

AbIdent Software

Version 5.2d



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1. Introduction

With the aid of this software, HLA class I and HLA class II antibody identification using the Bio-Rad Abldent can be easily and effectively evaluated. After data have been evaluated, results can be stored and printed out.

The software has interfaces to all standard microtiter plate readers and automated microtiter plate processors. With the aid of the user friendly setup menu, laboratory-specific adjustments can be carried out quickly and easily.

2. Installation

2.1 Minimum system requirements

Computer:

- operating system: Windows 2000 or better
- Screen resolution: 1024 x 768 or better
- Minimum free harddisk space: 20 MB
- For importing pipettor data: access to pipettor files (e.g. LAN connection)
- For importing photometer data: Serial interface

Note:

If your PC does not meet these minimum requirements, this may result in errors, extended waiting times and possibly program malfunctions.

Installation requires Administrator rights.

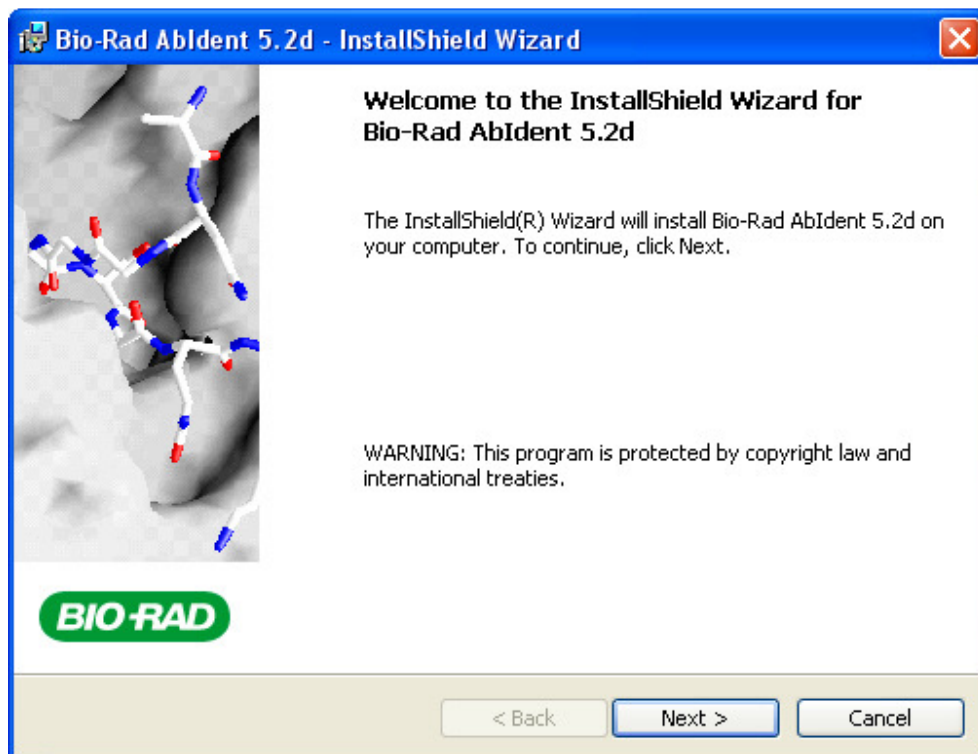
Recommendations for connecting up a photometer:

- Screened serial cable, length \leq 2m
- Transfer rate \leq 9600 baud
- Do not position cables close to electromagnetic fields
- Tuning the parameters in the reader

2.2 Installation instructions

Log on to the system as the Administrator. Insert the CD in the CD drive. Run the BABIDsetup.exe program from the CD.

1. First you will see the "Installation Wizard" followed by the welcome message:



Click <Next> to display the following sequence of windows:

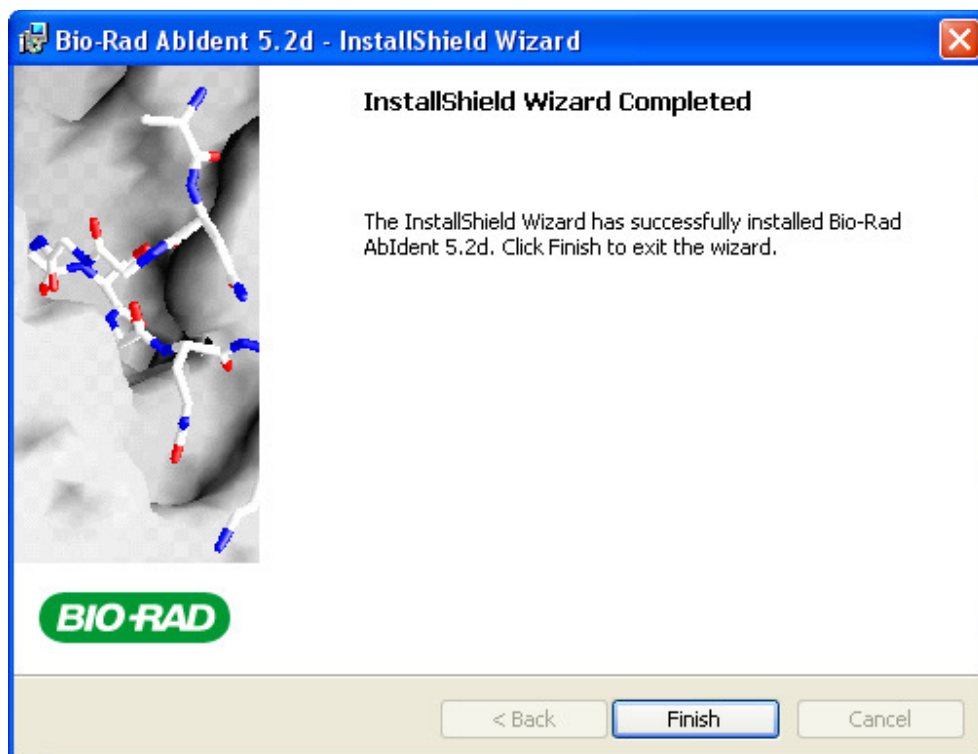
2. Licence agreement
Select "I accept the terms in the license agreement". The <Next> button cannot be clicked until you do so.
3. Note on installed files- <Next>
4. PC user and company- <Next>
5. Suggested target folder
The default folder is C:\Program Files\Bio-Rad\BABIDxxxx\ (xxxx = current version).

Changing the target folder:

Enter a changed folder name in this window or simply click <Next> to confirm the default.

6. Overview of settings before the installation itself begins
Click <Install> to confirm → the installation will then begin

8. When installation is complete, you will see the following message:



Click <Finish> to complete.

When installation has completed, you will see new entries as desktop icons and in the All Programs start menu called "Bio-Rad AbIdent xxxx (Reader)" and "Bio-Rad AbIdent xxxx (Pipettor)".

3. Data import

3.1 Importing updated lots

Lot specific software updates can be found on the internet at <http://www.medizinische-diagnostik-dreieich.de>. You can find new lot data by following these steps:

- Open "Download service"
- "Transplantation"
- "Transplantation AbScreen / AbIdent / AbCross"
 - A table with these products is shown. Choose the required product (AbIdent Class I or II). Click "SW" of the new lot and then <Save>. A zip folder is downloaded.


Extract the files in the zip folder to the C:\Program Files\Bio-Rad\BABIDxxxx\LotFiles folder.

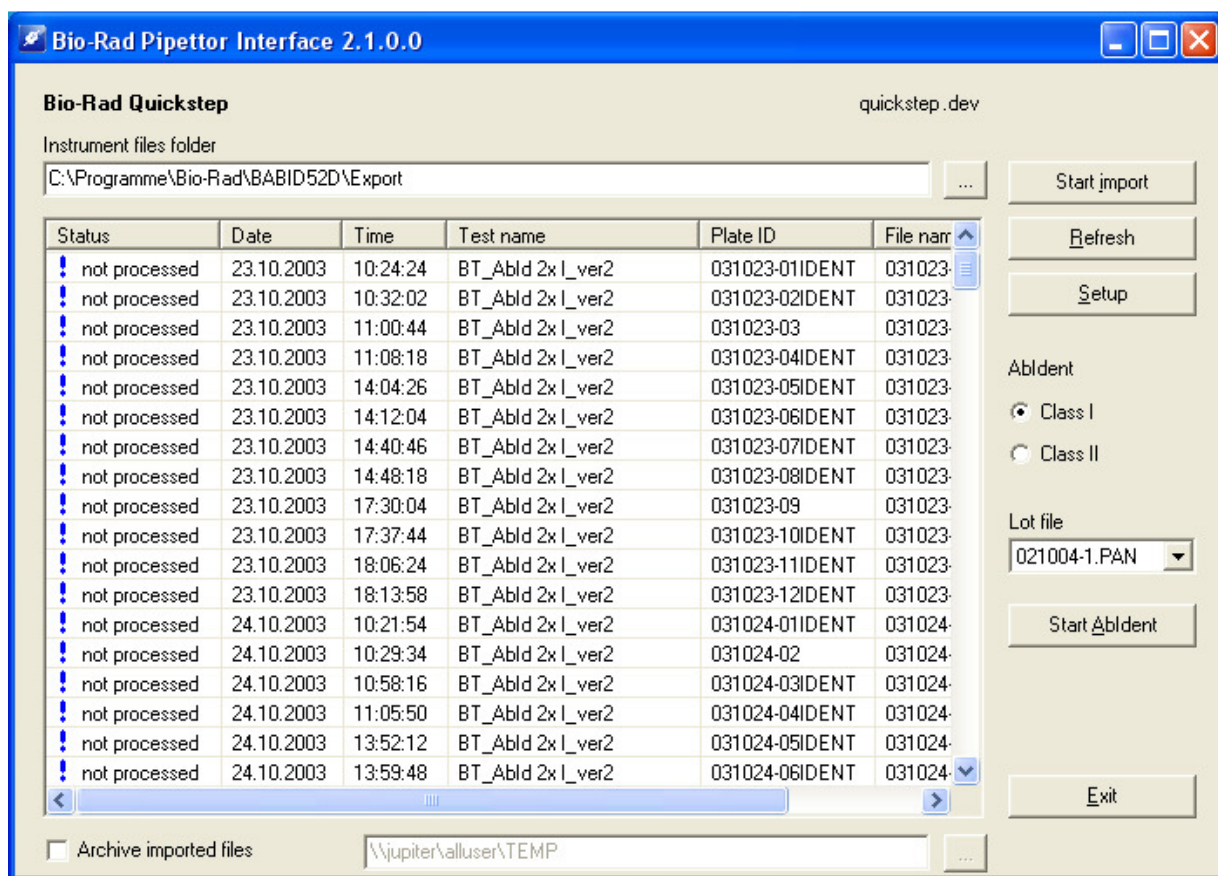
3.2 Importing pipettor files

To import pipettor data, run the "Bio-Rad AbIdent xxxx (Pipettor)" program. You will see the Bio-Rad Pipettor interface.

