User Manual
LABScreen® HLA Antibody Detection Software

LABScreen v. 3.1

2007/02

For In Vitro Diagnostic Use.

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Advancing Transplant Diagnostics

Manual_Template_R1

LABScreen_V3.1_IVD Rev 1.pdf supersedes LABScreen_V2.7_IVD Rev 0.pdf
OLI-UD-0176-022007 **3/7/07**

All One Lambda software products are designed to assist personnel experienced in HLA analysis by suggesting typing results. However, any clinical or diagnostic results must be carefully reviewed by a person qualified in HLA typing to assure correctness. The software may be used to aid in suggesting results, but should not be used as the sole method for determining reportable results. The software is meant as a laboratory aid, not as a source of definitive results. The software design does not mitigate hazards associated with the software. The laboratory director or technologist trained in histocompatibility testing is required to review all data to detect any problems with the software.

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Chapter 1: Introduction

Welcome to LABScreen® Software. LABScreen Software is an HLA screening application that aids users of One Lambda products with identifying the presence or absence of specific antibodies in an individual's serum sample.

This software imports data from the LABScan™ 100 analyzer for the purpose of suggesting HLA antibody specificities. LABScreen Software can generate a large variety of reports and is designed to store large amounts of patient demographic data and analysis result from previous testing to aid in the understanding of a patient's histocompatibility status.

Disclaimer

All One Lambda software products are designed to assist personnel experienced in HLA analysis by suggesting typing results. However, any clinical or diagnostic results must be carefully reviewed by a person qualified in HLA typing to assure correctness. The software may be used to aid in suggesting results, but should not be used as the sole method for determining reportable results. The software is meant as a laboratory aid, not as a source of definitive results. The software design does not mitigate hazards associated with the software. The laboratory director or technologist trained in histocompatibility testing is required to review all data to detect any problems with the software.

Product Description

One Lambda LABScreen antibody screening tests use color-coded microbeads coated with purified Class I or Class II HLA antigens to detect Class I or Class II HLA antibodies in human sera. Up to 100 beads may be combined in one suspension for a single test. The sera to be tested are incubated with LABScreen beads. HLA antibodies present in the test sera bind to the antigens and are then labeled with R-Phycoerythrin (PE)-conjugated goat anti-human immunoglobulin G (IgG). (IgG is the major circulating antibody in mammals and participates in many immune responses.) The LABScan 100 flow analyzer detects the fluorescent emission of the phycoerythrin from each bead as it is extracted from the tray well. The reaction pattern of the test serum is compared to the lot-specific worksheet defining the antigen array. From this comparison, percent PRA and HLA specificity can be made. If done by hand, this comparison is a tedious and error-prone undertaking. When done using LABScreen Software, the assignments can be made in seconds.

In a typical LABScan data collection session, the analyzer will continue to draw beads from the tray well until the minimum bead count threshold has been reached by all the beads. This minimum bead count threshold is generally set at 100 beads.

LABScreen products are available in three principal versions which are listed below in the order of increasing (i.e. finer) resolution:

LABScreen Mixed

The Mixed Antigen test contains a mix of both Class I and Class II antigens and is used to detect Class I and Class II antibodies in a single test. The bead set in a Mixed Antigen panel consists of 10 to 17 beads: 5 to 9 test beads with Class I antigens, 3 to 5 test bead with Class II antigens, plus negative and positive control beads. Each of the test beads is coated with cloned antigens of up to eight different cell line. The Mixed assay is typically used a preliminary screening test. For reasons of economy the bead set is restricted in number. If a Mixed analysis detects antibodies of interest, the researcher will then subject the serum to PRA testing.

LABScreen PRA

Panel Reactive Antibody tests detect antibodies and their specificities against the HLA antigens in panels of Class I or Class II antigens separately or Class I and Class II antigens simultaneously. Class I and Class II PRA panels contain approximately 55 and 35 beads, respectively, not counting a positive and negative control bead in each lot. In the PRA and Mixed assays, the antigens on a single bead may react with multiple antibodies, thus providing an ambiguous screening result by itself. By comparing the reactions of multiple beads, it is possible to determine which antibodies are present in the tested serum.

LABScreen SA

While the beads used in the other LABScreen tests may exhibit multiple specificities, each bead in a LABScreen Single Antigen assay is specific for a single antibody. Single Antigen assays are used to confirm the presence of specific antibodies suggested by an earlier PRA test.

About this Manual

This manual contains information helpful in using the LABScreen software and consists of the following chapters:

Chapter 1, *Introduction*, the current document, contains descriptions of the products in the LABScreen family, an overview of the manual, system requirements, and installation instructions.

Chapter 2, *Main Menu and Controls*, provides a survey of where to find various LABScreen analysis and maintenance functions.

Chapter 3, *Analysis*, provides step-by-step instructions for performing both Class I and Class II analyses. It includes details on changing computer-assigned specificities, adjusting cut-off values, and displaying reaction patterns.

Chapter 4, *File and Data Maintenance*, explains how to perform such routine housekeeping tasks as archiving and retrieving data, and packing and rebuilding the database.

Chapter 5, *Parameters and Algorithms*, provides instructions for setting up global program parameters and includes the statistical formulas used in the LABScreen analysis.

Appendix A, *LABScreen Reports*, provides examples of LABScreen analysis results reports, data reports and panel listings.

System Requirements

The following is required to successfully install and run LABScreen software.

- IBM compatible PC with Pentium III processor
- 5 Gigabyte hard drive
- 256 megabytes RAM
- VGA display
- Windows 2000 or Windows XP operating system
- Luminex interface software version 1.7 or IS2.2

Installing LABScreen Software

If you do not yet have the LABScreen software installed on your computer, follow these instructions to install it.

1 Close all other Windows applications before starting the installation process.

2 Insert the One Lambda CD in your CD ROM drive.

3 From the Windows Task Bar, select **Start** > **Run**.

4 From the Run window, in the **Open** box, type in the location of your CD ROM drive followed by a back slash and **Labscrn31.exe** (e.g., D:\Labscrn31.exe).

- Or, click the **Browse** button to select your drive, and then double-click the **Labscrn31.exe** file.

5 Click **OK** to begin the installation process. The install wizard usually places the LABScreen software in your C:\labscreen directory.

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Chapter 2: Main Menu and Controls

This section provides a brief overview of menu functions and links to detailed discussions in subsequent sections of the manual. All program functions can be accessed from the main menu or from interfaces accessible from the main menu. A small number of tasks can be executed directly from the tool bar below the main menu.

LABScreen Menus

File Menu

File options include database record archival and deletion, data exportation and database table rebuilding.

Archive and Delete Data – tools to save four categories of data to archive files with options to physically delete records marked for deletion. Archived data are saved to.arf files to a user-specified location. Data archival is intended to help the user reduce the number of samples present at any given time in the LABScreen database, thus facilitating sorting and analysis of the existing records.

Retrieve Data – tools to retrieve the archived data as described above.

Export Data – a utility that exports reaction data and Class I and Class II results for the specified sample IDs in dBase, Excel or ASCI text format. Mixed Assay data can be exported only in Excel format.

Print Setup – this is a standard system function that requires no comment.

Index Rebuild – this function reindexes database tables that may have become damaged as the result of a system malfunction.

Pack Database – this function packs the database to reduce storage requirements. Records that have been marked for deletion are physically deleted.

Edit Menu

The edit options access system text-editing functions that require no comment. They can be invoked with the keyboard combinations that are common to all Windows applications.

LABScan 100 Menu

Use these options to import data files generated by the LABScan 100 analyzer or to reprocess the data in files that have already been imported. Typically, the main purpose of reprocessing is to analyze imported data using a different product lot. From the user's perspective, the main distinction between data importation and data reprocessing is as follows:

- when importing data, the user locates and selects a Luminex .csv file by means of a standard Windows find file dialog;
- when reprocessing data, it is only necessary to select the session name of the file from a drop-down menu that contains session names of all the .csv files currently loaded into the database. Conveniently, the default session names of the imported files are the same as their original filenames.

In both cases, you must supply the appropriate catalog ID(s) and lot number(s) for the assay. In all other respects, import and reprocessing steps are identical.

These options are available:

Import/Reprocess LABScreen PRA/SA Data – import/reprocess .csv files for full PRA/SA assays. These may be either Class I, Class II, or combined Class I and Class II assays.

Import/Reprocess LABScreen Mixed (10-17 bead format) Data – import/reprocess .csv files for 10-17 Bead Mixed assays. Mixed assays always contain a mix of Class I and Class II antigens.

Import MESF Beads Data – import MESF (Molecules of Equivalent Soluble Fluorophores) beads data. This data is used to generate MFI to MESF conversion formulas.

Import/Reprocess LABScreen Mixed (4-bead format) Data – the 4-bead assay type is an obsolete product line. However, if you do have 4-beads data that you wish to revisit, the application can be configured to import/reprocess 4-bead .csv files as follows:

1 Exit the LABScreen application.

2 Assuming that LABScreen has been installed to the default C:\ location, navigate to the C:\labscrn\myflags folder. This folder contains a number of flag files that instruct the application to ignore data for obsolete products.

3 Locate the file NO_LSM_4BEADS.FLG and rename it to LSM_4BEADS.FLG. When you next launch LABScreen, the four-bead Import/Reprocess options will be enabled.

Analysis Menu

The Analysis options provide access to the LABScreen analysis functions. The options are briefly described below. See Chapter 3, *Analysis*, for usage details.

Serum Information – provides access to Class I and Class II Analysis Details (specificities tables) as well as Class I and Class II Antibody Histories (draw, test, reaction data and product tested against) for the samples in the database ordered by Serum ID. These tables contain data for all samples that have been imported into the LABScreen database.

A similar set of Serum Information tables can be accessed by selecting **Maintenance > Serum Information** from the main menu. These latter tables contain data only for those samples whose analyses have been reviewed and Saved (i.e. accepted) from within a Class I or Class II Analysis Results window (Figure 3-13).

HLA Typing Information – provides access to Class I and Class II serological typing results from LABScreen analyses and Class I and Class II DNA typing results from LABType analyses. Each record is identified by Cell ID.

The Serological and DNA panels are editable forms in which you can modify the current typing results by making different Class I and Class II antibody and allele assignments.

A similar set of Cell Information tables can be accessed by selecting **Maintenance > HLA Typing Information** from the main menu. These latter tables contain data only for those samples whose analyses have been reviewed and Saved (i.e. accepted) from within a Class I or Class II Analysis Results window (Figure 3-13).

Analyze by Batch – allows you to perform analyses on an entire previously imported PRA or Mixed Antigen batch or on selected sera contained in the database. Serum selection criteria include By Class within the specified batch or By Range of contiguous serum records in the database. Other search filters include by technician ID (the ID entered when imported a LABScan data file), test date, catalog ID/lot number, or sera that have not yet been reviewed, i.e. whose analysis results have not been accepted (Saved).

Note: When specifying multiple sera, your selection must observe the **Range Rule**: the end-of-range selection must follow the start-of-range selection in the Serum listing. To specify a single Patient, enter the

same Patient ID in both the start and start-of-range fields. The Range Rule applies throughout the selection functions of LABScreen.

Analyze Class I / Class II / Class I & II – use this to perform the respective analyses on a single serum.

Analyze Single Antigen – allows you to perform a batch analysis on a previously imported Single Antigen data file, or on selected sera within the database. Serum selection criteria are identical to those described above for PRA or Mixed Antigen **Analyze by Batch** option.

Analyze Epitopes – not yet implemented

Maintenance Menu

This menu provide tools for reviewing and editing a variety of records in the LABScreen database. Options include:

Readings – NIH scores, catalog lot data, and raw data for each tested serum in the database. The serum ID, catalog lot data, test date and reader ID can also be edited here.

Antigen – names, loci, pertinent leukocyte cell types for the antigen or allele (T or B) and allelic subtype information for each antigen and allele in the database.

Public Antigen – member antigens in CREGs (T or B) and edit fields for modifying or adding member antigens.

Serum Information – analysis results and demographic information for samples that have been Saved/Accepted upon analysis in the Class I or Class II Analysis Results window (Figure 3-13). Results for samples that have not yet been Saved/Accepted can be accessed by selecting **Analysis > Serum Information**.

HLA Typing Information – accesses tables that display cell demographic information, serological antibody typing from LABScreen analyses and DNA allele assignments from LABType analyses for samples that have been Saved/Accepted. Similar tables for samples that have not been Saved/Accepted can be accessed by selecting **Analysis > HLA Typing Information**.

Class I / Class II Analysis Results – the principal interfaces where analyses of Class I and Class II results are carried out. See *Analyzing Data*, *p. 24*.

LABScan 100 Raw Data – accesses panels that display raw data for each tested serum including PC and NC values, session ID and NIH scores.

Archive LABScreen Info – performs an archiving function for LABScreen catalog information similar to that described under the **File > Archive and Delete Data** topic.

Update LABScreen Info – performs a retrieval function for LABScreen catalog information similar to that described under the **File > Retrieve Data** topic.

Add New Lot for LABScreen Mixed – a tool for loading product catalog information into the database and assigning a new lot number to the product.

Reports Menu

Options allow to generate several of the most commonly produced LABScreen reports. A number of other reports types are available and are discussed in Appendix A, *LABScreen Reports*.

Update Parameters Menu

LABScreen program parameters can be set globally by selecting the appropriate Update Parameter functions accessed from the main menu. These settings are discussed in Chapter 5, *Parameters and Algorithms*.

Many of these parameters can be temporarily set to local values on the sample level in forms accessible from the Analysis windows (see *Class I Analysis Results Window, p. 26*).

Main Window Controls

The control panel icons and buttons allow you to move through records within a database table, to perform basic file management operations, and to access some of the most commonly used functions in the program. The Navigation, New record, Cancel and Save icons are enabled when you select the Analysis, Maintenance or Report menu options.

Many of these controls are conventional and require little comment.

Table 2-1: Main Menu Controls

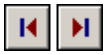
Control	Function	Comments
	Displays the first/last record of the currently active database table.	There are no keyboard equivalents for this button.
	Displays the previous/next record of the currently active database table.	The up/down arrows have the same effect.
	Creates a new record in an editable table.	If the table is read-only, this icon will be disabled.
	Discards all changes made since the last Save.	In many places in the program this button closes the current window and returns to the previous window; when analyzing multiple samples, it ends the current analysis and proceeds to the next.
	Saves any changes to the database.	Generally using the Save icon to commit a change avoids the Save confirmation dialog box. The **Alt + S** keystroke combination is equivalent to using an **OK** or **Save** button.
	Exits the program when in the home page.	Elsewhere in the program this button serves only to close the currently active window.
	Cell Info Update	Duplicates the function of **Maintenance > HLA Typing Information**.
	Sera Info Update	Duplicates the function of **Maintenance > Serum Information**.
	Analysis	Duplicates the function of **Analysis > Analyze Class I and Class II**.

Table 2-1: Main Menu Controls

Control	Function	Comments
	Import PRA	Duplicates the import function of **LABScan > Import/Reprocess LABScreen PRA Dat**a.
	Import Mixed	Duplicates the import function of **LABScan > Import/Reprocess LABScreen Mixed (10 - 17 bead format) Data**.
	Import SA	Duplicates the import function of **LABScan > Import/Reprocess LABScreen SA Dat**a.
	Update Parameters	Duplicates the function of **Update Parameters > Update Parameters**.

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Chapter 3: Analysis

File Naming Conventions for LABScreen Products

The first step in carrying out a LABScreen analysis is to import a batch file of serum information that has been generated by the LABScan™ 100 analyzer. In some cases, the LABScreen Catalog IDs and Lot numbers are not immediately obvious to the user.

The length of product names in LABScreen Software is limited to twelve characters. To accommodate product files name for LABScreen Single Antigen products which may consist of as many as three products with different Catalog IDs and Lot numbers, these file naming conventions are followed:

The Catalog IDs and Lot number combinations of two groups may include one of four alphanumeric characters. The absence of an alphanumeric character indicates that the lot number is the same for both groups.

- A = The lot as written for group one and the lot number as written plus one for group 2
- B = The lot as written for group one and the lot number as written plus two for group 2
- Z = The lot as written for group one and the lot number as written minus one for group 2
- Y = The lot as written for group one and the lot number as written minus two for group 2

Please refer to the following examples:

- LS1A1&2 007 - designates LS1A01 Lot #007 + LS1A02 Lot #007
- LS1A1&2 06A - designates LS1A01 Lot #006 + LS1A02 Lot #007
- LS1A2&3 05B - designates LS1A02 Lot #005 + LS1A03 Lot #007
- LS1A1&2 04Z - designates LS1A01 Lot #004 + LS1A02 Lot #003
- LS1A1&2 07Y - designates LS1A01 Lot #007 + LS1A02 Lot #005

The Catalog IDs and Lot number combinations of three groups consist of the character “C” plus three numbers which designate the lot numbers for each group.

- LS1A123 C557 - designates LS1A01 Lot #005 + LS1A02 Lot #005 + LS1A03 Lot #007
- LS1A123 C657 - designates LS1A01 Lot #006 + LS1A02 Lot #005 + LS1A03 Lot #007
- LS1A123 C775 - designates LS1A01 Lot #007 + LS1A02 Lot #007 + LS1A03 Lot #005

Importing PRA and Single Antigen Data

The importation of PRA and Single Antigen files is almost identical. Batch importation is a multi-step process (Figure 3-1).

1 Click the **?** to the right of the Luminex Data File field to open a standard Windows Find File dialog and locate the LABScan .csv file.

2 Enter a two-character **Technician ID** to identify you on the analysis reports. You will need this identification later when you want to search for and continue the analysis on samples that you personally have loaded into LABScreen.

3 Use the drop-down lists to specify the appropriate catalog ID(s) for the analysis. If carrying out both Class I and Class II analyses on the sera, specify a Class I catalog in one field and a Class II in the other. It does not matter which field you use for which Class. In any case, LABScreen will perform Class I analyses on each sample first.

- If you are analyzing samples using a Single Antigen product that combines two or three different catalog IDs and Lot numbers, see *File Naming Conventions for LABScreen Products, p. 15*, for guidelines on choosing the correct catalog ID.
- If you specify a catalog that does not match the assay, LABScreen will generate a bead conflict error message.
- If you specify a catalog that is not used, (say an unnecessary Class II catalog/lot when only Class I sample data are available), and then re-import the same batch later and specify the appropriate catalog(s), LABScreen will generate a data cleanup message indicating that the unusable or old catalog data is being purged from the database. You can ignore the message.

4 Select one or more report options:

- **Print Antigen Distribution Table with Report** – see Figure A-10, *Typical Antigen Distribution Listing* for an example.
- **Preview Report First** – displays a preview of the data report with results (pos/neg/grey area) for each bead for each Class. The report of each sample is on a separate page. See Figure A-4, *Mixed Data Full Report*. (This is the default.)
- **Analyze After Import** – imports all serum data, then performs the same function as when you select **Analysis > Analysis by Batch > By Session ID** which displays the Antibody Analysis Results Table for each sample in turn (Figure 3-13, *Class I Analysis Results Window*). You can move to the next serum by clicking the **Save** button, the **Return** button or by pressing <**Return**>.
- **Analyze non-prompt mode to printer** – imports all serum test data, performs analyses and sends reports for each serum directly to the printer without user intervention, thus printing reports for all imported samples automatically. Note that although the serum analyses have been performed by the program, the results have not yet been Saved (accepted) by a researcher. Use this option when you want LABScreen to carry out preliminary analysis of a batch of samples that you will review at a later time.

