of the strip also allows you to manually include or exclude each band from the test. To exclude a band from the test you must place the mouse cursor above the given band in the detailed view of the strip. The cursor changes into a hand symbol (**F**) and after clicking on the band a dialog box will appear which allows you to exclude the band from the test (**G**). The excluded band will be displayed in black colour (**H**) and a dash [-] will be assigned to it in the **Evaluation table**. Excluding a band from the strip means a new evaluation of the whole strip will be performed and the interpretation of the test results may change.

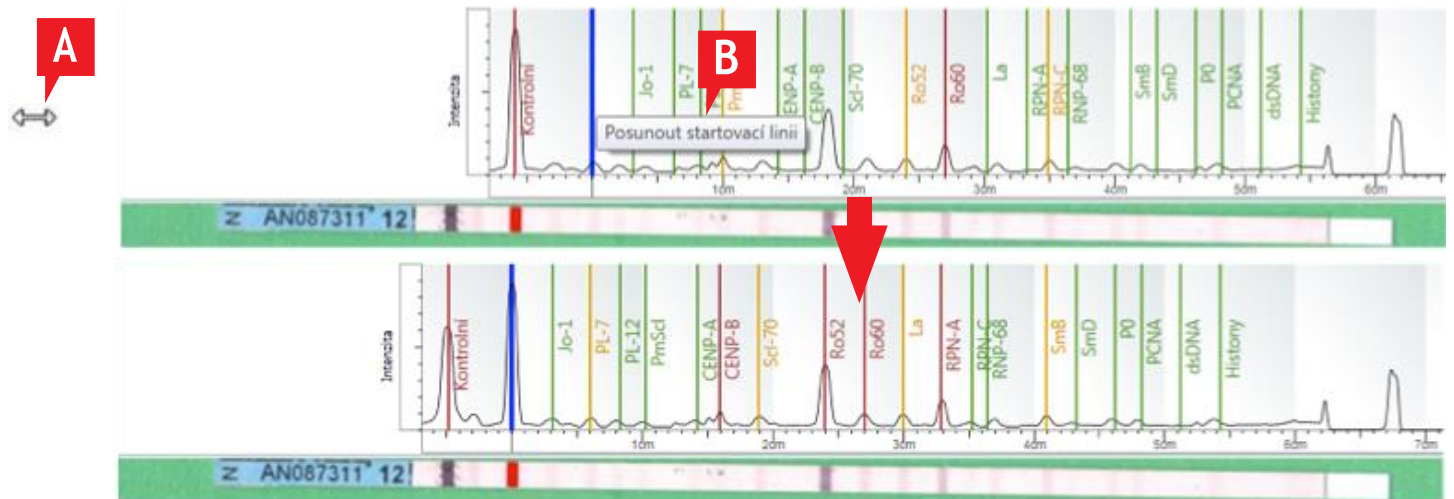


The excluded band can be included in the test again using the same procedure.



START LINE

The **Start line** and its correct location are vital for correct evaluation of the strip. The **Start line** is displayed in blue colour in the detailed view of the strip. If the band is not correctly located, you can correct its position manually. If the **Start line** is not found, the program informs you about the problem (see **Start line not found**).



Manual shift is possible after positioning the cursor on the **Start line**. The cursor changes into *horizontal arrows* (A) and a short instruction to move the band (B) appears. You can move the band to a new position by clicking on it and dragging it. After moving the **Start line** a new strip evaluation is performed.

By moving the **Start line** you accept responsibility for a potential change of the test evaluation. The change is indicated in the **Evaluation table** (C) and in the **Results** documents.

