

# BD HLA-B27

## Application Guide

### for BD FACSCanto Flow Cytometers

**IVD** For In Vitro Diagnostic Use



<http://www.bdbiosciences.com/>  
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## Patents

PE: US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942

BD Trucount tubes: US 5,723,218 and 5,187,288

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## History

Revision	Date	Change Made
Part No. 343366 Rev. A	11/05	Initial release

# Contents

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Introduction .....	5
Installing the BD HLA-B27 Application Module .....	6
Uninstalling the BD HLA-B27 Application Module .....	7
Running HLA-B27 Setup .....	7
Understanding the Application Setup Report .....	15
Acquiring Samples .....	16
Analyzing Samples .....	21
Understanding the HLA-B27 Lab Report .....	24
Lab Manager Options .....	26
Changing Acquisition Targets .....	26
Resetting Alarm Ranges .....	28
Troubleshooting .....	29
Performance .....	31



# Introduction

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The BD™ HLA-B27 assay is used to screen for the presence of the HLA-B27 antigen on lymphocyte surfaces. Presence of this antigen correlates with ankylosing spondylitis and other disorders, including Reiter's syndrome, psoriatic arthritis, and inflammatory bowel disease.

This guide provides instructions for installing and running the BD HLA-B27 application for BD FACSCanto™ clinical software. Before using this guide, make sure you are familiar with Cytometer Setup and general cytometer functions, as described in *BD FACSCanto Instructions for Use*.


This guide contains the following information:

- Installing the BD HLA-B27 Application Module on page 6
- Running HLA-B27 Setup on page 7
- Acquiring Samples on page 16
- Analyzing Samples on page 21
- Lab Manager Options on page 26
- Troubleshooting on page 29
- Performance on page 31

# Installing the BD HLA-B27 Application Module



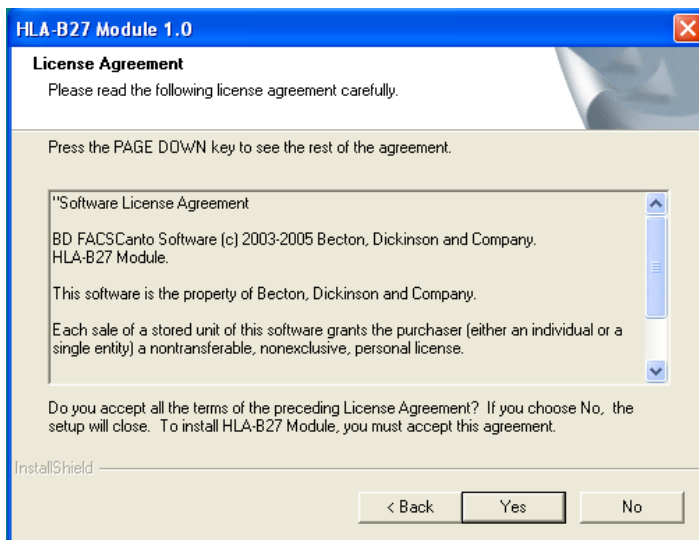
The BD HLA-B27 application module requires BD FACSCanto clinical software v2.0 or later to install and run properly. Installing the module with an earlier version of BD FACSCanto clinical software will not work properly (though no error message will be generated).



- 1 Verify that BD FACSCanto clinical software v2.0 or later is installed on your system.
- 2 Insert the BD HLA-B27 Software Module installation CD into the CD-ROM drive.
- 3 Use Windows Explorer to view the CD contents, and double-click the (  ) setup.exe icon.

The InstallShield Wizard appears.

- 4 Click  to begin installation.




The License Agreement appears.



- 5 Review and accept the license agreement; click .
- A progress message appears, and installation begins.
- 6 Click  when the installation complete message appears.
- 7 Return the CD to the pocket of this application guide for storage.

## Uninstalling the BD HLA-B27 Application Module

Uninstalling the BD HLA-B27 application module will not remove your data files.

- 1 From the Windows Start menu, choose Settings > Control Panel > Add or Remove Programs.
- 2 Select the HLA-B27 application module, and click .
- 3 Click  to confirm.
- 4 Follow the prompts on the screen to remove all installed components; click .
- 5 Close the Add or Remove Programs window.

## Running HLA-B27 Setup

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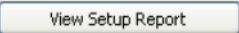
- 1 Start up the computer, cytometer, and software; log in to BD FACSCanto clinical software.
- 2 Prepare BD FACSTM 7-color setup beads and BD™ HLA-B27 calibration beads from the BD HLA-B27 kit as instructed in the appropriate package insert.

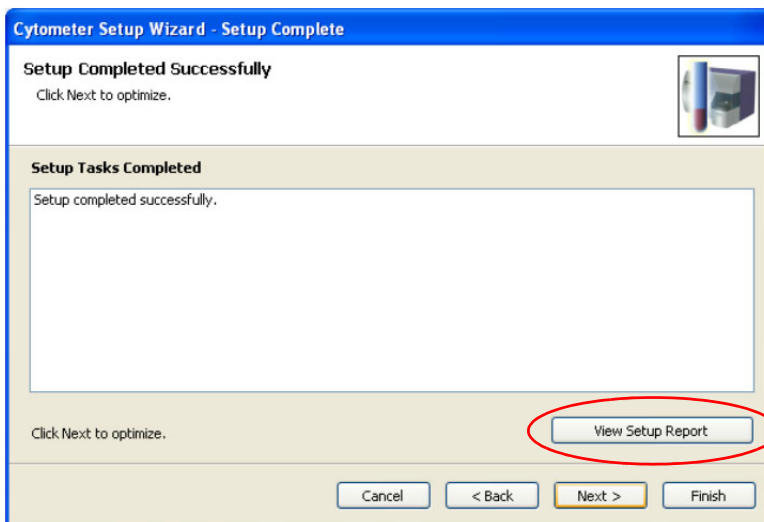



For dilution of the 7-color setup beads, use only the buffer provided with the beads. Incorrect setup of the instrument can result if other buffers are used.

