BD FACS™ Sample Prep Assistant III Instructions For Use
Notice

BD Biosciences delivers software and workstations that are intended for running the instruments supplied by BD Biosciences. It is the responsibility of the buyer/user to ensure that all added electronic files including software and transport media are virus-free. If the workstation is used for Internet access or purposes other than those specified by BD Biosciences, it is the buyer/user's responsibility to install and maintain up-to-date virus protection software. BD Biosciences does not make any warranty with respect to the workstation remaining virus-free after installation. BD Biosciences is not liable for any claims related to or resulting from buyer/user's failure to install and maintain virus protection.

History

<table>
<thead>
<tr>
<th>Revision</th>
<th>Date</th>
<th>Change Made</th>
</tr>
</thead>
<tbody>
<tr>
<td>23-13406-01</td>
<td>9/2013</td>
<td>Addition of the BD FACSCalibur system to the intended use statement.</td>
</tr>
<tr>
<td>23-13406-02</td>
<td>4/2016</td>
<td>Updated software version to 3.0 and welcome screen in Software Installation and Startup section</td>
</tr>
</tbody>
</table>
# Contents

## Chapter 1: Introduction
- About this Document .................................................. 12
- Conventions ............................................................... 13
- Technical Assistance .................................................. 14
- Intended Use ............................................................. 15
- Indications for Use .................................................... 15
- Validated Sample Types ............................................. 16

## Chapter 2: System Overview
- Introduction ............................................................. 18
- System Components .................................................. 20
  - Instrument ............................................................ 21
  - Primary Tube Rack .................................................. 23
  - Reagent Rack ........................................................ 25
  - Loader Carousel Rack .............................................. 27
  - Fluidics ............................................................... 28
  - Barcode Scanner ................................................... 29

## Chapter 3: Instrument Requirements, Installation, and Startup
- Required Equipment .................................................. 32
- Instrument Installation ............................................... 34
- Instrument Startup ................................................... 36
- Bulk Fluid Preparation .............................................. 37
- Fluid and Waste Tank Installation .............................. 38
## Chapter 5: Panels and Reagent Racks

<table>
<thead>
<tr>
<th>Pre-Programmed Panels and Reagent Racks</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Tritest Panels</td>
<td>82</td>
</tr>
<tr>
<td>BD Multitest Panels</td>
<td>84</td>
</tr>
<tr>
<td>BD Accuracy and Precision Panel</td>
<td>86</td>
</tr>
<tr>
<td>Pre-Programmed Reagent Racks</td>
<td>86</td>
</tr>
<tr>
<td>Tube Protocols</td>
<td>88</td>
</tr>
<tr>
<td>Creating a New Reagent Rack</td>
<td>89</td>
</tr>
<tr>
<td>Creating Multi-Rack Reagent Racks</td>
<td>91</td>
</tr>
<tr>
<td>Adding Controls to a New Reagent Rack</td>
<td>92</td>
</tr>
</tbody>
</table>

## Chapter 6: Sample Preparation

| Daily Startup                           | 96 |
| Setting Up for a Processing Run         | 100|
| Initializing a Worklist                 | 100|
| Preparing Primary Tubes                 | 101|
| Mounting the Primary Tube Rack          | 107|
| Panel Selection                         | 109|
| Carousel Rack Setup                     | 109|
| Reagent Rack Setup                      | 111|
| Removing and Replacing the Reagent Rack | 114|
| Entering Reagent Lot IDs                | 116|
| Offline Incubation                      | 118|
| Sample Processing                       | 119|
| LWA Information                         | 121|
| Ending a Run                            | 122|
| Pausing and Resuming a Run              | 124|
| Aborting a Run                          | 125|
Chapter 7: Shutdown and Maintenance

Daily Shutdown ......................................................... 128
Required Materials .................................................. 128
Performing Daily Cleaning ......................................... 128
Shutting Down the Instrument ..................................... 130
Scheduled Maintenance ............................................. 131
Weekly Cleaning ..................................................... 131
Calibrating the Probe Reference Point ......................... 134
Monthly Servicing of Waste In-Line Filters ..................... 141
Monthly Replacement of the Waste Tank Cap ................. 145
Unscheduled Maintenance ......................................... 146
Internal and External Surface Cleaning and Decontamination .. 147
Extended Periods of Non-Use .................................... 148
Startup Following Extended Non-Use ......................... 151
Replacing the Probe ............................................... 154
Replacing a Syringe ................................................. 166
Fuse Replacement ................................................... 175
Special Conditions .................................................. 179
Homing the Probe .................................................. 179
Initializing the Instrument ......................................... 179
Recovering from an X, Y, or Z-Axis Error ....................... 180
Recovering from a Reinitialization Error ......................... 181
Manually Replacing the Probe ................................... 183
Recovering from a Power Interruption ......................... 186

Chapter 8: Troubleshooting ........................................ 189

Instrument Troubleshooting ....................................... 190
Software Troubleshooting ......................................... 204
Software Error Messages ......................................... 205
This chapter covers the following topics:

- About this Document on page 12
- Conventions on page 13
- Technical Assistance on page 14
- Intended Use on page 15
- Indications for Use on page 15
- Validated Sample Types on page 16
About this Document

This document contains the information necessary to operate your BD FACSTM Sample Prep Assistant III (SPA III) instrument using BD FACSTM Sample Prep Assistant software (BD FACS SPA software).

If this equipment is used in manner not specified by BD, the protection provided by the equipment may be impaired.

The SPA III fully automates pipetting and dispensing of samples, reagents, and bulk fluids into secondary tubes, and prepares the samples for flow cytometry. Non-application-specific instructions are included in this instructions for use. For application-specific instructions, see the appropriate reagent package insert.

The BD FACSTM Sample Prep Assistant III Instructions For Use assumes that you have a working knowledge of basic Microsoft® Windows® operation. If you are not familiar with the Windows operating system, see the documentation provided with your computer.

Before using BD FACS SPA software, review the ReadMe file that was copied to the Program Files\BD FACS SPA Software folder on your hard drive during software installation. The ReadMe file contains information not printed in this instructions for use.
## Conventions

The following tables list conventions used in this instructions for use.

<table>
<thead>
<tr>
<th>Symbola</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>⚠️</td>
<td>CAUTION: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death</td>
</tr>
<tr>
<td>⚠️</td>
<td>Electrical danger</td>
</tr>
<tr>
<td>⚠️</td>
<td>Biological risk</td>
</tr>
</tbody>
</table>

a. Although these symbols appear in color on the instrument, they are in black and white throughout this instructions for use; their meaning remains unchanged.

<table>
<thead>
<tr>
<th>Convention</th>
<th>Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NOTE</strong></td>
<td>Provides information that supplements the topic material.</td>
</tr>
<tr>
<td><em>Italics</em></td>
<td>Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.</td>
</tr>
<tr>
<td>&gt;</td>
<td>The arrow indicates a menu choice. For example, “select File &gt; Print” means to select Print from the File menu.</td>
</tr>
<tr>
<td>Ctrl+X</td>
<td>When used with key names, a plus means to press two keys simultaneously. For example, “press Ctrl+P” means to press and hold down the Control key while pressing the letter P.</td>
</tr>
</tbody>
</table>
Technical Assistance

For technical questions or assistance in solving a problem:

- See Troubleshooting on page 189.
- Go to bdbiosciences.com.

If additional assistance is required, contact your local BD Biosciences technical support representative or supplier.

When contacting BD Biosciences, have the following information available:

- System Health report that includes the product name, part number, and serial number, and details of recent system performance.
- Error messages, if any.

For support, go to bdbiosciences.com.

Customers outside the US and Canada, contact your local BD representative or distributor.
Intended Use

The BD FACS Sample Prep Assistant III (SPA III) is intended to prepare human whole blood for flow cytometric analysis on BD FACSCanto™ II and BD FACSCalibur™ flow cytometry systems.

Indications for Use

Pipetting blood, reagents, and lysing solution using the following previously cleared assays for flow cytometric analysis on BD FACSCanto II flow cytometry systems:

- BD Multitest™ 6-Color TBNK reagent with or without BD Trucount™ tubes
- BD Multitest™ IMK kit with or without BD Trucount tubes
- BD Multitest™ CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC with or without BD Trucount tubes
- BD Multitest™ CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC with or without BD Trucount tubes

Pipetting blood, reagents, and lysing solution using the following previously cleared assays for flow cytometric analysis on BD FACSCalibur flow cytometry systems:

- BD MultiTest™ IMK kit with or without BD Trucount tubes
- BD MultiTest CD3 FITC/CD16+CD56 PE/CD45 PerCP/CD19 APC with or without BD Trucount tubes
- BD MultiTest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC with or without BD Trucount tubes
- BD Tritest™ CD3/CD16+56/CD45 with or without BD Trucount tubes
• BD Tritest CD3/CD19/CD45 with or without BD Trucount tubes
• BD Tritest CD3/CD4/CD45 with or without BD Trucount tubes
• BD Tritest CD3/CD8/CD45 with or without Trucount tubes
• BD Tritest CD4/CD8/CD3 with BD Trucount tubes

For in vitro diagnostic use.

Validated Sample Types

The following sample types have been validated for use with the SPA III (validated with the primary tube rack only):

• Human peripheral whole blood in K₂ or K₃ EDTA
• BD™ Multi-Check control
• BD™ Multi-Check CD4 low control
This chapter covers the following topics:

- Introduction on page 18
- System Components on page 20
Introduction

The SPA III instrument:

- Mixes the primary tube contents.
- Pipettes and aliquots samples from primary (blood) tubes to secondary (carousel) tubes.
- Pipettes antibody and lysing solution into the appropriate secondary tubes.
- Mixes the secondary tube contents.
- Cleans the probe and fluidic lines between sample and reagent dispenses.

The workstation:

- Tracks antibody and lysing incubation times (in default operation).
- Displays a message and sounds an alarm upon completion of the worklist.

You can process up to 40 secondary tubes at a time using one or more of the 16 pre-programmed panels provided with the instrument.
System Components

The SPA III consists of a sample preparation unit connected to an external computer workstation via an RS232 cable. A barcode reader enhances system functionality. The instrument ships with an accessory kit.