Positiv	✓	1.04	0.10	0.90	2.16
Positiv	✓	6.97	11.73	5.31	4.59
Positiv	✓	10.01	20.01	8.58	22.84

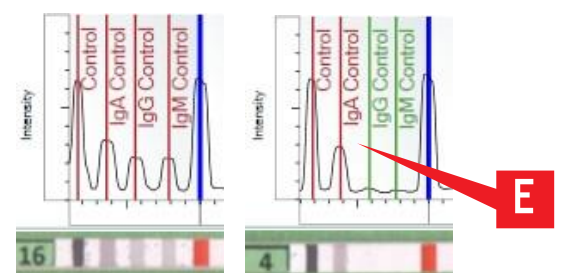
Start line position has been adjusted manually

CONJUGATE CONTROL

The analysis of the **Conjugate control** is available in version 1.3 and above, namely for QR variants of BLOT-LINE protocols (see chapter Basic protocol processing – Protocol, test and lot selection). If the user selects/loads this type of protocol, a new field will be added into the **Evaluation table** – **Conjugate control** (A).

No.	Description	Analysis ANA	Control	Conjugate control	Is 1
15		Negat	✓	✓	0
16		Borde	✓	✗	8

Strips with the **Conjugate control** contain, besides the **Control line**, also bands corresponding to relevant conjugates (IgG, IgA, IgM). These bands are indicated in the analysis in the same manner as the diagnostic bands (D), but just as the **Control line**, are not recorded in the **Evaluation table**. The outcome of the **Conjugate control** is shown as *valid* (B) or *invalid* (C).



CONTROL LINE

The **Control line** plays an important part in the BLOT-LINE protocols. Its intensity is crucial for correct determination of the intensity of all analysed antigenic bands. The **Control line** must be correctly located. An incorrect location is indicated by a cross (B) in the **Validity** column of the **Evaluation table**. Correctly located **Control line** is assigned a tick symbol (A).

Positiv	✓ (A)	10.01	20.01	8.58	22.84
Negativ	✗ (B)	0.04	0.10	0.40	0.21
Positiv	✓ (A)	0.70	0.53	0.79	1.24

BlueDiver MODE - EVALUATION TABLES

In the **BlueDiver mode**, the program allows evaluation of several kits simultaneously. Since the kits differ in the number and type of lines, it is no longer possible to summarize all the results into one table. The program solves this situation by using more tabs.

After the analysis several tabs appear next to the **Planner** in the **Evaluation table**. The number of the tabs corresponds to the number of used kits. The tabs are identified by kit abbreviations. A table in each tab displays all the strips which have been analysed by the kit.

No	Description	Analysis validity	conjugatecontrol	vlsebg	p83ba	p41bs
3		✓	✓	2.75	0.78	1.96
7		✓	✓	3.53	1.18	1.57
8		✓	✓	2.35	2.75	1.57
9		✓	✓	3.92	0.78	1.57
10		✓	✓	2.75	1.96	2.75
11		✓	✓	4.71	1.57	1.18
12		✓	✓	1.96	1.57	1.18

Essentials of all tables are same as in the **Immunoblot mode** (all marks, colours and symbols have the same meaning). If there has not been a kit assigned to some strips in the **Planner**, the program ignores those strips and they will not be found in any of the tables. The program will not highlight those strips in a **Scan field (A)**.

The screenshot shows the Immunoblot Software interface. On the left, a vertical strip of 24 test strips is visible. A red arrow labeled 'A' points to strip 19. The main window displays a scan field with a graph of intensity versus distance (0 to 6 cm). The graph shows peaks for various bands: reference, control, vlsae_bg, p83_ba, p41_bs, p39_ba, ospc_bs, ospc_bsp, ospc_bg, dbpa_p17_bg, filaplasma_p44, aniplasma_ompa, ebv_p18, and cutoff. Below the scan field is the **Planner** tab, which contains an evaluation table with columns for No, Description, Analysis validity, conjugatecontrol, vlsaebg, p83ba, p41bs, p39ba, ospcbs, ospcbsp, ospcbsp, ospcbsp, dbpap17_bg, and anap.