- 3 Run Cytometer Setup using the BD FACS 7-color setup beads.

For details, refer to *BD FACSCanto Instructions for Use*.

- 4 At the end of Cytometer Setup, click  and confirm that setup was completed successfully; close the report to return to the Wizard.



- 5 Click  to proceed with optimization.

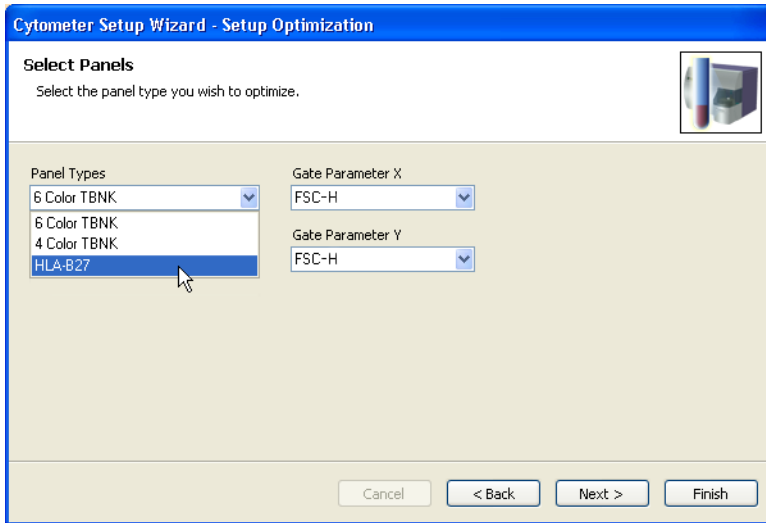
The Setup Optimization dialog appears.

- 6 Select HLA-B27 from the Panel Types drop-down menu.

See Figure 1 on page 9.

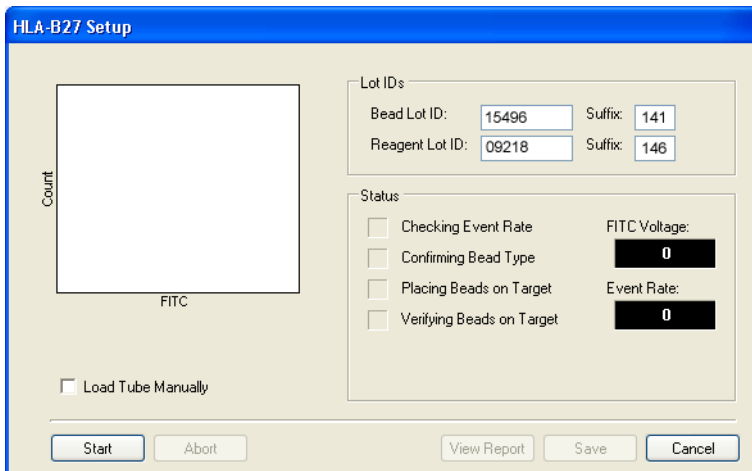


**Figure 1** Setup Optimization dialog



7 Click **Next >**.

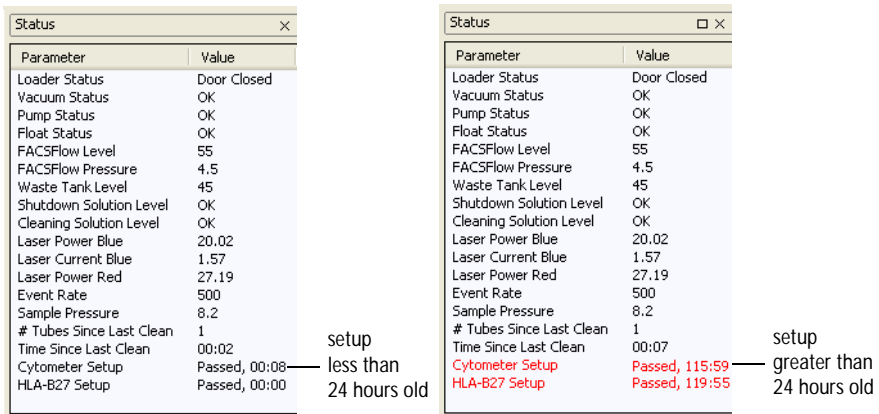
The HLA-B27 Setup dialog appears.



You can also access the HLA-B27 Setup dialog from the main menu by choosing Cytometer > Setup > HLA-B27 Setup. To run HLA-B27 Setup, the Cytometer Setup should have passed and should be less than 24 hours old. The Cytometer Setup and HLA-B27 Setup status and age appear in the Status window (Figure 2). The Cytometer Setup and the HLA-B27 Setup text turns red if the setup is greater than 24 hours old or if you accepted or saved a failed setup.

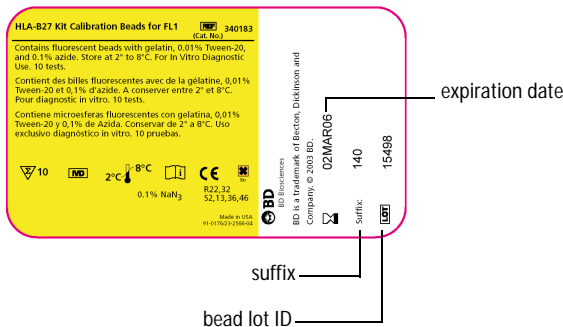
BD Biosciences recommends that you run Cytometer Setup every 24 hours.

**Figure 2** Status window and location of Cytometer Setup information





## 8 Enter a bead lot ID and suffix into the appropriate fields.

The bead lot ID and suffix are printed on the HLA-B27 bead label.