5 You can interrupt and terminate the analysis cycle by pressing <**S**> between samples.

Table 3-1: Test Figure Table

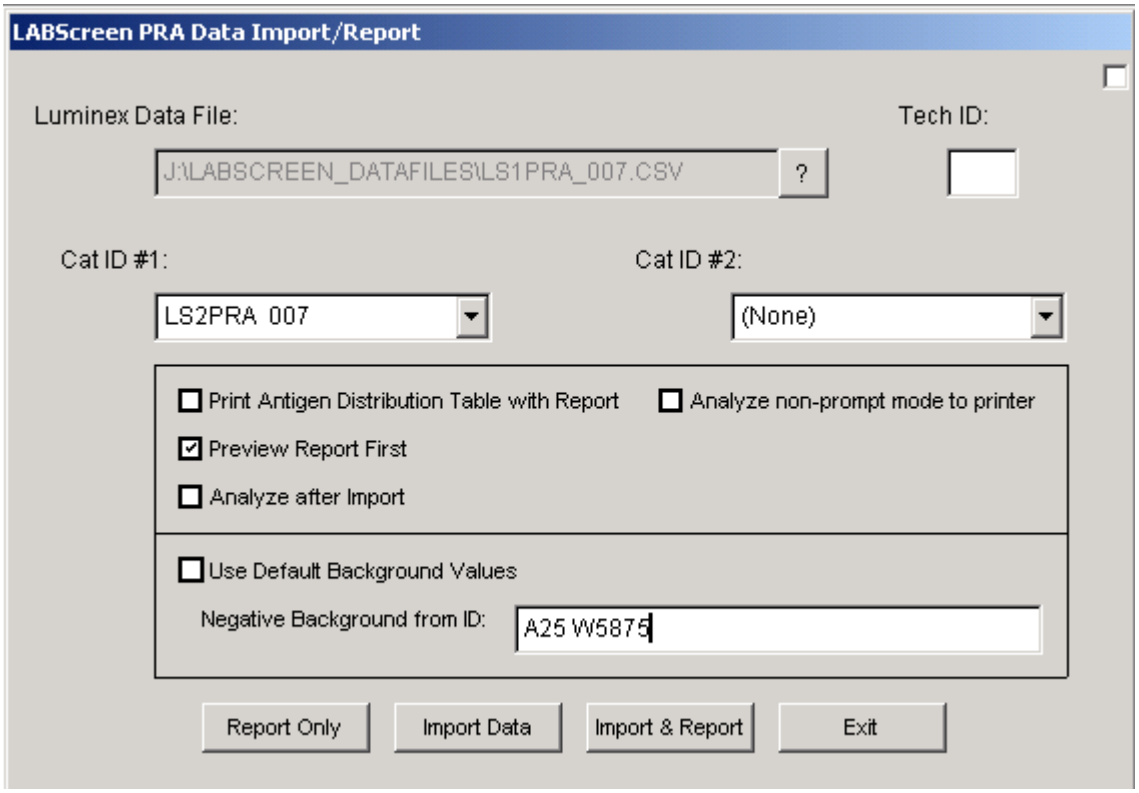
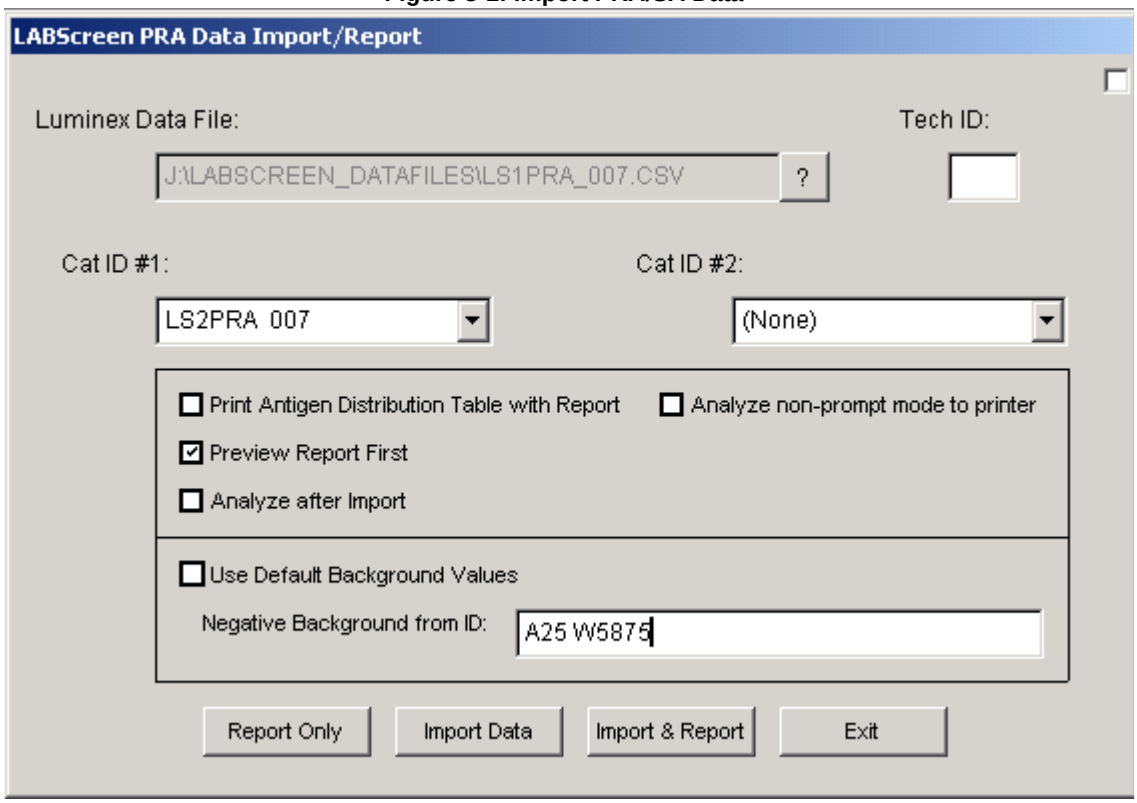


Figure 3-1: Import PRA/SA Data



6 Accept the default background values option. (These are the OLI defaults for the product).

Occasionally you may wish to specify an alternative set of background values from a specific tray well in the batch. This means that all analyses for the entire batch would use those data values as background values.

To specify an alternative set of background values using data values from one well of the batch:

- Clear the **Use Default Background Values** check box.
- Open the batch's LABScan .csv file in Excel and locate the column containing the Sample IDs. LABScreen will use the data values in the row corresponding to the data type which LABScreen has been set up to use. This is typically the median.

Figure 3-2: Batch Data File in Excel

358	Data Type:	Median				
359	Location	Sample	1	2	3	4
360	A1	A1 W4136	413.5	7396	587	366
361	B1	A2 X0957	329	7094.5	7643.5	9415.5
362	C1	A25 W5875	186	5777.5	6226	6030
363	D1	A30 W7614	315.5	7407	893	206
364	E1	A26 W5422	21	6884	332	271.5
365	F1	A24 W6726	309	5096.5	2822	15564
366	G1	A34 W6409	663	6063	8290.5	6537
367	H1	A24 W9527	144	6398	739	15250

- Copy the Sample ID from the .csv file into the **Negative Background from ID** field. If you have entered an invalid Sample ID, LABScreen will generate a warning message.
- Close the .csv file, making sure **not** to save it as an Excel file.

When you run an analysis of the sample whose values you have specified to use as background values, note that all the results are zero. This is expected inasmuch as all readings for that well are cancelled out by the background values you are now using for the entire batch.

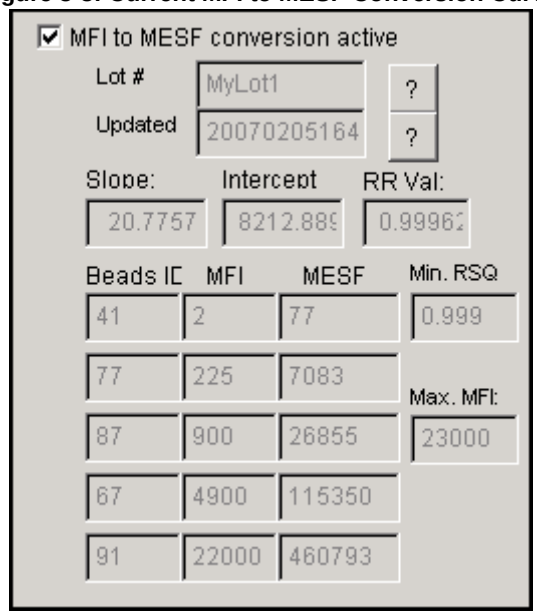
7 If you do not want to include MFI to MESF conversions in the reports, skip to Step 8.

In some cases you will want to use MESF calibration data for the Luminex machine that was used to collect the data for the samples you are analyzing in order to compare samples collected on different dates.

To activate MFI to MESF conversion:

- Check the unlabeled box at the upper right of the Import/Report dialog to display the MFI to MESF conversion table which shows the current values used in the regression routine that generates the conversion formula.
- The slope and intercept of the fitted curve (a straight line) are shown above the table. The RR value indicates the goodness of fit. By default the calibration curve is based on readings for the five beads listed in the first column of the table. (Figure 3-3)
- You can modify the values used to generate the conversion formula by selecting the **Update Parameters > MFI to MESF Conversion Settings** option from the main menu.

Figure 3-3: Current MFI to MESF Conversion Curve



8 Choose the execution mode:

- **Report Only** – generates the LABScreen SA or PRA Raw Data Report without creating any records to the database.
- **Import Only** – imports the data, creating records in the database without generating a report.
- **Import and Report** – imports the data, creating records in the database and generates reports.

9 When you first import the Luminex data, you have the option to Analyze After Import which starts the analysis automatically upon import. See *Analyzing Data, p. 24* for details.

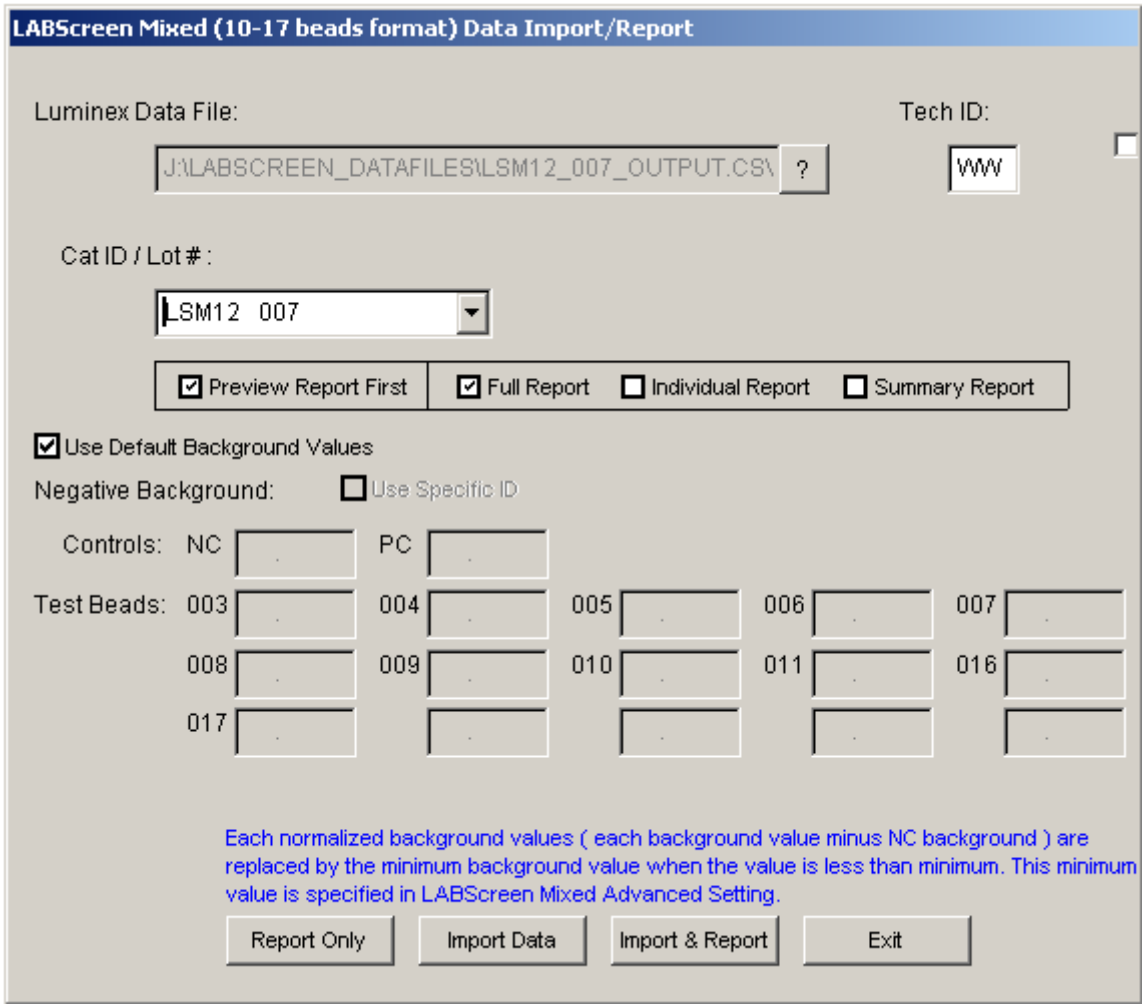
Importing Mixed Antigen Data

The steps followed when importing Mixed Antigen data are largely identical to those when importing PRA and Single Antigen data (Figure 3-4). There are, however, different reporting and background value specification options.

1 Just as when importing PRA or SA data:

- Locate the Luminex Data File
- Supply your two-character Technician ID
- Specify the LABScreen product by Catalog ID and Lot #.

Figure 3-4: Import Mixed Data



2 Choose the report output options:

- **Preview Report** – and/or one or more of the following:
- **Full Report** – Class I and Class II results (Overall and for each Bead) for **all** samples in the batch, four samples per page; includes raw data, NBG Ratio, and bead count; report indicates if background values are default, specified by Sample ID or manually entered. See Figure A-4, *Mixed Data Full Report*.
- **Individual Report** – same information as in the Full Report for **all** samples in the batch, but with each sample on a separate page. See Figure A-5, *Mixed Data Individual Report*.
- **Summary Report** – summary results for each sample on a single line; report includes overall result for Class I and Class II (Pos/Neg/Grey Area), Raw Data and counts for each sample's NC and PC beads, and the sample's PC/NC ratio. See Figure A-6, *Mixed Data Summary Report*.

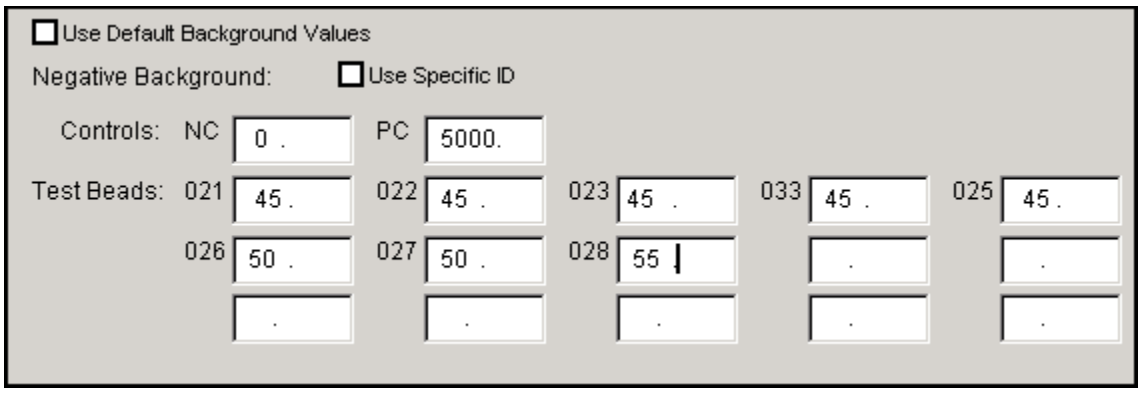
3 Choose to include MFI to MESF conversions as discussed above in Step 7.

4 Accept the default background values or specify Negative Background values by entering a Specific Sample ID as discussed above in Step 4.

5 Alternatively, clear both check boxes and manually enter background values for the Control Beads and Test Beads. Note that the numeric legends for the Test Beads to the left of the input fields change according to the definition of the product (Figure 3-5), but are updated only **after** you have pressed one of con-

trols at the bottom of the form (**Report Only, Import Data**, etc.). Check these legends to make sure you are dealing with the right product. The input fields are initially pre-populated with the default values for the product.

Figure 3-5: Manually Entering Negative Background Values



6 You can now proceed to the analysis. See *Analyzing Data, p. 24*.

Note: If you enter any Negative Background value for a test bead that is less than the minimum value threshold set globally in the LABScreen Mixed Advanced Settings, LABScreen will automatically bump the value up to the threshold value. By default this value is 50.
Manually entered Negative Background values are not persistent. Thus, if you reprocess the same batch at a later time, you must reenter the manual values.

Importing Quantiplex (MESF) Beads Data (For Investigational Use Only)

Note: The new Quantiplex features are a Beta version only, and may be subject to change when the actual OLI Bead product is released.

If you choose to include MFI to MESF data conversion, you will need initially to create manually a separate lot for each Luminex LABScan analyzer from which you import LABScreen batch data. Moreover, you will need to update the values for that lot on the days when it is in use so you can associate a time-stamped conversion formula with the data collected on that day by that analyzer.

In order to create a MFI to MESF conversion lot, carry out the following steps.

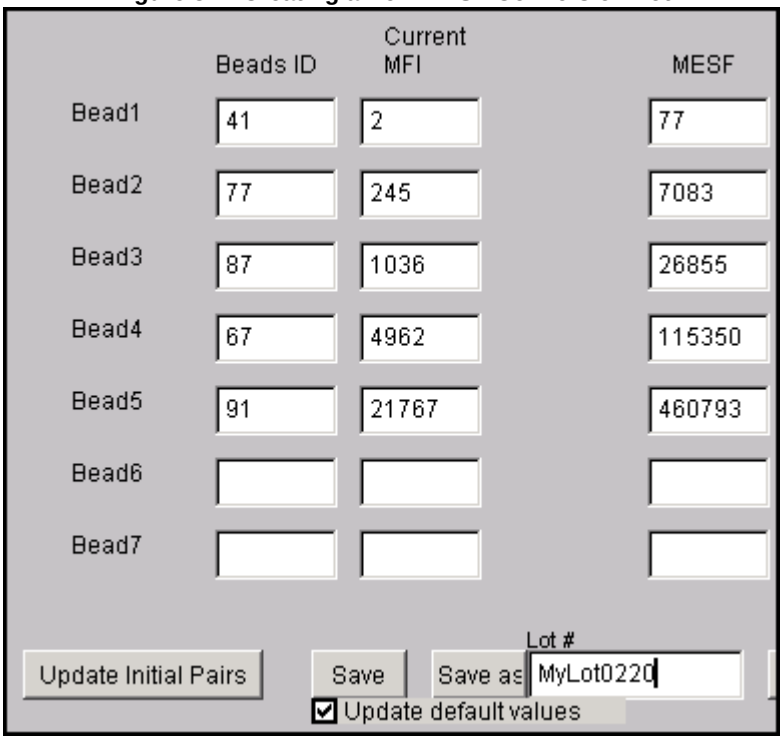
1 Locate a Quantiplex Bead Data sheet (Figure 3-6). This is an Excel file that will be provided to you by the laboratory staff who run the LABScan 100 analyzers.

2 Select **Update Parameters > LABScreen MFI to MESF Conversion Settings > Update Initial Pairs** to enable all the fields in the form.

Figure 3-6: Quantiplex Beads Data File

Quantiplex Beads Data Sheet Lot 1			12/8/2006
Beads ID	Trimmed Mean	Median	SFI Units
41	2	2	77
77	246	245	7083
87	1036	1025	26855
67	4932	4962	115350
91	21767	22219	460793
Use 80 ul per test, select regions 41, 67, 77, 87, and 91			
Set sample volume 50 ul, acquire100 beads			
Use the Trimmed Mean from Luminex for Calculation			

Figure 3-7: Creating a New MESF Conversion Lot



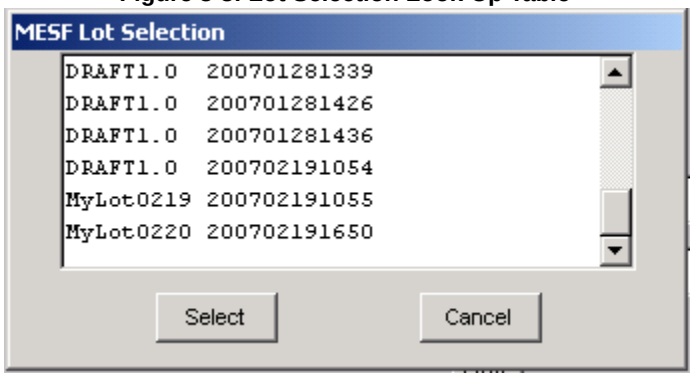
3 Transfer the data from the **Median** and **SFI** columns in the Quantiplex Beads Data Sheet (Figure 3-6) to the **Current MFI** and **MESF** columns in the **MFI to MESF Conversion Settings** form (Figure 3-7). Note that the Beads ID numbers are not necessarily in ascending order, but the EFI values are. Make sure that the order in the Conversion Settings fields is the same as in the Beads Data Sheet.

4 Enter a name in the **Save As Lot #** field and press **Save As**. (You don't need to clear the **Update Default Values** check box as this option applies only to the simple **Save** button directly above it.)