→ You will see a list of the microplates which can be read in.

If this list is blank even though you have pipetted some microplates, please check the following settings (also described in Chapter 5):

- Is the right path entered under "Instrument files folder"? It is necessary to enter a path here for the exported pipettor files (select with the browse button  on the right).
- Is the right pipettor selected? (check the Settings).
- Is the right instrument file template selected? (check the Settings).
- Is the right software selected? (check the Settings. The "AbIdent software" must be selected).
- Are the correct identification parameters for the test names in Class I/II selected? The words entered here (and separated from each other by commas) must all be contained in the test names in order to display the imported file (check Settings).



Select the corresponding files for import by clicking them once with the mouse, select the corresponding lot from the "Lot file" menu and import data by clicking <Start import>.

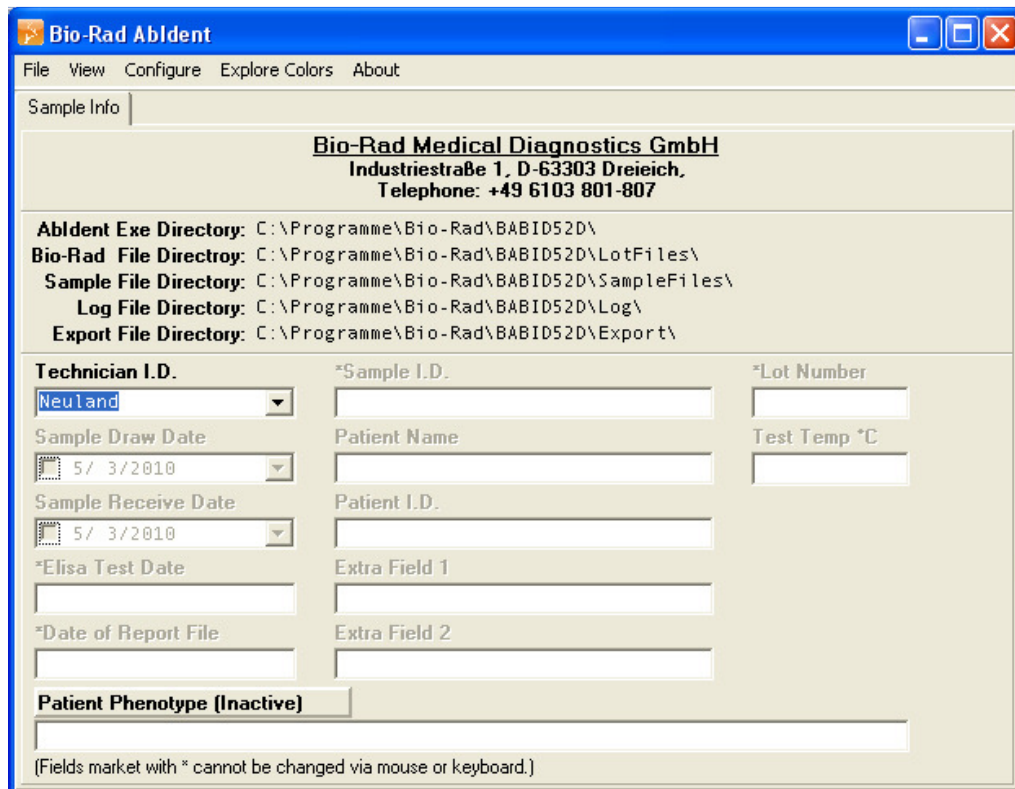
→ After the import, the names of the imported microplates are marked with a green tick and the name is modified. This is to prevent the same microplates being imported again:

✓	processed	23.10.2003	10:24:24	BT_Abld 2x I_ver2	031023-01IDENT	031023-
✓	processed	23.10.2003	10:32:02	BT_Abld 2x I_ver2	031023-02IDENT	031023-

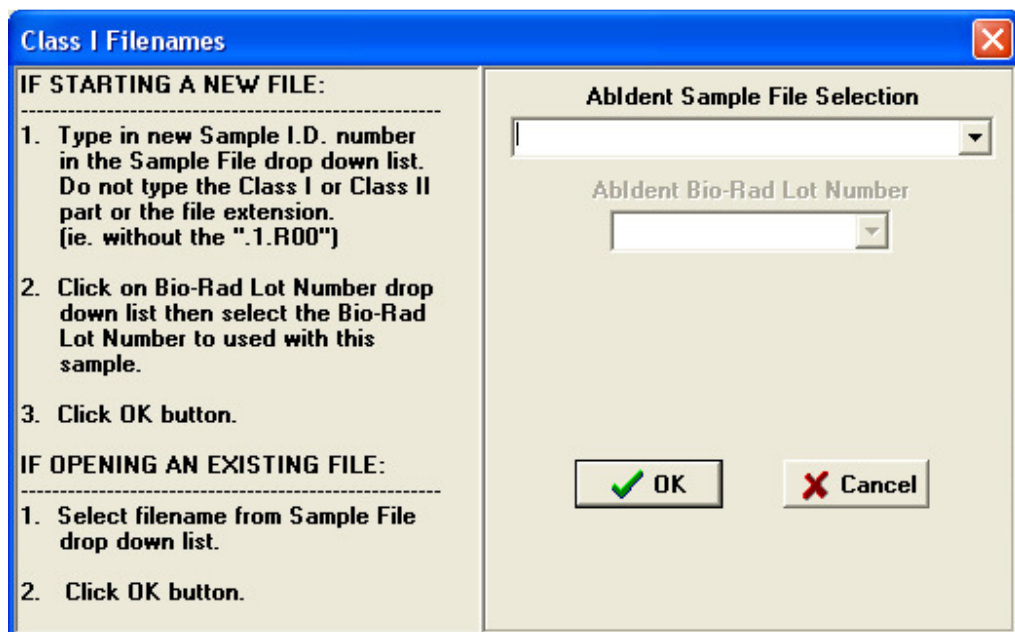
Note:

It is important to know the names of the patients which were used when the samples were entered because the software uses these names and sorts the data in the selection menu for samples to be imported by these names.

Click <Start Abldent > to run the Abldent software.



In the "File" menu, select <Start Class I Sample> or <Start Class II Sample>.



Abldent Sample File Selection

Select the sample names from a list.

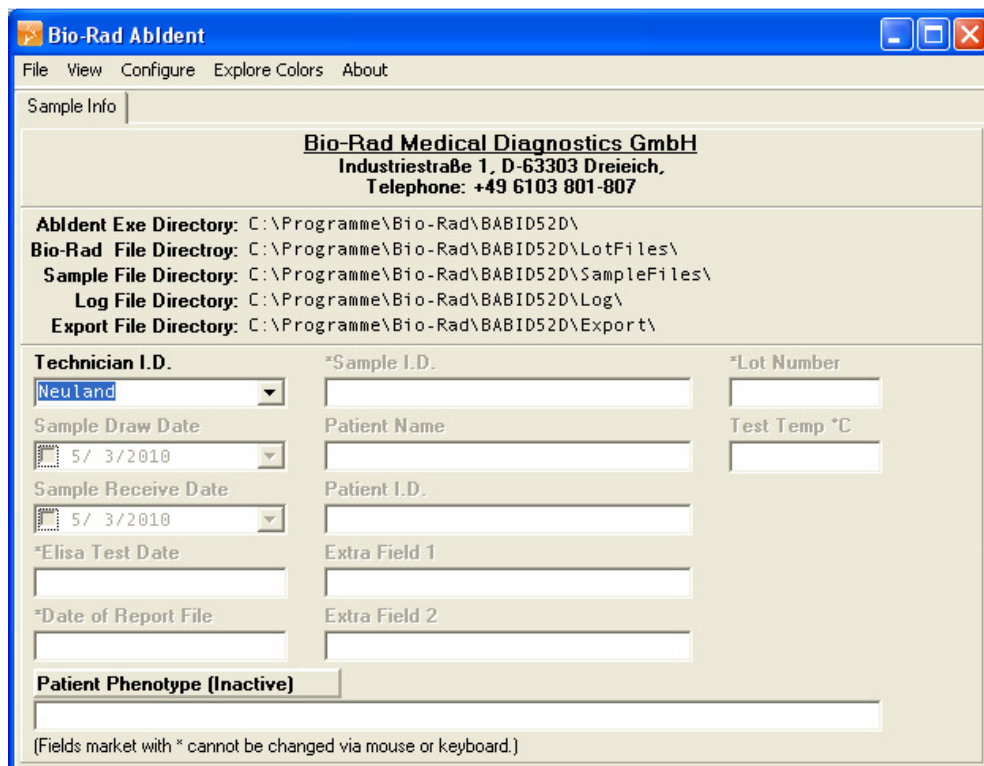
Abldent Lot Number

Select the Abldent lot you are using.

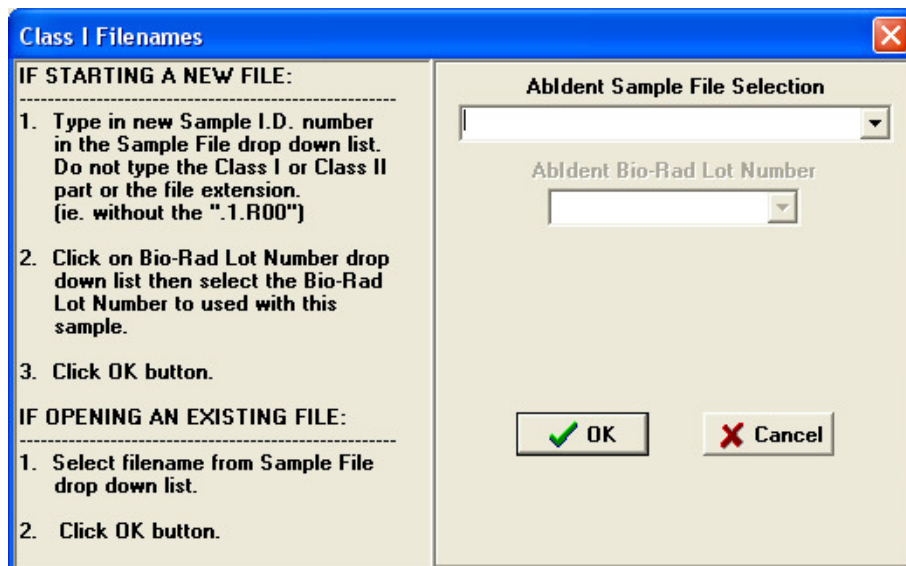
Click <OK> to confirm.