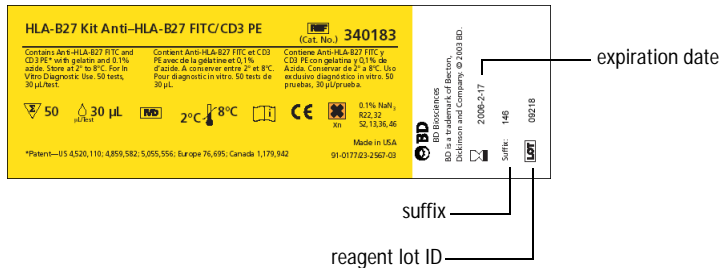
The bead suffix is the target value for setup with the HLA-B27 beads.

 Make sure you enter the correct bead suffix. Entering it incorrectly can cause erroneous test results.


 Always check the expiration date. Using a reagent beyond its expiration date can cause erroneous test results.


9 Enter a reagent lot ID and suffix into the appropriate fields.

The reagent lot ID and suffix are printed on the reagent label.



The reagent suffix is the marker that will separate HLA-B27-positive from HLA-B27-negative samples.

 Make sure you enter the correct reagent suffix. Entering it incorrectly can cause erroneous test results.

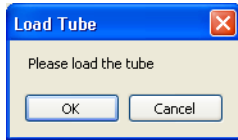
 Always check the expiration date. Using a reagent beyond its expiration date can cause erroneous test results.

10 (Optional) If you do not plan to use the BD FACS Loader, select *Load Tube Manually*.

11 Click  .

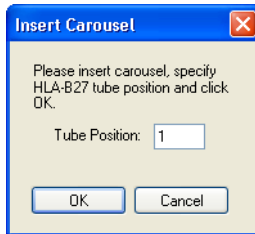
Follow the instructions for the loading method you are using.

- (Manual loading) The Load Tube dialog appears.



Load the HLA-B27 bead tube and click  .

- (Automatic loading) The Insert Carousel dialog appears.

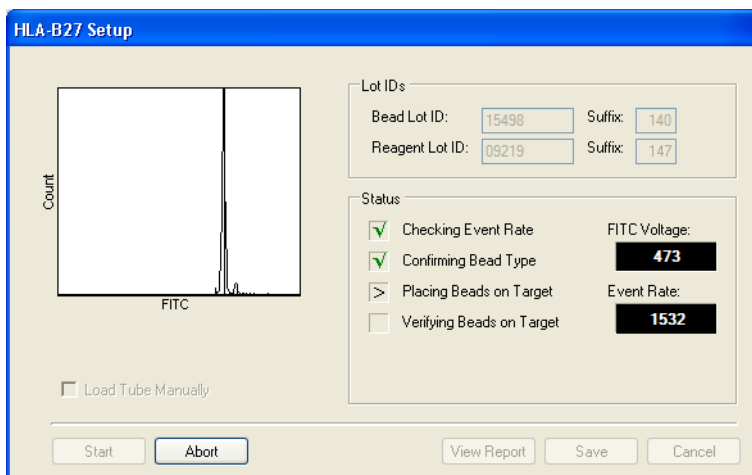


Specify the HLA-B27 bead tube position and click  .

12 Follow setup progress in the HLA-B27 Setup dialog.

See Figure 3 on page 13.

Figure 3 HLA-B27 Setup dialog

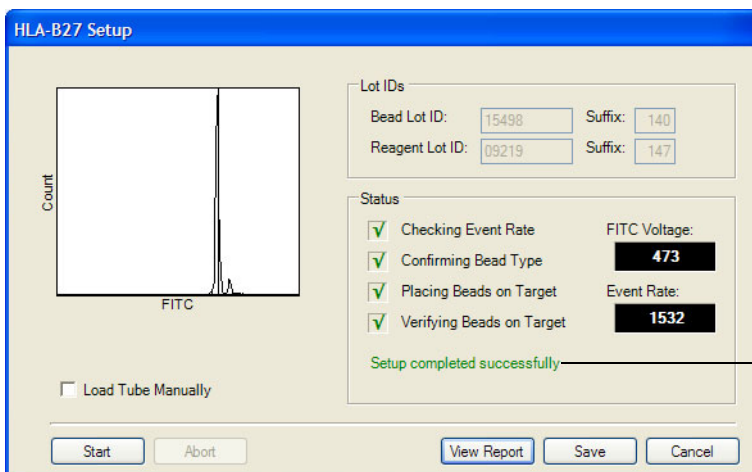


symbols key

- completed
- in process
- undone

Setup is completely automated, with no threshold or spectral overlap adjustments necessary. During setup, the software places the calibration beads at the required channel (target value) in the FITC detector and alters the FITC voltage from Cytometer Setup, recalculating compensation for the new FITC voltage.

When setup finishes, a message appears in the dialog.



completion message

If setup is not successful, note the message and see Troubleshooting on page 29.

- 13 If you are running samples manually, unload the tube when prompted.




**It is critical that you follow the tube removal sequence exactly.** Failure to follow this sequence could result in carryover between samples.

- Hold the tube while moving the aspirator arm all the way to the left.
- Remove the tube from the SIT.
- Release the aspirator arm and wait for SIT cleaning to finish.

- 14 (Optional) Click View Report to view the Application Setup Report for HLA-B27.

View the report now, or view it later. The software automatically saves setup reports to a PDF file and stores them at C:\Program Files > BD FACSCanto Software > SetupReports > HLAB27\_yyyyMMdd\_hhmm.pdf, where yyyy=year, MM=month, dd=date, hh=hour, and mm=minute. See Understanding the Application Setup Report for more information.

- 15 Click  to save the setup results.

The HLA-B27 setup results are saved to C:\Program Files\Common Files\BD\Setup Results\HLA-B27.opt.