5 You can confirm the existence of the new conversion lot in the LABScreen database table by accessing the MESF Lot Selection look-up (Figure 3-8). Do this by clicking the search button (**?**) to the right of the Lot

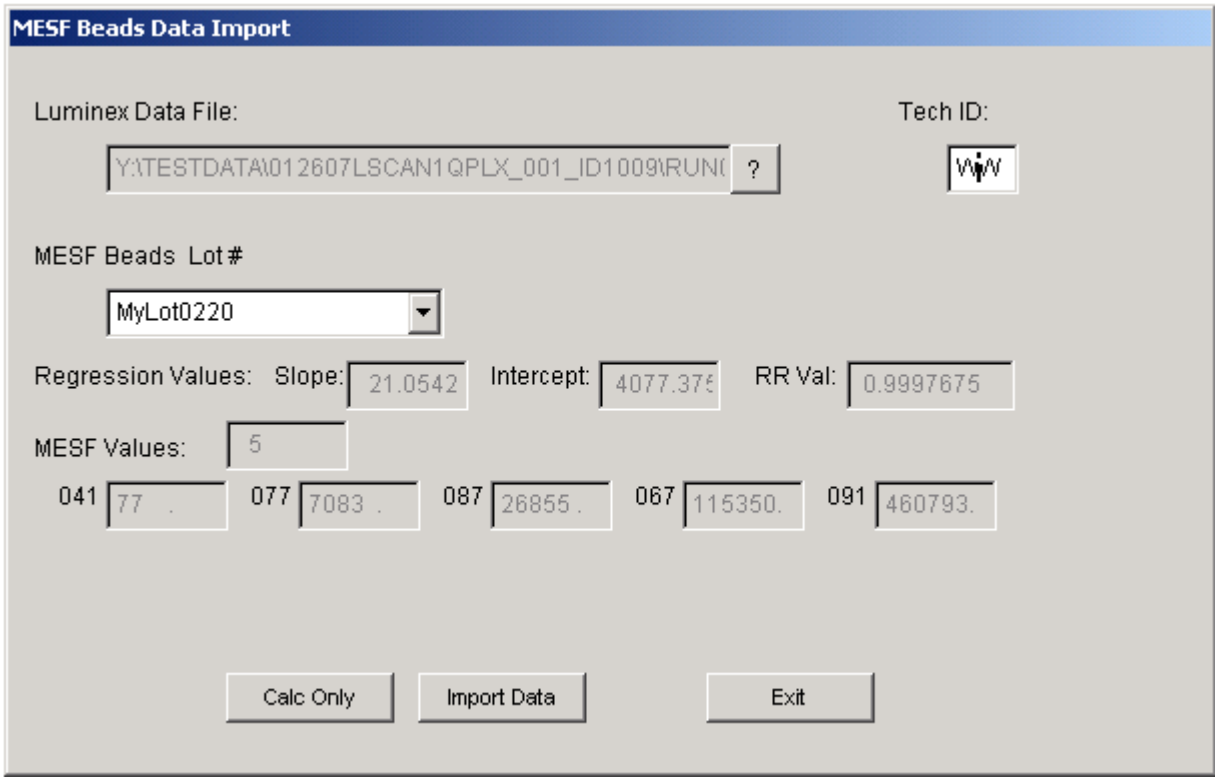
field at the top of the MFI to MESF Conversion Settings form. After you have confirmed that the new record is there, close this form and return to the main menu.

Figure 3-8: Lot Selection Look-Up Table



6 Select **LABScan 100 > Import MESF Bead Data** to access the like-named form. It should now display the newly created lot in the **MESF Beads Lot #** field (Figure 3-9). Click the browse button (**?**) to locate a Luminex .csv file containing LABScan calibration data. The filenname probably contains the characters **QPLX**, **Quantiplex**, or the like.

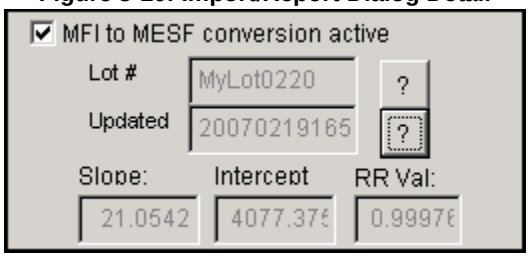
Figure 3-9: Importing MESF Beads Data



7 Click **Import Data**. If you have chosen a compatible conversion file, LABScreen will display a "Bead Number Matched" message, then briefly display the linear conversion formula associated with that lot.

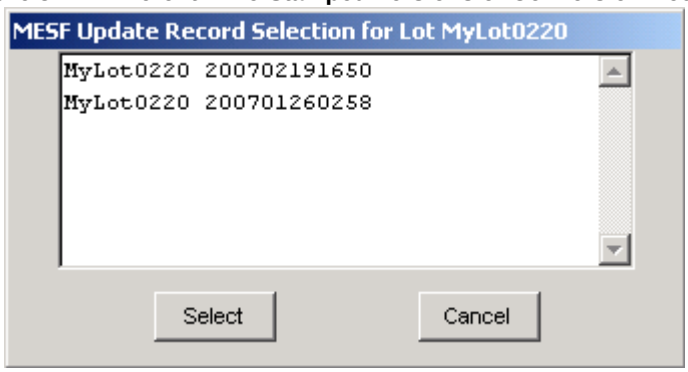
8 When you next import any kind of session data (PRA, Single Antigen or Mixed Antigen), note that the current MFI to MESF Conversion Lot appears in the **Lot #** field in the upper right corner of the **Import/ Report** form (Figure 3-10).

Figure 3-10: Import/Report Dialog Detail



9 You can use the **Lot #** browse button (**?**) to select a different calibration lot for a different Luminex machine, or you can use the **Updated** browse button to select an updated calibration lot with a different time stamp for the same machine (Figure 3-11).

Figure 3-11: Different Time-Stamped Versions of Conversion Record



Reprocessing a LABScan 100 File

If you wish to reanalyze the same LABScan data using a different product lot (usually a newer one), you can reprocess a LABScan 100 file that has been previously imported. Follow these instructions:

1 Instead of providing a Luminex data file name as in a regular import, select the Luminex session name from the pull-down.

2 Specify the different product(s).

3 All other processing options are the same as when importing.

Analyzing Data

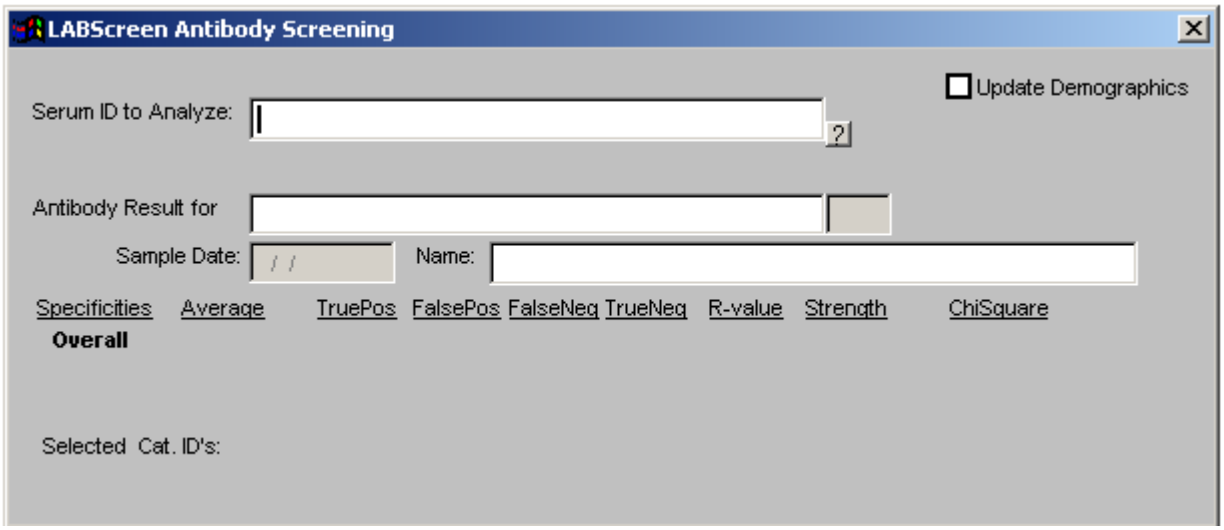
If you check the Analyze After Import option when importing the LABScan data, LABScreen immediately proceeds to analysis. If you choose to only import the data by leaving the **Analyze After Import** option unchecked, you will need to start up the analysis after the import.

1 On the Main menu, click **Analysis** and then select one of the following:

- Analyze by Batch – use this option to process multiple samples, one after another; See *Processing Multiple Samples, p. 37*.
- Analyze Class I – use this and the following options to analyze one sample at a time.
- Analyze Class II
- Analyze Class I and Class II

The three single-sample options invoke the LABScreen Antibody Screening window (Figure 3-12). When first accessed, it will not be populated with values. Later, if you decide to analyze another sample, this window will show the results of the just-completed analysis.

Figure 3-12: LABScreen Antibody Screening before Sample Selection



2 Select a **Sample ID** by clicking on the question mark (**?**) next to the Sample ID field (Figure 3-12), and selecting an ID from the pick list.

If you check the **Update Demographics** option, the program will open the Sample Information demographic information form before performing the analysis. The demographics form can also be accessed from other points in the program.

3 The analysis starts as soon as you select a Sample ID or finish updating the demographic information. The analysis results appear in the full Analysis Results window (Figure 3-13) which contains five panels. Each panel will be discussed separately.

- Specificities Table (Figure 3-14)
- Percent Positives Panel (Figure 3-15)
- Control Panel (Figure 3-16)
- Chi-Squares Table (Figure 3-17 and Figure 3-18)
- CREG Display (Figure 3-19)

Figure 3-13: Class I Analysis Results Window

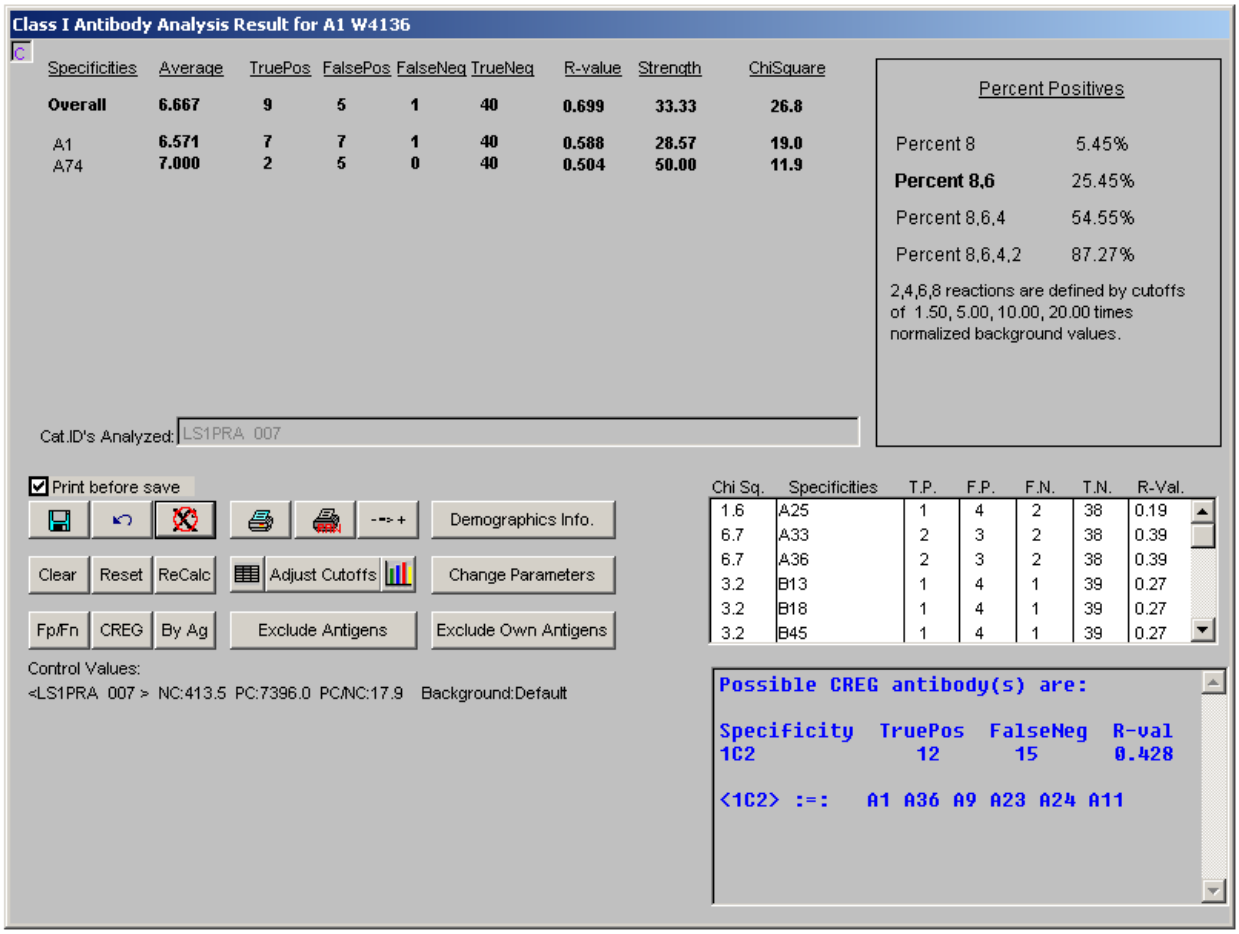
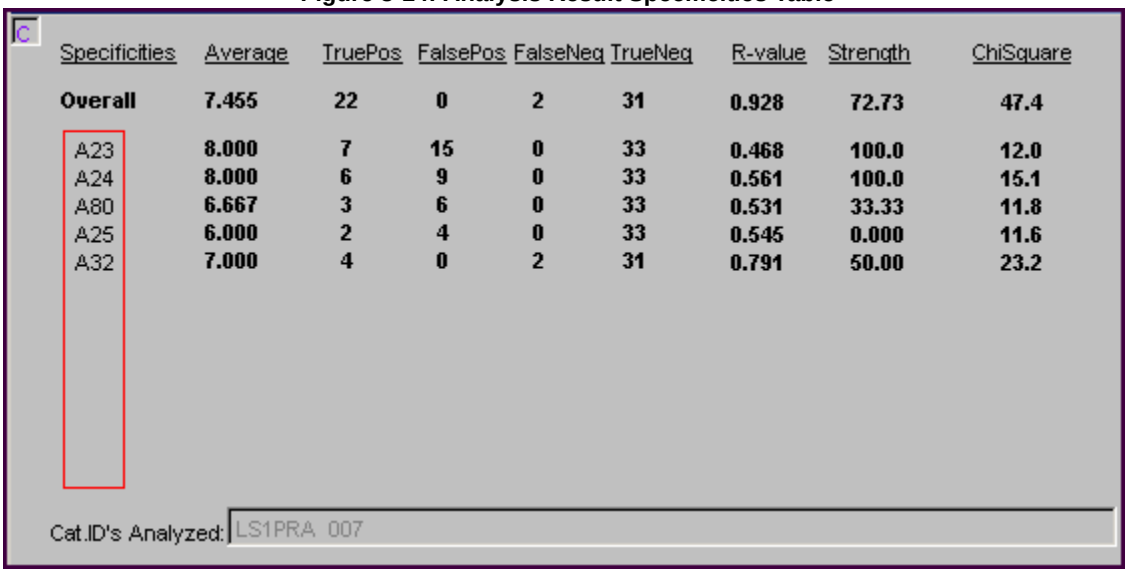


Figure 3-14: Analysis Result Specificities Table



Specificities Table – this table (Figure 3-14) displays statistical results for the antigens that qualify as specificities for the sample (R-Value > 0.25 and Score > 2). LABScreen displays its assignments in the **Overall** column.

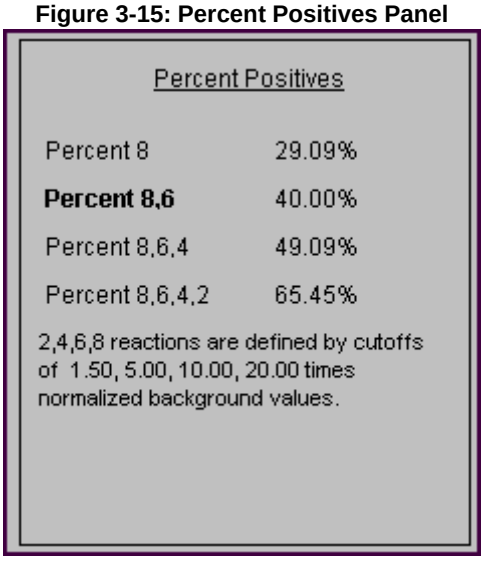
The area outlined in red contains editable fields. You can delete a computer assignment or add your own antigens to check the effect of the change on the statistics. If you attempt to include an antigen that does not belong to the panel, LABScreen will display an error message.

When you make any edit to the table, the small **C** (**Computer**) in the upper left changes to **M** (**Manual**). The product(s) used in the analysis are displayed in the **Cat IDs Analyzed** field beneath the table.

Percent Positives Panel – this panel displays cumulative percentages for each category of NIH scores (Figure 3-15).

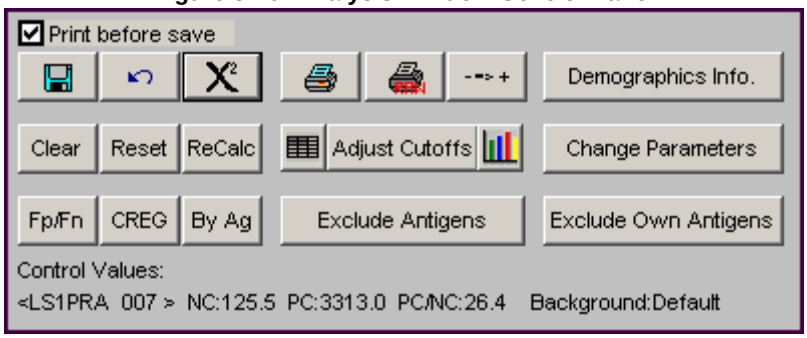
Note that the definitions of score assignment (2, 4, 6, 8) reflect the **global** settings made in the **Update Parameters > Settings** dialogs accessed from the main menu, and do not reflect any local or temporary settings made for the particular sample via the Adjust Cutoffs functions (Figure 3-16). Similarly, the reaction definitions reproduced in report footers reflect global and not local settings.

Figure 3-15: Percent Positives Panel



- **Control Panel** – the buttons in this panel (Figure 3-16) control the analysis functions of the Analysis Results window and are discussed in some detail in Table 3-2, *Analysis Window Controls*. A further set of controls used when adjusting cutoffs and setting background values is discussed in Table 3-3, *Adjust Cutoffs and Change Background Controls*.

Figure 3-16: Analysis Window Control Panel



- **Chi-Squares Table** – antigens that meet the R-Value and Score criteria for being classified as specificities are displayed in the Specificities Table (Figure 3-14). By default, the R-Value threshold is 0.25, and the Score must be greater than 2. (The Score, S, is defined as TP - FN.) The statistics of remainder antigens, i.e. those antigens that display false positives and which do not meet the R-Value and Score

criteria, are displayed in the Chi-Squares Table (Figure 3-17) when you press the Chi-Square button in the control panel. Many samples display no remainder antigens.

Figure 3-17: Chi-Square Table for Remainder Antigens

Chi Sq.	Specificities	T.P.	F.P.	F.N.	T.N.	R-Val.
1.6	A25	1	4	2	38	0.19
6.7	A33	2	3	2	38	0.39
6.7	A36	2	3	2	38	0.39
3.2	B13	1	4	1	39	0.27
3.2	B18	1	4	1	39	0.27
3.2	B45	1	4	1	39	0.27

If you access the Chi-Square table when the CREG display option has been invoked, the Chi-Square table displays the CREGs of the remainder antigens (Figure 3-18).

Figure 3-18: Chi-Square Table for Remainder Antigen CREGs

Chi Sq.	Specificities	T.P.	F.P.	F.N.	T.N.	R-Val.
2.2	1C3	2	0	12	14	0.28
1.0	21C	0	2	9	17	-0.19
3.3	2C1	0	2	17	9	-0.34
3.3	2C2	0	2	17	9	-0.34
1.6	8C	0	2	12	14	-0.24
6.5	B6	0	2	21	5	-0.48

- **CREG Display** – some antigens shown in the Specificities Table may be member antigens in Cross-Reactive groups. Possible CREGs, the complete listing of their known member antigens, and abbreviated statistics are displayed at the lower right of the Analysis Results window. More complete statistics for possible CREGs can be displayed in the Specificities Table by toggling the **CREG**/**Agn** buttons in the control panel.