3.3 Importing a reader file

To import reader data, run the "Bio-Rad Abldent xxxx (Reader)" program. The Bio-Rad Abldent software will start.



In the "File" menu, select <Start Class I Sample> or <Start Class II Sample>.



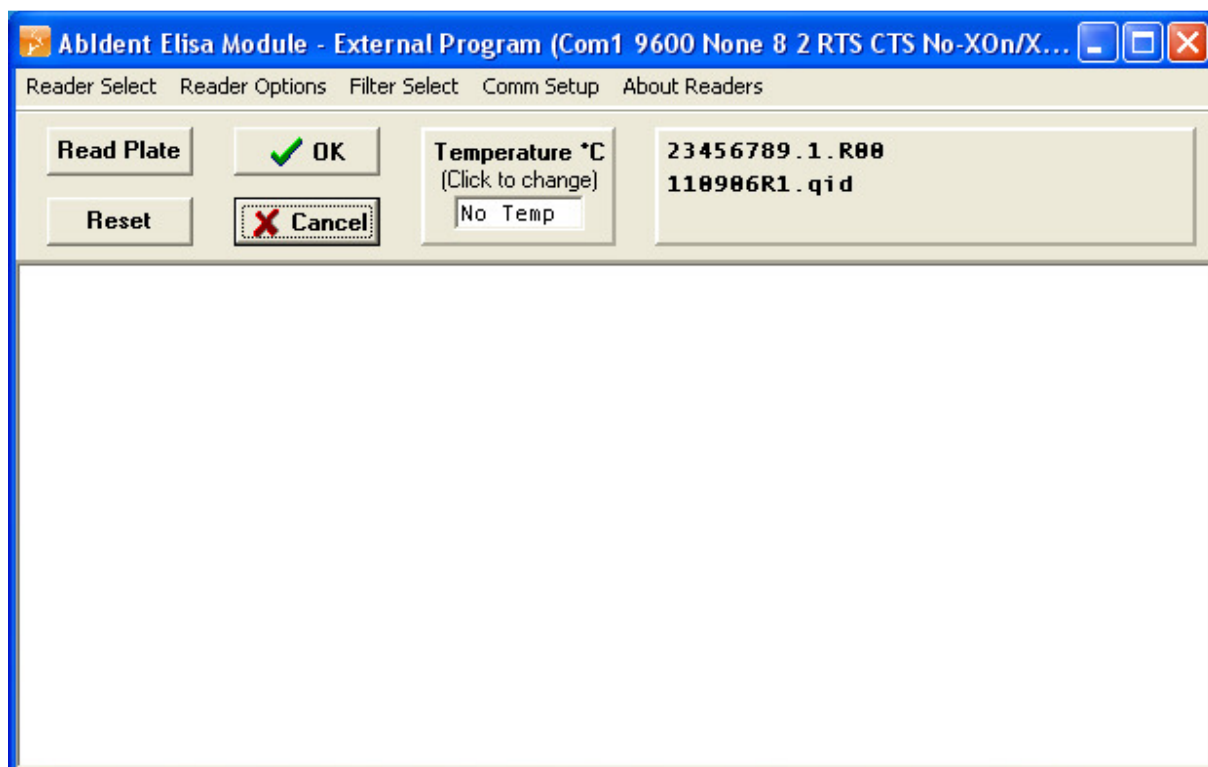
Abldent Sample File Selection

Enter a name for the results file manually.

Abldent Lot Number

Select the Abldent lot you are using.

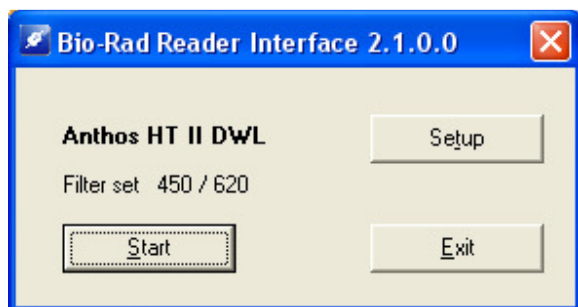
Click <OK> to generate an empty Reader file. You will see a dialogue window which enables you to control the Reader.



By default, an Anthos HT II DWL reader is used. Refer to chapter 5.1 to find out how to configure the software to use other types of readers.

Select <Read Plate> to initiate the read process. In the next window, you can enter the current ambient laboratory temperature during the Abldent ELISA tests. Select the temperature and click <OK>.

The Bio-Rad Reader interface opens. Start the read process by clicking <Start>.



Reader data is automatically read in by the software.

4. Working with the software

The workflow is as follows:

- Read in the microplate (as a file or using the Reader)
- If you use the Reader: enter the sample numbers
- Evaluate results and edit them.
- Save results
- Print results
- Optionally, Export results

After a pipettor or Reader file has been imported, the screen automatically displays the "Explore" tab showing these results.

The screenshot displays the Bio-Rad Abldent software interface. The top menu bar includes File, View, Configure, Explore Colors, and About. The main window is divided into several sections:

- Sample Info:** Plate [051849.1.R00], Test Parameters, Explore, Report.
- Antigen Short Tail Results -- PRA = 16wells/40wells => 40.0%**

Ant	Count	Sum	++	+-	-+	--	Incl	Cum Incl	r	Cum r	X ²	Cum X ²	Mean OD
1 A31	2	40	2	14	0	24	100%	100%	0.281	0.281	3.158	3.158	0.283
2 A28	4	38	3	11	1	23	75%	83%	0.271	0.372	2.797	5.523	0.204
3 C08	4	35	3	8	1	23	75%	80%	0.337	0.471	3.978	8.809	0.098
4 B41	1	32	1	7	0	24	100%	82%	0.311	0.526	3.097	11.055	0.122
- Independent Antigen Statistics -- R-Value Sort (X = Suppressed, * = Absent)**

X	Ant	Count	++	+-	-+	--	Incl	r	X ²	Mean OD
17+	A31	2	2	14	0	24	100.000	0.281	3.158	0.283
14+	A28	4	3	13	1	23	75.000	0.238	2.269	0.204
41+	B18	4	3	13	1	23	75.000	0.238	2.269	0.226
99+	C08	4	3	13	1	23	75.000	0.238	2.269	0.098
20+	A34	1	1	15	0	24	100.000	0.196	1.538	0.226
53+	B41	1	1	15	0	24	100.000	0.196	1.538	0.122
64+	B56	1	1	15	0	24	100.000	0.196	1.538	0.057
- Antigen Panel List -- Rel OD Sort (X = Suppressed)**

X	Well	Pan ID	Rel OD	= (RawOD) - (Factors)	ANTIGENS	Negative	Positive
73+	B10	18W	1.014	= (1.409) - (0.395)	A01 A26 B55 B57 C03 C06 W04 W06	1.014	
76+	E10	7HC	0.587	= (0.925) - (0.338)	A28 A30 B18 B63 C07 C-- W04 W06	0.587	
63+	H8	53LB	0.514	= (0.880) - (0.366)	A01 A31 B27 B44 C02 C05 W04 W--	0.514	
78+	G10	71LB	0.483	= (0.843) - (0.360)	A23 A66 B18 B35 C02 C04 W-- W06	0.483	
83+	D11	LR	0.433	= (0.803) - (0.370)	A02 A24 B08 B50 C06 C07 W-- W06	0.433	
69+	F9	47LB	0.253	= (0.670) - (0.417)	A03 A33 B42 B63 C05 C17 W04 W06	0.253	
68+	E9	12DFW	0.240	= (0.515) - (0.275)	A02 A32 B39 B64 C07 C08 W-- W06	0.240	
61+	F8	DE	0.226	= (0.673) - (0.447)	A29 A34 B44 B61 C04 C08 W04 W06	0.226	
52+	E7	95CBC	0.178	= (0.629) - (0.451)	A01 A28 B07 B60 C03 C07 W-- W06	0.178	
50+	C7	BF	0.156	= (0.590) - (0.434)	A03 A28 B27 B49 C02 C07 W04 W--	0.156	
67+	D9	69LB	0.122	= (0.454) - (0.332)	A11 A30 B41 B49 C07 C17 W04 W06	0.122	
70+	G9	36CBC	0.104	= (0.537) - (0.433)	A02 A33 B62 B65 C03 C08 W-- W06	0.104	
59+	D8	5DFW	0.080	= (0.517) - (0.437)	A03 A24 B13 B51 C06 C15 W04 W--	0.080	
53+	F7	2BW	0.057	= (0.469) - (0.412)	A02 A03 B13 B56 C01 C06 W04 W06	0.057	
55+	H7	19DFW	0.052	= (0.389) - (0.337)	A02 A31 B07 B51 C07 C15 W04 W06	0.052	
48+	A7	50CBC	0.010	= (0.457) - (0.447)	A24 A29 B18 B45 C12 C16 W-- W06	0.010	
81	B11	127CBC	-0.031	= (0.365) - (0.396)	A02 A24 B13 B44 C06 C16 W04 W--		-0.031
87	H11	28LB	-0.032	= (0.378) - (0.410)	A74 A80 B42 B44 C04 C17 W04 W06		-0.032
60	E8	51LB	-0.041	= (0.381) - (0.422)	A25 A33 B53 B60 C03 C04 W04 W06		-0.041
66	C9	82CBC	-0.043	= (0.355) - (0.398)	A02 A-- B13 B38 C06 C-- W04 W--		-0.043
64	A9	62LB	-0.068	= (0.341) - (0.409)	A74 A-- B50 B70 C02 C06 W-- W06		-0.068
84	E11	117CBC	-0.076	= (0.366) - (0.442)	A11 A26 B38 B60 C03 C12 W04 W06		-0.076
80	A11	98CBC	-0.080	= (0.348) - (0.428)	A03 A24 B35 B61 C02 C04 W-- W06		-0.080
82	C11	108CBC	-0.091	= (0.312) - (0.403)	A01 A25 B37 B44 C05 C06 W04 W--		-0.091
72	A10	59LB	-0.104	= (0.256) - (0.360)	A28 A74 B08 B70 C02 C07 W-- W06		-0.104
58	C8	21DFW	-0.115	= (0.284) - (0.399)	A29 A66 B07 B49 C07 C07 W04 W06		-0.115
49	B7	48LB	-0.119	= (0.382) - (0.501)	A23 A36 B07 B58 C04 C07 W04 W06		-0.119

Click the tabs visible at the top of the window to navigate between views.