For a description of system components, see the sections listed in the table.

<table>
<thead>
<tr>
<th>Component</th>
<th>Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tube rack (with tube adapters)</td>
<td>See Primary Tube Rack on page 23.</td>
</tr>
<tr>
<td>Reagent rack</td>
<td>See Reagent Rack on page 25.</td>
</tr>
<tr>
<td>Carousel rack</td>
<td>See Loader Carousel Rack on page 27.</td>
</tr>
<tr>
<td>Fluidics tower</td>
<td>See Fluidics on page 28.</td>
</tr>
<tr>
<td>Barcode reader</td>
<td>See Barcode Scanner on page 29.</td>
</tr>
<tr>
<td>PC workstation</td>
<td>See your PC workstation documentation.</td>
</tr>
</tbody>
</table>
Instrument

Safety Cover and Worktable

The SPA III safety cover protects the operator from moving parts and biological hazards.

The worktable is the work surface of the instrument, and is enclosed by the safety cover when the cover is closed. The instrument worktable holds the wash station, Loader carousel rack, reagent rack, and primary tube rack cage.
## Instrument Components

**Figure 2-1** SPA III instrument components

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Z-rack</td>
<td>Attaches the probe to the robotic arm.</td>
</tr>
<tr>
<td>b Insulation block</td>
<td>Provides an electrical connection to the probe for liquid level sensing of the reagent.</td>
</tr>
<tr>
<td>c Probe</td>
<td>Dispenses samples, reagents, and bulk fluids. Pierces the primary sample tube caps.</td>
</tr>
<tr>
<td>d Wash station</td>
<td>Washes the probe and disposes of waste.</td>
</tr>
<tr>
<td>e On/Off light</td>
<td>Signals on/off status (of the instrument only).</td>
</tr>
<tr>
<td>f Valves</td>
<td>Function as part of the fluidics system.</td>
</tr>
<tr>
<td>g Syringe pumps</td>
<td>Move fluids through the system.</td>
</tr>
<tr>
<td>h Primary tube rack cage</td>
<td>Holds the primary tube rack in place and rocks it.</td>
</tr>
</tbody>
</table>
Primary Tube Rack

Two removable primary tube (blood collection tube) racks ship with the SPA III:

- One 16-mm tube rack that holds:
  - 15-mm and 16-mm diameter tubes.
  - 13-mm diameter tubes using white tube adapters (see Tube Adapters on page 24).
  - 11-mm diameter tubes using black adaptors
- One 13-mm tube rack that holds 13-mm diameter tubes.

The primary tube racks fit into a primary tube rack cage, which locks and mixes the primary tubes during sample preparation. The primary tube rack cage fits onto the instrument worktable in only one orientation.
Tube Adapters

Figure 2-2 White tube adapters for primary tube rack

Tube adapters can be added to the 11-mm and 13-mm primary tubes so they can be used in the 16-mm tube rack. This allows the 16-mm rack to hold 11-mm, 13-mm, 15-mm, and 16-mm primary tubes.

- Use white tube adapters for 13-mm diameter tubes.
- Use black tube adapters for 11-mm diameter tubes.

⚠️ Do not leave empty tube adapters in the primary tube rack when running a worklist. The unused adapters can fall out and jam the sample mixer.
Reagent Rack

The removable reagent rack installs in only one orientation on the instrument worktable. It holds up to 24 standard amber reagent vials, three BD Trucount control vials, and one large 60-mL vial for a total of 28 uncapped vials.

Figure 2-3  Reagent rack

Each reagent rack position is numbered. The reagent vials sit in the rack at a slight angle to maximize reagent usage.

The R25 position on the reagent rack holds a 60-mL vial, which is included in the accessory kit, to contain bleach for cleaning.

⚠️ Use only the 60-mL vial in the R25 position. Vials with a smaller diameter can move in the well and cause problems with the system, including damage to the probe.
Reagent Cap Holder

Figure 2-4  Reagent cap holder

The reagent cap holder is numbered to match the reagent rack to keep caps organized and contaminant-free while you are preparing samples. Store the reagent cap holder on the benchtop during sample preparation.
Loader Carousel Rack

The carousel rack (Figure 2-5) holds up to 40 uncapped 12 x 75-mm polystyrene secondary tubes or BD Trucount tubes and is compatible with the BD FACSTM Loader. Each rack has an identification code stamped on top.

Discontinued Racks

⚠️ Do not use discontinued Loader racks with the SPA III. The SPA III is compatible only with Loader racks labeled Sample-Prep Ready.

Figure 2-5 Carousel rack

Tube Labels

⚠️ Use tube labels that are less than 5 mils (127 microns) thick to prevent tubes from jamming in Loader carousel racks. Do not use more than one label, overlap label edges, or allow a label to extend beyond a tube's surface. Make sure all labels are firmly and completely attached to the tubes before inserting the tubes into a rack.
Fluidics

The SPA III comes equipped with three fluid supply tanks. A fluidics tower houses the DI Water, Lyse, and DI Water 2 tanks. A standalone waste tank is also included. Sheath fluid can be used directly from the BD FACSFlow™ cubitainer.

**Figure 2-6** Fluidics tower (left) and waste tank (right)

Level sensors indicate when the tanks and sheath fluid need service. Remove the tanks from the tower before emptying and refilling them.

See Fluid and Waste Tank Installation on page 38 and Emptying the Waste Tank on page 45.
Waste In-Line Filter Assembly

The SPA III includes a waste in-line filter to prevent the pump from clogging and causing overflow at the wash station. The filter is contained in an assembly connected to the waste line, and is located to the right of the wash station at the back of the instrument worktable.

The waste in-line filter is serviced each time the instrument probe is replaced. See Monthly Servicing of Waste In-Line Filters on page 141 and Replacing the Probe on page 154.

Barcode Scanner

The Orbit® Presentation Laser Scanner supports many different barcode standards, and comes pre-programmed to read the ISBT 128 standard. The scanning window tilts up to 30° for easier scanning.
LEDs and audio signals indicate when the scanner is on and when a barcode has been successfully scanned.

<table>
<thead>
<tr>
<th>Status</th>
<th>Description</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Steady blue light, no white light</td>
<td>Ready to scan.</td>
</tr>
<tr>
<td></td>
<td>Steady blue light and single white flash accompanied by a beep</td>
<td>Barcode successfully scanned. If the white LED does not flash or the scanner does not beep once, then the barcode has not been successfully read.</td>
</tr>
<tr>
<td></td>
<td>Steady white light, steady blue light</td>
<td>Communicating with the PC.</td>
</tr>
<tr>
<td></td>
<td>Alternating blue and white lights</td>
<td>Scanner is in configuration mode. A buzzing tone indicates that an invalid barcode has been scanned in this mode.</td>
</tr>
<tr>
<td></td>
<td>No white or blue light</td>
<td>No power to scanner.</td>
</tr>
</tbody>
</table>

For more information about the barcode scanner, including how to program it for other standards, see the manufacturer's documentation.
This chapter covers the following topics:

- Required Equipment on page 32
- Instrument Installation on page 34
- Instrument Startup on page 36
- Bulk Fluid Preparation on page 37
- Fluid and Waste Tank Installation on page 38
- Maintaining the Fluid and Waste Tanks on page 44
Required Equipment

The tubes in Table 3-1 have been validated for use on the SPA III. See Validated Sample Types on page 16.

⚠️ Do not use Terumo® blood collection tubes (primary tubes). They are not compatible with the SPA III.

**Table 3-1** Supported primary tubes

<table>
<thead>
<tr>
<th>Tube Type</th>
<th>Tube Size</th>
<th>Primary Tube Rack</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Vacutainer®</td>
<td>13 x 75 mm</td>
<td>13 mm or 16 mm with white adapters</td>
</tr>
<tr>
<td></td>
<td>13 x 100 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 x 75 mm</td>
<td>16 mm</td>
</tr>
<tr>
<td></td>
<td>16 x 100 mm</td>
<td></td>
</tr>
<tr>
<td>Sarstedt S-Monovette®</td>
<td>2.7 mL, 11 x 66 mm</td>
<td>16 mm with black adapters</td>
</tr>
<tr>
<td></td>
<td>2.6 mL, 13 x 65 mm</td>
<td>13 mm or 16 mm with white adapters</td>
</tr>
<tr>
<td></td>
<td>3.4 mL, 13 x 65 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.9 mL, 13 x 90 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.0 mL, 15 x 75 mm</td>
<td>16 mm</td>
</tr>
<tr>
<td></td>
<td>5.5 mL, 15 x 75 mm</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3-1  Examples of supported tube types

The primary tube racks support up to 40 capped tubes.

The bulk fluids and cleaning supplies listed in Table 3-2 are required to run the instrument.

Table 3-2  Required materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary tube types (up to 40 uncapped secondary tubes)</td>
<td>Corning Falcon® polystyrene tubes, 12 x 75-mm BD Trucount tubes, 12 x 75-mm</td>
</tr>
<tr>
<td>Bulk fluids</td>
<td>BD FACSTM lysing solution</td>
</tr>
<tr>
<td></td>
<td>BD FACSFlowSTM solution</td>
</tr>
<tr>
<td></td>
<td>DI water</td>
</tr>
<tr>
<td>Other fluids</td>
<td>BD FACSClean solution or 10% bleach</td>
</tr>
<tr>
<td></td>
<td>70% ethanol</td>
</tr>
<tr>
<td></td>
<td>Sigma Antifoam A Concentrate</td>
</tr>
</tbody>
</table>
Instrument Installation

A BD Biosciences service representative will initially install your SPA III. The following instructions are included if you need to reattach system components.

To install the SPA III:

1  Plug the following into their ports in the instrument.
   •  RS232 cable (connects to computer workstation)
   •  Power cord

   Figure 3-2  Power switch on instrument left side panel

   Leave sufficient room on the side of the instrument so you can easily access the power switch, which is located directly above the power cord.

2  Connect the fluid level sensor cable to the instrument right side panel.
3 Attach the waste tubing to the left side of the fluidics tower (Figure 3-4).