No	Description	Analysis validity	conjugatecontrol	vlsaebg	p83ba	p41bs	p39ba	ospcbs	ospcbsp	ospcbsp	ospcbsp	dbpap17_bg	anap
3		✓	✓	2.75	0.78	1.96	1.57	0.39	0.78	3.14	5.49	1.18	1.18
7		✓	✓	3.53	1.18	1.57	1.57	1.57	2.35	3.92	1.57	3.14	3.14
8		✓	✓	2.35	2.75	1.57	1.57	0.78	2.35	1.18	1.96	2.35	0.78
9		✓	✓	3.92	0.78	1.57	1.57	1.18	1.18	2.35	0.39	0.78	2.35
10		✓	✓	2.75	1.96	2.75	3.53	1.57	0.78	1.57	1.18	1.96	1.96
11		✓	✓	4.71	1.57	1.18	1.57	2.35	1.96	1.57	0.78	1.18	1.96
12		✓	✓	1.96	1.57	1.18	2.35	0.78	1.18	0.78	1.57	2.75	1.18
13		✓	✓	4.71	2.75	1.96	2.75	0.78	1.18	3.14	7.06	2.75	5.49
14		✓	✓	4.31	1.96	1.18	2.35	2.35	1.18	2.35	3.53	1.18	4.31
15		✓	✓	5.88	1.18	0.78	1.96	0.39	0.78	1.18	4.31	0.78	1.57
16		✓	✓	2.35	1.18	3.14	2.35	1.96	2.35	1.57	1.18	2.75	1.96
17		✓	✓	3.14	1.18	1.57	1.18	2.35	4.31	4.31	6.67	1.57	1.96
19		✓	✓	3.14	2.35	0.78	0.78	0.78	0.78	0.78	0.39	0.39	1.96
20		✓	✓	2.75	0.78	0.39	1.57	0.39	0.39	1.18	1.57	1.18	1.18
21		✓	✓	2.35	0.78	1.96	2.75	1.18	1.18	1.57	3.53	1.57	2.35

SUMMARY RESULTS AND TEST (X) RESULTS

You can save and print two types of **Results** documents – summary or individual. The **Summary results** protocol includes all analysed strips and their evaluation. The **Test (X) results** protocol includes detailed information of an individual strip.

The **Summary results** protocol includes:

- A) Heading with lab identification
- B) Protocol type and kit type
- C) Lot, date and time of the process, and user identification
- D) Table of strip images
- E) Table of band evaluation and general results of individual tests
- F) Analysis notes
- G) Footer with the path to the saved protocol scan

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Summary results

BLot-LINE ANA

Lot: AN078311
Performed by: Lab
Evaluation time: 6/2/2013 10:10:25 AM

Sample no. 1	Sample no. 2	Sample no. 3	Sample no. 4	Sample no. 5	Sample no. 6	Sample no. 7 (*)	Sample no. 8	Sample no. 9 (**)	Sample no. 10
3a-1	0.67(N)	3.38(N)	1.00(N)	0.06(N)	1.04(N)	(X)	(X)	0.04(N)	0.70(N)
PL-7	0.79(N)	3.11(N)	14.2(N)	0.32(N)	0.10(N)	11.73(B)	20.01(P)	0.10(N)	0.53(N)
PL-12	0.50(N)	2.16(N)	1.00(N)	0.09(N)	0.00(N)	(X)	(X)	0.40(N)	0.79(N)
PosSci	0.15(N)	1.00(N)	23.07(P)	0.09(N)	2.16(N)	(X)	22.84(P)	0.21(N)	1.24(N)
CENP-A	0.30(N)	1.88(N)	(X)	0.46(N)	87.58(P)	(X)	(X)	0.04(N)	69.11(P)
CENP-B	0.53(N)	1.53(N)	(X)	0.48(N)	108.99(P)	(X)	16.23(P)	(X)	130.34(P)
Scl-70	0.00(N)	1.50(N)	3.10(N)	0.33(N)	0.34(N)	0.01(N)	12.89(B)	(X)	0.16(N)
RuS2	0.00(N)	50.84(P)	10.47(N)	0.00(N)	88.41(P)	0.00(N)	62.38(P)	(X)	0.25(N)
RuS0	0.00(N)	2.25(N)	3.25(N)	0.00(N)	108.14(P)	0.00(N)	15.30(B)	16.43(P)	0.00(N)
La	0.00(N)	0.53(N)	21.30(P)	0.00(N)	130.68(P)	0.00(N)	15.16(B)	17.37(P)	0.08(N)
RNP-A	0.00(N)	22.22(P)	0.30(N)	0.30(N)	24.07(P)	0.30(N)	28.03(P)	(X)	0.02(N)
RNP-C	0.00(N)	0.04(N)	0.39(N)	0.00(N)	0.21(N)	0.00(N)	(X)	(X)	0.04(N)
RNP-68	0.00(N)	0.21(N)	0.19(N)	0.00(N)	0.25(N)	0.00(N)	11.13(B)	16.97(P)	0.13(N)
SclB	0.13(N)	1.00(N)	0.18(N)	0.00(N)	0.20(N)	0.39(N)	13.97(B)	(X)	0.73(N)
SclD	0.00(N)	0.00(N)	0.10(N)	0.00(N)	0.24(N)	0.00(N)	3.76(N)	(X)	0.09(N)
JO	26.62(P)	0.07(N)	0.17(N)	0.00(N)	0.33(N)	36.96(P)	(X)	19.12(P)	0.64(N)
PCNA	0.00(N)	0.01(N)	0.37(N)	0.12(N)	0.35(N)	0.21(N)	(X)	18.24(P)	0.01(N)
Histone	0.00(N)	0.30(N)	0.04(N)	0.00(N)	0.26(N)	0.16(N)	(X)	(X)	2.06(N)
ANA	Positive	Positive	Positive	Negative	Positive	Positive	(X)	Positive	Positive