4.1 "Sample Info" tab

The "Sample Info" tab contains details of the patients examined and additional details of the lab technician performing the test or analysis, the kit lots used and the test parameters in the laboratory.

Note:

Those fields marked with an asterisk can not be edited using mouse or keyboard.

Technician I.D.:

If required, enter the name of the person in the laboratory performing the analysis. By default, the computer enters the name of the current Windows user. This can be changed.

Sample Draw Date:

The date when the blood sample was taken can be entered here.

Sample Receive Date:

The date when the sample reached the laboratory can be entered here.

***ELISA Test Date:**

The date the Abldent test was performed appears here.

***Date of Report File:**

The date the report is generated appears here once it has been generated.

***Sample I.D.:**

The sample number appears here

Patient Name / Patient I.D.:

The name or lab ID of the patient may be entered here.

Extra Fields 1 and 2:

Freeform text comments can be entered here.

***Abldent Lot Number:**

The Lot Number of the Abldent lot used is displayed here.

Test Temp °C:

The ambient temperature in the laboratory during the test can be entered here.

Patient Phenotype:

Click <Patient Phenotype> to enter the patient's HLA type. To do so, you may mark the corresponding specificities in the list. Click <OK> to confirm.

If the patient's phenotype is entered here, these specificities may be suppressed on the "Test Parameters" tab.

4.2 "Plate" tab

The "Plate" tab contains the microplate layout of the Abldent lot used. The file name is shown in brackets (e.g. 7135.1.R00). The "R" stands for "Raw Data".

Bio-Rad Abldent

File View Configure Explore Colors About

Sample Info Plate (051849.1.R00) Test Parameters Explore Report

Comment Portion of Elisa File (Begin each line with at least one space)

```
021004R1.QID 051849.1.R00
Bio-Rad Pipettor Interface version 2.1.0.0

Test limit: 3.000
Test wavelengths: 405nm/492nm
Created from file: 031023-11IDENT.TXT
Assay name: BT_AbId 2x 1_ver2

Blanking well (*) found at G:12 OD = 0.004
Blanking well (*) found at H:12 OD = 0.005
Blank average = 0.005
OD values not calculated
```

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.054	1.085	1.157	2.267	1.322	0.242	50CBC ✓ 0.918 0.457	LA ✓ 0.877 0.250	62LB ✓ 0.839 0.341	59LB ✓ 0.740 0.256	98CBC ✓ 0.879 0.348	-NC ✓ 1.000 0.257
B	0.320	0.418	1.320	2.129	0.265	0.238	48LB ✓ 1.028 0.382	7LB ✓ 0.930 0.319	27LB ✓ 0.919 0.270	1BW ✓ 0.811 1.409	127CBC ✓ 0.813 0.365	-NC ✓ 1.000 0.250
C	2.507	2.070	1.612	1.124	1.866	0.232	BF ✓ 0.891 0.590	21DFW ✓ 0.820 0.284	82CBC ✓ 0.818 0.355	2HC ✓ 0.987 0.339	108CBC ✓ 0.828 0.312	-NC ✓ 1.000 0.256
D	1.849	0.918	1.510	2.194	1.310	0.209	15LB ✓ 0.816 0.258	5DFW ✓ 0.897 0.517	69LB ✓ 0.682 0.454	19LB ✓ 0.792 0.248	LR ✓ 0.760 0.803	-NC ✓ 1.000 0.211
E	1.687	1.950	2.081	2.249	0.240	1.721	95CBC ✓ 0.926 0.629	51LB ✓ 0.866 0.381	12DFW ✓ 0.564 0.515	7HC ✓ 0.695 0.925	117CBC ✓ 0.908 0.366	+PC ✓ 1.000 1.856
F	1.856	1.756	1.809	0.228	1.391	0.075	2BW ✓ 0.847 0.469	DE ✓ 0.918 0.673	47LB ✓ 0.856 0.670	7CBC ✓ 0.960 0.305	DB ✓ 0.833 0.218	=NAW ✓ 1.000 0.100
G	1.164	1.422	1.179	1.313	1.827	0.005	17LB ✓ 0.789 0.234	11CBC ✓ 1.081 0.270	36CBC ✓ 0.890 0.537	71LB ✓ 0.739 0.843	58CBC ✓ 0.934 0.239	*BLAN ✓ 1.000 0.004
H	2.075	1.901	1.728	1.959	0.350	0.005	19DFW ✓ 0.691 0.389	53LB ✓ 0.752 0.880	103CBC ✓ 0.852 0.288	3CBC ✓ 0.863 0.269	28LB ✓ 0.842 0.378	*BLAN ✓ 1.000 0.005

The information on the individual wells on the microplate is shown below by reference to one well, well A7:

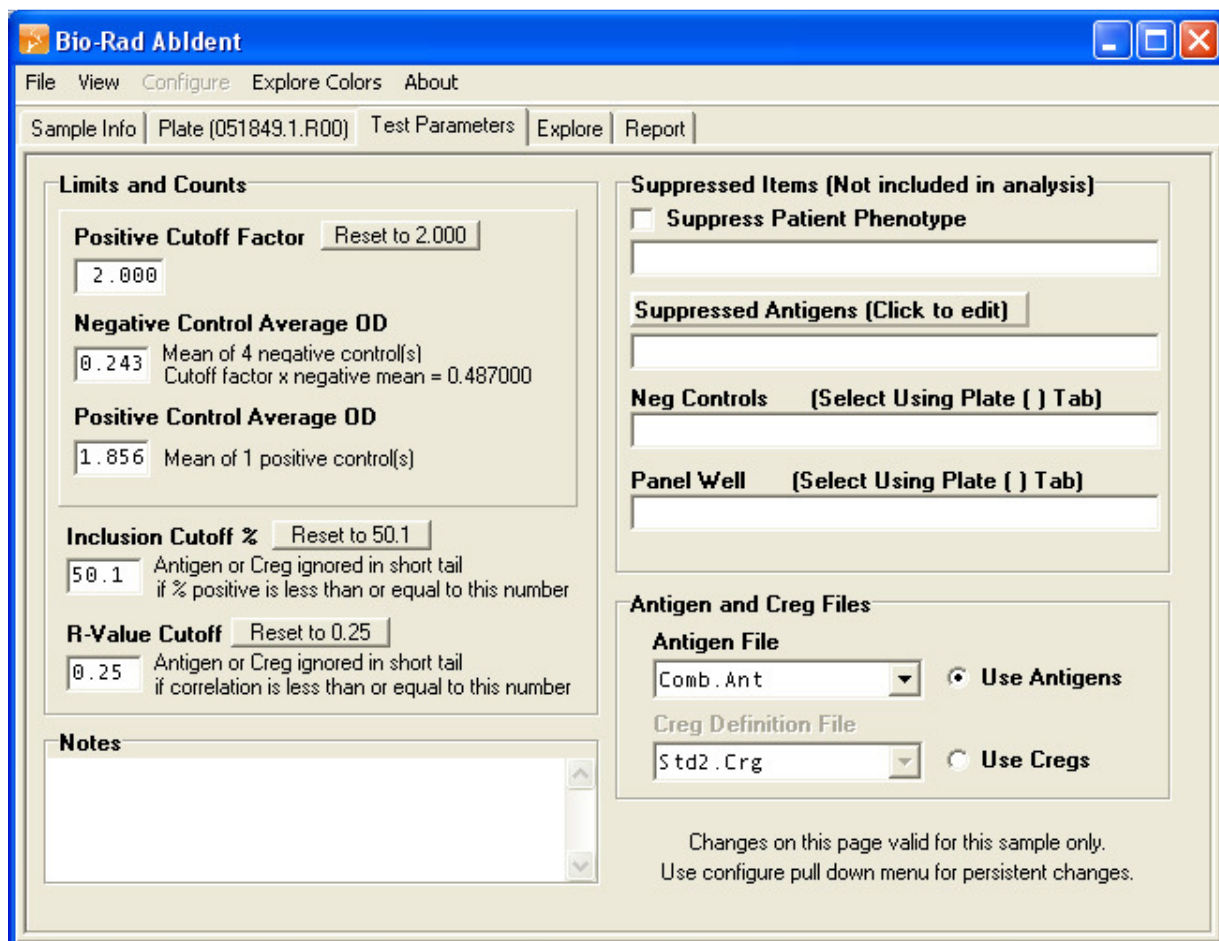
Line 1: "50CBC": internal information on the well

Line 2: The tick indicates that the well was included in the analysis. If more than three wells are excluded from the analysis, the software will not be able to evaluate this sample.
The number "0.918" describes the background adjustment factor (BAF) for this well. The cut-off for this well is defined by 2 x the mean of the negative controls multiplied by the BAF. This factor compensates for varying reaction strengths in the cells used.

Line 3: The number "0.457" describes the OD value of the sample after deducting the blank.

4.3 "Test Parameters" tab

Test settings are made on the "Test Parameters" tab.



Test-specific parameters can be set under "Limits and Counts".

Positive Cut-off Factor:

The value by which the mean value of the negative controls is to be multiplied in order to calculate the cut-off.

Negative Control Average OD:

Calculation of the mean values from four negative controls after deducting the blank.

Positive Control Average OD:

Calculation of the positive control sample after deducting the blank.

Inclusion of cut-off:

The inclusion cut-off is recalculated for each sample and indicates that an antigen or Creg (=Cross reactive group) is no longer included in the short tail list if this would mean that the overall percentage of 50.1% is not attained.

R value cut-off:

The R value cut-off indicates that if the R value is e.g. 0.25 or less, that antigen or Creg is no longer included in the short tail list.

In the "Suppressed Items" window, certain specificities can be excluded from the analysis.

Suppress Patient Phenotype:

Here, the patient's HLA specificities can be excluded from the analysis.

Note:

For this purpose, the patient's HLA type data must be entered on the "Sample Info" tab.

Suppressed Antigens:

Certain HLA specificities can be excluded from the analysis here.

Neg Controls / Panel Well:

This option displays the microplate positions deactivated in the "Plate" tab.