⚠️ Always attach the waste line (from the instrument) to the fluidics tower before starting the software to prevent a biohazardous spill.

**Figure 3-3** Instrument, right side panel

![Image of instrument with waste tubing and fluid level sensor cable]

**Figure 3-4** Fluidics tower (left side)

![Image of fluidics tower with waste tubing]
4 Connect the barcode reader to the PC.

**NOTE** To connect the barcode reader and other workstation components, see the manufacturer’s instructions.

5 Plug the instrument power cord into a properly grounded electrical outlet.

6 Plug the barcode reader into a properly grounded electrical outlet.

7 Plug the computer and the monitor into properly grounded electrical outlets.

**Instrument Startup**

⚠️ Before starting the instrument, visually inspect it to ensure that all fluid lines and sensors are connected. If the sensor wires are connected but the fluid lines are not, the SPA III will continue to operate, but no fluid will move through the affected lines.

⚠️ Before starting the instrument, visually inspect it to ensure that the waste sensor is connected. If the sensor wire is connected but the waste fluid sensor is not installed, the instrument will continue to operate and the waste station will overflow with biohazardous waste.

To start up the instrument:

1 Open the safety cover.

2 Close and lock the primary tube rack cage.

3 Close the safety cover.

4 Turn on the instrument power.

   The power switch is on the left side panel above the power cord port (Figure 3-2 on page 34).
5 Turn on the computer and monitor.

6 Start the software as described in Starting SPA Software on page 56.

**Bulk Fluid Preparation**

Table 3-3 lists the bulk fluids required for the SPA III, including compatible fluids and capacities.

**Table 3-3 Bulk fluids required for the SPA III**

<table>
<thead>
<tr>
<th>Item</th>
<th>Compatible Fluid or Type(s)</th>
<th>Capacity</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water tank</td>
<td>Deionized (DI) water</td>
<td>1 L</td>
<td>N/A</td>
</tr>
<tr>
<td>Lyse tank</td>
<td>BD FACS lysing solution (1X)</td>
<td>1 L</td>
<td>Dilute 10X solution. See the <em>BD FACS Lysing Solution</em> package insert for warnings and instructions.</td>
</tr>
<tr>
<td>DI water 2 tank</td>
<td>DI water</td>
<td>1 L</td>
<td>Provides extra DI water through a separate fluidics pathway for probe cleaning.</td>
</tr>
<tr>
<td>Flow container</td>
<td>BD FACSFlow solution</td>
<td>20 L</td>
<td>BD FACSFlow cubitainer</td>
</tr>
<tr>
<td>Waste tank</td>
<td>Household bleach, or equivalent</td>
<td>10 L</td>
<td>Add 1 L undiluted bleach for a final concentration of 10%.</td>
</tr>
<tr>
<td></td>
<td>Sigma Antifoam A Concentrate</td>
<td></td>
<td>Add 500 µL of antifoam concentrate.</td>
</tr>
</tbody>
</table>
Fluid and Waste Tank Installation

⚠️ For accurate results, fill fluid tanks with only the indicated bulk fluid (see Table 3-3 on page 37). Dilute BD FACS lysing solution as described in the package insert before filling the lyse tank.

To fill the fluid and waste tanks:

1. Fill the DI water and lyse tanks completely.

2. Add approximately 1 L of undiluted household bleach (or equivalent) to the waste tank.

3. Add 500 µL of Sigma Antifoam A Concentrate to the waste tank to prevent foaming of potentially biohazardous waste around the cap.

4. Replace the caps on each tank and hand tighten.
Fluidics Tower

After you fill the DI water and lyse tanks with the appropriate fluid, install them in the instrument fluidics tower. The tank fluid connectors and tank labels are color-coded for easy installation.

Figure 3-5  Color-coded labels and fluid connectors on tanks

To install the tanks:

1. Place the tanks in their appropriate locations in the fluidics tower.

⚠️ To ensure the appropriate solutions are dispensed, do not switch tank positions.
The following table lists the tanks and their corresponding label and quick-release connector colors.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Label Color</th>
<th>Connector Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI water (B)</td>
<td>Blue</td>
<td>Blue</td>
</tr>
<tr>
<td>Lyse (C)</td>
<td>Tan</td>
<td>Black</td>
</tr>
<tr>
<td>DI water 2 (D)</td>
<td>Green</td>
<td>Green</td>
</tr>
</tbody>
</table>

2 Push the color-coded, quick-release connectors into the matching tank ports.

3 Firmly push the sensor connectors into the ports on the fluidics tower.
Flow Container and Waste Tank

The fluid sensors for the flow container and waste tank are part of the cap assemblies.
To install the flow container and waste tank:

1. Install the fluid sensors in the flow container and waste tank.
2. Replace each cap and hand-tighten.

⚠️ Do not use tools to tighten the waste tank cap. Firmly tighten the cap with only your hands to avoid damaging it. Failure to firmly tighten the waste tank cap can result in accidental overflow of waste.

The table below lists the tanks and their corresponding label and connector colors.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Label Color</th>
<th>Connector Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow</td>
<td>Yellow</td>
<td>White</td>
</tr>
<tr>
<td>Waste</td>
<td>Orange</td>
<td>Orange</td>
</tr>
</tbody>
</table>

3. Firmly push the sensor and fluid connectors into the ports on the fluidics tower.

Make sure the tubing does not crimp or bend at the workbench edge.

⚠️ You must attach the waste tank to the fluidics tower before starting the software to prevent a biohazardous spill.

**Priming the Fluid Lines**

The SPA III will automatically self-prime:

- At software startup
- After a completed worklist
- During the daily and weekly cleaning procedures
Manually Priming the Instrument

**NOTE** You must manually prime the instrument if you have to change the BD FACSFlow cubitainer during a run. See During a Run on page 44.

Under other typical circumstances you do not need to manually prime the instrument. However, you can initiate a prime whenever the instrument is idle. For example, if you notice air in the fluidics line, you should prime the instrument with the appropriate fluid.

1. From the main menu in the Prep Worklist window, select **Instrument > Prime**.

   The Prime Fluidics dialog opens.

2. Select one of the fluid types from the Prime Fluidics menu:

   - **FLOW**: BD FACSFlow solution
   - **LYSE**: BD FACS lysing solution
   - **DI WATER**: Deionized water
   - **DI WATER 2**: Deionized water

3. Click **Yes**.

   The instrument prime begins.
Maintaining the Fluid and Waste Tanks

Each fluid tank has a built-in level sensor. When any fluid tank runs low or when the waste tank fills:

- Instructions appear in the worklist Messages field.
- The corresponding Fluid Tank Status indicator turns from blue to red.
- A beep sounds.

See Emptying the Waste Tank on page 45 for more information.

Before Beginning a Run

Before beginning a run, the SPA III displays a message if a tank needs service. You must empty or refill the indicated tank before the SPA III will process the rack.

During a Run

During a run, the SPA III pauses if it senses that the waste tank is full or that BD FACSFlow solution is low. It does not resume processing until you service the flow or waste tanks.

**NOTE** When you change the BD FACSFlow cubitainer during a run, you must perform a manual prime with Flow before resuming operation, to clear any bubbles in the fluid line. See Manually Priming the Instrument on page 43.
If the SPA III senses that the lyse or either DI water tank fluid level is low during a run, processing continues. The indicators turn on, a message is displayed, and an alarm sounds. To turn off the alarm, you must acknowledge the message.

The tank indicators will clear after you service the tanks.

**Emptying the Waste Tank**

Empty the waste tank:

- When the fluid level sensor indicates the tank is full.
- At the beginning of a run if the tank is more than 3/4 full.

⚠️ The waste tank contents could be biohazardous. Expose contents to bleach (10% of the tank’s total volume) for a minimum of 30 minutes before disposal. Dispose of with proper precautions in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

⚠️ Every time you empty the waste tank, you must add 500 µL of Sigma Antifoam A Concentrate to the tank to prevent foaming of potentially biohazardous waste up around the cap. Add Sigma Antifoam A Concentrate to the waste tank in addition to bleach.

⚠️ Change the waste tank cap every month to prevent tank pressurization. To order new replacement caps (Part Number 338347), contact your local BD Biosciences representative.

To empty the waste tank:

1. Disconnect the waste fluid and waste sensor lines from the instrument.

⚠️ The waste tank can become pressurized when the SPA III is running. Always disconnect the tank from the fluidics tower before you empty it. Wait at least 1 minute for pressure to dissipate before you remove the waste cap or sensor.
2 Take the waste tank to the appropriate location for emptying.

3 Remove the disposable waste cap (large-sized cap) and attached trap from the container. Place the cap and trap on a disposable cloth with the label side up.

⚠️ Do not wet the cap on top of the trap. If you see liquid inside the trap, remove the drain plug and fully drain the liquid before you replace the plug.

4 Empty the waste tank according to your standard laboratory procedures for biohazardous waste.

⚠️ Dispose of the waste using precautions in accordance with local regulations. Wear suitable protective clothing, eyewear, and gloves.

5 Add approximately 1 L of undiluted household bleach to the empty tank.

6 Add 500 µL of Sigma Antifoam A Concentrate to the empty tank.

7 Replace the trap and attached filter cap.

⚠️ To prevent over-pressurization during fluidics startup, do not overtighten the waste tank baffle or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants such as Teflon® tape or other adhesives.
If one month has passed since you last changed the cap, replace the filter cap with a new one.

Change the waste tank cap every month to prevent tank pressurization. To order new replacement caps (Part Number 338347), contact your local BD Biosciences representative.

When you replace the cap, write the date on the new cap as a reminder.

8 Hand-tighten the waste tank trap and cap until they are fully closed.

Failure to tighten the waste tank cap can result in accidental overflow of waste. Hand-tighten only to avoid damage to the cap.

9 Reconnect the waste fluid and waste sensor lines to their respective ports on the fluidics tower.
Software Installation and Startup

This chapter covers the following topics:

- Software Requirements on page 50
- Software Installation on page 52
- Starting SPA Software on page 56
- Prep Worklist Window on page 58
- User Accounts on page 70
- Entering LWA Information on page 74
- Log Files on page 76
- Shutting Down the Software on page 77
Software Requirements

To run SPA software, you will need the following computer hardware, operating system, and additional software.