Strip assigned (*): Start line shifted manually
Strip assigned (**): Control line located incorrectly, SW evaluation result is not valid
Bands assigned (X): the assignment was altered because of an interference with the strip background

Immunoblot Software, version 1.0.0 TestLine Clinical Diagnostics s. r. o. www.testlinecd.com
C:\test_line\testngpurn_3601\testngpurn.BLOT-LINE\deflab.0.jpg

The **Test (X) results** protocol includes:

- A) Heading with lab identification
- B) Protocol type and kit type
- C) Lot, date and time of the process, and user identification
- D) Detailed image of the strip showing the intensity of each band and the intensity curve of the bands (like in the **Analysis field**)
- E) Table of detailed evaluation of each band and general results of the strip
- F) Identifications of detected antibodies (if defined for a given kit)
- G) Disease association (if defined for a given kit)
- H) Analysis notes
- I) Footer with the path to the saved protocol scan
- J) Serial number of the strip
- K) Sample label

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Test results(5)
sample S5-x

BLot-LINE ANA

Lot: AN078311
Performed by: Lab
Evaluation time: 6/4/2013 10:02:10 AM

Scan image

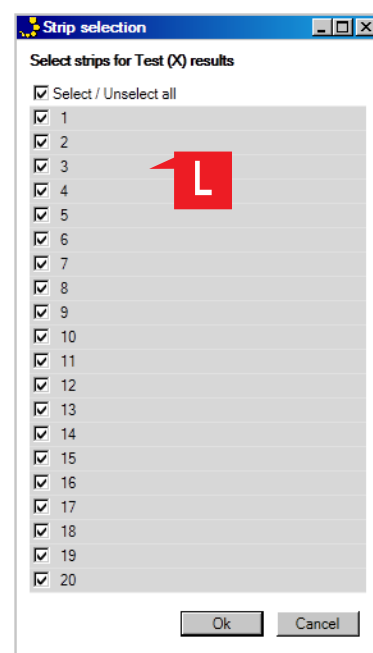
Band order	Band name	Position (mm)	Intensity (%)	Evaluation	Antibodies found against
1	Control	1.5	100.00	Positive	
2	3a-1	3.2	0.67	Negative	
3	PL-7	6.2	0.79	Negative	
4	PL-12	8.2	0.50	Negative	
5	PosSci	10.0	0.15	Negative	
6	CENP-A	13.8	58.99	Positive	Centromeres
7	CENP-B	15.83	108.02	Positive	Centromeres
8	Scl-70	19.13	0.39	Negative	
9	RuS2	23.88	86.50	Positive	SS-A antigen
10	RuS0	26.92	107.72	Positive	SS-B antigen
11	La	29.80	132.83	Positive	SS-B antigen
12	RNP-A	32.77	24.41	Positive	UI-RNP antigen
13	RNP-C	35.22	0.38	Negative	
14	RNP-68	37.25	0.14	Negative	
15	SclB	41.23	0.14	Negative	
16	SclD	43.26	0.43	Negative	
17	JO	46.23	0.42	Negative	
18	PCNA	48.26	0.44	Negative	
19	Histone	54.27	0.14	Negative	