Figure 3-19: CREG Display

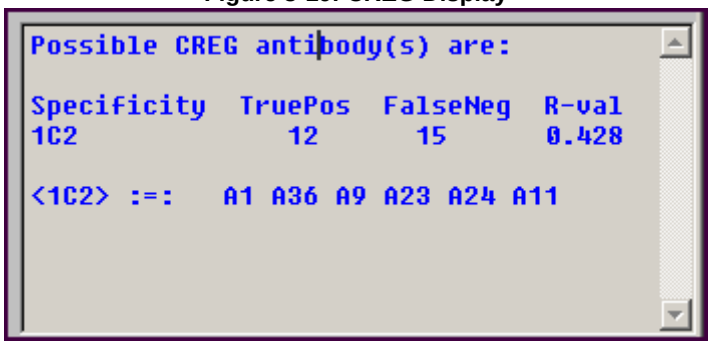


Table 3-2: Analysis Window Controls

Control	Function	See
	Generates a Class I or Class II Antibody Screening Report when you Save the analysis of the current sample	Figure 3-16, *Analysis Window Control Panel*
	Saves any manual edits to the analysis and Accepts the sample analysis; Saved/Accepted samples appear in the Maintenance > Class I/II Results tables; before being Saved/Accepted the samples appear only in the Analysis > Serum Information tables	Figure 3-12, *LABScreen Antibody Screening before Sample Selection*
	Exits the Analysis Results window without saving/accepting the sample analysis results; when doing batch analyses, moves to the next analysis or sample	
	Performs and displays Chi-Sq. analyses for remainder antigens (antigens not included in the Specificities Table); if the CRGE options is selected, the Chi-Sq. tables shows the CREGs of which the remainder antigens are members	Figure 3-17, *Chi-Square Table for Remainder Antigens*
	Toggle for the Chi-Sq. button (above), hiding the Chi-Sq. display	
	Prints analysis report; right-click previews the report	Figure A-13, *Class I and Class II Antibody Screening Reports*
	Prints raw data report; right-click previews the report	Figure A-11, *Sample Raw Data Report*
	Inverts NIH scores: 8s become 1s, 6s become 2s, 2s become 6s and 1s become 8s. LABScreen recalculates the statistical analysis using the inverted scores, thus testing for the absence of antigens rather than their presence.	Figure 3-14, *Analysis Result Specificities Table*
	Toggles with the -/+ button (above), restoring the original NIH scores	
	Accesses the Sample demographic Information form; this form can be accessed from several places within the program.	
	Clears all entries from Specificities Table including computer assignments	Figure 3-14, *Analysis Result Specificities Table*
	The “Eraser” resets the entries in the Specificities Table to the original computer assignments	
	Recalculates sample statistics after user edits to the Specificities Table.	

Table 3-2: Analysis Window Controls (cont.)

Control	Function	See
	Displays Revise Cutoff/Raw Data grid only	Figure 3-28, *Color Coding in Revise Cutoff/Raw Data Grid*
Adjust Cutoffs	Displays Revise Cutoff/Raw Data grid and Reaction Histogram. Note that local modifications to cutoffs are not indicated in Report footers and in the Percent Positives panel legend.	Figure 3-27 and Figure 3-28
	Displays Reaction Histogram only	
Change Parameters	Allows user to temporarily change the Reaction Definition, R-Value and Score parameters, and report options for the Antibody Screening Report for the current sample (Figure A-13). Global parameter changes must be made using the Update Parameters form from the main menu.	*Changing Parameters Locally (Temporarily)*, *p. 36*
Fp/Fn	Displays the Reaction Listing by antigens table; by default only FPs and FNs are shown; TPs and TNs can optionally be displayed as well; when the CREG option is invoked, the Reaction Listing shows the CREGs of the member antigens present on each bead	Figure 3-21, *Reaction Listing with CREGs*
CREG	Displays any CREG with member antigens among the detected specificities in the Specificities Table	Figure 3-21, *Reaction Listing with CREGs*
Agn	Causes the Specificities Table to display detected antigen specificities (default display)	
By Ag	Accesses a pick list containing all antigens and alleles currently in the LABScreen database; upon selection of an antigen, displays a Reaction Table listing all beads in the product that exhibit the antigen	*Viewing Results by Antigen*, *p. 31*
Exclude Antigens	Excludes the specified antigens from analysis (permanently or temporarily), thus reducing the masking effect of those antigens on other antigens	*Excluding Antigens*, *p. 36*
Exclude Own Antigens	Excludes the patient's own antigens from the analysis if they have been entered in the patient information.	

Figure 3-20: Reaction Listing with CREGs

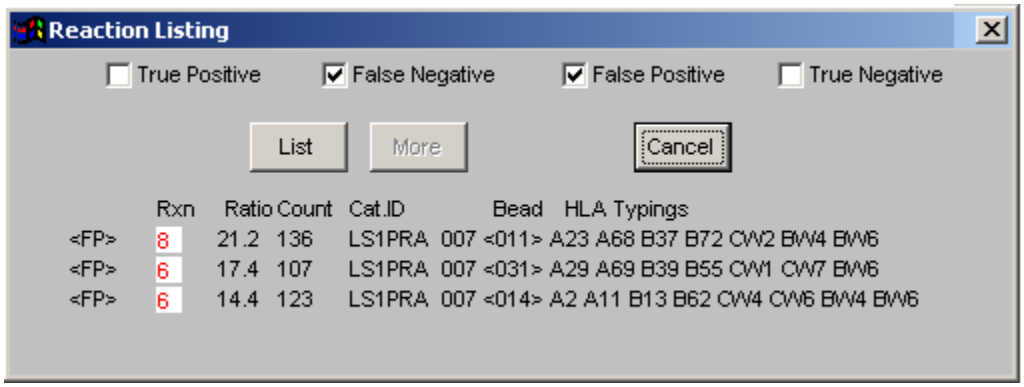
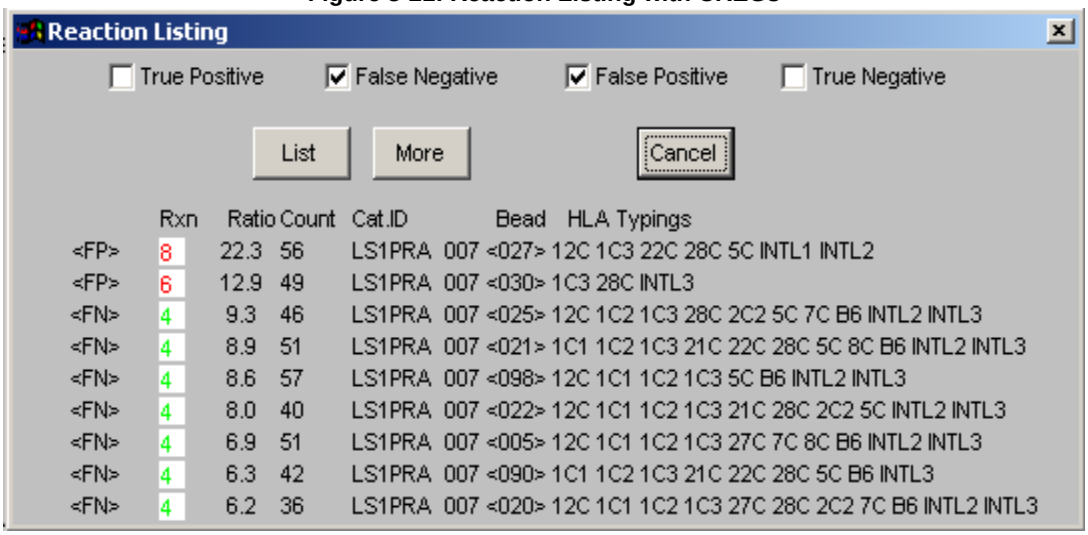


Figure 3-21: Reaction Listing with CREGs

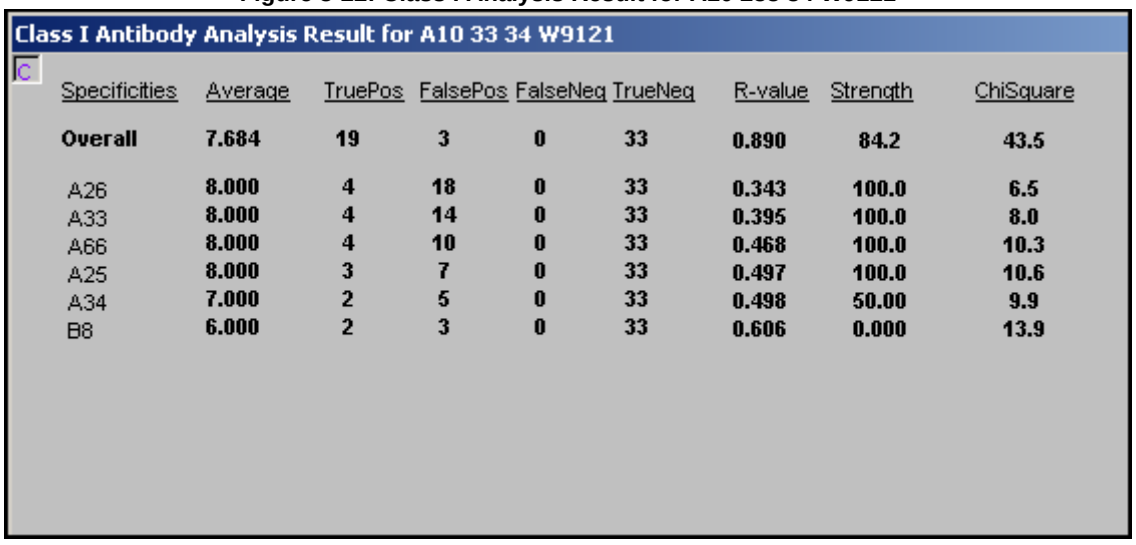


Viewing Results by Antigen

This option allows you to inspect the behavior of antigens that do not meet the R-Value and Score criteria. Consider the analysis results of the sample shown in Figure 3-22.

- In this example, the six antigens that meet the R-Value and Score criteria are shown in the Specificities Table (see *Antigen Specificity Calculation*, *p. 51*):

Figure 3-22: Class I Analysis Result for A10 233 34 W9121

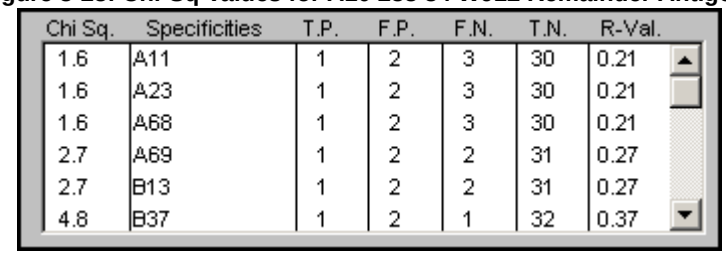


Class I Antibody Analysis Result for A10 33 34 W9121

Specificities	Average	TruePos	FalsePos	FalseNeg	TrueNeg	R-value	Strength	ChiSquare
Overall	**7.684**	**19**	**3**	**0**	**33**	**0.890**	**84.2**	**43.5**
A26	**8.000**	**4**	**18**	**0**	**33**	**0.343**	**100.0**	**6.5**
A33	**8.000**	**4**	**14**	**0**	**33**	**0.395**	**100.0**	**8.0**
A66	**8.000**	**4**	**10**	**0**	**33**	**0.468**	**100.0**	**10.3**
A25	**8.000**	**3**	**7**	**0**	**33**	**0.497**	**100.0**	**10.6**
A34	**7.000**	**2**	**5**	**0**	**33**	**0.498**	**50.00**	**9.9**
B8	**6.000**	**2**	**3**	**0**	**33**	**0.606**	**0.000**	**13.9**

- If we consult the Chi-Square display for the remainder antigens in this sample, we can see a listing of all the other antigens that have at least 2 FPs and an R-Value over 0.20 (Figure 3-23).

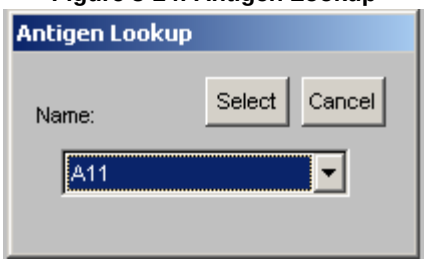
Figure 3-23: Chi-Sq Values for A10 233 34 W912 Remainder Antigens



Chi Sq.	Specificities	T.P.	F.P.	F.N.	T.N.	R-Val.
1.6	A11	1	2	3	30	0.21
1.6	A23	1	2	3	30	0.21
1.6	A68	1	2	3	30	0.21
2.7	A69	1	2	2	31	0.27
2.7	B13	1	2	2	31	0.27
4.8	B37	1	2	1	32	0.37

- We can use the **Reaction by Antigen** function to see how the remainder antigens reacted in the test by clicking the **By Agn** button and choosing a remainder antigen from the **Antigen Lookup** pick list.

Figure 3-24: Antigen Lookup



- The Reaction Listing for the A11 antigen is shown in Figure 3-25. Since the point of this feature is to let us view all beads carrying the antigen, all reaction types are displayed by default. The complete specificities of all beads that also have the A11 specificity are listed.

Figure 3-25: Reaction Listing for A11 in A10 233 34 W912

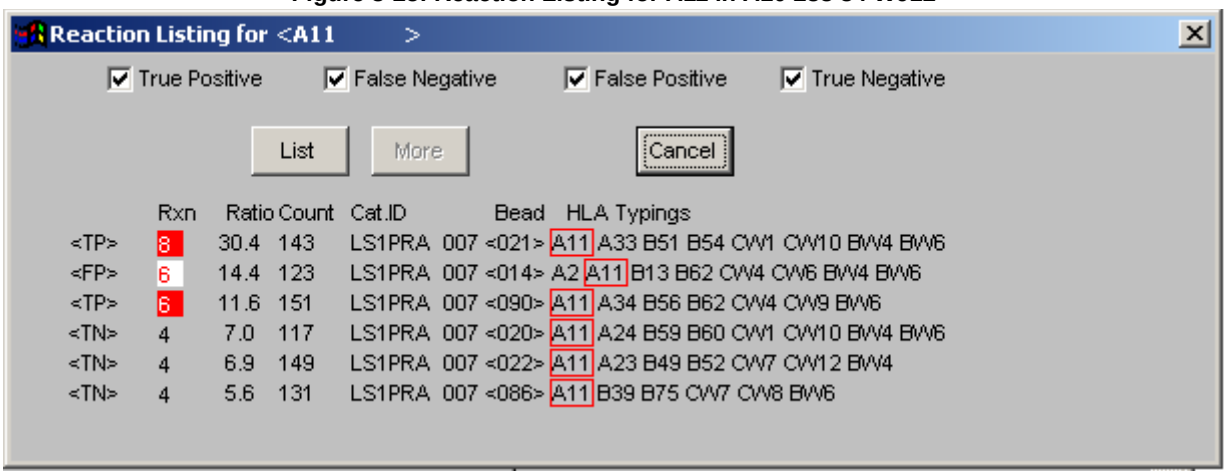


Table 3-3: Adjust Cutoffs and Change Background Controls

Control	Function	For Details, See
10.00	Spin Box - increments/decrements all NBG Background Values by the same amount	
Apply	Applies value specified in the Spin Box	
Defaults	Resets BG values to Default values (before any user edits)	
Update	Applies any changes and recalculates the NIH scores; applies yellow alert color to any NBG ratio that is +/- 0.25 of the cutoff between NIH scores of 1 and 2.	
	Restore NBG Values to previous values	
Cutoff	Display Cutoff Adjustment entry fields for Weak Positive, Positive, Strong Positive & Very Strong Positive (PRA) or Negative, Grey Area & Positive (Mixed)	
	Saves changes, returns to Analysis Results window	
	Cancels; returns to Analysis Results window	
Save to	Saves background values for current sample to a .BGV file.	
Update from	Loads background values from a saved .BGV file for analysis of current sample.	

Table 3-3: Adjust Cutoffs and Change Background Controls

Control	Function	For Details, See
Copy	Replaces the NBG values with the Raw Data Values for the sample. Used when processing a sample as a negative control serum. The user would copy the sample results into the local background values, then use the Save to function to save the NBG values to a .BGV file.	
NBG Ratio	Sorts entries in Data Grid/Reaction Histogram by descending NBG Ratio score	
Reaction	Sorts entries in Data Grid/Reaction Histogram by descending NIH score; sub-sorts by descending bead number	
Bead ID	Sorts entries in Data Grid/Reaction Histogram by ascending bead number	
RawData	Sorts entries in Data Grid/Reaction Histogram by descending raw data value	

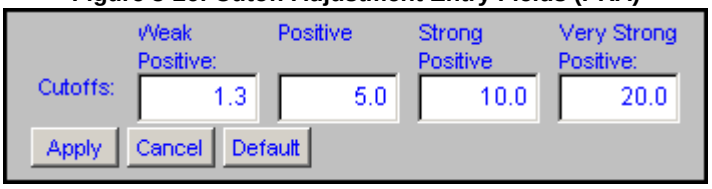
Adjusting Cutoffs

You can adjust cutoff thresholds numerically or graphically.

To adjust cutoffs numerically:

- Click the **Cutoff** button at the bottom of the Revise Cutoff/Raw Data window to display the cutoff adjustment entry fields (Figure 3-26).
- Enter the desired values for each threshold. The smallest increment the fields accept is 0.1.
- Click **Apply** to save your adjustments. The new threshold values will be reflected in the Reaction Histogram (Figure 3-27).
- Finally, click **Save** in the tool bar at the bottom left to make the changes persistent. The adjusted cutoff values apply only to the current sample.

Figure 3-26: Cutoff Adjustment Entry Fields (PRA)



To adjust cutoffs graphically:

- Grab a cutoff line and drag it to the desired location. The coordinates in the tool tip are [Bead Position, NBG Ratio] (Figure 3-27). The revised values also appear in the Cutoff Adjustment Entry Fields (Figure 3-26).
- Click the **Save File** icon at the bottom left to preserve the changes

Color Coding in Revise Cutoff/Raw Data Grid

When you adjust cutoff values, beads in the data grid that have undergone a change in NIH score are marked with a red **X**, and their new NIH scores are also shown in red. (See bead #17 in Figure 3-28).

LABScreen applies the yellow alert color to any NBG ratio that is +/- 0.25 of the cutoff between NIH scores of 1 and 2.