Hardware

- SPA III workstation (purchased from BD Biosciences)
- Serial (RS232) cable connection from the PC workstation to the SPA III instrument via Com Port 1
- Keyboard wedge (a cable included with the barcode scanner)
- (Upgrade customers) 10-mL and 1-mL syringes installed

Operating System and Additional Software

- Windows® XP operating system with Service Pack 2
- Adobe® Acrobat® Reader® v8.1 or later to use electronic documentation provided on the SPA software CD

Software Compatibility Issues

You must quit all other applications before running SPA software.

Turn off all screen savers and the energy saver on your computer. They could interfere with the performance of the software.

To turn off the screen saver and the energy saver:

1 Right-click on the desktop.
2 Select Properties from the shortcut menu.
3 In the Display Properties dialog, click the Screen Saver tab.
4 From the Screen Saver menu, select None.
The screen saver is now disabled.

5 Click the Power button to access the Power Option Properties dialog.

6 Click the Power Schemes tab.

7 From the Power Schemes menu, select Home/Office Desk.

8 Under Settings for Home/Office Desk power scheme, specify the following:
   - Turn off monitor: Never
   - Turn off hard disks: Never
   - System standby: Never
   - System hibernates: Never

9 Click OK in the Power Options Properties dialog.

10 Click OK in the Display Properties dialog.
Software Installation

SPA software is already installed on your computer. Follow these steps if you need to reinstall the software.

Before you begin, close any open programs.

To install the software:

1. Insert the SPA software version 5.0 installation CD into the CD drive.
   
   The installer should start up automatically. If it doesn’t, use Windows Explorer to view the CD contents, and double-click the Setup.exe icon.

2. Click Next to begin the installation.

3. Confirm that the SPA III system has the correct syringes installed as shown in the next dialog.
4 Click **Yes** to begin the installation.

5 Review and accept the license agreement, review the setup information, and specify the destination folder.

By default, the installer copies all component files to the Program Files\BD FACS SPA Software folder on the C drive. You can specify another local drive, but you must keep the same folder names and path.

6 Click **Next**.

A confirmation dialog opens.

7 Click **Next** again to begin copying files.

The installer loads the software and its support files on the selected hard drive. A dialog opens when the installation is complete.

8 Click **Finish** to exit the installer.

The installer places SPA software, the ReadMe file, and a PDF file version of this instructions for use in the Start menu. It also creates a shortcut to SPA software, a SPA III tutorial (in English only), and this instructions for use on the desktop.
When to Install the Worktable Database File

Each SPA III system has an instrument-specific worktable database file that is shipped with the system on a CD. This file is installed on the computer by your BD service engineer and fine-tuned during initial system installation. The file is located at C:\Program Files\BD FACS SPA Software\DataFiles. The file name is BD-HwWktb.mdb. Under normal circumstances, you do not have to do anything related to this file.

If you need to reinstall SPA software, see the use cases in Table 4-1 for the various instructions about the worktable database file.

Table 4-1

<table>
<thead>
<tr>
<th>Situation</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upgrading SPA software to a new version.</td>
<td>No action. The installer keeps the existing worktable file.</td>
</tr>
<tr>
<td>Re-installing SPA software because the previous version is not functioning.</td>
<td>No action is required if the existing worktable file is available and not damaged. If the existing file is not usable, contact BD Customer Support.</td>
</tr>
<tr>
<td>Re-installing SPA software on a new computer or a computer with a new hard drive.</td>
<td>Load the existing worktable file if a backup copy is available. If not, contact BD Customer Support.</td>
</tr>
</tbody>
</table>
Uninstalling the Software

1. From the Windows Start menu, select Settings > Control Panel > Add or Remove Programs.
2. Select SPA software Version 5.0 from the list.
3. Click Remove.
4. Click OK to confirm the uninstall.
5. Click Finish to return to the previous dialog.
6. Click Close.
Starting SPA Software

To start the software, either:

- From the desktop, select **Start > Programs > BD FACS SPA Software > BD FACS SPA Software**.

- Double-click the shortcut icon on the desktop.

The welcome screen opens, followed by the Login dialog.
Administrative Login

The software is installed with a default User Name: Administrator. A BD Biosciences service representative provides the Administrator account password.

The lab administrator should be the first user to log in to the software. The Administrator account has administrative privileges and is used by the lab administrator to set up additional user accounts. See Passwords on page 70.

Logging in to the Software

1. Select a user name from the User Name menu.
2. Type the password, and then click OK.

The main application window (Prep Worklist) is displayed.
Prep Worklist Window

Most of your interaction with the SPA software is done in the Prep Worklist window. The following figure and Table 4-2 provide a brief orientation to the Prep Worklist window and its features.

Table 4-2 Prep Worklist window

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Main menu</td>
<td>File, Setup, Instrument, Administration, and Help menus.</td>
</tr>
<tr>
<td>b Worklist</td>
<td>Enter primary tube identification, tube type, position, panel, and secondary tube positions here (for a full description, see Worklist on page 62).</td>
</tr>
<tr>
<td>c Reagent Rack ID Multi-rack number</td>
<td>Select the reagent rack for a panel from the menu. The current rack is indicated below the menu.</td>
</tr>
</tbody>
</table>
### Table 4-2  Prep Worklist window

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>d Skip Initial Sample Mix checkbox</td>
<td>Skips the initial 5-minute mix of samples in the primary tube rack. The checkbox is cleared by default.</td>
</tr>
<tr>
<td>e Incubate Offline checkbox</td>
<td>Enables the option of performing the final incubation offline. The checkbox is cleared by default.</td>
</tr>
<tr>
<td>f Worktable</td>
<td>Graphic representation of the placement of all primary and secondary tubes and reagent vials.</td>
</tr>
<tr>
<td>g Carousel ID</td>
<td>Type an ID (serial number) for the carousel. Use a number from 1 to 16.</td>
</tr>
<tr>
<td>h Unique Carousel ID</td>
<td>Scan the carousel barcode or type the unique carousel ID in this field. Limited to a maximum of 22 characters.</td>
</tr>
<tr>
<td>i Probe Counter</td>
<td>Tracks the total number of cap piercings.</td>
</tr>
</tbody>
</table>
| j Minimize, Maximize, and Close buttons (in title bar) | **Minimize.** Reduces the application to a button on the task bar.  
**Maximize.** Expands the Prep Worklist window to full screen.  
**Close.** Exits the application and prompts the Daily Clean procedure. |
| k Worklist tabs | Display names of all open worklists. Click a tab to display the selected worklist. |
| l Function buttons | Control instrument functions. The available function buttons depend on processing context, and include:  
  - **Run.** Start processing a rack.  
  - **Pause.** Pause processing.  
  - **Abort.** Cancel a rack.  
  - **Shutdown.** Exit the software and end a run.  
  - **Close.** Close the selected worklist. |
| m Secondary Tubes Carousel Position and Name field | Shows the secondary tubes associated with each panel for a selected Sample ID. |
Table 4-2  Prep Worklist window

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>n  Status and Messages fields</td>
<td>Display current instrument processing and diagnostic messages.</td>
</tr>
<tr>
<td>o  Fluid Tanks Status</td>
<td>Indicates when the waste and fluid tanks need filling or emptying (See Fluid Tanks Status on page 69.)</td>
</tr>
<tr>
<td>p  Status bar</td>
<td>Displays the percentage of completion when processes are running.</td>
</tr>
</tbody>
</table>

Worktable

The worktable display is a graphic representation of the placement of all primary and secondary tubes and reagent vials. It consists of the following components.
### Component Function

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
</tr>
</thead>
</table>
| a Carousel Rack Map         | Displays all secondary tube positions and highlights the secondary tubes for a selected primary tube. Allows you to type a carousel identification number and unique identification number. During the run, tube positions are colored according to their status:  
  - Gray: No tube present  
  - White: Tube present  
  - Blue: Tube in sample selected or lyse being dispensed  
  - Red: Sample being dispensed  
  - Green: Reagents or controls being dispensed |
| b Reagent Rack Map           | Displays reagent positions. When the cursor is placed over an assigned position, the reagent name is displayed. When “Other Panel” is selected in the Panel Editor, L (low), M (medium), and H (high) control positions change to S1, S2, and S3 (samples 1, 2, and 3) for open primary sample tubes. |
| c Primary Tube Rack Map      | Displays the primary tube positions. |
**Worklist**

All sample, panel, rack, and tube information for a run is entered into the worklist.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Case Num</th>
<th>Primary Tube Type</th>
<th>Primary Rack</th>
<th>Panel Name</th>
<th>Carousel Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrueCount Control</td>
<td>BD Vacutainer</td>
<td>1</td>
<td>3/4/4S/4 Control</td>
<td>1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MultiCheck</td>
<td>BD Vacutainer</td>
<td>2</td>
<td>4 Color TRMK + TruC</td>
<td>4-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MultiCheck CD1 Lo</td>
<td>BD Vacutainer</td>
<td>3</td>
<td>4 Color TRMK + TruC</td>
<td>6-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse Hist</td>
<td>BD Vacutainer</td>
<td>4</td>
<td>4 Color TRMK + TruC</td>
<td>8-9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Worklists can be:

- **Saved and re-used.** See Initializing a Worklist on page 100 and Ending a Run on page 122.
- **Printed.** See Printing a Worklist on page 66.
- **Exported.** See Using SPA III Worklists with Other BD Software on page 68.

**Entering Information in the Worklist**

Use the keyboard or the barcode scanner to enter sample information into the worklist. Use the keyboard and mouse to enter information into the Sample ID field first and then select the Primary Tube Type. Then enter information for Primary Rack Position, Panel Name, and Carousel Position fields.