ANA Positive

Possible association with:
CREST, systemic scleroderma
Sjögren's syndrome, SLE, neonatal SLE
SLE, MCTD

Immunoblot Software, version 1.0.0 TestLine Clinical Diagnostics s. r. o. www.testlinecd.com
C:\test_line\testngpurn_3601\testngpurn.BLOT-LINE\deflab.0.jpg

You can select strips for generating individual **Test (X) results**. Use **Print test (X) results** or **Save individual test results as PDF** to open a dialog box **(L)** where you can select individual strips.



PROGRAM SETTINGS AND UPDATE

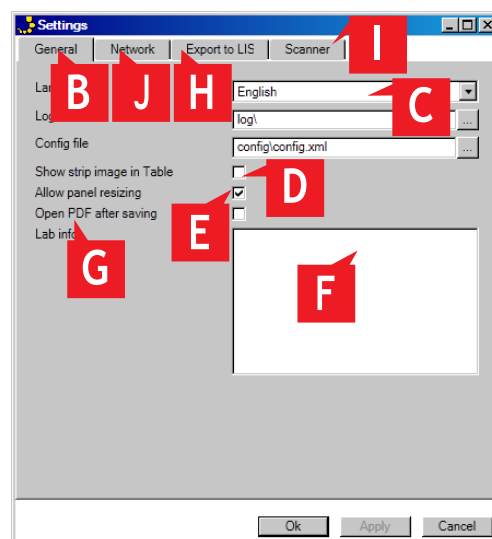
Functions of certain parts of the program can be customized according to your needs.

PROGRAM SETTINGS

The program settings can be found in the **Menu Edit – Settings (A)**.

Select an item to display a window with settings. You can choose between **General (B)** and **Network (J)**:

- C)** *Language* – you can change the language of the application.
- D)** *View strip* – you can switch off the view in the **Evaluation table** to make this area more clear.
- E)** *Panel size* – you can change the size of the **Protocol field**, the **Analysis field** and the **Evaluation table**.
- F)** *Lab info* – you can identify the lab which performs the examination. This information will be displayed in the heading of final **Results** documents.
- G)** *Automatic opening of PDF* – saved PDF files will appear in a set browser.
- H)** *Export to LIS* – allows you to export results to the laboratory information system.
- I)** *Scanner calibration* – allows you to calibrate the scanner.



The **Network settings** also allow you to set a website for downloading program updates.

LOT UPDATE

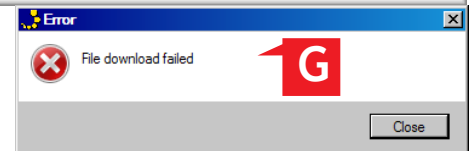
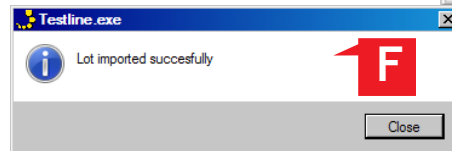
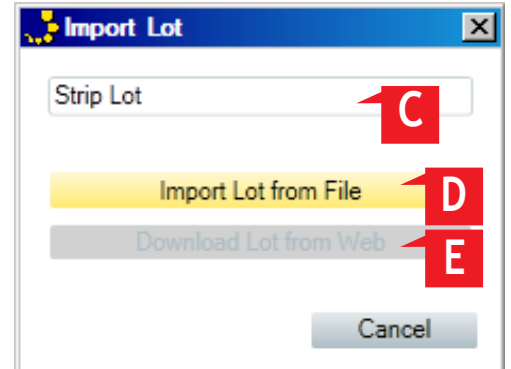
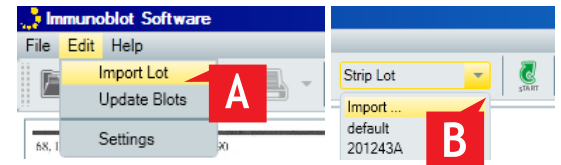
If the lot of the analysed strips is not available in the system while processing BLOT protocols, you can download it from the web or import it from the CD. The lot can be imported in the following way:

- A) Select *Edit – Import Lot* in the **Menu**
- B) Select *Import...* in the **Lot** drop-down menu

The dialog box *Import lot* allows you to enter a source and a number (C) of the lot to be imported. The source can be the CD (D) or the website (E). You can change the website used for importing the lots in the **Network settings**.

The program will inform you whether the operation was successful (F), or failed (G):

If the lot was imported successfully, it can be immediately selected and used in the analysis.

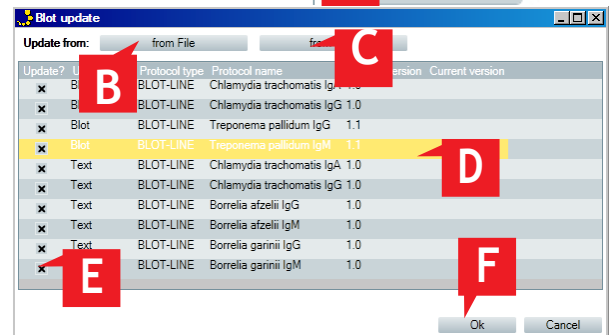
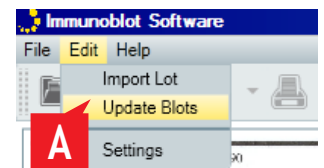


KIT UPDATE

You can update kit type by selecting *Edit – Update Blots* (A) in the **Menu**. A dialog box appears where you can select the update source.

Click either **from File** (CD) (B) or from **Web** (C) and a list of available kits will appear (D). After selecting a kit (E) and clicking on the OK button (F) the program will import the kit.

The program will inform you whether the operation was successful or failed. If the kits were imported successfully they are immediately available in the **Tool bar** and can be used in the analysis.

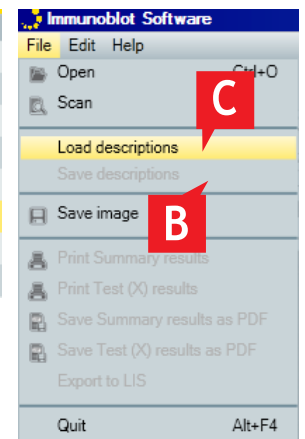


SAVING AND LOADING DESCRIPTIONS

You can describe individual strips, and save and open the descriptions. Such procedure can be helpful, for example, in situations when more than one examinations are assigned to a patient. Strip descriptions are filled in in advance and saved in a file. Then you can just open them from the file for following analyses.

- A) *Strip descriptions*, filled in in advance by a user.
- B) To save descriptions select *File – Save descriptions* in the **Menu** and specify a path to the file where the descriptions will be saved.
- C) To load descriptions select *File – Load descriptions* in the **Menu** and select a source file. The descriptions will be loaded and displayed in the **Evaluation table**.