Figure 3-27: Reaction Histogram with Cutoff Lines

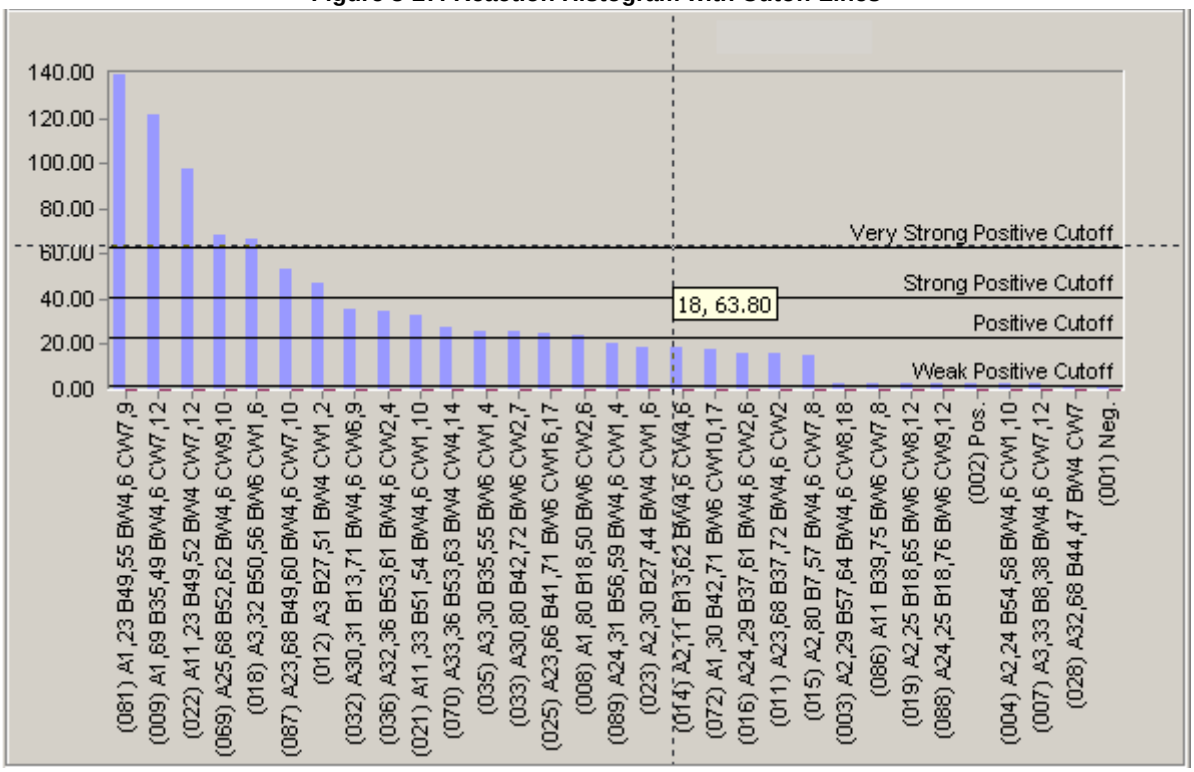


Figure 3-28: Color Coding in Revise Cutoff/Raw Data Grid

	007	2	221.000	1.930	104.79	114.53	114.53	91.26	A3,33 B8,38 BW4,6 CW7,12
	090	2	444.500	1.661	244.94	267.69	267.69	213.30	A11,34 B56,62 BW6 CW4,9
	017	1	165.000	1.359	111.07	121.38	121.38 X	96.72	A2,31 B39,48 BW6 CW7,8
	010	1	191.500	1.248	140.35	153.39	153.39	122.22	A30,69 B41,73 BW6 CW15,17
	021	1	170.000	1.233	126.13	137.85	137.85	109.84	A11,33 B51,54 BW4,6 CW1,10
	003	1	147.000	1.124	119.64	130.76	130.76	104.19	A2,29 B57,64 BW4,6 CW8,18
	082	1	216.000	1.118	176.74	193.16	193.16	153.91	A2,66 B63,65 BW4,6 CW8,16
	013	1	159.000	1.090	133.44	145.84	145.84	116.21	A2,29 B7,46 BW6 CW1,15
	097	1	275.000	1.038	242.43	264.94	264.94	211.11	A2 B46,67 BW6 CW1,7
	077	1	187.500	1.020	168.17	183.80	183.79	146.45	A2,26 B51,64 BW4,6 CW8,14
	070	1	167.000	1.006	151.85	165.96	165.96	132.24	A33,36 B53,63 BW4 CW4,14
	001	-	125.500	1.000	114.83	125.50	125.50 X	100.00	
	098	1	369.000	0.985	342.62	374.44	374.44	298.36	A3,36 B45,53 BW4,6 CW4,16
	076	1	177.000	0.961	168.58	184.25	184.25	146.81	A2,26 B47,65 BW4,6 CW6,8

Changing Parameters Locally (Temporarily)

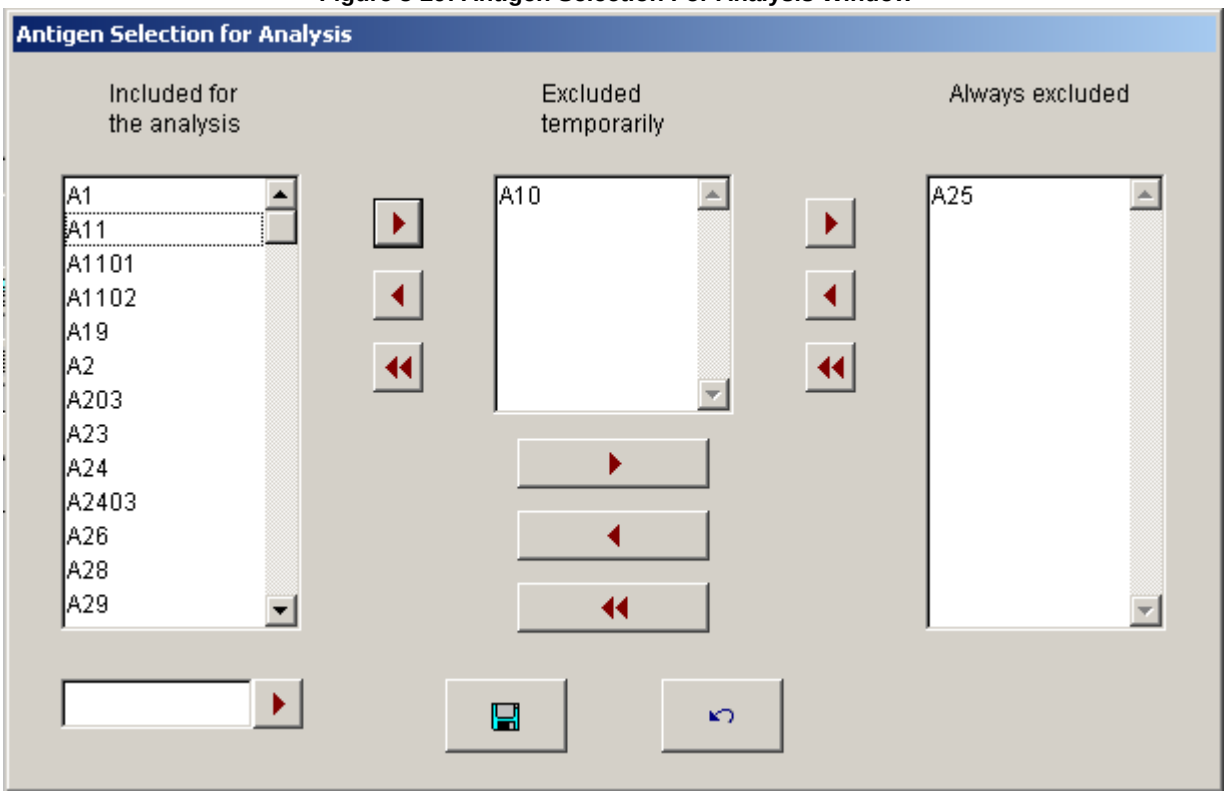
You can change parameters for the current sample from the Analysis window. Temporary parameter setting are not persistent. Thus, if you return to the sample at a later time, you must reset the parameters to the desired values. See *Updating Global Parameters, p. 41* for details on the parameter settings.

Excluding Antigens

1 To exclude antigens from the Analysis, click **Agn Exclude** to access the Antigen Selection for Analysis form. The usual selection conventions apply:

- Shift-click to select adjacent antigens
- Ctrl-click to select non-contiguous antigens
- A button with single arrow moves selected antigens
- A button with double arrow moves all antigens in the field

Figure 3-29: Antigen Selection For Analysis Window

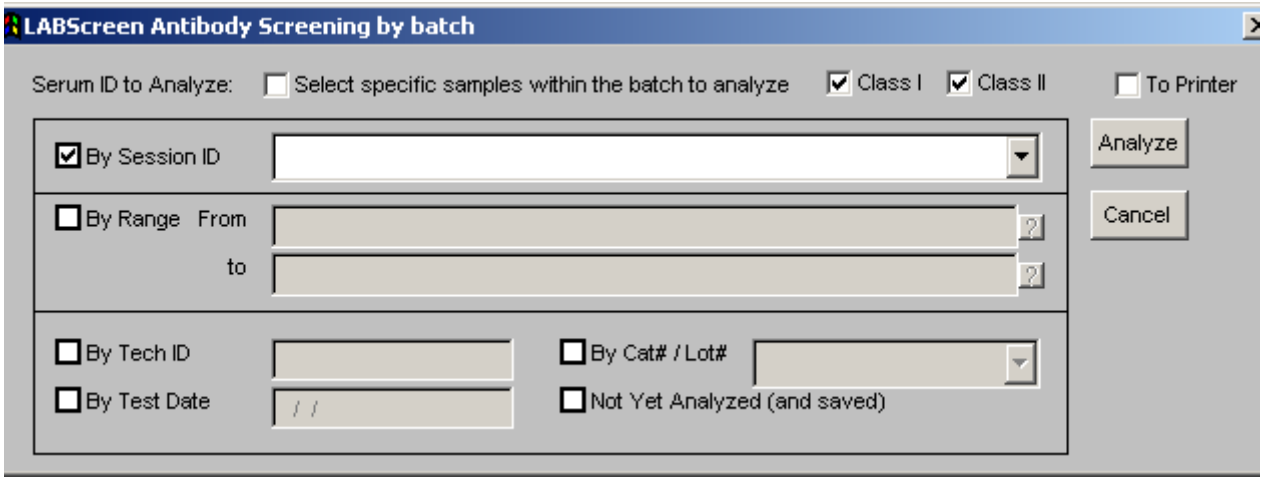


2 A temporary exclusion applies only to the sample currently under analysis. If you return to the same sample at a later time, you must reapply the temporary exclusion.

3 A permanent exclusion applies to the current sample and to other samples in other batches at all times.

Processing Multiple Samples

Figure 3-30: Antibody Screening By Batch



You can process multiple samples after importing as follows:

1 Select **Analysis** > **Analyze By Batch** from the Main menu to access the LABScreen Antibody Screening By Batch dialog (Figure 3-30).

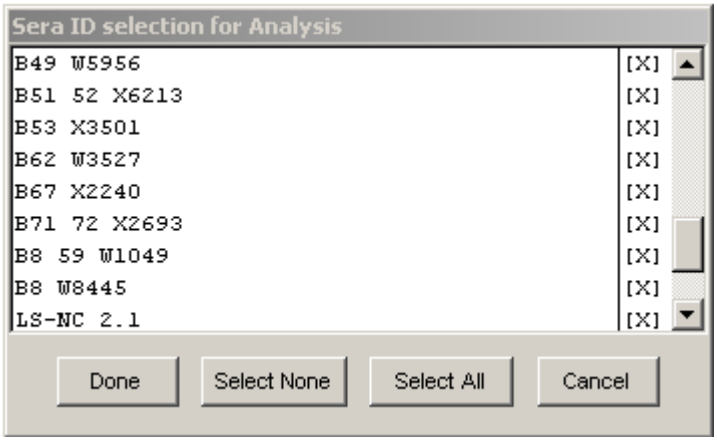
2 Choose from among these subtractive filters to specify how you want to process the batch.

- **By Session ID** – processes a batch based on the session ID.
- **By Range** – processes a specified range of samples with adjacent Sample IDs
- **By Tech ID** – processes samples loaded into LABScreen by the user with the specified ID; this ID is the one supplied when you Import a LABScan file; it can be viewed and edited in the **Maintenance > Reading > Data Entry** form
- **By Cat# /Lot#** – as stated; Cat IDs and Lot# can be viewed and edited in the **Maintenance > Reading > Data Entry** form
- **By Test Dat**e – as stated; this ID can be viewed and edited in the **Maintenance > Reading > Data Entry** form
- **Not Yet Analyzed** – only those samples that have not yet been Saved?Accepted by the user during analysis are presented. You can revisit a Saved sample by selecting the appropriate single-sample Analyze option and entering its Sample ID.

3 Specify whether you are analyzing Class I, Class II, or both, by checking the appropriate boxes.

- If you want to select specific Sample IDs within each batch, check the **Select Specific Samples** check box to access the pick-list shown in Figure 3-31:

Figure 3-31: Sera ID Selection Window



- Select samples by selecting or clearing the "X" next to each sample ID. Click **Select All** to include all samples in the range. Click **Select None** to clear all check boxes.

4 Click **Analyze** to proceed.

Note: If your criteria for selecting multiple samples produces more than 96 matches, the pick list in Figure 3-31 will appear even if you have not checked the **Select Specific Samples** option. You can deselect samples to reduce the size of the group and thus reduce the processing time.

Chapter 4: File and Data Maintenance

This chapter provides instructions for completing these tasks:

- Archive and retrieve serum, reaction, and results information
- Export reaction and results information
- Pack the database
- Rebuild the database

Archiving Data

The Archive Data option is available from the File menu. You can archive:

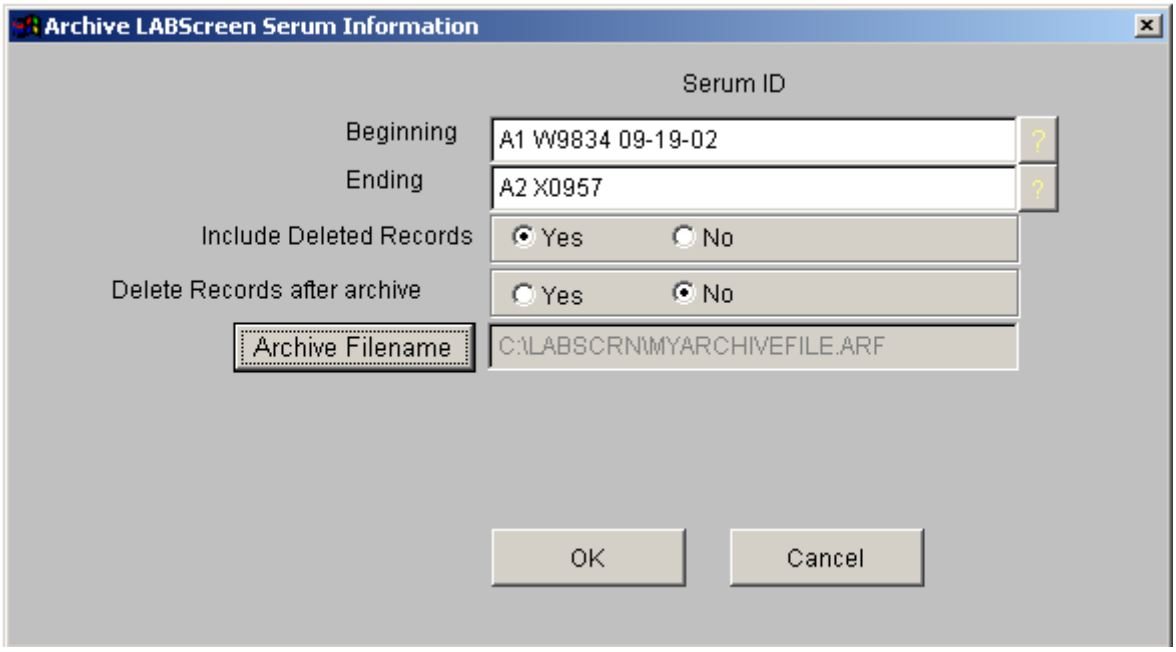
- Serum information
- Reaction information
- Class I or Class II analysis results

1 From the **Main** menu, select **File** > **Archive and Delete Data.**

2 Select one of the data options listed above.

3 Although the windows for archiving each type of data differ in some respects, the procedure for archiving and deleting records from these tables is basically the same for each type of data. The examples in this chapter use the Serum Information screens.

Figure 4-1: Archive LABScreen Serum Information Dialog Box



4 Specify the beginning and ending serum IDs of the range you want to archive or delete. To archive a single record, specify the same value for both the beginning and ending serum ID in the range. Click the **?** button to select specific serum IDs.

5 Specify whether to include records marked for deletion.

6 Specify whether to premanently delete records marked for deletion after they have been archived.

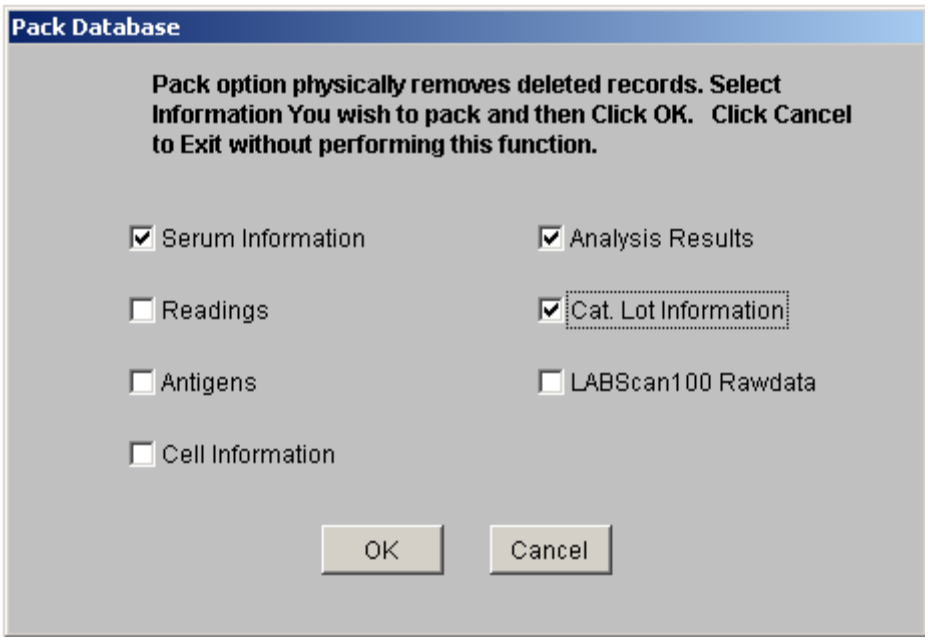
7 Supply a name for the archived file. LABScreen automatically adds the **.arf** extension. Click **OK** to create the file. A message confirms the count of the records archived.

Retrieving Archived Data

The Retrieve Data option is also accessed from the File Menu. It is effectively the same as the Archive Data function but only in reverse. As such, it requires no separate discussion.

Packing the Database

Figure 4-2: Pack Database Options



Use the Pack Database function to compress the tables shown in Figure 4-2.

Note: Using the Pack Database option permanently deletes all records that are marked for deletion. Make sure that you want to remove these records before you use this option.

Rebuilding the Index

If you experience circumstances that affect the integrity of your database tables, you may be able to use the Index Rebuild function to restore your database tables. This function is completely analogous to the Index Retrieval function and requires no separate comment.

Chapter 5: Parameters and Algorithms

Updating Global Parameters

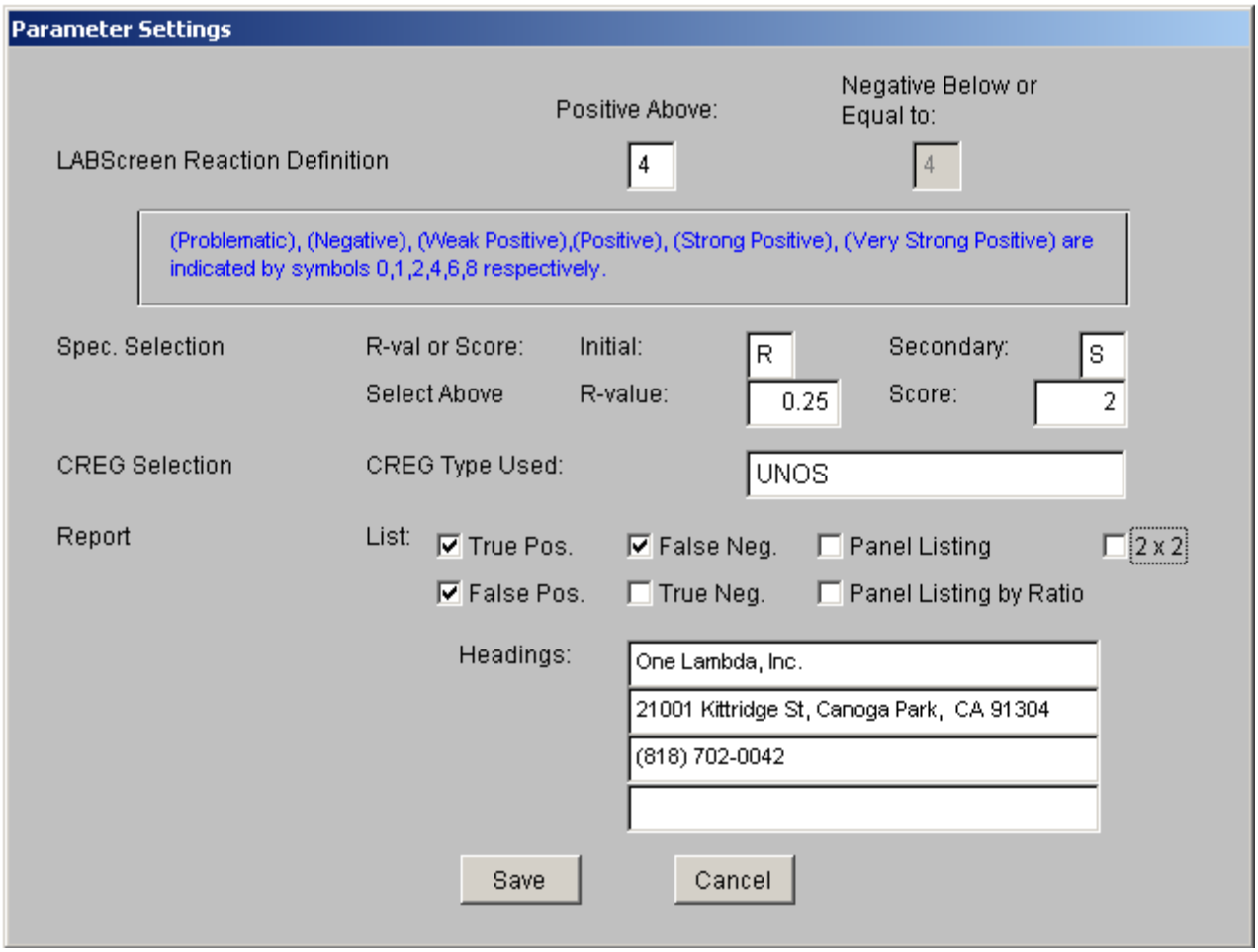
The Parameters Settings form (Figure 5-1) is used to set global analysis parameters and report defaults. It is accessed by selecting **Update Parameters** > **Update Parameter** from the Main menu.