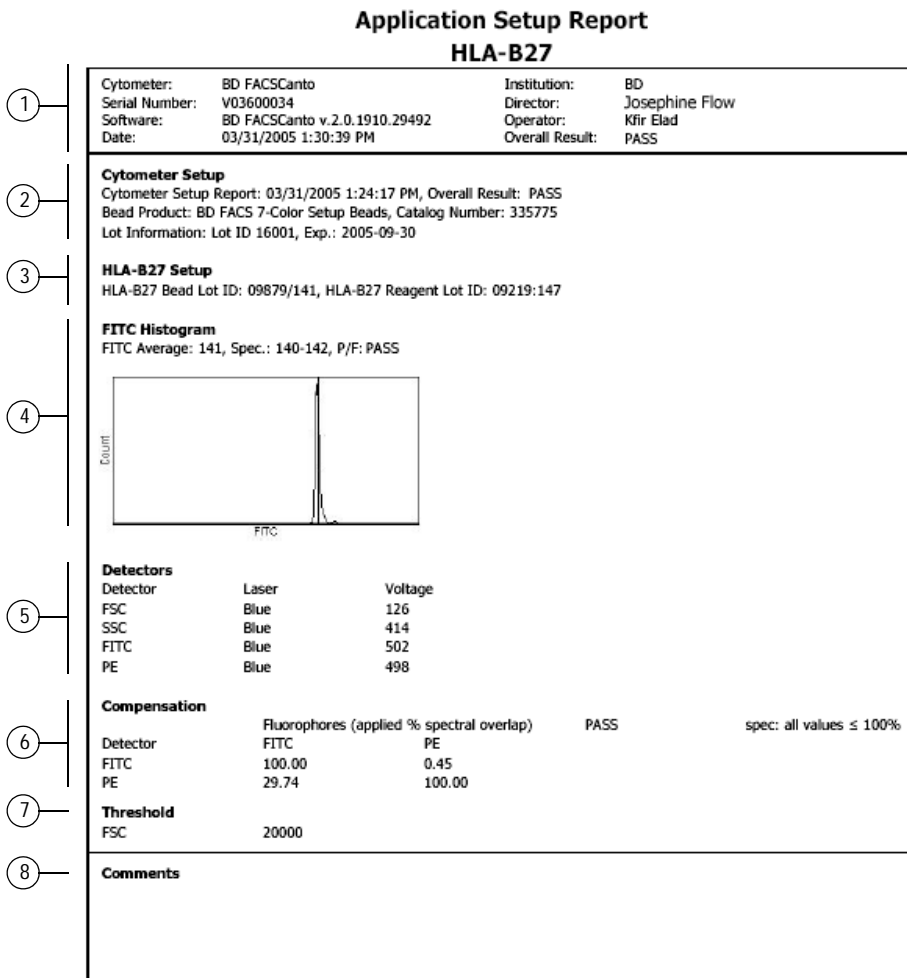
The software uses the HLA-B27.opt file to initiate HLA-B27 setup. Do not move or rename the file.

- 16 Click  after setup is complete.

# Understanding the Application Setup Report

The HLA-B27 Setup generates an application-specific setup report. For an example setup report and an explanation of setup report elements, see Figure 4.

**Figure 4** Example Application Setup Report for HLA-B27



①	The <b>Report Header</b> contains basic cytometer and operator information, as well as an overall Pass or Fail. For a Pass, both parts of HLA-B27 Setup must pass (FITC Histogram and Compensation sections).
②	<b>Cytometer Setup</b> contains information about the Cytometer Setup, upon which HLA-B27 Setup is based. The Pass/Fail status of the Cytometer Setup is shown here.
③	<b>HLA-B27 Setup</b> uses BD HLA-B27 beads and contains bead and lot information.
④	<b>FITC Histogram</b> shows the placement of HLA-B27 beads after setup.
⑤	<b>Detectors</b> shows final voltages resulting from setup.
⑥	<b>Compensation</b> displays spectral overlap values automatically calculated for the voltages listed in the Detectors section.
⑦	<b>Threshold</b> indicates the detector and intensity value used to exclude unwanted events during sample acquisition.
⑧	<b>Comments</b> provides an area to write additional information on an Application Setup Report after you print the report.

## Acquiring Samples

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For details about cytometer behavior or options during acquisition, refer to *BD FACSCanto Instructions for Use*.

- 1 Stain samples with Anti-HLA-B27 FITC/CD3 PE reagent (from the BD HLA-B27 kit) as instructed in the appropriate package insert.



Use only the Anti-HLA-B27 FITC/CD3 reagent for this assay, to prevent incorrect results.

- 2 Choose appropriate assay options, including Lab Manager options.

See page 26 for information on setting Lab Manager options.



### 3 Prepare a worklist.

- Enter information into the required fields.
- From the Panel menu, select HLA-B27 for all appropriate samples.


See Figure 5. For details, refer to *BD FACSCanto Instructions for Use*.

**Figure 5** Example worklist with HLA-B27 samples

Worklist   Levey-Jennings											
#	Name	Demographics			Panel Information			Acquisition			
		ID	Case Number	Panel	Col...	Col...	Col...	Carousel	Posi...	Status	FCS File
001	Baggins, Bilbo	Sample 01		HLA-B27				1	1 - 1	Ready To Run	
002	Sauron, Melvin	Sample 02		HLA-B27				1	2 - 2	Ready To Run	
* 003	Smigel, Rufus T	Sample 03		HLA-B27				1	3 - 3	Ready To Run	
*											

↓                          ↓                          |  
\*                                    \*                          enter additional information here (optional)  
\* required fields

### 4 (Automatic loading only) Put the tubes into the Loader according to the order listed in the worklist.

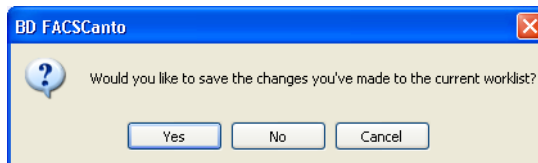
 **To prevent incorrect results, make sure the tube order in the carousel matches the worklist.**

### 5 (Automatic loading only) Install the Loader carousel with sample tubes.

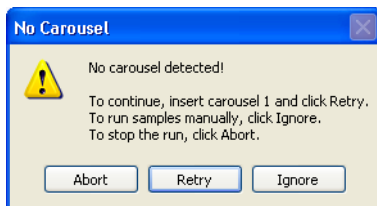
The Loader mixes the sample tubes at the start of a carousel. After that, it mixes periodically.