In the "Antigen and Creg Files" window, you can select whether you wish to analyse using individual HLA antigens or Cregs (Cross Reactive Groups).

4.4 "Explore" tab

The "Explore" tab is subdivided into 3 sections describing the results of the Abldent ELISA.

Antigen Short Tail Results -- PRA = 16wells/40wells => 40.0%													
Ant	Count	Sum	++	+	-	--	Incl	Cum Incl	r	Cum r	X ²	Cum X ²	Mean OD
1 A31	2	40	2	14	0	24	100%	100%	0.281	0.281	3.158	3.158	0.283
2 A28	4	38	3	11	1	23	75%	83%	0.271	0.372	2.797	5.523	0.204
3 C08	4	35	3	8	1	23	75%	80%	0.337	0.471	3.978	8.889	0.098
4 B41	1	32	1	7	0	24	100%	82%	0.311	0.526	3.097	11.055	0.122

Independent Antigen Statistics -- R-Value Sort (X = Suppressed, * = Absent)										
X	Ant	Count	++	+	-	--	Incl	r	X ²	Mean OD
17 +	A31	2	2	14	0	24	100.000	0.281	3.158	0.283
14 +	A28	4	3	13	1	23	75.000	0.238	2.269	0.204
41 +	B18	4	3	13	1	23	75.000	0.238	2.269	0.226
99 +	C08	4	3	13	1	23	75.000	0.238	2.269	0.098
20 +	A34	1	1	15	0	24	100.000	0.196	1.538	0.226
53 +	B41	1	1	15	0	24	100.000	0.196	1.538	0.122
60 +	B56	1	1	15	0	24	100.000	0.196	1.538	0.057

Antigen Panel List -- Rel OD Sort (X = Suppressed)													
X	Well	Pan ID	Rel OD	= (RawOD) - (Factors)	ANTIGENS --> --> --> --> --> --> -->								
73 +	B10	1BW	1.014	= (1.409) - (0.395)	A01	A26	B55	B57	C03	C06	W04	W06	Negative
76 +	E10	7HC	0.587	= (0.925) - (0.338)	A28	A30	B18	B63	C07	C--	W04	W06	Positive
63 +	H8	53LB	0.514	= (0.880) - (0.366)	A01	A31	B27	B44	C02	C05	W04	W--	Positive
78 +	G10	71LB	0.483	= (0.843) - (0.360)	A23	A66	B18	B35	C02	C04	W--	W06	Positive
83 +	D11	LR	0.433	= (0.803) - (0.370)	A02	A24	B08	B50	C06	C07	W--	W06	Positive
69 +	F9	47LB	0.253	= (0.670) - (0.417)	A03	A33	B42	B63	C05	C17	W04	W06	Positive
68 +	E9	12DFW	0.240	= (0.515) - (0.275)	A02	A32	B39	B64	C07	C08	W--	W06	Positive
61 +	F8	DE	0.226	= (0.673) - (0.447)	A29	A34	B44	B61	C04	C08	W04	W06	Positive
52 +	E7	95CBC	0.178	= (0.629) - (0.451)	A01	A28	B07	B60	C03	C07	W--	W06	Positive
50 +	C7	BF	0.156	= (0.590) - (0.434)	A03	A28	B27	B49	C02	C07	W04	W--	Positive
67 +	D9	69LB	0.122	= (0.454) - (0.332)	A11	A30	B41	B49	C07	C17	W04	W06	Positive
70 +	G9	36CBC	0.104	= (0.537) - (0.433)	A02	A33	B62	B65	C03	C08	W--	W06	Positive
59 +	D8	5DFW	0.080	= (0.517) - (0.437)	A03	A24	B13	B51	C06	C15	W04	W--	Positive
53 +	F7	2BW	0.057	= (0.469) - (0.412)	A02	A03	B13	B56	C01	C06	W04	W06	Positive
55 +	H7	19DFW	0.052	= (0.389) - (0.337)	A02	A31	B07	B51	C07	C15	W04	W06	Positive
48 +	A7	50CBC	0.010	= (0.457) - (0.447)	A24	A29	B18	B45	C12	C16	W--	W06	Positive
81	B11	127CBC	-0.031	= (0.365) - (0.396)	A02	A24	B13	B44	C06	C16	W04	W--	Positive
87	H11	28LB	-0.032	= (0.378) - (0.410)	A74	A80	B42	B44	C04	C17	W04	W06	Positive
60	E8	51LB	-0.041	= (0.381) - (0.422)	A25	A33	B53	B60	C03	C04	W04	W06	Positive
66	C9	82CBC	-0.043	= (0.355) - (0.398)	A02	A--	B13	B38	C06	C--	W04	W--	Positive
64	A9	62LB	-0.068	= (0.341) - (0.409)	A74	A--	B50	B70	C02	C06	W--	W06	Positive
84	E11	117CBC	-0.076	= (0.366) - (0.442)	A11	A26	B38	B60	C03	C12	W04	W06	Positive
80	A11	98CBC	-0.080	= (0.348) - (0.428)	A03	A24	B35	B61	C02	C04	W--	W06	Positive
82	C11	108CBC	-0.091	= (0.312) - (0.403)	A01	A25	B37	B44	C05	C06	W04	W--	Positive
72	A10	59LB	-0.104	= (0.256) - (0.360)	A28	A74	B08	B70	C02	C07	W--	W06	Positive
58	C8	21DFW	-0.115	= (0.284) - (0.399)	A29	A66	B07	B49	C07	C07	W04	W06	Positive
49	B7	48LB	-0.119	= (0.382) - (0.501)	A23	A36	B07	B58	C04	C07	W04	W06	Positive

PRA:

The PRA (Panel Reactive Antibodies) indicates the percentage of all antigen wells on the entire panel that reacted positively. The total number of positive wells (the example shows 16) is shown as a percentage of the entire panel (= 40 wells). $16 / 40 \times 100\% = 40.0\%$.

Antigen Short Tail Results:

The "Antigen Short Tail Results" show a statistically calculated HLA antigen identification for the patient. The main criterion is the number of correct positive wells for a certain specificity in relation to the total number of wells containing that specificity. If this ratio concludes with the specifications in the "Test Parameters" tab (Inclusion Cutoff, R-Value Cutoff), the respective specificity appears in the list. The specificity with the most correct positive reactions appears on top of the list.

Second place is taken by the specificity with the next best match of correct positive wells in relation to the number of all positive wells for the same specificity. Those wells included in the first line are no longer included and are subtracted from the total number of reactions.

Explanation using patient 21849 as an example: 2 of a total of 40 wells contain HLA antigens against A31. There was a positive reaction in these two wells. The Incl is therefore 100%. The columns marked "++", "+-", "-+", "-" and "--" show the distribution of the reactions. This totals 40. The sample is then analysed for further HLA antibodies present in significant numbers. One more specificity is listed: A28. 4 of the remaining 38 wells ($40 - 2 = 38$) on the microplate contain this specificity. 3 wells of the sample react as "correct positive". Ten more positive reactions are evaluated as false-positives and one is evaluated as false negative because one A28 reaction did not take place. The total number of reactions for specificity A28 is 38 (see calculation). Since the sample reacts with A28 in 3 out of a total of 4 wells, the Incl is 75. The cumulative Incl is higher because it includes the positive reactions of wells covering A31: the total of A31 and A28 specific wells is 6, of which 5 are detected as reactions with correct positives.

Explanations of the "Antigen Short Tail Results" table from left to right:

- "Antigen" = name of the specificity or Creg (cross reactive group)
- "Count" = the total number of wells covering the respective specificity
- "Sum" = the total number of wells for identifying one patient (Class I: 40 wells, Class II: 30 wells)
- "++" = "correct positive"; indicates how many wells reacted
- "+-" = "false positive"; shows how many wells reacted positive with other antigens
- "-+" = "false negative"; shows how many reactions for the respective specificity did not take place
- "- -" = "correct negative"; shows how many reactions were negative for other specificities
- "Incl" = if the number "Cnt" matches with the number "++", this makes 100 %; "Incl" is calculated using the following equation: $\text{"Incl"} = \frac{\text{"++"}}{\text{"Cnt"}} \times 100\%$
- "Cum Incl" = cumulated "Incl"; this value is equal to the Incl value for the first position of the list but higher in line two because it includes the reaction of the specificity in line one and so on.
- "r" = shows the reproducibility of test results. An R value of e.g. 0.6 indicates that 60% of the results are correct positive and 40% non-specific.
Explanation using an example: If 13 false positive reactions were included with 2 correct ones, the value of R is 0.296. If there are no non-specific, false positive or false negative reactions, the value of R will be exactly 1.000.
- "Cum r" = cumulative value for R; is the same as the specificity at the top of the table; with the second specificity, the reactions of the first specificity are included and so on.
- "X²" = means chi squared, which is a statistical measurement of the credibility of the test results. The higher the value of chi squared, the more accurate and credible the meaning of the test results.
- "Cum X²" = cumulative value of chi squared
- "Avg.OD" = average OD. The difference between Rel.OD and Avg.OD can be seen from the following formula: $\text{Rel.OD} = \text{Avg.OD} - \text{well cut-off}$

Independent Antigen Statistics:

"Independent Antigen Statistics" means that each specificity is considered and evaluated independently. There are therefore no cumulative results.

This table lists all the specificities on the microplate, beginning with the largest number of positive reactions in the sample having a certain specificity. There is no relation between the specificities listed (as is the case with the Antigen Short Tail Results table) and the reactivity of each specificity is assessed individually.

Antigen Panel List:

"Antigen Panel List" means that each well is assessed individually. Wells are listed in the order of their "relative OD values". The letter "X" shows whether or not the user has omitted wells from examination. "Well" shows the position of the well and "PanID" is its internal designation. The "Relative OD" shows how close the OD value is to the cut-off value and whether it is above (= positive rel. OD value) or below it (= negative rel. OD value). A positive rel. OD value which is above the cut-off value and is still positive after deduction of the cut-off value is a positive reaction. "Antigens" shows all the specificities in a well.

Menu functions in the Antigen Panel List:

A right-click in the Antigen Panel List opens the Panel List Menu window.

The screenshot displays the Bio-Rad Abldent software interface. The main window shows the 'Antigen Short Tail Results' for plate 051849.1.R00, with a PRA of 16wells/40wells => 40.0%. Below this, the 'Independent Antigen Statistics' are shown, sorted by R-Value. The 'Antigen Panel List' is displayed, sorted by Relative OD. A right-click context menu is open over the 'Antigen Panel List', showing options for sorting and viewing data.