To use the barcode scanner, place the cursor in the appropriate field (Sample Name, Sample ID, or Case Number) and swipe the barcoded tube past the scanner. The Sample ID is a required field. The Sample Name and Case Number are optional. For more information on using the scanner, see Barcode Scanner on page 29, and Preparing Primary Tubes on page 101.
As you enter information into the worklist, symbols are displayed to indicate the entry status:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Symbol" /></td>
<td>Indicates which line is selected.</td>
</tr>
<tr>
<td><img src="image2" alt="Symbol" /></td>
<td>Indicates the line being edited.</td>
</tr>
<tr>
<td><img src="image3" alt="Symbol" /></td>
<td>Indicates the next line for which there are no current entries.</td>
</tr>
<tr>
<td><img src="image4" alt="Symbol" /></td>
<td>Indicates a problem with a worklist entry. Place your cursor over the symbol for more information about the problem. For example, in the following figure, a pop-up with the information <em>Invalid Sample ID</em> indicates that the ID is missing.</td>
</tr>
</tbody>
</table>

### Using One Primary Sample for Multiple Panels

SPA software allows you to use one primary sample for multiple panels. If you create multiple panels from one sample, you must ensure that each panel entry in the Worklist is unique. Each panel must follow the rules for unique identification. It can be a combination of the Sample Name, Sample ID, and Case Number that makes it unique.

When using this option, make sure that the primary sample tube is not pierced more than four times. The software displays a warning if you attempt to pierce the same sample tube more than four times.
Entering the Last Line of a Worklist

A Sample ID must be entered for each line of the worklist. The Sample ID of the last line of a worklist must be entered for that line to be processed when the worklist is run.

To assure traceability of samples processed by the SPA III, save and print the worklist before running samples.

The following figure illustrates a situation where the last line is being edited, but does not have a Sample ID entered. The worktable display indicates an active primary tube rack position 4, but if the worklist is run at this point, the last line and the sample associated with primary tube rack position 4 will NOT be processed.
Chapter 4: Software Installation and Startup

Resizing Worklist Fields

You can resize fields in the worklist by placing the cursor in the column title and dragging to the right or left.
Deleting Samples from a Worklist

To delete a sample line from the worklist:

1. Select a sample by clicking in the far left column (header) as shown in Figure 4-1.
2. Press the Delete key.

**Figure 4-1** Deleting a line of information

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Case Num</th>
<th>Primary Tube Type</th>
<th>Primary Rack</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trucount control</td>
<td></td>
<td></td>
<td>BD Vacutainer</td>
<td>1</td>
</tr>
<tr>
<td>▶</td>
<td>Mult-Check</td>
<td></td>
<td>BD Vacutainer</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mult-Check CD4 Lo</td>
<td></td>
<td>BD Vacutainer</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Patient #1</td>
<td></td>
<td>BD Vacutainer</td>
<td>4</td>
</tr>
</tbody>
</table>

Printing a Worklist

To print the currently open worklist:

1. Select File > Print from the main menu in the Prep Worklist window.

   The Print dialog opens.

2. Click OK.

   A print version of the open worklist is sent to the printer.
The rows of the Prep Worklist printout contain the same fields found in the Prep Worklist window. An icon to the left of each sample entry describes sample status:

<table>
<thead>
<tr>
<th>Icon</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>No icon</td>
<td>The sample has not yet been processed</td>
</tr>
<tr>
<td>✓</td>
<td>The sample was successfully processed</td>
</tr>
<tr>
<td>✗</td>
<td>The sample processing was incomplete because of an error or because the Prep Worklist was aborted</td>
</tr>
</tbody>
</table>

Use Page Setup to adjust your paper orientation and size, and Print Preview to view the worklist prior to printing it.
Using SPA III Worklists with Other BD Software

Saved SPA III worklists can be read directly by BD FACSCanto™ clinical software, BD FACSDiva™ software, and BD™ Worklist Manager software on a system with Mac OS® X.

When using a SPA III worklist with other BD software, panel names must match exactly (panel names are case sensitive, and spaces in the panel names must also match). Preserving carousel rack positions depends on matching panel names and panel definitions between software applications.

Exporting the Worklist for Mac OS 9

Saved worklists can be exported in tab-delimited text format. If you are using BD Worklist Manager on a system running Mac OS® 9, you must export your SPA III worklist to make it available for use.

Exported worklists contain only Sample Name, Sample ID, Case Number, Panel Name, and Assay (MultiSET or CELLQuest). Additional information required by BD WorklistManager must be entered manually.

To export a saved worklist:

1. Select File > Export Prep Worklist from the main menu.
   The Select Worklist to Export dialog opens.

2. Locate and select the worklist file to export.

3. Click Open.
   The exported text file is saved in the same folder, and with the same name as the XML file, but it has a .txt extension.
Fluid Tanks Status

The fluid tank status indicators in the Prep Worklist window keep you informed of tank status.

- Blue: Fluid level okay
- Red: Service needed

The following table lists the points where the indicators change from blue to red.

<table>
<thead>
<tr>
<th>Tank</th>
<th>Fluid Level Indicator Trigger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waste</td>
<td>80% full (at 8 L)</td>
</tr>
<tr>
<td>Bulk fluids</td>
<td>20% (of full) remaining</td>
</tr>
</tbody>
</table>

When a tank needs service, an error message is displayed and a continual alarm sounds. To turn off the alarm, you must acknowledge the message.
User Accounts

Passwords

Passwords do not expire.

To change the password on your user account:

1. Select Administration > Password from the main menu.
   
   The Change Password dialog opens.

2. Type your old password.

3. Type a new password and confirm it.
   
   Passwords are case-sensitive and cannot contain more than 10 characters.

4. Click OK.
Managing User Accounts

The lab administrator (or another user with administrative privileges) can create, edit, and delete SPA III user accounts.

1. Select Administration > Users from the main menu.

The User Administration dialog opens. The text box at the top of the dialog contains the current list of User Names.

2. Create, configure, edit, and delete user accounts as described in the following sections.

3. To exit the dialog, click Close.
Creating User Accounts

To create a user account:

1. Type user information for the first new account (see table) in the User Administration dialog.

   **NOTE** Use the mouse or Tab key to move from field to field. Pressing the Enter key is equivalent to clicking Apply, and Enter should not be pressed when entering the value for a field.

<table>
<thead>
<tr>
<th>Field</th>
<th>Status</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Optional</td>
<td>0–32 characters</td>
</tr>
<tr>
<td>Account Name</td>
<td>Required</td>
<td>1–10 characters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Account Name is the identifier that appears in the User Name menu at login.</td>
</tr>
<tr>
<td>Password</td>
<td>Optional</td>
<td>0–10 characters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Passwords are optional. You can leave the password field blank.</td>
</tr>
<tr>
<td>Confirm Password</td>
<td>Optional</td>
<td>0–10 characters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>The Password and Confirm Password entries must match exactly.</td>
</tr>
<tr>
<td>Task Level</td>
<td>Required</td>
<td>Normal. Does not include administrative privileges (see below).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Administrator. Includes administrative privileges: creating panels and reagent racks, and creating and editing user accounts.</td>
</tr>
<tr>
<td>Institution</td>
<td>Optional</td>
<td>0–32 characters</td>
</tr>
<tr>
<td>BD FACS SPA</td>
<td>Optional</td>
<td>0–32 characters</td>
</tr>
<tr>
<td>Serial Number</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Click Apply.

   The account name is added to the current list of user names.
To create each additional user account:

a  Click New to clear all fields.
b  Type information for the new account.
c  Click Apply.
d  Click Close to exit the dialog.

Modifying User Accounts

To modify a user account:

1  Click on an account name in the text box at the top of the User Administration dialog.

2  Edit user information as needed (see Creating User Accounts on page 72 for details about the user information fields).

3  Click Apply.

4  Click Close to exit the dialog.

Deleting User Accounts

To delete a user account:

1  Click on an account name in the text box at the top of the User Administration dialog.

2  Click Remove.

3  Click Close to exit the dialog.
Entering LWA Information

To enter information for the BD FACS™ Lyse Wash Assistant (LWA), follow these steps. You can enter a serial number for each LWA in your laboratory.

1. Select Administration > LWA Info from the main menu.
   The LWA Info dialog opens.

2. Select the serial number and user name from the menus.

3. (Optional) Click the Modify link to add serial numbers or user names to the lists.
   The Modify LWA Info dialog opens.
• To add a new serial number or user name, type the new item in the corresponding text field and click Add.

• To delete an existing serial number or user name, select the item and press the Delete key.

4 (Optional) Select the Remind to proceed to LWA at the end of sample prepping checkbox. When this option is enabled, a confirmation dialog opens after all materials have been dispensed during a run (see LWA Information on page 121).

5 Click OK to dismiss the dialog.
Log Files

SPA software maintains the following information:

- **Login files.** Account name, and time and date of each login by month. These files have names of the form `year Month.txt`, and are located in the `Program Files\BD FACS SPA Software\DataFiles` folder.

- **Log files.** An instrument log file (instrument`datetimecode.log`) is created each time you start the software and log in. The files are stored in the `Program Files\BD FACS SPA Software\LogFiles` folder. These files track instrument operations. You may want to periodically delete these files when they are no longer needed.

- **Error messages.** These are saved to a file (spa_error.log) located in the `Program Files\BD FACS SPA Software` folder. The error message file is overwritten in each session that an error occurs.

Double-click a .log or .txt file to open it.

**Copying Log Files**

To copy a log file, use the standard Windows file system copy and paste functions.

**Viewing and Printing Log Files**

To view a log file, open the file in a text editor. To print the file, use the Print function of the text editor.

For example, open the log file in Notepad. To print, select File > Print.

**Deleting Log Files**

Use the standard Windows file system functions to delete a log file.
Shutting Down the Software

To quit the software:

1. Do one of the following:
   - Select File > Shutdown.
   - Click the Shutdown button on the right side of the application window.

   The shutdown dialog opens.

2. Leave the Daily Clean checkbox cleared.

3. Click OK.
Panels and Reagent Racks

This chapter includes the following topics:

- Pre-Programmed Panels and Reagent Racks on page 80
- Tube Protocols on page 88
- Creating a New Reagent Rack on page 89
- Creating Multi-Rack Reagent Racks on page 91
- Adding Controls to a New Reagent Rack on page 92
**Pre-Programmed Panels and Reagent Racks**

A panel consists of one or more secondary tubes. Each secondary tube specifies reagents and controls, as well as a tube protocol (see Tube Protocols on page 88).