### 6 Click .

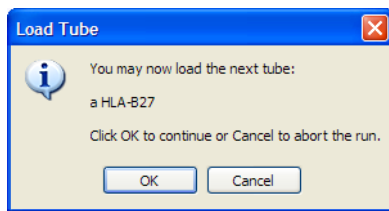
A dialog appears.



- Click  to save the worklist changes. In the Save As dialog that appears, name the worklist and navigate to a storage folder. Click Save.
  - Click  No to continue without saving.
  - Click  Cancel to discontinue the worklist run.
- 7** (Manual loading only) If the Loader is installed, you will see the No Carousel dialog; click . Otherwise, go to step 8.



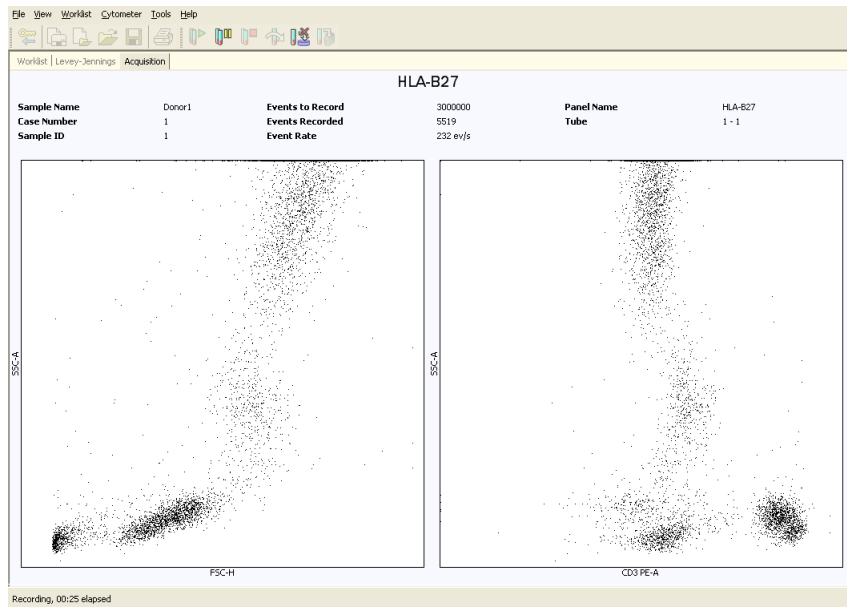
- 8** (Manual loading only) At the Load Tube dialog, mix the first sample tube manually, and then install it on the cytometer; click .



- 9** Observe as the cytometer begins acquiring samples.







See Figure 6 on page 19.

**Figure 6** Acquisition view



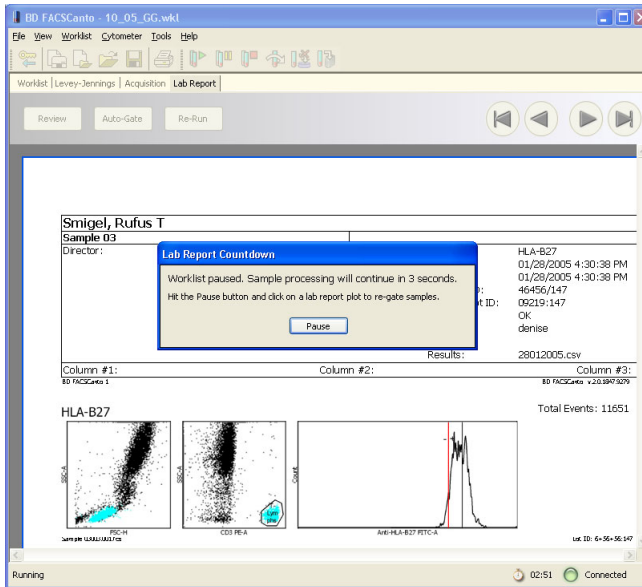
- 10 The cytometer uses a medium flow rate (60  $\mu\text{L}/\text{min}$ ). When the minimum number of T lymphocytes (default 2000) have been recorded or the maximum time (default 250 seconds) has been reached, the software stops recording.

Use buttons to perform the following functions.

Button	Function	Button	Function
	pause a sample		skip a sample
	end recording of a sample		stop the worklist
	resume acquisition		optimize instrument settings button disabled when running samples

After the sample has been recorded, the Lab Report view appears with an automated analysis. See Figure 7.

**Figure 7** Lab Report view with Lab Report Countdown dialog



You can pause the Lab Report countdown, check the sample, and manually re-gate the sample now, or you can wait until all samples have been recorded, and analyze them later, if required (see Analyzing Samples on page 21).

**!** Manually re-gating can alter sample results.

For instructions on pausing the Lab Report countdown, refer to *BD FACSCanto Instructions for Use*.

- 11 (Manual loading) When prompted, remove the sample tube and install the next tube; repeat until data for all tubes has been recorded.



**It is critical that you follow the tube removal sequence exactly.** Failure to follow this sequence could result in carryover between samples.

- Hold the tube while moving the aspirator arm all the way to the left.
- Remove the tube from the SIT.
- Release the aspirator arm and wait for cleaning to finish.