Antigen Short Tail Results -- PRA = 16wells/40wells => 40.0%

Ant	Count	Sum	++	+	-	--	Incl	Cum Incl	r	Cum r	X ²	Cum X ²	Mean OD
1 A31	2	40	2	14	0	24	100%	100%	0.281	0.281	3.158	3.158	0.283
2 A28	4	38	3	11	1	23	75%	83%	0.271	0.372	2.797	5.523	0.204
3 C08	4	35	3	8	1	23	75%	80%	0.337	0.471	3.978	8.889	0.098
4 B41	1	32	1	7	0	24	100%	82%	0.311	0.526	3.097	11.055	0.122

Independent Antigen Statistics -- R-Value Sort (X = Suppressed, * = Absent)

X	Ant	Count	++	+	-	--	Incl	r	X ²	Mean OD
17+	A31	2	2	14	0	24	100.000	0.281	3.158	0.283
14+	A28	4	3	13	1	23	75.000	0.238	2.269	0.204
41+	B18	4	3	13	1	23	75.000	0.238	2.269	0.226
99+	C08	4	3	13	1	23	75.000	0.238	2.269	0.098
20+	A34	1	1	15	0	24	100.000	0.196	1.538	0.226

Antigen Panel List -- Rel OD Sort (X = Suppressed)

X	Well	Pan ID	Rel OD	= (RawOD) - (Factors)	ANTIGENS	Negative	Positive
73+	B10	1BW	1.014	= (1.409) - (0.395)	A01 A26 B55 B57	1.014	
76+	E10	7HC	0.587	= (0.925) - (0.338)	A28 A30 B18 B63		
63+	H8	53LB	0.514	= (0.880) - (0.366)	A01 A31 B27 B44		
78+	G10	71LB	0.483	= (0.843) - (0.360)	A23 A66 B18 B35		
83+	D11	LR	0.433	= (0.803) - (0.370)	A02 A24 B08 B50		
69+	F9	47LB	0.253	= (0.670) - (0.417)	A03 A33 B42 B63		
68+	E9	12DFW	0.240	= (0.515) - (0.275)	A02 A32 B39 B64		
61+	F8	DE	0.226	= (0.673) - (0.447)	A29 A34 B44 B61		
52+	E7	95CBC	0.178	= (0.629) - (0.451)	A01 A28 B07 B60		
50+	C7	BF	0.156	= (0.590) - (0.434)	A03 A28 B27 B49		
67+	D9	69LB	0.122	= (0.454) - (0.332)	A11 A30 B41 B49		
70+	G9	36CBC	0.104	= (0.537) - (0.433)	A02 A33 B62 B65		
59+	D8	5DFW	0.080	= (0.517) - (0.437)	A03 A24 B13 B51		
53+	F7	2BW	0.057	= (0.469) - (0.412)	A02 A03 B13 B56		
55+	A7	19DFW	0.052	= (0.389) - (0.337)	A02 A31 B07 B51		
48+	A7	50CBC	0.010	= (0.457) - (0.447)	A24 A29 B18 B45		
81	B11	127CBC	-0.031	= (0.365) - (0.396)	A02 A24 B13 B44		
87	H11	28LB	-0.032	= (0.378) - (0.410)	A74 A80 B42 B44		
60	E8	51LB	-0.041	= (0.381) - (0.422)	A25 A33 B53 B60		
66	C9	82CBC	-0.043	= (0.355) - (0.398)	A02 A-- B13 B38		
64	A9	62LB	-0.068	= (0.341) - (0.409)	A74 A-- B50 B70		
84	E11	117CBC	-0.076	= (0.366) - (0.442)	A11 A26 B38 B60		
80	A11	98CBC	-0.080	= (0.348) - (0.428)	A03 A24 B35 B61		
82	C11	108CBC	-0.091	= (0.312) - (0.403)	A01 A25 B37 B44	C05 C06 W04 W--	-0.091

PANEL LIST MENU

- PANEL LIST -- SORT BY:
 - Tail Antigens/Cregs Ctrl+T
 - Selected Antigens/Cregs Ctrl+S
 - Relative Optical Density Ctrl+O
 - Raw Optical Density Ctrl+R
 - Unsorted Ctrl+U
- View +/- Blank Control Wells Ctrl+V
- View Naw & Mab Wells Ctrl+M
- Display Raw Optical Density Ctrl+Z
- Normalize Histogram Ctrl+N
- Increase Cutoff Factor from 2.000 Ctrl+I
- Decrease Cutoff Factor from 2.000 Ctrl+D
- Reset Cutoff Factor to 2.000 Ctrl+F
- Use Antigens Ctrl+A
- Use Cregs Ctrl+C
- Print Panel Window Ctrl+P

- "Tail Antigens/Cregs" highlights the specificities shown in the Short Tail Results.
- Under "Selected Antigens/Cregs", individual antigens or Cregs can be highlighted.
- "Relative Optical Density" sorts antigen wells by their measured relative optical density.
- "Raw Optical Density" sorts antigen wells by their measured values for optical density.
- "Unsorted" sorts antigen wells the way they are arranged on the microplate.
- Selecting "View +/- / Blank Control Wells" will include the OD values for control wells in the list.
- Selecting "View Naw & Mab" will include the No-Antigen Well (Naw) and the well with only the monoclonal capture antibody (Mab – only for HLA Class II) in the list.

-
- "Display Raw Optical Density" shows the measured OD values.
 - "Normalize Histogram" shows all the OD values in relation to the OD value of the positive control sample. If this option is not activated, all the OD values will be shown relative to 4.0.
 - "Increase or Decrease Cut-off Factor from ..." raises or lowers the cut-off factor in such a way that the last positive microplate position becomes negative or the first negative microplate position becomes positive.
 - "Reset Cut-off Factor to 2.000" resets the cut-off factor to its default value of 2.000.
 - Selecting either "Use Antigens" or "Use Cregs" allows the user to choose between the evaluation method.
 - "Print Panel Window" prints the Antigen Panel List.

4.5 "Report" tab

Sample result reports can be generated in the "Report" tab. To do so, do a right-click on the white field. The following menu appears:

Bio-Rad AbIdent

File View Configure Explore Colors About

Sample Info | Plate (051849.1.R00) | Test Parameters | Explore | Report

ANTIGEN REPORT - 051849.1.R00 - 05/03/2010 14:16

Bio-Rad Medical Diagnostics GmbH
Industriestraße 1, D-63303 Dreieich,
Telephone: +49 6103 801-807

Preliminary HLA Class I Antigen Report - Subject to Supervisor Review

Sample ID - 051849.1.R00 **Technical**

Patient Name: NONE **Patient ID:** NONE
Extra Field 1: NONE **Extra Field 2:** NONE
Patient Phenotype: NONE

DATES **FILES**
Draw Date: Not Used **Lot #:** 021004R1.QID
Receive Date: Not Used **Antigen File:** Comb.Ant
Elisa Date: 23/10/2003 19:06:13 **Creg File:** Std2.Crg

Mean of 4 negative control(s) = 0.243 -> A12=0.257, B12=0.250, C12=0.250
Mean of 1 positive control(s) = 1.856 -> E12=1.856
Suppr. Phenotype: NO
Suppr. Antigens: NO
Test Temp °C = NONE **NAW** = F12 =NAW -0.387 **MAb** = NONE

Test Notes
 NONE

 • AbIdent Exe Directory: C:\Programme\Bio-Rad\BABID52D\ (Rev. 52.4.3.877)
 • Bio-Rad File Directory: C:\Programme\Bio-Rad\BABID52D\LotFiles\
 • Sample File Directory: C:\Programme\Bio-Rad\BABID52D\SampleFiles\

Antigen Short Tail Results
 PRA = 16wells/40wells => 40.0%

Antigen	Cnt	Sum	++	+-	-+	--	Inc%	CIn%	r	Cum r	X2	Cum X2	Avg. OD
A31	2	40	2	14	0	24	100%	100%	0.281:r	0.281:r	3.16:X2	3.16:X2	0.283:OD
A28	4	38	3	11	1	23	75%	83%	0.271:r	0.372:r	2.80:X2	5.52:X2	0.204:OD
C08	4	35	3	8	1	23	75%	80%	0.337:r	0.471:r	3.98:X2	8.89:X2	0.098:OD
B41	1	32	1	7	0	24	100%	82%	0.311:r	0.526:r	3.10:X2	11.06:X2	0.122:OD
B18	3	31	2	5	1	23	67%	79%	0.345:r	0.578:r	3.69:X2	13.35:X2	0.105:OD
B56	1	29	1	4	0	24	100%	80%	0.414:r	0.632:r	4.97:X2	16.00:X2	0.057:OD
B57	1	28	1	3	0	24	100%	81%	0.471:r	0.688:r	6.22:X2	18.91:X2	1.014:OD

(Remaining inclusions and/or r-values are <= their cutoff values)

There are five possible displays to choose from:

Report Style A and Report Style B:

These report types contain first of all the "Antigen Panel List" and, below that, the "Antigen Short Tail Results". The difference between Type A and Type B is the target printer (laser or colour printer).

Report Style C

This report type contains "Antigen Short Tail Results" first, followed by the "Antigen Panel List".

Report Style D

This report type only includes the "Antigen Short Tail Results".

Printout

Results are printed by right-clicking and selecting <Print Report>.

4.6 Other tabs

In the "View" menu further tabs can be displayed besides the tabs shown by default (chapter 4.1 to 4.5).

- "Lot Number.Qid/Pan" tab

The lot-specific microplate assembly is displayed.