SPA software includes the following types of pre-programmed panels:

- BD Tritest
- BD Multitest
- BD Accuracy and Precision

To view the pre-programmed panels:

1. Select **Setup > Panel Editor** from the main menu in the Prep Worklist window.

   The Panel Editor opens (Figure 5-1).

   Panel category is at the top level of the Panel Editor browser hierarchy (for example, BD Multitest, BD Tritest, and BD Accuracy and Precision). Panel categories contain panels. Panels contain secondary tubes.

2. Expand the categories and panels by clicking the plus sign next to each item.
A reagent rack is a set of reagents and their positions in a rack (rack map). SPA software also includes pre-programmed reagent racks that correspond to the reagents and controls required by the pre-programmed panels.

Table 5-1, Table 5-2, and Table 5-3 list the pre-programmed panels and their secondary tube reagents, as well as the corresponding pre-programmed reagent racks. See Reagent Rack Setup on page 111 for specific reagents associated with each rack.

Table 5-4 lists the pre-programmed reagent racks and their reagents and controls.
## BD Tritest Panels

### Table 5-1 BD Tritest panels

<table>
<thead>
<tr>
<th>Panel</th>
<th>Secondary Tube</th>
<th>Reagents</th>
<th>Reagent Rack ID Choices</th>
</tr>
</thead>
<tbody>
<tr>
<td>MultiReagentControl</td>
<td>Tube 1</td>
<td>CD4/CD8/CD3 TruC Ctrl L</td>
<td>BD MultiReagent Control</td>
</tr>
<tr>
<td>(BD Trucount tubes)</td>
<td>Tube 2</td>
<td>CD3/CD19/CD45 TruC Ctrl M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD16+/CD56/CD45 TruC Ctrl H</td>
<td></td>
</tr>
<tr>
<td>4/8/3 Control</td>
<td>Tube 1</td>
<td>CD4/CD8/CD3 TruC Ctrl L</td>
<td>3-Tube T cell Control</td>
</tr>
<tr>
<td>(BD Trucount tubes)</td>
<td>Tube 2</td>
<td>CD4/CD8/CD3 TruC Ctrl M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD4/CD8/CD3 TruC Ctrl H</td>
<td></td>
</tr>
<tr>
<td>3/4/45 Control</td>
<td>Tube 1</td>
<td>CD3/CD4/CD45 TruC Ctrl L</td>
<td>BD Tritest Control or</td>
</tr>
<tr>
<td>(BD Trucount tubes)</td>
<td>Tube 2</td>
<td>CD3/CD4/CD45 TruC Ctrl M</td>
<td>3-Tube T cell Control</td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD4/CD45 TruC Ctrl H</td>
<td></td>
</tr>
<tr>
<td>4-4-8 Control</td>
<td>Tube 1</td>
<td>CD3/CD4/CD45 TruC Ctrl L</td>
<td>BD Tritest Control or</td>
</tr>
<tr>
<td>(BD Trucount tubes)</td>
<td>Tube 2</td>
<td>CD3/CD4/CD45 TruC Ctrl M</td>
<td>3-Tube T cell Control</td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD8/CD45 TruC Ctrl H</td>
<td></td>
</tr>
</tbody>
</table>
### Table 5-1  BD Tritest panels

<table>
<thead>
<tr>
<th>Panel</th>
<th>Secondary Tube</th>
<th>Reagents</th>
<th>Reagent Rack ID Choices</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-4-8 Control (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD8/CD45 TruC Ctrl L</td>
<td>BD Tritest Control or 3-Tube T cell Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD4/CD45 TruC Ctrl M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD8/CD45 TruC Ctrl H</td>
<td></td>
</tr>
<tr>
<td>3 Color TBNK</td>
<td>Tube 1</td>
<td>CD3/CD4/CD45</td>
<td>BD Tritest or BD Tritest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD8/CD45</td>
<td>BD Tritest or BD Tritest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD19/CD45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 4</td>
<td>CD3/CD16+CD56/CD45</td>
<td></td>
</tr>
<tr>
<td>3 Color TBNK TruC (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD4/CD45</td>
<td>BD Tritest or BD Tritest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD8/CD45</td>
<td>BD Tritest or BD Tritest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD19/CD45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 4</td>
<td>CD3/CD16+CD56/CD45</td>
<td></td>
</tr>
<tr>
<td>2 4/8/3 and 3/4/45</td>
<td>Tube 1</td>
<td>CD4/CD8/CD3</td>
<td>BD Tritest 3-Tube T cell</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD4/CD45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD8/CD45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD8/CD45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD4/CD8/CD3</td>
<td></td>
</tr>
</tbody>
</table>
## BD Multitest Panels

**Table 5-2** BD Multitest panels

<table>
<thead>
<tr>
<th>Panel</th>
<th>Secondary Tube</th>
<th>Reagents</th>
<th>Reagent Rack ID Choices</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/8/45/4 Control (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD8/CD45/CD4 TruC Ctrl L</td>
<td>BD Multitest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD8/CD45/CD4 TruC Ctrl M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD8/CD45/CD4 TruC Ctrl H</td>
<td></td>
</tr>
<tr>
<td>3/16+56/45/19 Control (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD16+CD56/CD45/CD19 TruC Ctrl L</td>
<td>BD Multitest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD16+CD56/CD45/CD19 TruC Ctrl M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD16+CD56/CD45/CD19 TruC Ctrl H</td>
<td></td>
</tr>
<tr>
<td>4 Color TBNK</td>
<td>Tube 1</td>
<td>CD3/CD8/CD45/CD4</td>
<td>BD Multitest or BD Multitest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD16+CD56/CD45/CD19</td>
<td></td>
</tr>
<tr>
<td>4 Color TBNK + TruC (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD8/CD45/CD4</td>
<td>BD Multitest or BD Multitest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD16+CD56/CD45/CD19</td>
<td></td>
</tr>
<tr>
<td>6 Color TBNK + TruC (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD16+CD56/CD45/CD4/CD19/CD8</td>
<td>BD 6 Color</td>
</tr>
<tr>
<td>6 Color TBNK</td>
<td>Tube 1</td>
<td>CD3/CD16+CD56/CD45/CD4/CD19/CD8</td>
<td>BD 6 Color</td>
</tr>
<tr>
<td>3/8/45/4</td>
<td>Tube 1</td>
<td>CD3/CD8/CD45/CD4</td>
<td>BD Multitest or BD Multitest Control</td>
</tr>
</tbody>
</table>
### Table 5-2  BD Multitest panels

<table>
<thead>
<tr>
<th>Panel</th>
<th>Secondary Tube</th>
<th>Reagents</th>
<th>Reagent Rack ID Choices</th>
</tr>
</thead>
<tbody>
<tr>
<td>3/16+56/45/19</td>
<td>Tube 1</td>
<td>CD3/CD16+CD56/CD45/CD19</td>
<td>BD Multitest or BD Multitest Control</td>
</tr>
<tr>
<td>3/8/45/4 + TruC</td>
<td>Tube 1</td>
<td>CD3/CD8/CD45/CD4 TruC</td>
<td>BD Multitest or BD Multitest Control</td>
</tr>
<tr>
<td>3/16+56/45/19 + TruC</td>
<td>Tube 1</td>
<td>CD3/CD16+CD56/CD45/CD19 TruC</td>
<td>BD Multitest or BD Multitest Control</td>
</tr>
<tr>
<td>4-19-4 Control (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD8/CD45/CD4 TruC Ctrl L</td>
<td>BD Multitest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD16+CD56/CD45/CD19 TruC Ctrl M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD8/CD45/CD4 TruC Ctrl H</td>
<td></td>
</tr>
<tr>
<td>19-4-19 (BD Trucount tubes)</td>
<td>Tube 1</td>
<td>CD3/CD16+CD56/CD45/CD19 TruC Ctrl L</td>
<td>BD Multitest Control</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td>CD3/CD8/CD45/CD4 TruC Ctrl M</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td>CD3/CD16+CD56/CD45/CD19 TruC Ctrl H</td>
<td></td>
</tr>
</tbody>
</table>
BD Accuracy and Precision Panel

Table 5-3  BD Accuracy and Precision panel

<table>
<thead>
<tr>
<th>Panel</th>
<th>Secondary Tube</th>
<th>Reagents</th>
<th>Reagent Rack ID Choices</th>
</tr>
</thead>
<tbody>
<tr>
<td>A &amp; P Dispense</td>
<td>Tube 1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Tube 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tube 5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pre-Programmed Reagent Racks

Table 5-4  Pre-programmed reagent racks

<table>
<thead>
<tr>
<th>Reagent Rack ID</th>
<th>Reagents</th>
<th>Reagent Rack Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Multitest</td>
<td>CD3/CD8/CD45/CD4</td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td>CD3/CD16+CD56/CD45/CD19</td>
<td>R2</td>
</tr>
<tr>
<td>BD Multitest Control</td>
<td>CD3/CD8/CD45/CD4</td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td>CD3/CD16+CD56/CD45/CD19</td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td>BD Trucount Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Ctrl L</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>• Ctrl M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>• Ctrl H</td>
<td>H</td>
</tr>
<tr>
<td>BD Tritest</td>
<td>CD3/CD4/CD45</td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td>CD3/CD8/CD45</td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td>CD3/CD19/CD45</td>
<td>R3</td>
</tr>
<tr>
<td></td>
<td>CD3/CD16+CD56/CD45</td>
<td>R4</td>
</tr>
</tbody>
</table>
Table 5-4  Pre-programmed reagent racks (continued)

<table>
<thead>
<tr>
<th>Reagent Rack ID</th>
<th>Reagents</th>
<th>Reagent Rack Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Tritest Control</td>
<td>CD3/CD4/CD45</td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td>CD3/CD8/CD45</td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td>CD3/CD19/CD45</td>
<td>R3</td>
</tr>
<tr>
<td></td>
<td>CD3/CD16+CD56/CD45</td>
<td>R4</td>
</tr>
<tr>
<td></td>
<td>BD Trucount Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Ctrl L</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>• Ctrl M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>• Ctrl H</td>
<td>H</td>
</tr>
<tr>
<td>BD Tritest 3-Tube T cell Controls</td>
<td>CD4/CD8/CD3</td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td>CD3/CD4/CD45</td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td>CD3/CD8/CD45</td>
<td>R3</td>
</tr>
<tr>
<td>BD Tritest 3-Tube T cell Controls</td>
<td>CD4/CD8/CD3</td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td>CD3/CD4/CD45</td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td>CD3/CD8/CD45</td>
<td>R3</td>
</tr>
<tr>
<td></td>
<td>BD Trucount Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Ctrl L</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>• Ctrl M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>• Ctrl H</td>
<td>H</td>
</tr>
<tr>
<td>BD MultiReagent Control</td>
<td>CD4/CD8/CD3</td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td>CD3/CD19/CD45</td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td>CD3/CD16+CD56/CD45</td>
<td>R3</td>
</tr>
<tr>
<td></td>
<td>BD Trucount Controls</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Ctrl L</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>• Ctrl M</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td>• Ctrl H</td>
<td>H</td>
</tr>
<tr>
<td>BD 6 Color</td>
<td>CD3/CD16+CD56/CD45/CD4/CD19/CD8</td>
<td>R1</td>
</tr>
</tbody>
</table>
Tube Protocols

Each secondary tube has a set of tube protocols associated with it for sample, lyse, and reagent amounts, and incubation times. You cannot edit a pre-programmed panel and the tube protocol settings associated with its secondary tubes.