## Analyzing Samples

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To view and analyze files after acquisition and recording has finished, use the following steps.

- 1 Inspect the Lab Report for each sample in the worklist.

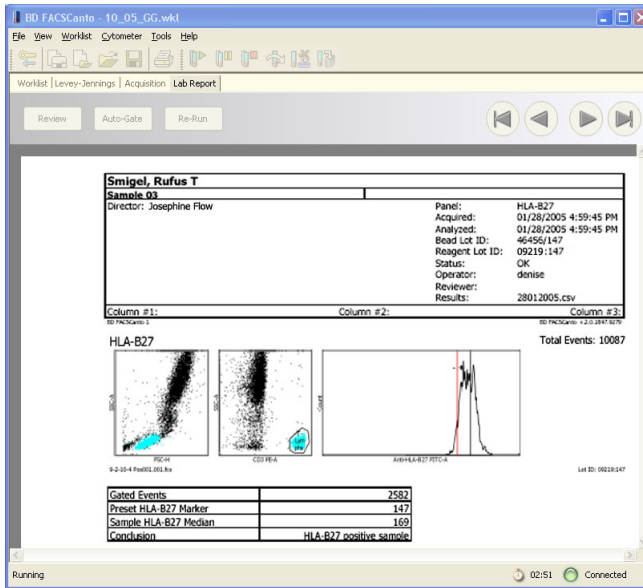
Double-click a Status field to view the Lab Report.

Worklist   Levey-Jennings										
#	Name	ID	Case Number	Panel	C	C	Carousel	Position	Status	FCS File
001	Beggins, Bilbo	Sample 01		HLA-B27			1	1 - 1	OK	Sample 01_b001.001.fcs
002	Sauron, Melvin	Sample 02		HLA-B27			1	2 - 2	OK	Sample 02_b002.001.fcs
003	Smigel, Rufus T	Sample 03		HLA-B27			1	3 - 3	OK	Sample 03_b003.001.fcs

double-click to see Lab Report for sample 001

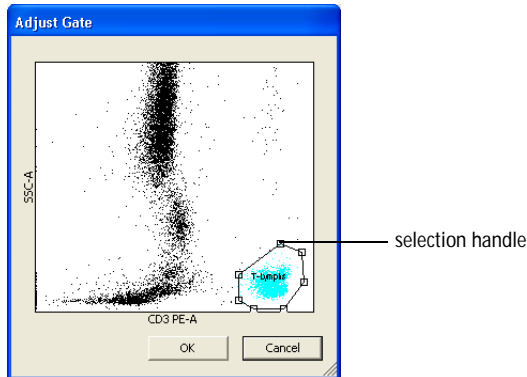
The Lab Report view appears. See Figure 8 on page 22.

**Figure 8** Lab Report view



- 2 Inspect the plots for expected patterns.
- 3 (Optional) Click the second plot to adjust its expert (automatic) lymph gate, if it is necessary.

The selected plot appears in an enlarged view.



4 Adjust the gate.




Manually re-gating can alter sample results.

- Select the gate by clicking on the gate boundary.
- Drag the selection handle to adjust the shape or size of the gate.
- Drag the gate boundary between selection handles to move the gate.

Gate changes apply only to the current tube.

5 Click .

6 Click  to advance to the next sample's Lab Report.

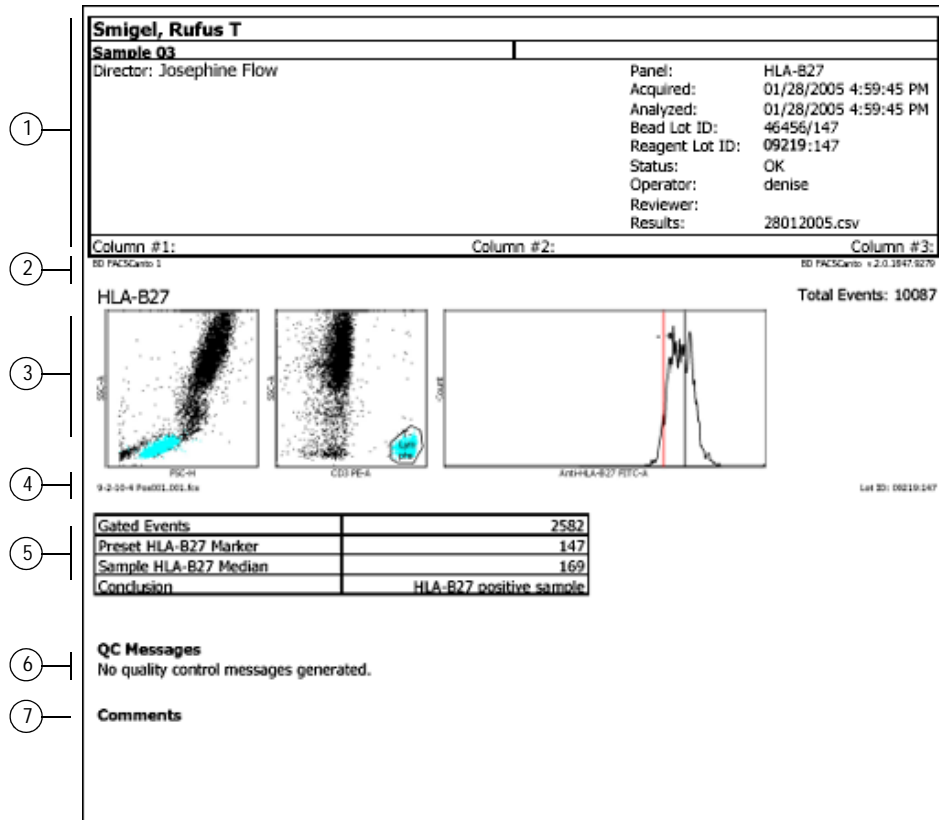
7 Print Lab Reports for any re-gated samples, if necessary.