Reaction Definitions, Specificity Selection Methods and Report Header Information

Table 5-1: Update Parameters > Parameter Settings

Control	Function	See
Reaction Definition	Specifies NIH score threshold for determining positive reactions in Class I and Class II analyses.	
Legend	Displays currently defined symbols for different strengths of reactions in PRA and SA which are set in the Advanced Settings forms.	
Specificity Selection	Determines whether R-Value or Score is to be used to make the first specificity assignment and then subsequent assignments. The software calculates R-Values for all possible antigens, then selects the antibody specificity with the highest R-Value. Once the first specificity has been select by the R-Value method, the Score calculation is used to assign the rest of the specificities. Be advised that it may be admissible to use the R-Value calculation for both Primary and Secondary assignments, but it is not recommended to use the Score method for the Primary assignment. If you choose to change the threshold values, you should perform validation testing based on the frequencies in your population.	*R-Value*, p. 50, *Score*, p. 50
CREG Selection	Determines whether the analysis will use UNOS or FULLER CREGs. If no entry is made, the analysis uses both. If one or the other is entered, the analysis uses only that one. By default both types are used.	Figure 3-13, *Class I Analysis Results Window*
Report	Specifies which report elements are included by default in the Antibody Screening Report; the preset default is TP, FN and FP only. The 2 X 2 option generates a separate report that lists the statistics for all antigens in the assay in the order of the highest R-Value to the lowest.	Figure A-13, *Class I and Class II Antibody Screening Reports*; Figure A-15, *Class I and Class II Antibody Screening Reports (2 x 2 Listing)*
Headings	Institution-specific information that is included in report headers.	

Figure 5-1: Global Parameter Settings Form



Changing the Date Format

The Select Data Format function accessed from the Update Parameters menu is self-explanatory and requires no comment.

Advanced Settings

PRA Parameter Settings

The PRA Advanced Settings form (Figure 5-2) is used to set cutoff levels and NIH score symbols for LABScreen PRA analyses. It is accessed by selecting **Update Parameters** > **LABScreen PRA Adv Settings** from the Main menu.

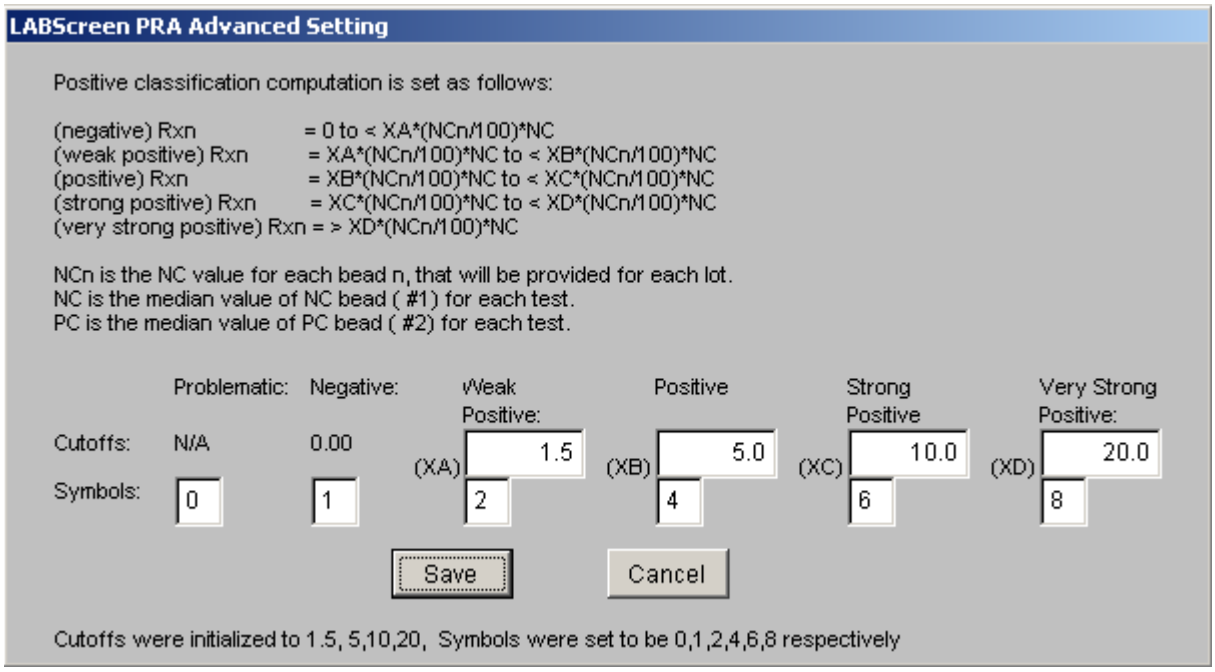
Table 5-2: Update Parameters > PRA Advanced Settings

Control	Function	See
Legend	States the formulas used to determine reaction strengths in assigning NIH scores.	*Reaction Positive Classification Guidelines and Formulas*, p. 49
Cutoffs	Cutoff constants used in the Reaction Classification formulas.	

Table 5-2: Update Parameters > PRA Advanced Settings

Control	Function	See
Symbols	NIH score symbols used the reports and database tables. By default these are 1, 2, 4, 6 and 8. A bead is designated as “Problematic” is it does not have a reading. These fields accept any characters in the extended ASCI character set.	
Legend (at bottom)	States the LABScreen default values for reference.	

Figure 5-2: LABScreen PRA Advanced Setting Dialog Box



SA Advanced Parameter Settings

The SA Advanced Settings form (Figure 5-3) is used to set cutoff levels and NIH score symbols for Single Antigen analyses. It is accessed by selecting **Update Parameters** > **LABScreen SA Adv Settings** from the Main menu.

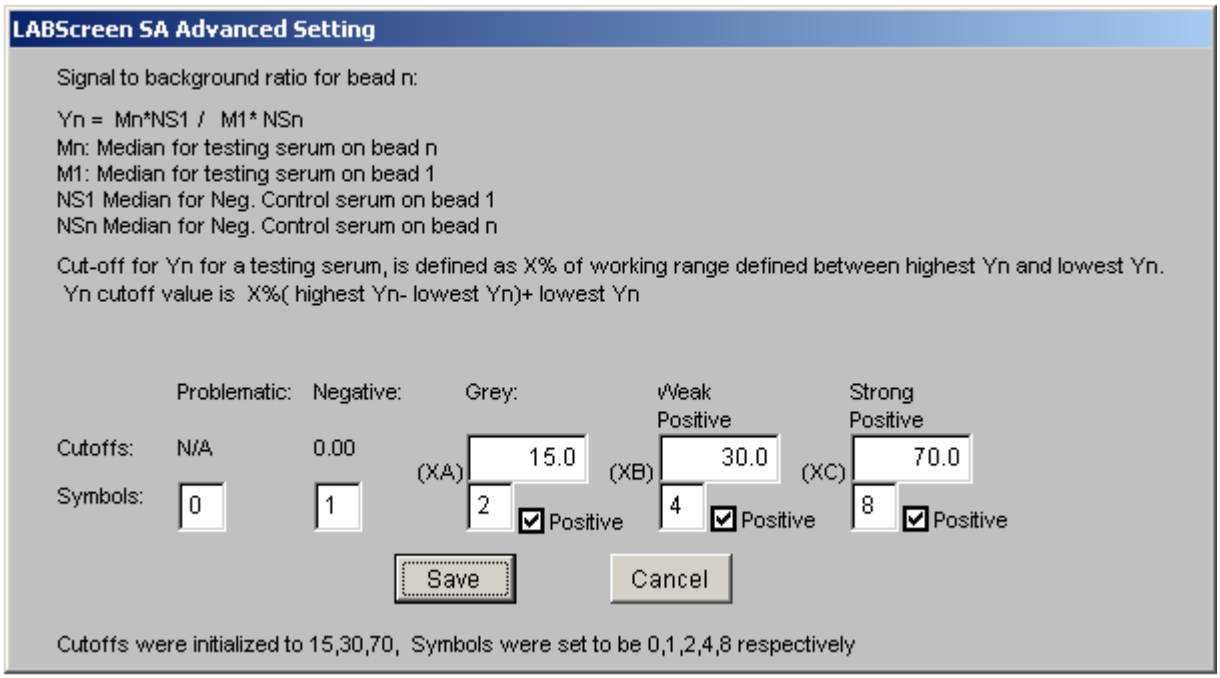
Table 5-3: Update Parameters > SA Advanced Settings

Control	Function	See
Legend	States the formulas used to determine reaction strengths in assigning NIH scores based on the Working Range of the reaction.	*Reaction Positive Classification Guidelines and Formulas*, p. 49
Cutoffs	Cutoff constants used in the Reaction Classification formulas.	

Table 5-3: Update Parameters > SA Advanced Settings

Control	Function	See
Symbols	NIH score symbols used the reports and database tables. By default these are 1, 2, 4, and 8. A bead is designated as “Problematic” if it does not have a reading. In contrast to PRA scoring, Single Antigen NIH scoring does not include a “6” reaction classification. These fields accept any characters in the extended ASCI character set.	
Legend	The legend at the bottom of the form states the LABScreen default values for reference.	

Figure 5-3: LABScreen SA Advanced Settings Form



Mixed Advanced Parameter Settings

The Mixed Advanced Settings form (Figure 5-4) is used to set Grey Area and Positive Reaction criteria for Mixed analyses. It is accessed by selecting **Update Parameters** > **LABScreen Mixed Adv Settings** from the Main menu.

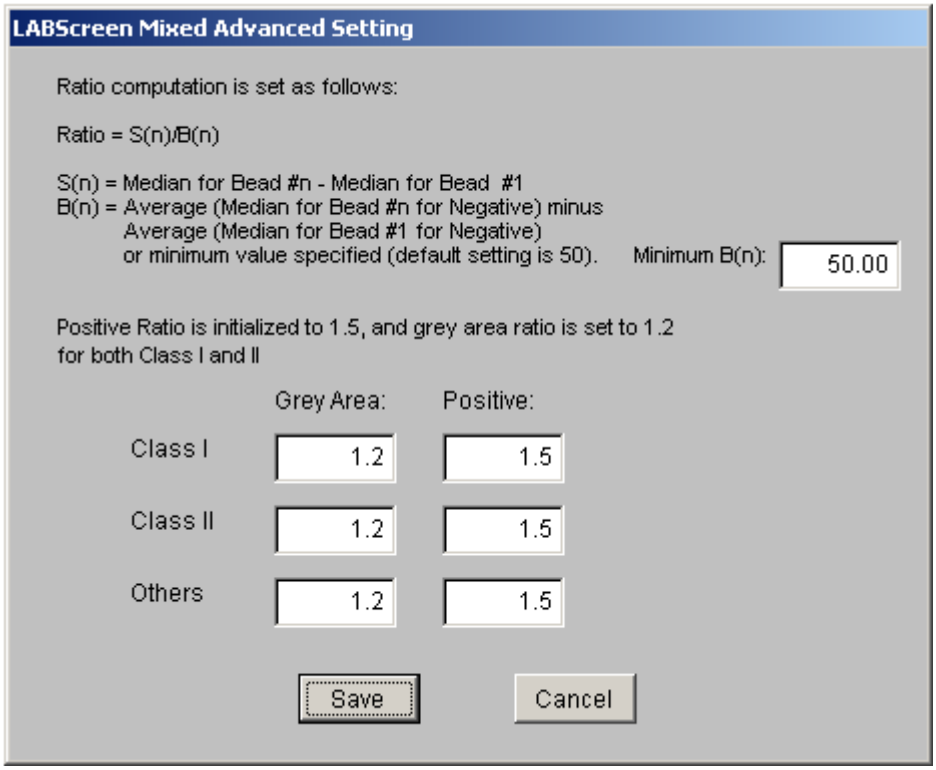
Table 5-4: Mixed Advanced Settings

Control	Function	See
Legend	State how Background values are defined.	*Reaction Positive Classification Guidelines and Formulas*, p. 49
Minimum	Sets minimum default background value for test beads.	

Table 5-4: Mixed Advanced Settings

Control	Function	See
Legend	States the initial default values LABScreen for reference for Class I, Class II and Other (e.g. MICA). Note that these are the initial and not the previous settings.	
Class I, Class II, Other	Current cutoff values for Class I, Class II and Other (e.g. MICA).	

Figure 5-4: LABScreen Mixed Advanced Setting Dialog Box



MFI to MESF Conversion Settings

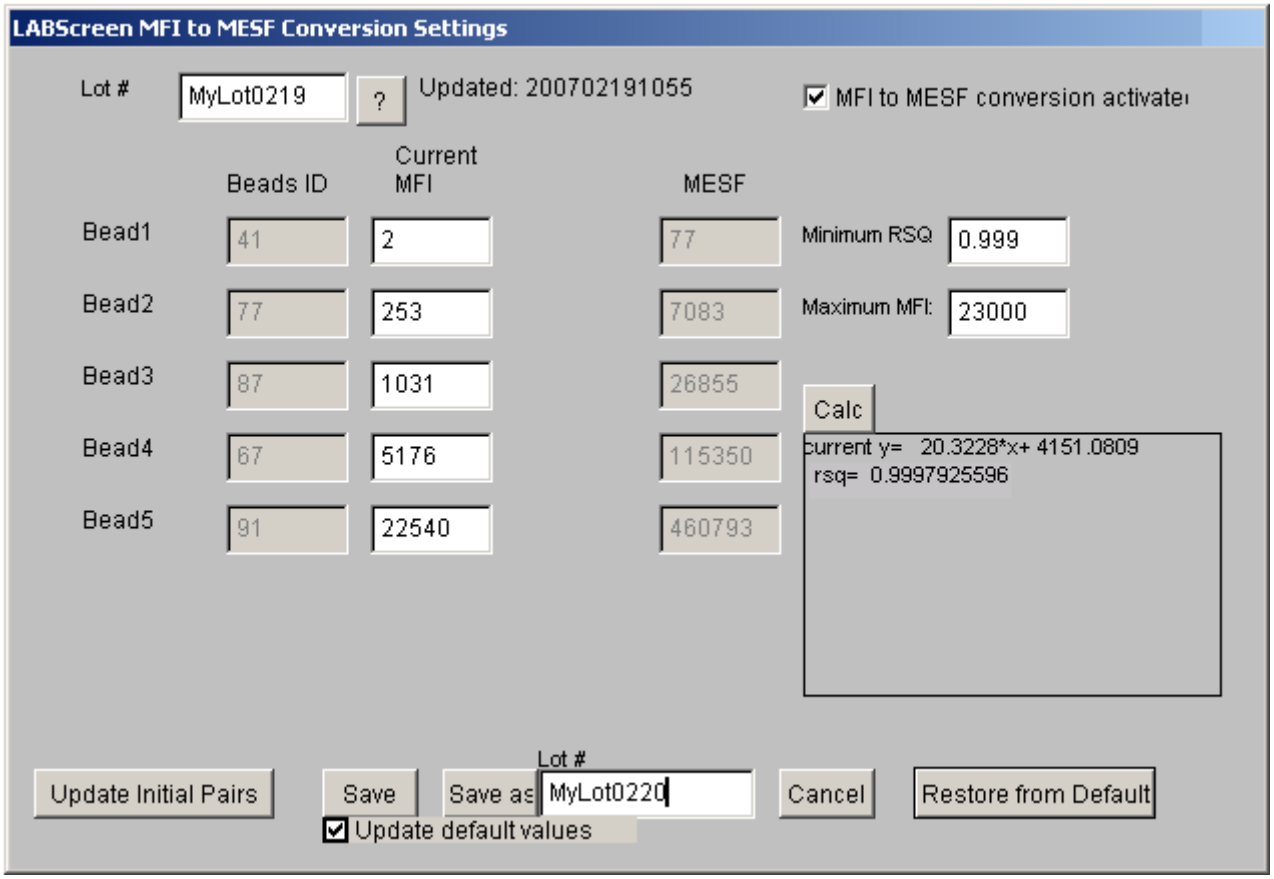
Table 5-5: MFI to MESF Conversion Settings

Control/Field	Function	See
Lot #	Use to locate database record containing conversion curve slope and intercept.	
Update legend	Timestamp for selected record (YYYYMMDDhhmm format)	
MFI to MESF conversion activate	Activates conversion globally; when selected, MFI to MESF conversion panel (Figure 3-3) is automatically opened during PRA/SA and Mixed Data Import/Reprocess.	Figure 3-1, Figure 3-4

Table 5-5: MFI to MESF Conversion Settings

Control/Field	Function	See
Bead ID	Data pairs table accommodates up to 7 beads. Note that the Bead ID is not necessarily always in ascending order although the MFI values are. Initially these fields are read only.	
Current MFI	MFI values used together with MESF values for generate the Slope and Intercept of the conversion formula.	
MESF	See above	
Minimum RSQ	Minimum threshold for goodness of fit. The conversion formula is generated from the MFI/ MESF data paris using a least squares fit. If a linear formula cannot be generated within this threshold, the RSQ value in the Calc panel is flagged with asterisks.	
Maximum MFI	Data pairs of beads with MFI values above this limit are discarded before the equation fit is made.	
Calc	Reruns the least squares fit using the valid values in the cells; then displays the updated MFI to MESF conversion formula.	
Update Initial Pairs	Enables Bead ID and MESF entry fields	
Save As	Saves new conversion formula to database table after user enters name for database record (up to 9 characters)	
Restore from Default	Reloads values from currently selected data-base record	

Figure 5-5: MFI to MESF Conversion Settings



Algorithms

Calculation of the Normalized BackGround Ratio

The reactivity of a test sample is calculated based on the median fluorescence values which are one of the data type in the LABScan .csv file. The strength of the reactivity is defined as the ratio (R) of the normalized sample median to the normalized background median.

The abbreviations used in this section are defined below:

NBG	**N**ormalized **B**ack**G**round
S_N	Median fluorescence value for **S**ample bead **N**
S_{NC} bead	**S**ample-specific median fluorescence value for **N**egative **C**ontrol bead
BG_N	**B**ack**G**round fluorescence value for bead **N** is by default a predetermined background value (from OLI reference data)
BG_{NC} bead	**B**ack**G**round fluorescence value for **N**egative **C**ontrol bead; the negative control bead is not coated with HLA antigen and is used to determine the non-specific binding for each bead

- For LABScreen PRA or LABScreen Single Antigen, the Normalized BackGround, NBG, for each bead is calculated as follows:

$$NBG = S_{NC}\frac{BG_N}{BG_{NC}}$$ **Eq. 5-1**

The Normalized BackGround *Ratio*, NBG *Ratio*, for each bead is the ratio of the median raw data value for each bead, S_N, to its Normalized BackGround, NBG.

$$NBGRatio = \frac{S_N}{NBG} = \frac{S_N/S_{NC}}{BG_N/BG_{NC}}$$ **Eq. 5-2**

- For LABScreen Mixed, a subtractive normalization is used in which the normalized median is defined as the median value of the Class I or Class II coated bead minus the median value of the NC bead. The NBG ratio is thus:

$$NBGRatio = \frac{S_N - S_{NC}}{BG_N - BG_{NC}}$$ **Eq. 5-3**

Determination of Positive/Negative Cutoff

1 For LABScreen PRA and Mixed:

1.1 Select the NBG ratio that gives a significant shift over background fluorescent value when the background value is obtained using the negative serum in 3 - 5 replicate tests. If you prefer, test 5 - 10 serum samples from non-transfused, non-transplanted male donors to obtain an average background value.