QID File

	1	2	3	4	5	6
A	50CBC	LA	62LB	59LB	98CBC	-NC
A	0.918	0.877	0.839	0.740	0.879	1.000
B	48LB	7LB	27LB	1BW	127CBC	-NC
B	1.028	0.930	0.919	0.811	0.813	1.000
C	BF	21DFW	82CBC	2HC	108CBC	-NC
C	0.891	0.820	0.818	0.987	0.828	1.000
D	15LB	5DFW	69LB	19LB	LR	-NC
D	0.816	0.897	0.682	0.792	0.760	1.000
E	95CBC	51LB	12DFW	7HC	117CBC	+PC
E	0.926	0.866	0.564	0.695	0.908	1.000
F	3DFW	6F	47LB	3CBC	80	NAU

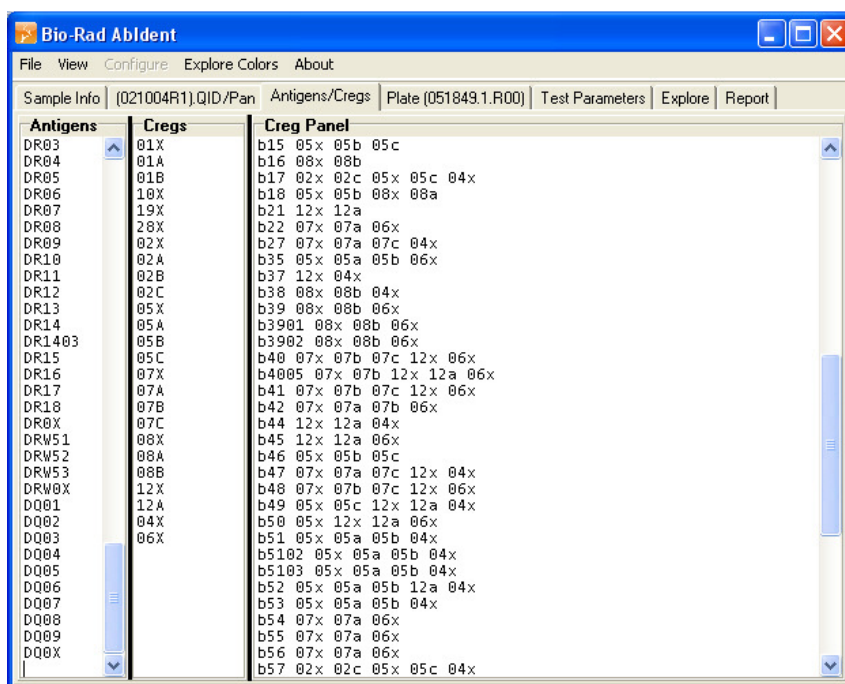
Panel File

This blank line is needed.

021004-1	AAA	AAA	BBB	BBB	CCC	CCC	BW4	BW6	COMMENTS CAN BE PUT IN THESE COLUMNS
50CBC	A24	A29	B18	B45	C12	C16	W--	W06	
48LB	A23	A36	B07	B58	C04	C07	W04	W06	
BF	A03	A28	B27	B49	C02	C07	W04	W--	
15LB	A23	A80	B45	B81	C16	C18	W--	W06	
95CBC	A01	A28	B07	B60	C03	C07	W--	W06	
2BW	A02	A03	B13	B56	C01	C06	W04	W06	
17LB	A01	A66	B52	B58	C07	C12	W04	W--	
19DFW	A02	A31	B07	B51	C07	C15	W04	W06	
LA	A03	A32	B18	B37	C05	C06	W04	W06	
7LB	A11	A26	B27	B58	C02	C03	W04	W--	
21DFW	A29	A66	B07	B49	C07	C07	W04	W06	
5DFW	A03	A24	B13	B51	C06	C15	W04	W--	
51LB	A25	A33	B53	B60	C03	C04	W04	W06	
DE	A29	A34	B44	B61	C04	C08	W04	W06	

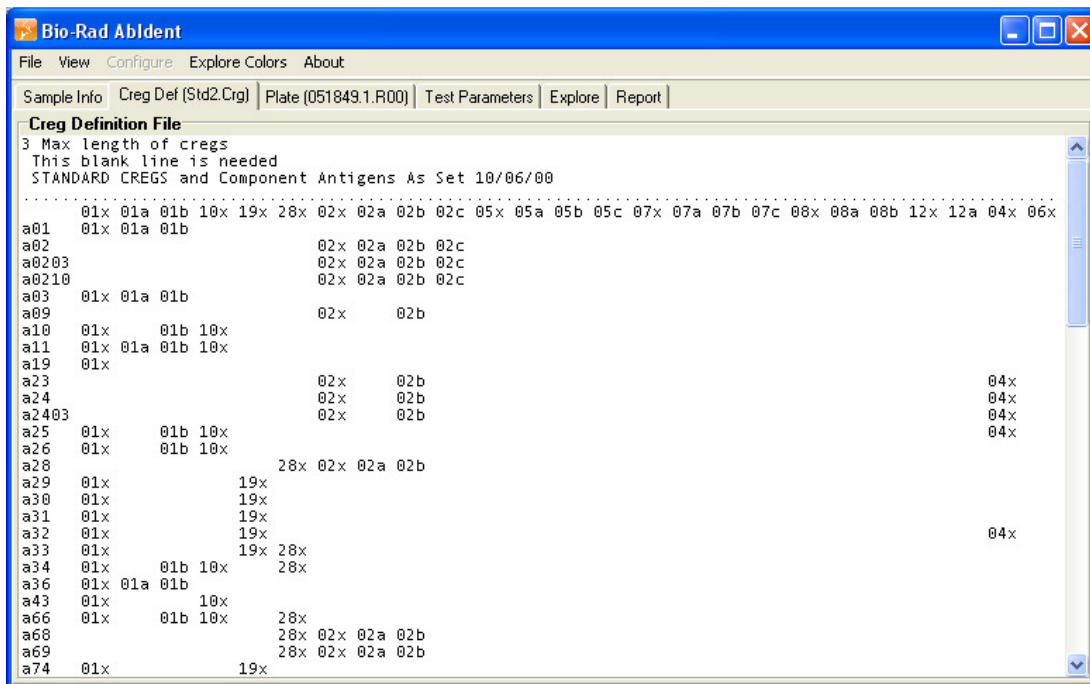
- "Antigens/Cregs" tab

Antigens and Cregs are listed.



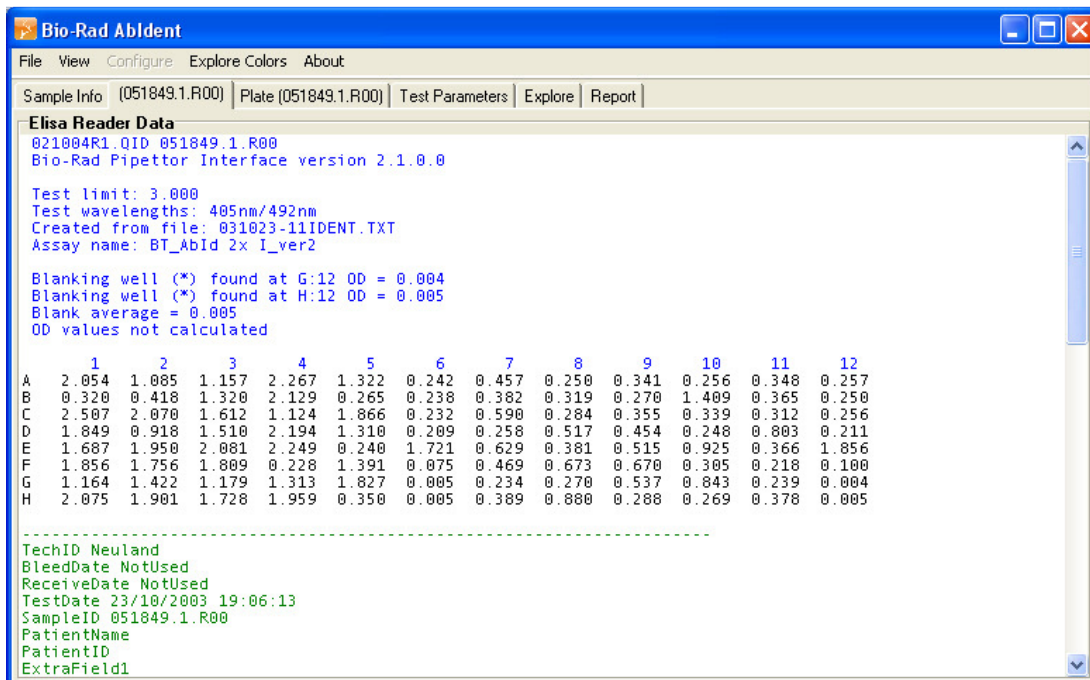
- "Creg Definition" tab

Antigens are assigned to the respective cregs.



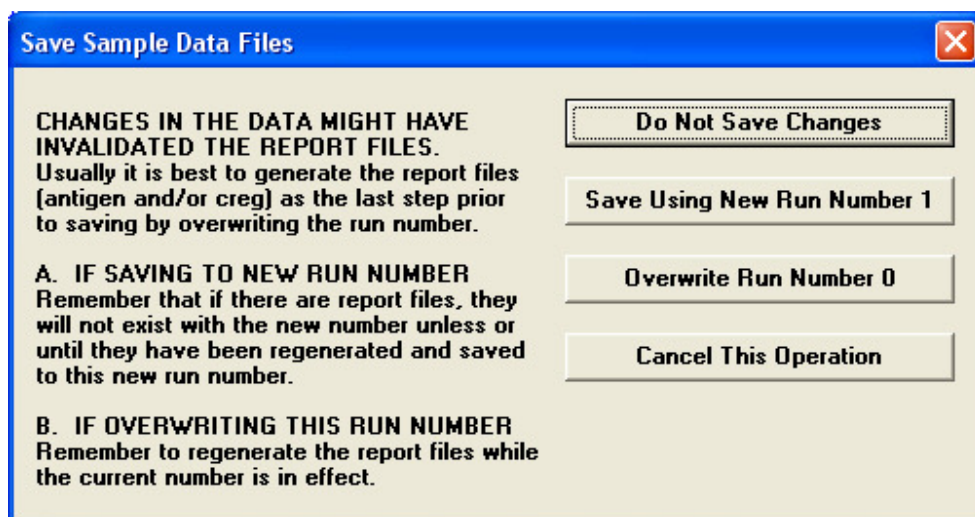
- "ELISA Data File" tab

Reader data for the tested sample is shown.



4.7 Saving results

If a new sample is imported or the program is closed, the user is automatically asked whether the changes are to be saved and in what format:



Do Not Save Changes:

Changes are not saved. If the sample is reopened, the user will be presented with the default settings as they were when the sample file was imported.

Save Using New Run Number 2:

The number of the run is incremented by one. Modifications are saved and the original file is left untouched.


Overwrite Run Number 0:

Modifications are saved under the same file name, which means the original file will be overwritten.

Cancel This Operation:

Aborts the action without saving modifications.