The tube protocol settings for the secondary tubes of pre-programmed BD Multitest and BD Tritest panels are listed in the following table.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Amount</td>
<td>50 µL</td>
</tr>
<tr>
<td>Reagent Amount</td>
<td>20 µL</td>
</tr>
<tr>
<td>Post-Reagent Dispense Mix</td>
<td>At end of dispense</td>
</tr>
<tr>
<td>Reagent Incubation</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Lyse Amount</td>
<td>450 µL</td>
</tr>
<tr>
<td>Post-Lyse Dispense Mix</td>
<td>At end of dispense</td>
</tr>
<tr>
<td>Lyse Incubation</td>
<td>15 minutes</td>
</tr>
<tr>
<td>BD Trucount Controls</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

**Maximum Number of Secondary Tubes Per Panel**

⚠️ Primary tube caps should be pierced no more than four times. With additional piercings, particles from the rubber cap are more likely to clog the probe, resulting in blocked or inaccurate sample dispense.

There is also a limit to the volume of sample that can be dispensed from a single draw. Together, these constraints limit the total sample that can be dispensed from a capped primary tube.

**BD MultiTEST/BD TriTEST/Absolute Count Panels**

From a single draw, a maximum of 200 µL of sample can be dispensed for BD MultiTEST/BD TriTEST/Absolute Count panels. With a four cap-pierce
limit, a maximum of 800 µL of sample can be dispensed to the secondary tubes of these panels.

Since each secondary tube of a BD MultiTEST/BD TriTEST/Absolute Count panel receives a 50 µL sample dispense, a maximum of 16 secondary tubes can be used.

**NOTE** The carousel rack holds up to 40 secondary tubes.

### Creating a New Reagent Rack

The BD Reagent Racks category contains pre-programmed reagent racks.

To create a new reagent rack, you must have SPA software administrator privileges.

Reagent rack categories are indicated by the folder icon. Reagent rack categories contain reagent racks, which are indicated by the rack icon. You can create additional reagent rack categories to organize your reagent racks.

New reagent racks and categories for reagent racks are displayed in blue. Pre-programmed reagent racks and the BD Reagent Racks category are displayed in bold black text.

The rack icon ( ) indicates that the reagent rack is available for editing. When a reagent rack is being used in a running worklist, it is displayed in the Reagent Rack Editor with a pointer icon ( ), to indicate that it is currently read-only.

**NOTE** In this section, you use the Reagent Rack Editor to create a new reagent rack. The Reagent Rack Editor can also be used to copy and edit existing reagent racks (see Adding Controls to a New Reagent Rack on page 92).

To create a new reagent rack:

1. Select **Setup > Reagent Rack Editor** from the Prep Worklist window.

   The Reagent Rack Editor opens.
2 Click the New Category button to create a new reagent rack category.
   The default name New Category is assigned.

3 Type a name for the new panel category.
   a Right-click the new category and select Rename.
   b Type the new name.

4 Select a new reagent rack category, and click New Rack.
   The default name New Rack is assigned.

5 Type a name for the new reagent rack.
   a Right-click the new rack and select Rename.
   b Type the new name.

6 Use one of the following methods to open the Rack Map Editor:
   • Select the new rack and click the Edit Rack button.
   • Right-click the new rack and select Properties.
   • Double-click the new rack.

7 Click the Reagent List link.
   The Reagent List window is displayed. The Reagent List contains all of the
   BD pre-programmed reagents.
   The Rack Map Editor remains open. Any reagent name in the Reagent List
   can be dragged to the Reagent/Position table in the Rack Map Editor.

8 Drag a reagent from the Reagent List to the Reagent/Position table in the
   Rack Map Editor.
9 Assign a rack position for the reagent by selecting the reagent in the Rack Map Editor, then clicking on a map position.

![Rack Map Editor](image)

10 Continue to add reagents and assign their positions until your reagent rack is complete.

11 Click OK to save the reagent rack.

12 The Rack Map Editor and the Reagent List close.

13 (Optional) Click Print in the Reagent Rack Editor to print a copy of the reagent rack.

14 Click OK to close the Reagent Rack Editor.

15 Verify that your new reagent rack is available in the Reagent Rack ID menu of the Prep Worklist window.

### Creating Multi-Rack Reagent Racks

You can create a rack in the software that consists of multiple physical racks. This is useful when you have more than 24 reagents to process. The system processes the racks in order, prompting you to replace each rack upon completion of aspiration of all reagents in the rack.

To create a multi-rack reagent rack:

1 In the Rack Map Editor, click Add Rack under Multi-rack Functions.
A new rack is created.

2 Move between racks with the Previous and Next buttons.

3 Add reagents to the added racks as described in Creating a New Reagent Rack on page 89.

Adding Controls to a New Reagent Rack

To add BD Trucount controls to a new reagent rack:

1 Select Setup > Reagent Rack Editor from the main menu.

The Reagent Rack Editor opens.

2 Select a new reagent rack without BD Trucount controls.

If none exists, create one using the instructions of Creating a New Reagent Rack on page 89.

3 Click Edit Rack.

The Rack Map Editor opens.

4 Select all of the BD Trucount Controls checkboxes.
5 Click OK to save the rack map changes.
   The Rack Map Editor closes.

6 Click OK to save the reagent rack changes.
   The Reagent Rack Editor closes.
6

Sample Preparation

This chapter covers the following topics:

- Daily Startup on page 96
- Setting Up for a Processing Run on page 100
- Offline Incubation on page 118
- Sample Processing on page 119
Daily Startup

Perform the daily startup procedure each day before processing samples.

1. Remove reagents from the refrigerator to allow them to warm up to room temperature.

2. Open the safety cover.

3. Close and lock the primary tube rack cage.

4. Close the safety cover.

5. Turn on the instrument power and the computer power.

   The power switch is located on the lower-left side of the instrument.

⚠️ Do NOT start SPA software at this time.

   To prevent a biohazardous fluid spill, you must service the waste tank and reattach it to the fluidics tower before you start the software.

6. Empty the waste tank, and then add 1 L of undiluted household bleach and 500 µL of Sigma Antifoam A Concentrate to the tank. See Emptying the Waste Tank on page 45.

7. Ensure that the waste tank fluid and sensor connectors are properly attached to the fluidics tower (front and side connections).

8. Empty any DI water from the previous day.

⚠️ Empty the DI water tanks every day to keep the instrument fluidics system free of mineral deposits and clogs.

   To service a bulk fluid container:

   a. Detach the quick-release connector on the fluid tank by pressing the metal clip.
b Disconnect the wire connector from the fluidics tower.

9 Fill all the bulk fluid tanks in the fluidics tower.

See Fluid and Waste Tank Installation on page 38.

10 Reconnect the quick-release connectors to the fluid tanks and the wire connectors to the fluidics tower. Ensure that the fluid tanks are in the correct positions and that all connections are correct.

11 Start the software and log in.

The Prep Worklist window opens and the instrument initializes.

12 Open the safety cover.
Perform the Daily Inspection (see next section).

**Daily Inspection**

Perform a visual inspection every day to keep your instrument running properly. If you find any of the symptoms noted here, consult Troubleshooting on page 189 for help with identifying and solving the problem.

⚠️ Instrument hardware could be contaminated with biohazardous material. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Wear protective clothing, eyewear, and gloves.

Check for the following:

- Leaks or mineral deposits (white powder or crystals) around the syringes, valves, and pumps

In Figure 6-1, the valve fittings are circled in blue, the pump fittings are circled in green, and the syringe seals are circled in magenta.

**Figure 6-1** Valve, syringe, and pump fittings on fluidics panel

- Crimps in the fluidics tubing

- Bubbles in the fluidics tubing. If there are bubbles, see Manually Priming the Instrument on page 43.
• Leaks or mineral deposits around the waste port (left side of fluidics tower)

• Kinks or twists in the insulation block cable

• Signs of probe wear (the probe is no longer straight or the Teflon coating is gone). See Figure 7-8 on page 155.
Setting Up for a Processing Run

Perform the following procedures to prepare for a sample processing run:

- Initializing a Worklist on page 100
- Preparing Primary Tubes on page 101
- Sample Entry on page 102
- Panel Selection on page 109
- Carousel Rack Setup on page 109
- Reagent Rack Setup on page 111

Initializing a Worklist

When you start the software, a new worklist is created. You can either use the new worklist or you can open a previously saved worklist.

To open a previously saved worklist:

1. Select File > Open Prep Worklist.

   The Open Prep Worklist dialog opens.