1.2 Validate the cut-off using 5 - 10 reference alloserum samples with defined HLA antibody specificity. The NGB ratio values for the expected positive antigen reactions should be above the cut-off.

1.3 Additional positive/negative reactions may be noted. If necessary, adjust the LABScreen assay cut-off to match the sensitivity of a previously accepted antibody detection assay.

2 For LABScreen Single Antigen:

2.1 Test negative control serum or several negative serum samples by following Step 1.1 above.

2.2 Define Working Range, WR:

$$WR = NBG_{max} - NBG_{min}$$ **Eq. 5-4**

2.3 Define cut-off points within the Working Range:

$$\text{Relative Cutoff} = X\%\ WR(NBG_{max} - NBG_{min}) + NBG_{min}$$ **Eq. 5-5**

where X% = user-defined percent cut-off point within the Working Range for negative(1), gray area(2), weak positive(4) or strong positive(8).

The cut-off points are relative to the maximum and minimum NBG ratios. Therefore, the definition of Negative, Weak Positive and Strong Positive will change for every sample.

2.4 Set criteria to define positive vs. negative reactions, for example, where **R** is the reading value:

- If the ratio of $\mathbf{R_{max}/R_{min} > 8}$, then apply the calculation in Step 2.3. If the serum test value is greater than a given % cut-off, assign the corresponding NIH score for that particular bead reaction.

- If the ratio of $R_{max}/R_{min} < 8$ AND

  If $R_{max} > 5$, then R_{min} should be adjusted to one half of the R_{max} and the relative NBG ratio cutoff should be re-computed based on the adjusted R_{min}. The reaction is then scored as above.

  If $R_{max} < 5$, then the reaction of the test serum with that bead is negative. Assign a score of "1".

Reaction Positive Classification Guidelines and Formulas

General parameters for LABScreen assays

- Bead counts should not be lower than 50 events per bead
- Patient samples with high background may be due to
  - Hydrophobicity of the plastic or the antigen binding process can effect non-specific binding
  - Patient therapies such as immunoglobulin (IVIG), Rituxan, or thymoglobulin (ATG)
- Negative Control bead (#001) median fluorescence should be lower than 1500; NC beads are blank (not coated with HLA antigen)

  NC bead fluorescence indicates the level of non-specific binding

  Abnormally low NC values can be due to high albumin/IgG ratios observed in dialysis and plasma pheresis patients
- Positive Control bead (#002) median fluorescence should be higher than 500 and at least twice the NC value; PC beads are coated with purified human IgG

  PC bead fluorescence indicates saturation of binding sites

  Low PC values may be due to washing techniques
  - Free IgG left in the assay can prevent the secondary antibody from binding to target
  - IgG-PE signal can be diluted

  Low PC values may be due to dilution of IgG-PE

PRA Reaction Formulas

- NBG Ratio < 1.5 = NIH score of "1" (negative)
- NBG Ratio between 1.5 and 5 = NIH score of "2" (weak positive)
- NBG Ratio between 5 and 10 = NIH score of "4" (positive)
- NBG Ratio between 10 and 20 = NIH score of "6" (positive)
- NBG Ratio above 20 = NIH score of "s" (strong positive)

Single Antigen Formulas

- % of Working Range < 15 = NIH score of "1" (negative)
- % of Working Range between 15 and 30 = NIH score of "2" (grey area)
- % of Working Range between 30 and 70 = NIH score of "4" (weak positive)
- % of Working Range above 70 = NIH score of "8" (positive)

Mixed Antigen Formulas

- NBG Ratio < 1.2 = Negative
- NBG Ratio between 1.2 and 1.5 = Grey area
- NBG Ratio above 1.5 Positive

Statistical and Scoring Formulas

TPs, FPs, TNs and FNs

True Positive (**TP**), **False Positive** (**FP**), **True Negative** (**TN**) and **False Negative** (**FN**) are defined as shown in Table 5-6. For example, if an antigen is present, but there no serum reaction detected, the reading would be considered a False Negative. To make the formulas more concise, the cell designations (A, B, C, D) are used in place of TP, FN, FP and TN.

Table 5-6: TP, FP, TN and FN Reaction Designations

	Reaction	
	Pos	**Neg**
Antigen Present	TP (A)	FN (B)
Antigen Absent	FP (C)	TN (D)

R-Value

R-Value – the Correlation Coefficient, R, is a measurement of the interdependence of two random variables. R ranges in value from -1 to +1, indicating perfect negative correlation at -1, absence of correlation at zero, and perfect positive correlation at +1.

The R-Value is calculated using the formula:

$$R = \frac{AD - DC}{\sqrt{(A+B)(A+C)(B+D)(C+D)}} \qquad \textbf{Eq. 5-6}$$

Values range from -1 to +1.

Chi-Square

Chi-Square – the Chi Square estimate of confidence, X^2, is calculated using the formula:

$$\chi^2 = \frac{N(AD - DC)^2}{(A+B)(A+C)(B+D)(C+D)} \qquad \textbf{Eq. 5-7}$$

Values range from 0 to infinity.

Score

Score – the Score, S, is the difference: TP - FN. The higher this number, the stronger the correlation.

Strength

Strength - the percent Strength is calculated by the formula:

$$Str = 100\frac{N_8}{N_{TP}}$$ **Eq. 5-8**

Inclusion Index

Inclusion Index– the Inclusion Index is the ratio:

$$Incl = \frac{N_{TP}}{N_{TP} + N_{FN}}$$ **Eq. 5-9**

Values range from 0 to 1.

Average

Average – this is the weighted Average of all reactions that are classified as true positives. If both 8s and 6s are considered positive, the Average is calculated as:

$$Avg = \frac{8N_8 + 6N_6}{N_8 + N_6}$$ **Eq. 5-10**

where N is the number of reactions in the true positive group. If 4s are also considered positive, both the numerator and the denominator contain corresponding terms for the 4s:

$$Avg = \frac{8N_8 + 6N_6 + 4N_4}{N_8 + N_6 + N_4}$$ **Eq. 5-11**

Fisher's Two-Tail

Fisher's Two-Tail – Tail analysis refers to the analysis of the low-frequency parts of a distribution, for example, the tails or ends of a distribution curve. Tail analysis is an appropriate statistic tool to use when analyzing antigen frequencies because of the large number of antigens and the small number of times any given antigen is detected during the assay.

The Fisher probability test is used to examine the significance of the association between two variables in a 2 x 2 contingency table. The need for the Fisher test arises when we have data that are divided into two categories in two separate ways (reaction positive or negative; antigen present or absent), and the sample sizes are small, or the data are very unequally distributed among the cells of the table (e.g. Table 5-6).

The question posed regards the probability of the distribution of the observed reactions presented in a 2 x 2 table such as shown in Table 5-6. The probability is calculated using the formula:

$$F = \frac{(A+B)!(A+C)!(B+D)!(C+D)!}{NA!B!C!D!}$$ **Eq. 5-12**

Note that 0! = 1.

Values range from 0 to +1, with 0 indicating no probability of occurrence and 1.00 complete probability.

Antigen Specificity Calculation

The antigen specificity table is generated as shown in Figure 5-6:

1 Retrieve all data needed for the selected sample and product combination: reactions, associated raw and normalized data and the antigens associated with each bead; compile a list of candidate antigens from which the specificities will be determined.

2 Set up a 2 x 2 table of TPs, TNs, FPs and FNs for each candidate antigen as shown above in Table 5-6, *TP, FP, TN and FN Reaction Designations*. Compute appropriate statistics including Chi-Square, Fisher's Two-Tail, Score, Strength, and Inclusion Index for each antigen.

3 Check the statistics against the specified threshold. Currently the user may choose either the R-Value or the score as the primary threshold criterion and the other as the secondary criterion. If no antigen meets the minimum, exit the loop.

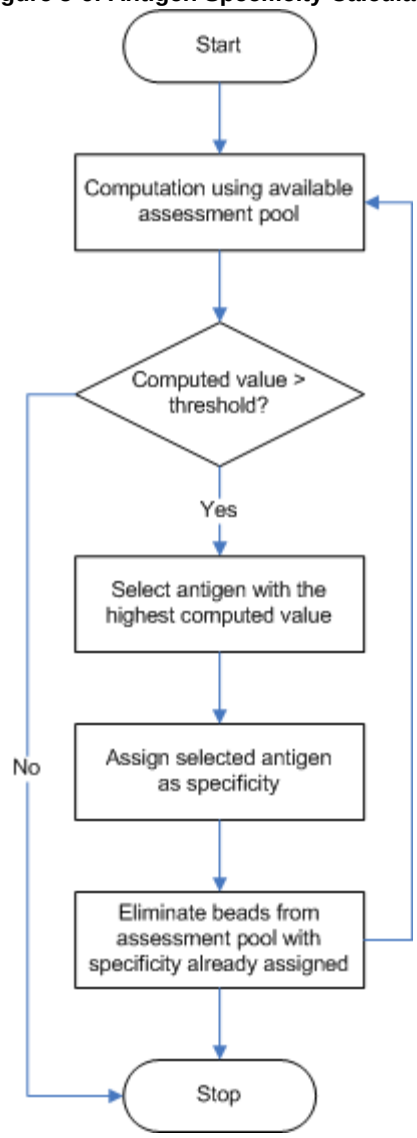
4 Check the candidate antigens to see which has the highest value in the selected statistics. In the case of a tie, the first-occurring antigen is ranked higher (or highest).

5 Add the antigen with the highest valuation to the list of selected specificities; include associated statistics in the tabulation.

6 Eliminate the selected antigen from the candidate list and remove all beads with that specificity from the data pool.

7 Go back to Step 2 for the next iteration.

Figure 5-6: Antigen Specificity Calculations



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Appendix A: LABScreen Reports

LABScreen generates raw data, analysis results, and panel listing reports. Many of the reports can be accessed from more than one point in the application. Depending on layout requirements, reports are generated either in portrait or landscape orientation.

When generating a report, you can preview the report by right-clicking on the appropriate print icon.

- Grey area results are indicated by three asterisks (***)
- Low PC/NC ratios are indicated by double left angle brackets (<<)
- Low bead counts are indicated by double pound signs (##)

LABScreen Reports

Reports Menu

Use these menu options to generate several of the most commonly produced LABScreen reports. A number of other reports types are available and are discussed later in this appendix.

Patient Antibody Summary – generates a summary Patient Sera Report (see Figure A-1, *Patient Antibody Summary Report*) for each patient specified by Patient ID. A summary report lists just the % positive Rxns and the specificities from all LABScreen analyses associated with that patient. The user can specify a single patient ID or a range of contiguous patient IDs.

Patient Antibody Report – generates a detailed specificity report with overall statistics for the analysis (# Rxns, # of positive Rxns, # of strong Rxns, etc.) and detailed data for each specificity (**TP**, **FN**, **FP** and **TN**) and statistics (**Inclusion**, **Strength**, **R-Value** and **Chi-Sq**). The user can specify a single patient ID or a range of contiguous patient IDs.

Patient HLA Report – generates a summary report of HLA typings for all the cells associated with the specified patient(s). The user can specify a single patient ID or a range of contiguous patient IDs.

Panel Listing – generates a panel listing for the specified product Cat ID and Lot #.

CREG Listing – generates a listing of the currently defined member antigens in Class I CREGs (Cross-Reacting Groups). The modification of CREG definitions is done in the Public Antigen Maintenance Data Entry form accessible via the main menu Maintenance options.

Patient Antibody Summary Report

Collected typing results for all samples linked with the specified Patient ID showing only percent positive reactions and specificities.

- Generated by selecting Report > Patient Antibody Summary Report from the main menu.

Figure A-1: Patient Antibody Summary Report

Patient Sera Report for (MARK5678) MARKOV, SOREN *02/07/2007*

Sera ID	Sample Date	Cat.ID Lot	Test Date	%Pos	Specificity
BIHI 4.1 LOT 7.1	12/12/2004	LS2PRA 007	05/18/2004	97.1	
A1 W4136	12/12/2004	LS1PRA 007	06/22/2004	25.5	A1 A74

Patient Antibody Report

Collected detailed reaction data and specificities for all samples linked with the specified Patient ID.

- Generated by selecting Report > Patient Antibody Report from the main menu.

Figure A-2: Patient Antibody Report

Patient Sera Report for (GREN2443) GRENFELD, MAXIM *02/07/2007*

Cat.ID Lot	Sera ID	Specificity	Avg	Sample Date Tpos	ABO M/C Fneg	#Rxn Fpos	#Pos Tneg	# (8) Rxn Incl	%Pos Strg	Rval	Chsq
LS1PRA 008	PA1			/ /	C	34	20	11	58.8		
		Overall	7.294	17	6	3	8	73.9	64.71	0.443	6.7
		A23	6.800	5	0	15	14	100.0	40.00	0.347	4.1
		BW4	7.400	10	6	5	8	62.5	70.00	0.239	1.7
		A1	8.000	2	0	3	8	100.0	100.0	0.539	3.8

Patient Cell Typing Report

Collected detailed HLA Typing for all samples linked with the specified Patient ID.

- Generated by selecting Report > Patient HLA Report from the main menu.

Figure A-3: Patient Cell Typing Report

Patient Cell Typing Report for (SMIT3413) SMITHERS, WAYLON *02/07/2007*

Cell ID	Sample Date	ABO	HLA_A HLA_DRB1	HLA_B HLA_DQB1	HLA_C HLA_DRB3/4/5	HLA_Bw
A1 W4136	12/12/2004	B				
			A74			
BIHI 4.1 LOT 7.1	12/12/2004		A*0101	B*07022	CW*0501	

LABScreen Mixed Data Full Report

Lists the results of all samples within a selected batch. Due to space restrictions, MESF values are not shown on the Mixed Data Full Report.

- Generated by selecting **LABScan 100 > Import/Reprocess Mixed** batch, then selecting the **Full** Report option in the Data Import/Report dialog.

Figure A-4: Mixed Data Full Report

02/07/2007 — **LABScreen Mixed Data** — Page 1

Cat.ID/Lot LSM12 012

Session ID J:\LABSCREEN_DATAFILES\LSM12_012_OUTPUT.CSV
Test Date 05/26/2006 03:17:57 **Tested By** WW

Default Background Values												
	NC	0.0	003	50.0	006	50.0	009	50.0	016	50.0		
	PC	5642.0	004	50.0	007	50.0	010	50.0	017	50.0		
			005	50.0	008	50.0	011	50.0				

	<---------- Class I ---------->						<---------- Class II ---------->								
Sample ID	Overall		Result	Raw data	Ratio	Count	Overall		Result	Raw data	Ratio	Count			Raw data	Count
16030	Pos.	003	Pos.	23562.0	471.1	119	Pos.	009	Pos.	542.5	10.7	290	NC		6.0	337
		004	Pos.	23639.0	472.7	13##		010	Pos.	466.0	9.2	227	PC		1082.0	127
		005	Pos.	22504.0	450.0	152		011	Pos.	508.0	10.0	263				
		006	Pos.	23769.5	475.3	110							PC/NC Ratio		180.3	
		007	Pos.	23814.0	476.2	118										
		008	Pos.	23707.0	474.0	101										
							Pos.	016	Pos.	19451.0	388.9	120				
								017	Pos.	397.0	7.8	180				
16031	Pos.	003	Pos.	142.5	2.6	150	Pos.	009	Pos.	23856.0	476.9	290	NC		11.0	248
		004	Pos.	148.0	2.7	174		010	Pos.	23840.5	476.6	227	PC		243.0	111
		005	***	75.0	1.3	183		011	Pos.	23650.5	472.8	263				
		006	Pos.	119.0	2.2	198							PC/NC Ratio		22.1	
		007	Neg.	69.0	1.2	152										
		008	Pos.	138.0	2.5	172										
							Pos.	016	Pos.	19451.0	388.8	120				
								017	Pos.	397.0	7.7	180				

Mixed Data Individual Report

MESF conversion values are shown only on Mixed Individual Reports.

- Generated by selecting **LABScan 100 > Import/Reprocess Mixed** > **Individual** in the Data Import/Report dialog.

Figure A-5: Mixed Data Individual Report

02/07/2007 **LABScreen Mixed Data** Page 1

Cat.ID/Lot LSM12 012

Sample ID 16030

Test Date 05/26/2006 03:17:57 **Tested By** WW

Session ID J:\LABSCREEN_DATAFILES\LSM12_012_OUTPUT.CSV

Default Background Values

NC	0.0	003	50.0	006	50.0	009	50.0	016	50.0	
PC	5642.0	004	50.0	007	50.0	010	50.0	017	50.0	
		005	50.0	008	50.0	011	50.0			

	Overall	Beads	Result	Raw data		Ratio	Count
Class I	Pos.	003	Pos.	23562.0	(590403.5)	471.1	119
		004	Pos.	23639.0	(592260.2)	472.7	13 ##
		005	Pos.	22504.0	(564891.5)	450.0	152
		006	Pos.	23769.5	(595407.0)	475.3	110
		007	Pos.	23814.0	(596480.0)	476.2	118
		008	Pos.	23707.0	(593899.9)	474.0	101
Class II	Pos.	009	Pos.	542.5	(35325.3)	10.7	290
		010	Pos.	466.0	(33480.6)	9.2	227
		011	Pos.	508.0	(34493.4)	10.0	263
MICA	Pos.	016	Pos.	19451.0	(491273.3)	388.9	120
		017	Pos.	397.0	(31816.8)	7.8	180
Controls		NC		6.0	(22388.5)		337
		PC		1082.0	(48334.5)		127
PC/NC Ratio						180.3	

Mixed Data Summary Report

Summarizes the results of all samples within a selected batch on one line per sample.

- Generated by selecting **LABScan 100 > Import/Reprocess Mixed** batch, then selecting the **Summary** option in the Data Import/Report dialog.

Figure A-6: Mixed Data Summary Report

02/07/2007 **LABScreen Mixed Data Summary** Page 1

Cat.ID/Lot LSM12 012

Session ID J:\LABSCREEN_DATAFILES\LSM12_012_OUTPUT.CSV
Test Date 05/26/2006 03:17:57 **Tested By** WW

Default Background Values

NC	0.0	003	50.0	006	50.0	009	50.0	016	50.0
PC	5642.0	004	50.0	007	50.0	010	50.0	017	50.0
		005	50.0	008	50.0	011	50.0		

SampleID	Class I	Class II	MCA	<----- NC -----> Rawdata	Count	<----- PC -----> Rawdata	Count	PC/NC Ratio
16030	Pos.	Pos.	Pos.	6.0	337	1082.0	127	180.3
16031	Pos.	Pos.	Pos.	11.0	248	243.0	111	22.1
16032	Pos.	Neg.	Pos.	89.0	201	10033.5	13##	112.7
16033	Pos.	Pos.	Pos.	8.0	266	9142.5	13##	1142.8
16034	Neg.	Neg.	Pos.	87.0	256	6226.5	13##	71.6
16035	Neg.	Neg.	Pos.	188.0	292	10423.5	13##	55.4
16036	Neg.	Neg.	Pos.	1.0	205	9537.5	13##	9537.5
16037	Neg.	Pos.	Pos.	3.0	184	56.0	13##	18.7
16038	Pos.	Pos.	Pos.	11.0	433	1068.0	191	97.1
16039	Pos.	Pos.	Pos.	11.0	302	217.0	159	19.7
16040	Pos.	Pos.	Pos.	96.0	234	9631.5	13##	100.3
16041	Pos.	Pos.	Pos.	7.0	240	10364.5	13##	1480.6
16042	Neg.	Neg.	Pos.	86.5	252	5951.0	13##	68.8
16043	Neg.	Pos.	Neg.	186.5	256	9965.0	13##	53.4
16044	Neg.	Neg.	Pos.	1.0	214	9877.5	13##	9877.5
16045	Pos.	Pos.	Pos.	1.0	218	33.0	13##	33.0
16046	Pos.	Pos.	Pos.	15.0	366	1070.0	177	71.3

PRA Data Report

This report can be printed from the Analysis or Import windows. When MEFS conversion is activated, it contains MESF values.