4.8 Exiting the program

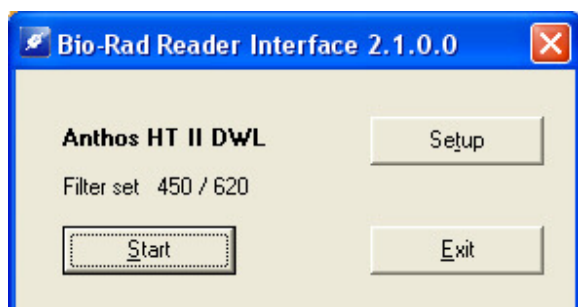
- Either use the standard Windows method by clicking the  in the top right-hand corner or
- Navigate to "File" - "Exit"

5. Appendix

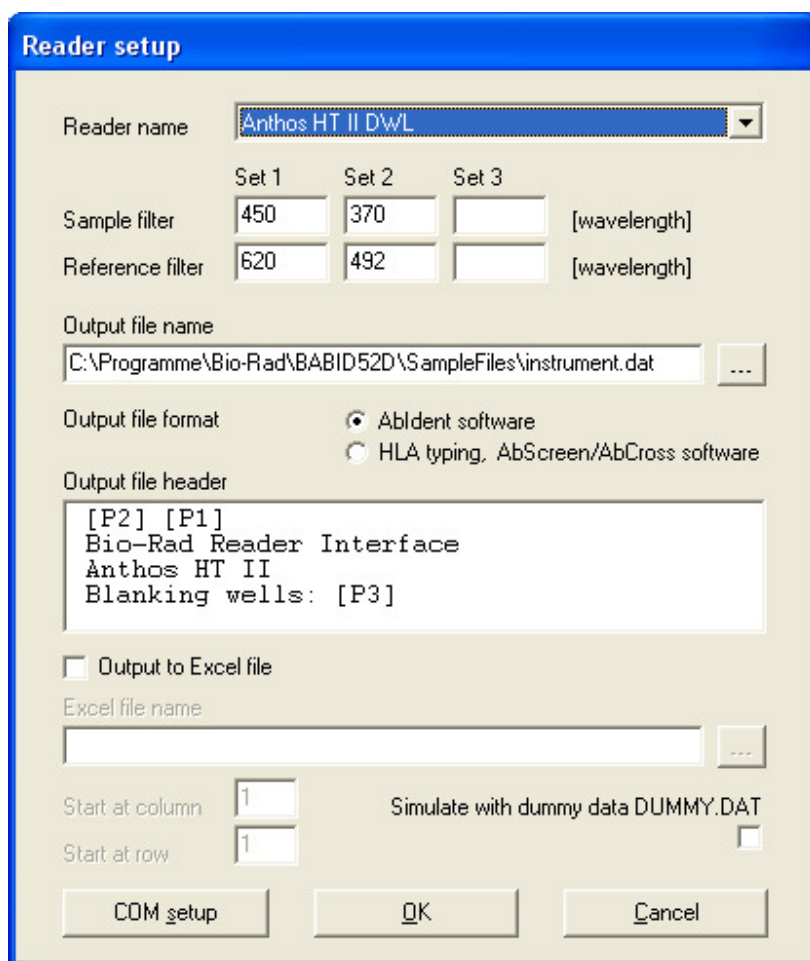
5.1 Configuring the reader

You may configure the Reader settings. **This is normally the task of the Bio-Rad product specialists!** If you modify these settings without contacting Bio-Rad first, Bio-Rad cannot accept any guarantee for error-free functioning of the interface with the Reader!

Navigate to "File – Start Class I Sample or Start Class II Sample – manual entry of the microplate number and selection of the lot number" – select <Read Plate> - you will see the Reader window:

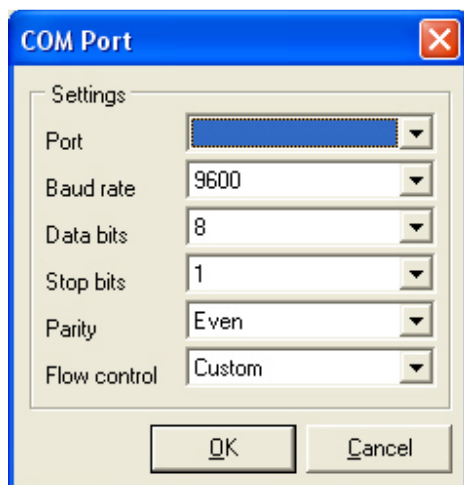


Select <Setup> to access the setup menu.



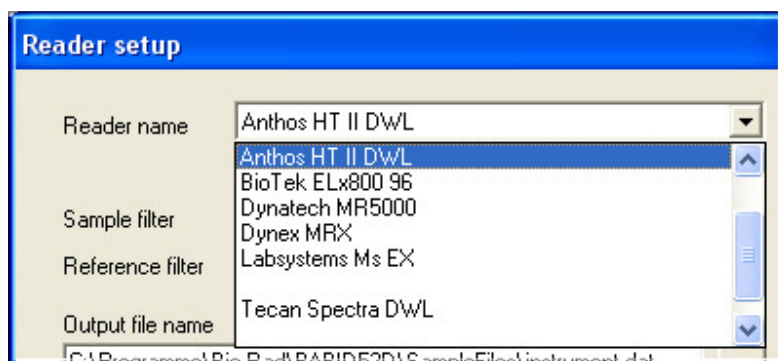
The required settings for the filter and output file(s) can be set here.

The COM settings can be accessed via a different button:



Save and leave the Reader changes by clicking <OK>

You currently have a choice of 7 readers.



Note:

Please also refer to the recommendations relating to Readers in the chapter on System Requirements.

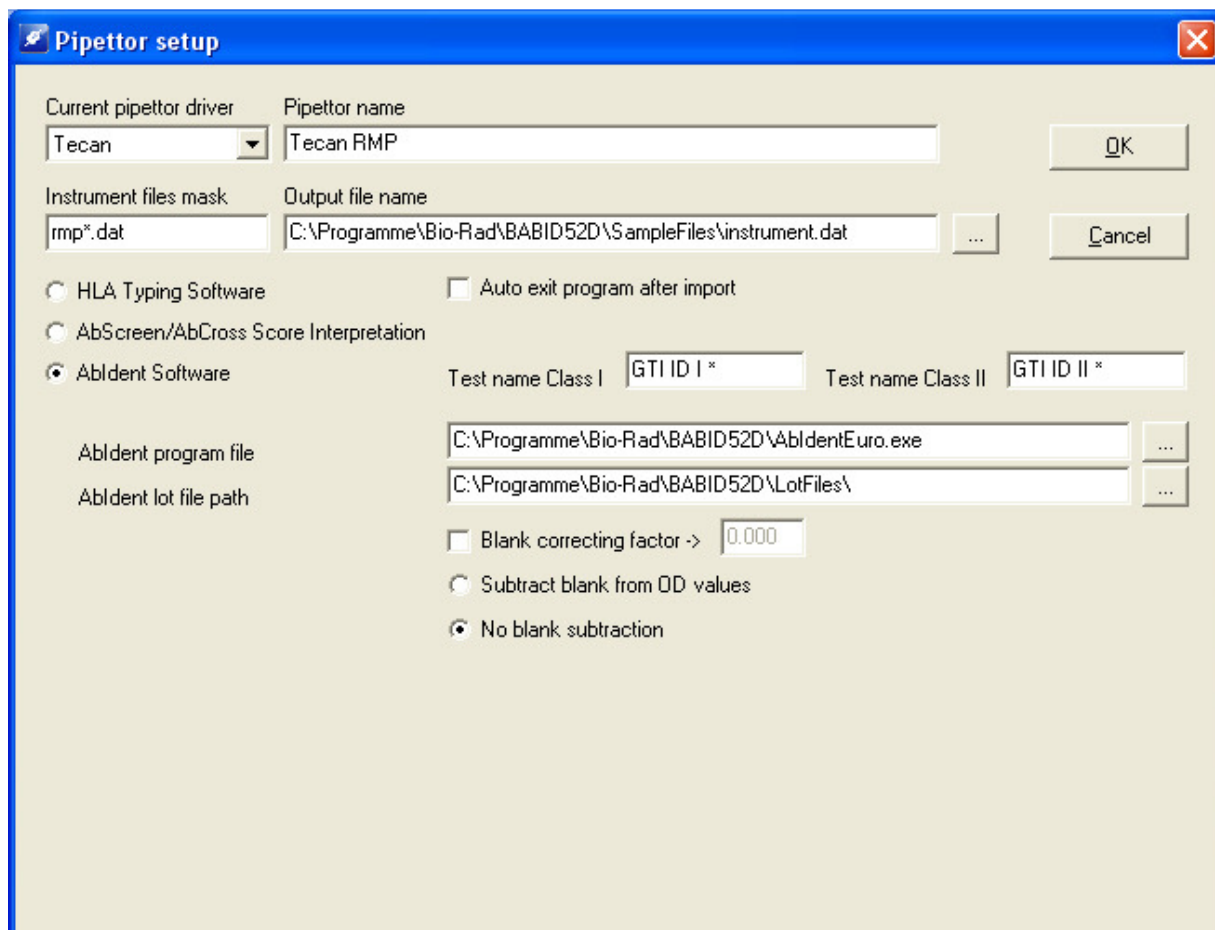
5.2 Configuring the pipettor

Before you can take over pipettor data, the Abldent software has to be configured in such a way that the paths, microplate names and microplate assignments tally with the results produced by the pipettor. When the software is installed, the most typical settings are selected. However, changes may be made as required.

This is normally the task of the Bio-Rad product specialists! If you modify these settings without contacting Bio-Rad first, Bio-Rad cannot accept any guarantee for error-free functioning of the interface with the pipettor! Our customer services staff will be pleased to assist you.

5.2.1 Settings: Tecan

After starting the program version Bio-Rad Abldent xxxx (pipettor), selecting the <Setup> option takes the user to the dialogue screen for pipettor settings. The required settings can now be made:



Pipettor name: Enter the name of the pipettor

Data input mask: Tecan: rmp*.dat
Quickstep: *.txt
BepIII: *.

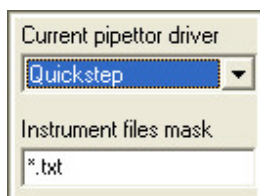
Input file path: normally the import folder of the Abldent software

Output file name: This file is generated by the software itself. It is expected to be in the import folder and must be named "instrument.dat".

5.2.2 Settings: Quickstep

The settings for Quickstep are identical to those for Tecan, with two minor exceptions:

- The driver is called "Quickstep" and the file name is *.txt



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