During analysis, you can also reapply the Auto-Gate (default expert lymph gate) and Review samples. For directions, refer to *BD FACSCanto Instructions for Use*.

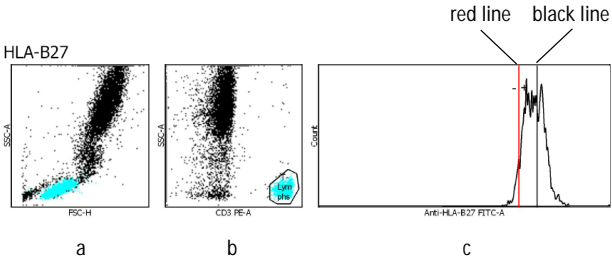
# Understanding the HLA-B27 Lab Report

You can view, export as a PDF file, or print a Lab Report for every analyzed sample in a worklist. Figure 9 shows a sample HLA-B27 Lab Report.

**Figure 9** Lab Report example





①	The <b>Report Header</b> contains basic operator and sample information chosen by a lab manager. Values entered in Column 1, 2, or 3 of the worklist will appear here as well.
②	The <b>Cytometer</b> and <b>Software</b> fields (printed in small type) show the cytometer serial number and BD FACSCanto clinical software version.
③	<p>This section shows the plots and the analyzed data. The gating strategy is as follows:</p>  <p>HLA-B27</p> <p>a                      b                      c</p> <ul style="list-style-type: none"> <li>• Plot a—(FSC v SSC) shows all events; blue events are T lymphocytes (from the gate in plot b)</li> <li>• Plot b—(CD3 PE v SSC) shows an expert gate around the T lymphocytes</li> <li>• Plot c—(histogram) shows the Anti-HLA-B27 FITC events from the T-lymphocyte expert gate; the histogram uses a Log Median Fluorescence (LMF) scale of 0 to 256. <ul style="list-style-type: none"> <li>- Black line—the median fluorescence for the sample</li> <li>- Red line—the marker that determines if a sample is positive or negative; the reagent suffix that you entered during HLA-B27 Setup sets this marker; samples with a median fluorescence on or to the right of the marker are positive, to the left, negative</li> </ul> </li> </ul>
④	The <b>FCS</b> and <b>Reagent Lot ID</b> fields (printed in small type) show which FCS file is displayed in the Lab Report and reagent lot ID used.

⑤	<p>This section reports the analysis results for each tube.</p> <p>If a result falls outside the alarm range, the text is highlighted in red, and the message “One or more results are outside the alarm range” appears in the QC Messages section of the Lab Report. A lab manager can alter alarm ranges (see Resetting Alarm Ranges on page 28).</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%;">Gated Events</td> <td style="width: 50%; text-align: right;">208</td> <td style="font-size: small;">— out of range result</td> </tr> <tr> <td>Preset HLA-B27 Marker</td> <td style="text-align: right;">147</td> <td></td> </tr> <tr> <td>Sample HLA-B27 Median</td> <td style="text-align: right;">170</td> <td></td> </tr> <tr> <td>Conclusion</td> <td colspan="2" style="text-align: center;">HLA-B27 positive sample</td> </tr> </table>	Gated Events	208	— out of range result	Preset HLA-B27 Marker	147		Sample HLA-B27 Median	170		Conclusion	HLA-B27 positive sample	
Gated Events	208	— out of range result											
Preset HLA-B27 Marker	147												
Sample HLA-B27 Median	170												
Conclusion	HLA-B27 positive sample												
⑥	<p><b>QC Messages</b> show all QC messages generated during sample acquisition. See Troubleshooting on page 29 for a table QC messages.</p>												
⑦	<p><b>Comments</b> provides an area to enter additional information on the Lab Report.</p>												

## Lab Manager Options

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For the BD HLA-B27 application, Lab Managers can make the following alterations to software defaults:

- change acquisition targets (this page)
- reset alarm ranges (page 28)

If you do not have Lab Manager privileges, you will not be able to access these features.

### Changing Acquisition Targets

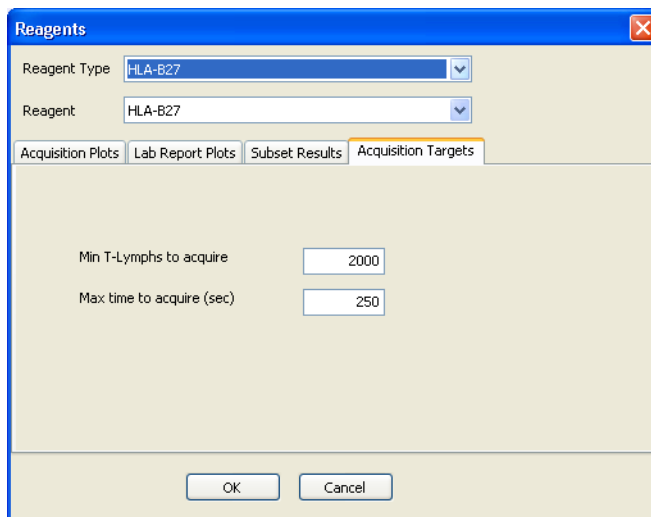
To change the acquisition targets, follow these steps.

- 1 From the main menu, choose Tools > Reagents.
- 2 From the Reagent Type and Reagent menus, choose HLA-B27.

3 Click the Acquisition Targets tab.

See Figure 10.

**Figure 10** Default values for HLA-B27 acquisition targets



The screenshot shows a dialog box titled "Reagents" with a close button in the top right corner. It contains two dropdown menus: "Reagent Type" and "Reagent", both set to "HLA-B27". Below these is a tabbed interface with four tabs: "Acquisition Plots", "Lab Report Plots", "Subset Results", and "Acquisition Targets". The "Acquisition Targets" tab is selected and active. It contains two input fields: "Min T-Lymphs to acquire" with the value "2000" and "Max time to acquire (sec)" with the value "250". At the bottom of the dialog are "OK" and "Cancel" buttons.