No.	Description	Analysis	ANA	Control
1	Pokus			
2				
3	sample 3			
4				
5				
6	pokus 6			
7				
8				

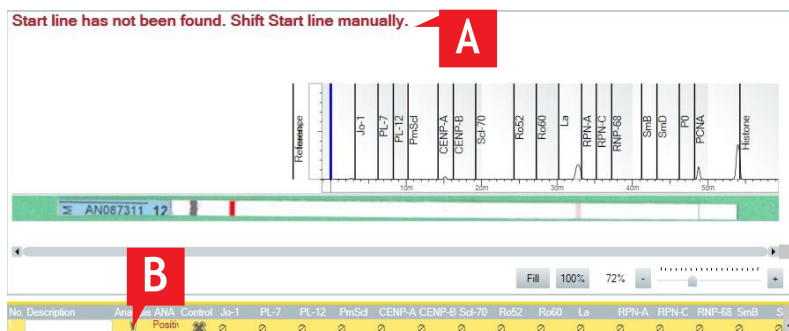


In the **Bluediver mode**, the program allows to fill in descriptions (patient ID) in the **Planner**. Loading and saving of these descriptions from/to the file works the same way as in the **Immunoblot mode**.

COMMON ISSUES

▶ START LINE NOT FOUND (BLOT/BLOT-LINE PROTOCOL)

If the **Start line** of a strip is not found, antigenic bands cannot be properly located and the test will not be correctly evaluated. Such a problem is indicated in two ways – by a text information in the **Analysis field (A)** and by an exclamation mark **(B)** in the **Analysis column** in the **Evaluation table**. The strip is automatically marked as non-assessable and all its bands are displayed in black colour. In such case you must place the **Start line** manually into the correct position. The **Start line** of BLOT-LINE protocols must be positioned on the red stripe near the upper margin of the strip.



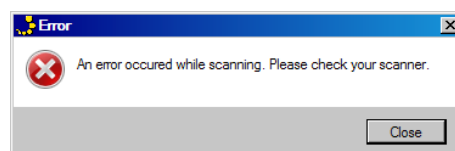
▶ CONTROL LINE NOT FOUND (BLOT-LINE PROTOCOLS)

Usually the **Control line** of BLOT-LINE strips is not found when the **Start line** is not found or is incorrectly located. Such problem is indicated by a cross in the **Validity** column in the **Evaluation table (A)**. You can usually solve the problem by moving the **Start line** to a correct position. However, if the **Start line** is located correctly, you can usually solve the problem by resetting slightly its position by approx. 0.5 mm.

No.	Description	Analysis	ANA	Control	Jo-1	PL-7	PL-12	PmScd	CENP-A	CENP-B
2		!	Positiv	✘	3	0.17	0.15	0.21	0.00	0.00
			Positiv	✔	3	3.11	2.16	1.93	1.88	1.53

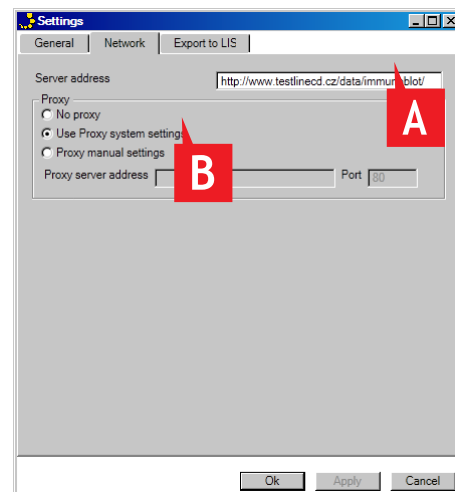
▶ UNABLE TO SCAN

When scanning of the protocol fails, the program will report the failure. In such case, you must contact a system integrator. The scanner has not been correctly installed in the operating system and the program cannot detect it.



▶ UNABLE TO DOWNLOAD UPDATES

When the **Kit update** or the **Lot update** from the web fails repeatedly, there may be a problem with **Network settings**. Check settings of the TestLine web address (<http://testlinecd.cz/data/immunoblot>) used for update importing **(A)**. If the web address is entered correctly but the problems remain, contact a system integrator to check the proxy server settings **(B)** in the **Network settings**.



▶ NOT LOADED QR CODE

In the case of scanning/opening a protocol/clamp with printed QR code, the program automatically chooses the protocol type and the kit type. If automatic loading for some reason (damaged QR code) fails, continue with the selection of the protocol type and the kit type as in previous versions of the program.

ATTENTION: In the case of a manual choice, select always the QR variant of the protocol after loading protocol with QR code!!!