Figure A-7: PRA Raw Data Report

02/26/2007 **LABScreen Data** Page 1

Cat.ID/Lot LS1PRA 010

Session ID Y:\TESTDATA\LS1PRA010.CSV
Test Date 04/24/2006 03:20:22 PM **Tested By** WW **Nc** 37.0 **Pc** 3,096.0 **Pc/Nc** 83.7
Sample ID <UNNAMED> (9106)
PRA Percent 8 : 98.18% Percent 8,6,4 : 100.00%
Percent 8,6 : 100.00% Percent 8,6,4,2 : 100.00%
MESF MESF Beads Lot# (1234567890) Processed on [2007022211054] Y=1372.1835+23.03863X

Beads ID	Rxn	Reagent ID	Rawdata	Ratio (*)	Count	Specificities
001	-	NC	37.0 (2224.6)	1.0	295	
010	8	E18278	24189 (558653.6)	31.9	137	A1,2 B57,8201 BW4,6 CW6,10
011	8	G0229	23905 (552110.6)	129.2	146	A1,80 B18,50 BW6 CW2,6
012	8	E5061	23509 (542975.8)	98.9	150	A11,23 B49,52 BW4 CW7,12
013	8	G0269	23936 (552813.3)	134.7	140	A11,24 B27,48 BW4,6 CW1,8
014	8	E5482	20311 (469309.8)	106.3	243	A11,24 B54,59 BW6,4 CW1
015	8	G0106	23454 (541720.2)	89.0	155	A11,24 B59,60 BW4,6 CW1,10
016	8	G0363	22241 (513774.4)	21.9	161	A11,29 B18,38 BW6,4 CW12
017	8	C4613	24090 (556361.3)	53.1	110	A11,33 B51,54 BW4,6 CW1,10
018	8	E12635	23992 (554115.0)	27.6	145	A11 B39,75 BW6 CW7,8
019	8	G0086	23461 (541870.0)	44.3	166	A2,11 B13,62 BW4,6 CW4,6
002	+	PC	3096.0 (72699.8)	0.1	257	
020	8	G0286	24036 (555128.7)	76.9	101	A2,11 B51,76 BW4,6 CW10,14
084	8	E3825	24274 (560611.9)	22.8	100	A32,68 B44,47 BW4 CW7
085	8	E14072	23540 (543701.5)	47.7	155	A33,36 B53,63 BW4 CW4,14
086	8	E20388	23853 (550912.6)	79.0	245	A33,74 B63,42 BW4,6 CW14,17
087	8	E18401	21178 (489284.3)	55.1	186	A33,74 B72,78 BW6 CW2,16
088	8	G0238	23405 (540591.3)	41.3	167	A34 B56,61 BW6 CW1,15
089	8	E18066	24084 (556234.5)	54.4	155	A68,74 B45,72 BW6 CW2, CW16
009	8	E17776	23596 (544991.7)	47.4	150	A1,69 B35,49 BW6,4 CW7,12

SA Raw Data Report

This report can be printed from the Analysis or import windows. When MEFS conversion is activated, it contains MESF values.

Figure A-8: Single Antigen Raw Data Report

02/16/2007 **LABScreen Data** Page 1

Cat.ID/Lot LS1A1&2 001

Session ID J:\LABSCREEN_DATAFILES\LS1A1_2.CSV

Test Date 09/30/2002 03:12:03 PM **Tested By** WW **Nc** 84.5 **Pc** 4,597.0 **Pc/Nc** 54.4

Sample ID PC1 09-30-02

% Positive	Percent 8	:	5.00%	Percent 8,4	:	28.75%
				Percent 8,4,2	:	43.75%
Ratio	Maximum Ratio	:	202.38	Minimum Ratio	:	1.69
	Cutoff for 8	:	142.173	Cutoff for 4	:	61.897
				Cutoff for 2	:	31.794

MESF MESF Beads Lot# (DRAFT1.0) Processed on [200702161558] Y=4176.2178+20.30519X

Beads ID	Rxn	Reagent ID	Rawdata	Ratio (*)	Count	Specificities
001	-	NC	84.5 (5892.0)	1.0	562	
002	+	PC	4597.0 (97519.2)	4.0	572	
003	2	RA0101	7362.5 (153673.2)	57.2	100	A1
004	2	RA0201	8582.0 (178435.4)	58.5	248	A2
005	4	RA0206	14006 (288570.7)	129.6	167	A2
006	1	RA0301	964.0 (23750.4)	6.9	146	A3
007	1	RA1101	993.0 (24339.3)	7.8	203	A11
008	1	RA2301	3158.0 (68300.0)	28.4	236	A23
077	1	RB7301	119.0 (6592.5)	1.1	378	B73 BW6
078	1	RB1512	113.0 (6470.7)	1.0	301	B76 BW6
079	1	RB1513	277.0 (9800.8)	2.6	273	B77 BW4
080	1	RB7801	111.0 (6430.1)	1.1	301	B78 BW6
081	1	RB8101	123.0 (6673.8)	1.2	261	B81 BW6
082	1	RB8201	131.0 (6836.2)	0.9	187	B8201 BW6
083	1	RA2902	537.5 (15090.3)	2.1	276	A29
084	1	RA6801	600.0 (16359.3)	3.6	292	A68

SA Analysis Report

This report can be printed from the Analysis window. When MEFS conversion is activated, the report also contains MESF values.

Figure A-9: Single Antigen Analysis Report

02/16/2007 **LABScreen Data** Page 1

Cat.ID/Lot LS1A1&2 001

Session ID J:\LABSCREEN_DATAFILES\LS1A1_2.CSV
Test Date 09/30/2002 03:12:03 PM **Tested By** WW **Nc** 248.0 **Pc** 5,456.0 **Pc/Nc** 22.0
Sample ID A19 Z9003 09-30-02

% Positive	Percent 8	: 5.000	Percent 8,4	: 18.750	
			Percent 8,4,2	: 31.250	
Ratio	Maximum Ratio	: 51.625	Minimum Ratio	: 0.616	
	Cutoff for 8	: 36.322	Cutoff for 4	: 15.919	
			Cutoff for 2	: 8.267	

Specificity A31(1/0),B7(2/0),A30(1/0),A33(2/0),A32(1/0),B81(1/0),B60(1/0),B27(2/0),A66(2/0),A43(1/0),A25(1/0),A74(1/0),B41(1/1),A23(1/0),B48(1/0),A29(1/0),B67(1/0),A11(1/1),B42(1/0),B73(1/0)
Overall True Positives: 25 False Positives: 0 False Negatives: 2 True Negatives: 53

CREG Possible CREG antibody(s) are:

Specificity	TruePos	FalseNeg	R-val
27C	6	2	0.315
28C	8	6	0.343

<27C> := B7 B27 B13 B40 B60 B61 B47
<28C> := A10 A25 A26 A34 A66 A11 A28 A68 A69 A30 A31 A33

MESF MESF Beads Lot# (DRAFT1.0) Processed on [200702161558] Y=4176.2178+20.30519X

Beads ID	Rxn	Reagent ID	Rawdata	Ratio (*)	Count	Specificities
015	8	RA3101	14160 (291697.7)	51.6	175	A31
019	8	RB0702	13786 (284103.6)	51.3	174	B7 BW6
014	8	RA3001	12825 (264590.3)	48.9	237	A30
017	8	RA3301	10478 (216934.0)	39.6	223	A33
016	4	RA3201	12079 (249442.6)	33.6	198	A32
081	4	RB8101	11793 (243635.3)	30.6	323	B81 BW6
035	1	RB5301	340.5 (11090.1)	0.9	178	B53 BW4
063	1	RB4701	452.0 (13354.2)	0.9	273	B47 BW4
076	1	RB1510	347.0 (11222.1)	0.9	235	B71 BW6
071	1	RB5901	456.0 (13435.4)	0.9	239	B59 BW4
065	1	RB5001	290.0 (10064.7)	0.8	225	B50 BW6
058	1	RB3905	258.0 (9415.0)	0.8	351	B39 BW6
080	1	RB7801	278.0 (9821.1)	0.7	351	B78 BW6
062	1	RB4601	244.0 (9130.7)	0.6	276	B46 BW6
001	-	NC	248.0 (9211.9)	1.0	520	
002	+	PC	5456.0 (114961.3)	1.6	555	

Antigen Distribution Listing

The Antigen Distribution Listing shows how many beads in the product would react with a given antigen. To learn the specificities of a given bead, consult the Panel Listing for that product (Figure A-12). Class I and Class II Antigen Distribution listings are similar in format. A Distribution Listing can be generated when importing or reprocessing PRA data by selecting the Include Panel with Report option.

Figure A-10: Typical Antigen Distribution Listing

Class II Antigen Distribution for LS2PRA Lot 007

Antigen	Count	Antigen	Count	Antigen	Count
DQ2	14	DQ4	5	DQ5	19
DQ6	10	DQ7	10	DQ8	4
DQ9	4	DR1	6	DR10	2
DR103	2	DR11	4	DR12	4
DR13	5	DR14	5	DR15	7
DR16	3	DR17	7	DR18	3
DR4	7	DR51	10	DR52	21
DR53	15	DR7	7	DR8	4
DR9	4				

Sample Raw Data Report

Contains raw data values, statistics and specificities for the sample currently under analysis.

- To generate, select **Raw Data** button from within the Class I/Class II Analysis Results window or from within the **Maintenance > Readings > Raw Data** panel.

Figure A-11: Sample Raw Data Report

02/07/2007 **LABScreen Data** Page 1

Cat.ID/Lot LS1PRA 007

Session ID J:\LABSCREEN_DATAFILES\LS1PRA_007.CSV
Test Date 06/22/2004 10:08:14 AM **Tested By** WW **Nc** 413.5 **Pc** 7,396.0 **Pc/Nc** 17.9
Sample ID A1 W4136
PRA Percent 8 : 5.45% Percent 8,6,4 : 54.55%
Percent 8,6 : 25.45% Percent 8,6,4,2 : 87.27%

Beads ID	Rxn	Reagent ID	Rawdata	Ratio (*)	Count	Specificities
001	-	NC	413.5	1.0	42##	
002	+	PC	7396.0	0.4	62	
003	1	G0102	587.0	1.4	34##	A2,29 B57,64 BW4,6 CW8,18
004	1	C5014	366.0	0.8	36##	A2,24 B54,58 BW4,6 CW1,10
005	4	E6448	3488.0	6.9	51	A1,32 B60,64 BW6 CW8,10
006	6	E22237	9234.0	13.8	48##	A1,23 B8,27 BW4,6 CW1,7
007	2	C5001	912.0	2.4	51	A3,33 B8,38 BW4,6 CW7,12
008	8	C5009	11690	24.0	43##	A1,80 B18,50 BW6 CW2,6
009	6	E17776	7369.0	13.8	32##	A1,69 B35,49 BW4,6 CW7,12
010	2	E16092	2462.0	4.9	38##	A30,69 B41,73 BW6 CW15,17
011	2	E15254	900.0	2.0	32##	A23,68 B37,72 BW4,6 CW2
012	2	C5003	1055.0	2.3	42##	A3 B27,51 BW4 CW1,2
013	1	G0099	696.5	1.4	32##	A2,29 B7,46 BW6 CW1,15
089	2	C5016	1112.0	1.8	32##	A24,31 B56,59 BW4,6 CW1,4
090	4	G0138	5534.5	6.3	42##	A11,34 B56,62 BW6 CW4,9
095	1	E2934	576.0	0.7	40##	A2,24 B54,67 BW6 CW1,7
096	4	C4630	4228.0	5.7	49##	A3,66 B7,81 BW6 CW15,18
097	1	E12572	998.5	1.1	38##	A2 B46,67 BW6 CW1,7
098	4	E18858	10641	8.6	57	A3,36 B45,53 BW4,6 CW4,16

Class and Class II Panel Listings

Panel listings can be generated by selecting **Report** > **Panel Listing** from the main menu, then specifying the LABScreen product from a pick list. You can preview the listing by right-clicking on the print button, or export the listing to an Excel file.

Figure A-12: Typical Antigen Panel Listing

s ReagentID	HLA DRB1	HLA DQ	HLA DRB3/4/5	Others
NC				
PC				
E17776	DR1 DR4	DQ5 DQ8	DR53	
C4630	DR12 DR18	DQ4 DQ5	DR52	
G0099	DR9 DR10	DQ5 DQ9	DR53	
C4613	DR13 DR15	DQ5 DQ6	DR52 DR51	
G0087	DR8 DR17	DQ2 DQ6	DR52	
C4615	DR4 DR7	DQ2 DQ8	DR53	
E5610	DR103 DR17	DQ2 DQ5	DR52	
G0069	DR9 DR15	DQ5 DQ9	DR53	
C4722	DR4 DR14	DQ7 DQ8	DR52 DR53	
E6448	DR1 DR7	DQ2 DQ5	DR53	
E4538	DR11 DR13	DQ6	DR52	

Class and Class II Analysis (Antibody Screening) Reports

Contains complete analysis data for the sample currently under analysis. NIH scores, NBG ratios and specificities for FPs, FNs and TNs are also included in the report, although only the TPs are shown in Figure A-13.

- Generated from within the Class I/Class II Analysis Result window by selecting the Print option.

Figure A-13: Class I and Class II Antibody Screening Reports

LABScreen Class I Antibody Screening

Patient's name :
Serum Id. No. : Al W4136
Patient Id :
Race :
Center Id :
Date sampled : / /
Analysis : Computer Assignment
Date tested : 06/22/2004
HLA Typing :

Percent positive : 25.45 %
Total valid reactions : 55
Number of positives : 14
Positive reactions are : 6,8
Overall strength index : 21.43 %

Control Values :

<LS1PRA 007 > NC:413.5 PC:7396.0 PC/NC:17.9 Background:Default

Percent 8 : 5.45%
Percent 8,6 : 25.45%
Percent 8,6,4 : 54.55%
Percent 8,6,4,2 : 87.27%

Specificity	Avg Score	TP	FP	FN	TN	N	R Value	% Incl.	Str. Index	Chi Square	Fisher's 2-Tail
Al	6.5	7	7	1	40	55	0.588	87.50	28.57	18.99	0.000118
A74	7.0	2	5	0	40	47	0.504	100.00	50.00	11.94	0.019426
Overall	6.6	9	5	1	40	55	0.699	90.00	33.33	26.80	0.000003

Possible CREG antibody(s) are:

Specificity	TruePos	FalseNeg	R-val
1C2	12	15	0.428

<1C2> :=: Al A36 A9 A23 A24 All

(True Positive)

	CatID	Lot	Bead	Reagent	HLA A, B, C and Bw4/Bw6				Rxn	Ratio	Count
(Al)	LS1PRA	007	008	C5009	Al, A80	B18, B50	CW2, CW6	BW6	8	24.0	43###
(Al)	LS1PRA	007	081	E19109	Al, A23	B49, B55	CW7, CW9	BW4, BW6	8	23.7	32###
(Al)	LS1PRA	007	073	E17990	Al, A74	B72, B8201	CW2, CW10	BW6	6	18.6	30###
(Al)	LS1PRA	007	071	E18149	Al, A29	B13, B81	CW6	BW4, BW6	6	16.8	28###
(Al)	LS1PRA	007	072	G0079	Al, A30	B42, B71	CW10, CW17	BW6	6	15.8	39###
(Al)	LS1PRA	007	009	E17776	Al, A69	B35, B49	CW7, CW12	BW4, BW6	6	13.8	32###
(Al)	LS1PRA	007	006	E22237	Al, A23	B8, B27	CW1, CW7	BW4, BW6	6	13.8	48###
(A74)	LS1PRA	007	027	G0146	A34, A74	B44, B57	CW4, CW7	BW4	8	22.3	56
(A74)	LS1PRA	007	073	E17990	Al, A74	B72, B8201	CW2, CW10	BW6	6	18.6	30###
(A74)	LS1PRA	007	030	E13060	A26, A74	B78, B81	CW16, CW18	BW6	6	12.9	49###

Class I and Class II Analysis (Antibody Screening) Reports (Reversed)

Analysis report generated after reversing the NIH scores for the sample: 8s become 1s, 6s become 2s, etc. The score reversal (or inversion) in effect tests for the absence of antigens rather than their presence.

- Generated from within the Class I/Class II Analysis Result window by clicking the +/- button, then selecting the Print option. A detail of the report is shown below.

Figure A-14: Class I and Class II Antibody Screening Reports (Reversed)

LABScreen Class I Antibody Screening (Positive and Negative Reversed)

Patient's name : MARKOV, SOREN
Serum Id. No. : Al W4136
Patient Id : MARK5678
Race :
Center Id : GLENDALE
Date sampled : 12/12/2004
Analysis : Manual Assignment
Date tested : 06/22/2004
HLA Typing :

Percent positive : 45.45 %
Total valid reactions : 55
Number of positives : 25
Positive reactions are : 6,8
Overall strength index : 28.00 %

Control Values :

<LSIPRA 007 > NC: 413.5 PC: 7396.0 PC/NC: 17.9 Background: Default

Percent 8 : 12.73%
Percent 8,6 : 45.45%
Percent 8,6,4 : 74.55%
Percent 8,6,4,2 : 94.55%

Specificity	Avg Score	TP	FP	FN	TN	N	R Value	% Incl.	Str. Index	Chi Square	Fisher's 2-Tail
CW1	6.8	10	15	4	26	55	0.305	71.43	40.00	5.11	0.031971
A68	6.5	4	11	1	25	41	0.336	80.00	25.00	4.63	0.051365
B39	7.0	2	9	0	25	36	0.366	100.00	50.00	4.81	0.087302
B58	6.0	2	7	0	25	34	0.417	100.00	0.00	5.90	0.064171
Overall	6.6	18	7	5	25	55	0.559	78.26	33.30	17.20	0.000042

Class I and Class II Analysis (Antibody Screening) Reports (2 x 2 Listing)

This report contains statistics for all the antigens in the assay, including those not selected as specificities for the sample. You can select Patient Antibody Report to generate analysis results reports for one or more patients with contiguous Patient IDs.

- Generated after selecting 2 x 2 Report output option for the specific sample in the Temporary Parameters Setting form accessible from the Analysis Window or in the Update Parameters form (Figure 5-1, *Global Parameter Settings Form*). In the detail of the report shown below only the beginning and ending antigens are included.

Figure A-15: Class I and Class II Antibody Screening Reports (2 x 2 Listing)

LABScreen Class I Antibody Screening

Patient's name :
Serum Id. No. : <UNNAMED> (9107)
Patient Id :
Race :
Center Id :
Date sampled : / /
Analysis : 2x2 Listing
Date tested : 04/24/2006
HLA Typing :

Percent positive : 30.91 %
Total valid reactions : 55
Number of positives : 17
Positive reactions are : 6,8
Overall strength index : 5.88 %

Control Values :

<LS1PRA 010 > NC:3.0 PC:564.0 PC/NC:188.0 Background:Default

Percent 8 : 1.82%
Percent 8,6 : 30.91%
Percent 8,6,4 : 70.91%
Percent 8,6,4,2 : 100.00%

Specificity	Avg Score	TP	FP	FN	TN	N	R Value	% Incl.	Str. Index	Chi Square	Fisher's 2-Tail
A24	6.4	5	12	1	37	55	0.397	83.33	20.00	8.67	0.008538
CW17	6.0	4	13	1	37	55	0.336	80.00	0.00	6.21	0.027777
B59	6.0	2	15	0	38	55	0.290	100.00	0.00	4.64	0.091582
B48	6.0	2	15	0	38	55	0.290	100.00	0.00	4.64	0.091582
B41	6.0	2	15	0	38	55	0.290	100.00	0.00	4.64	0.091582
B76	6.0	1	16	0	38	55	0.203	100.00	0.00	2.28	0.309091
CW9	8.0	1	16	4	34	55	-.075	20.00	100.00	0.31	0.670200
A30	6.0	1	16	4	34	55	-.075	20.00	0.00	0.31	0.670200
A23	6.0	1	16	4	34	55	-.075	20.00	0.00	0.31	0.670200
A1	6.0	2	15	7	31	55	-.083	22.22	0.00	0.38	0.704759
A3	6.0	1	16	5	33	55	-.108	16.67	0.00	0.64	0.653710
CW8	6.0	2	15	8	30	55	-.111	20.00	0.00	0.68	0.481952
CW4	6.0	1	16	6	32	55	-.137	14.29	0.00	1.04	0.416254

Changing Report Paper Size

You can change the report paper size from the default U.S. letter size to A-4 as follows:

1 Exit the LABScreen application.

2 Assuming that LABScreen has been installed in the default C:\ location, navigate to C:\labscrn.

3 Sort the files by type and locate the two MS-DOS batch files, **A4_size_report** and **Letter_size_report**.

4 Launch the **A4_size_report** batch file. This replaces the contents of the folder **C:\labscrn\Reports** with the contents of the folder **C:\labscrn\REPORTS_A4**. The next time you generate a LABScreen report, it will be in the A-4 format.

5 To restore the report size back to the default letter size, launch the **Letter_size_report** batch file.

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