4 Type a value into the *Min T-Lymphs to acquire* or the *Max time to acquire (sec)* fields.

- For *Min T-Lymphs to acquire*, enter a value from 600–300,000.

BD Biosciences recommends that you use the default of 2000 as the minimum T lymphocytes to acquire.

- For *Max time to acquire (sec)*, enter a value from 0–900.

Entering 0 will cause the software to ignore time as a stopping criterion.

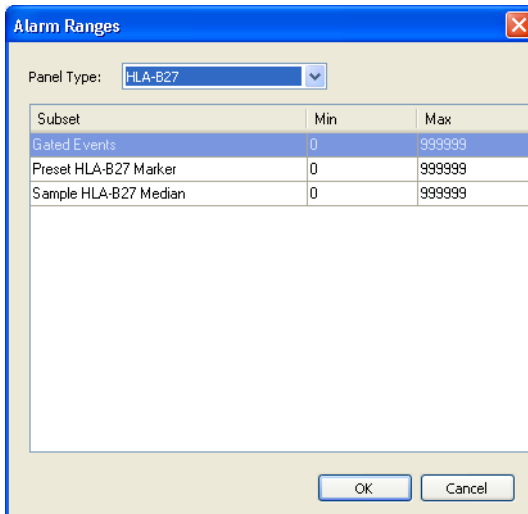
# Resetting Alarm Ranges

Alarms for out-of-range results appear as red text and as a QC message on the Lab Report.

To change the alarm ranges, follow these steps.

- 1 From the main menu, choose Tools > Alarm Ranges.
- 2 From the Panel Type menu, choose HLA-B27.

**Figure 11** Default values for HLA-B27 alarm ranges







- 3 Select and enter new alarm ranges.


# Troubleshooting

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This section provides assistance for BD HLA-B27-specific problems. For instrument or general software troubleshooting, refer to *BD FACSCanto Instructions for Use*. If additional assistance is required, contact your local BD Biosciences technical support representative. Refer to our website, [www.bdbiosciences.com](http://www.bdbiosciences.com), for up-to-date contact information.

Observation or Error Message	Possible Causes	Recommended Solutions
Any Cytometer Setup message	Multiple	Refer to Troubleshooting in <i>BD FACSCanto Instructions for Use</i> and the <i>BD FACS 7-Color Setup Beads</i> package insert.
HLA-B27 Setup too old (Worklist error)	HLA-B27 Setup more than 24 hours old	Click Cancel to stop the worklist, and then run a new HLA-B27 Setup.  While the software allows you to ignore the warning and run the worklist with an old setup, BD Biosciences does not recommend you do so.
HLA-B27 Setup older than last Cytometer Setup (Worklist error)	HLA-B27 Setup not run after Cytometer Setup	Run HLA-B27 Setup before running a worklist with HLA-B27 samples.
HLA-B27 Setup Failed (Worklist error)	Refer to the <i>BD HLA-B27</i> package insert	Click Cancel to stop the worklist, and then run a new HLA-B27 Setup.  While the software allows you to ignore the warning and run the worklist with a failed setup, BD Biosciences does not recommend you do so.

Observation or Error Message	Possible Causes	Recommended Solutions
“Cytometer settings were generated from a failed HLA-B27 setup.” (Lab Report, QC Message)	Samples run using a failed HLA-B27 Setup	<p><b>1</b> Run HLA-B27 Setup until it passes.</p> <p><b>2</b> Rerun your samples.</p> <p> While the software allows you to run the samples with a failed setup, BD Biosciences does not recommend you do so.</p>
	Samples run when HLA-B27 Setup more than 24 hours old	<ul style="list-style-type: none"> <li>• Repeat HLA-B27 Setup again with a fresh tube of beads.</li> <li>• Verify the setup by using the QC methods described in the <i>BD HLA-B27 Kit</i> package insert.</li> </ul>
“T-Lymphs gate failure: Gate manually.” (Lab Report, QC Message)	Patient abnormality	<p>Manually gate the sample.</p> <p> Manually re-gating can alter sample results.</p>
	Wrong reagent used	Make sure you used the BD Anti-HLA-B27 FITC/CD3 PE reagent when preparing samples.
	Improper sample preparation method used	Restain the samples according to the instructions in the <i>BD HLA-B27 Kit</i> package insert, and rerun them on the cytometer.
	Tubes switched in Loader, tube from another panel in HLA-B27 sample position	Re-run the switched samples again, this time making sure that the worklist and sample order in carousel match.

Observation or Error Message	Possible Causes	Recommended Solutions
“No HLA-B27 signal detected.” (Lab Report, QC Message)	No events in gate	Review the report and manually gate the sample.  Manually re-gating can alter sample results.
“Could not acquire the user-requested number of T-Lymphs.” (Lab Report, QC Message)	Leukopenic sample	Manually gate the sample.
	Acquisition targets set too high	<b>1</b> Change the acquisition targets. See <i>Changing Acquisition Targets</i> on page 26. <b>2</b> Rerun the sample.
	Improper sample preparation method used	Restain the samples according to the instructions in the <i>BD HLA-B27</i> package insert, and rerun them on the cytometer.
“Manual gate is in effect.” (Lab Report, QC Message)	Expert Lymph gate overridden, gate changed manually	Inspect the dot plots and gating.
Any other Lab Report QC Message not listed here	Multiple	Refer to the QC Messages section of <i>Troubleshooting in BD FACSCanto Instructions for Use</i> .

## Performance

For assay performance information, refer to the *BD HLA-B27 Kit* package insert.

For instrument performance information, refer to the *BD FACSCanto Instructions for Use*.