2. Select a worklist and click Open.

   The selected worklist opens.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Case Num</th>
<th>Primary Tube Type</th>
<th>Primary Rack</th>
<th>Panel Name</th>
<th>Carousal Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>TruCount cell</td>
<td>BD Vacutainer</td>
<td>1</td>
<td>3/6/12/4 Connect</td>
<td>1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MultiCheck</td>
<td>BD Vacutainer</td>
<td>2</td>
<td>4 Color TBNK + TruC</td>
<td>4-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MultiCheck CD4 Lo</td>
<td>BD Vacutainer</td>
<td>3</td>
<td>4 Color TBNK + TruC</td>
<td>6-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient 1</td>
<td>BD Vacutainer</td>
<td>4</td>
<td>4 Color TBNK + TruC</td>
<td>8-9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

100  BD FACS Sample Prep Assistant III Instructions For Use
Preparing Primary Tubes

⚠️ To prevent damage to the probe, use only tubes that have been validated for use on the instrument. For a list of acceptable primary tube sizes see Required Equipment on page 32.

⚠️ Before running on the SPA III, ensure blood samples have been adequately mixed according to the tube manufacturer’s instructions.

This document contains the information necessary to operate your BD FACS™ Sample Prep Assistant III (SPA III) instrument using BD FACS™ Sample Prep Assistant software (BD FACS SPA software), and the appropriate reagent package insert for acceptable sample types.

You can mix different tube types in a rack using the tube adapters provided.

If you are running BD Multi-Check controls, see Running BD Multi-Check Controls on page 106.

Loading the Primary Tube Rack

1. Unlock the primary tube rack cage and remove the primary tube rack.
   Squeeze the lock mechanism together to unlock and open the cage.

2. Prepare samples for loading into a primary tube rack.
See Minimum Sample Volumes on page 232, to determine the minimum volume required in the capped primary tubes.

⚠️ To prevent biohazardous spills inside the instrument during sample mixing, DO NOT place uncapped blood collection tubes in the primary tube rack.

**Sample Entry**

In this section, you enter information about the samples into the worklist, and load the sample tubes into their assigned positions in the primary tube rack.

⚠️ To ensure correspondence between worklist sample entries and primary tubes, position each primary tube in the rack immediately after you complete its sample entry.

When reusing a saved worklist, verify that each primary tube position corresponds with its assigned position on the worklist.

To enter sample information:

1. (Optional) Type a Sample Name.

2. Scan a primary tube with the barcode scanner, or manually type a unique identification code (Sample ID) into the worklist.

   You must enter the Sample ID first, then select the Primary Tube Type.

   Each tube must have a unique Sample ID.

   - To enter the sample identification using the barcode scanner, place the cursor in the appropriate **Sample ID** field (see Figure 6-2) and then swipe the label past the scanning window.
The white indicator light on top will flash once and a beep will sound when a barcode has been successfully scanned.

- To manually enter sample identification, type it into the **Sample ID** field (Figure 6-2). The **Sample ID** field accepts up to 22 alphanumeric characters.

**Figure 6-2** Sample ID field

When you first click in a row, default values for the following fields are displayed: **Primary Rack Position**, **Panel Name**, and **Carousel Position**. These fields are editable.

The default value for a given field is the last value entered for that field. The first time you use the software, there are no default values for these fields.

> (Optional) Type a case number.
4 Select a tube type from the **Primary Tube Type** menu. The tube types are BD Vacutainer and Sarstedt. See Table 3-1 on page 32 for more details on supported tubes.

**Warning:** Possible exposure to biohazardous material. Verify that the correct tube type is selected. A wrong tube type can cause incorrect results and/or damage to the probe. This is especially important when using Sarstedt tubes because they are a different size than the other types. Selecting a wrong tube type can cause a probe crash at the bottom of the tube.

You can mix different tube types in a rack using the tube adapters provided. It is good practice to group similar tube types together in the primary tube rack to make it easier to visually ensure you have selected the correct tube type from the **Primary Tube Type** menu.

⚠️ Use a tube adapter when necessary to prevent damage to the probe and sample tube.

⚠️ Do not leave empty tube adapters in the primary tube rack when running a worklist. The unused adapters can fall out and jam the sample mixer.

5 If the primary tube corresponding to the sample entry has been sitting for an extended period, or if it has not been on a tube rocker, mix the tube by inverting it.

6 Load the primary tube into the primary rack position assigned in the worklist (each position in the primary tube rack has a unique map number).
You can override the software-assigned primary rack position and load a sample into any available primary rack.

**NOTE** Leave gaps in the rack to organize the samples into groups.

To override the software-assigned position:

a. Type a primary tube rack map number in the **Primary Rack Position** field.

b. Ensure that you load the sample tube into the corresponding position.

c. Repeat steps 1 to 6 until all required samples have been specified and loaded.

**NOTE** To advance to the next sample entry, click in any field in the next row.
Running BD Multi-Check Controls

⚠️ Primary tube caps should be pierced no more than four times.

Depending on how often you run controls, you might reach the cap-piercings limit (4) when using BD Multi-Check controls in the primary sample rack. See Maximum Number of Secondary Tubes Per Panel on page 88. If this occurs, we recommend replacing the BD Multi-Check controls tube cap. Obtain identical caps from BD Vacutainer Plus plastic serum tubes (Part Number 367812).

⚠️ Do not transfer the contents of the BD Multi-Check control into the BD Vacutainer tube.

To replace the cap on a BD Vacutainer tube:

1. Place the BD Vacutainer tube cap on the BD Multi-Check control tube.
2. Discard the BD Vacutainer tube.
3. Follow the procedure in Venting Primary Sample Tubes on page 106 before running the control on the SPA III.

Re-capping Primary Sample Tubes

When you re-cap a primary sample tube, positive pressure is created within the tube. Then, when the sampling probe pierces the cap during processing, the pressure is dispelled into the probe, affecting the sampling fluidics distribution. The resulting pressure change might cause sample dilution or inaccurate results.

If running re-capped primary sample tubes is standard operating procedure in your lab, vent the tubes prior to loading them on the SPA III. We recommend using a device such as the BD Vacutainer blood transfer device (Part Number 364880). See Figure 6-4 on page 107.

Venting Primary Sample Tubes

Under a biosafety hood, insert the internal needle of the blood transfer device into the tube cap just far enough to break the septum. The pressure above the
sample is released within the biosafety hood. The tube is now ready for loading on the SPA III.

**Figure 6-4** BD Vacutainer blood transfer device

Mounting the Primary Tube Rack

To mount the primary tube rack:

1. Place the primary tube rack into the primary tube rack cage (Figure 6-5).
Figure 6-5  Placing the primary tube rack in the primary tube rack cage

The primary tube rack should slip easily into the cage. If it does not, try turning it around. The numbers 1–40 should appear right-side up when the rack is in the correct position.

2 Close and lock the primary tube rack cage.

Pinch the latch to lock it.

⚠️ If you can see any part of the red label with the caution triangle, the primary tube rack cage is not closed and tubes can spill onto the instrument worktable during mixing.
Panel Selection

1. Select a panel from the Panel Name menu.

   **Figure 6-6 Panel Name menu**

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Case Name</th>
<th>Primary Tube Type</th>
<th>Primary Rack Pos</th>
<th>Panel Name</th>
<th>Carousel Pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trucount control</td>
<td>1</td>
<td>BD Vacutainer</td>
<td>1</td>
<td>3/18/45/44 Control</td>
<td>1 - 3</td>
<td></td>
</tr>
<tr>
<td>MultChek</td>
<td>2</td>
<td>BD Vacutainer</td>
<td>2</td>
<td>4 C50x TRN + T1</td>
<td>4 - 5</td>
<td></td>
</tr>
<tr>
<td>MultChek CD4 Lo</td>
<td>3</td>
<td>BD Vacutainer</td>
<td>3</td>
<td>4 C50x TRN + T1</td>
<td>4 - 5</td>
<td></td>
</tr>
<tr>
<td>Patient #1</td>
<td>4</td>
<td>BD Vacutainer</td>
<td>4</td>
<td>4 C50x TRN + T1</td>
<td>4 - 5</td>
<td></td>
</tr>
</tbody>
</table>

2. Note the changes that occur in the Carousel Position field, the worktable display, and the Secondary Tubes Carousel Position and Name field.

   These changes reflect the new panel choice.

Carousel Rack Setup

Before setting up a carousel rack, consider the following points:

- If you plan to acquire from your secondary tubes using a BD FACS Loader, ensure that there are no empty positions between tubes in the carousel rack.
- You cannot split a panel between two carousel racks.

⚠️ Uncap all secondary tubes to prevent damage to the probe. Use only 12 x 75-mm Corning Falcon polystyrene tubes or 12 x 75-mm BD Trucount tubes.

To set up a carousel rack:

1. (Optional) Enter a Carousel ID number (1 to 16) in the Carousel ID field in the center of the carousel rack map (Figure 6-7).
2 (Optional) Scan the carousel barcode or type a unique carousel ID in the **Unique Carousel ID** field.

The ID is limited to a maximum of 22 characters.

3 Label the uncapped secondary tubes.

4 (Optional) Print the worklist and use it for help in loading the carousel rack. See Printing a Worklist on page 66.

5 Load the labeled secondary tubes into the positions indicated by the **Carousel Position** field (Figure 6-7), and the **Secondary Tubes Carousel Position and Name** field (Figure 6-8).

**Figure 6-7** Secondary tube position in carousel rack

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample ID</th>
<th>Case Name</th>
<th>Primary Tube Type</th>
<th>Primary Rack</th>
<th>Panel Name</th>
<th>Carousel Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracant controls</td>
<td>BD Vacutainer</td>
<td>2</td>
<td>3/5/4/8 Control</td>
<td>1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multi Check</td>
<td>BD Vacutainer</td>
<td>2</td>
<td>4 Color Nikon + TruC</td>
<td>4-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multi Check CD4 low</td>
<td>BD Vacutainer</td>
<td>3</td>
<td>4 Color Nikon + TruC</td>
<td>6-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient III</td>
<td>BD Vacutainer</td>
<td>4</td>
<td>4 Color Nikon + TruC</td>
<td>8-8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 6-8** Secondary Tubes Carousel Position and Name field

04. CD3/CD8/CD45/CD4 TruC
05. CD3/CD16/CD56/CD45/CD19 TruC
Mounting the Carousel Rack

To mount the carousel rack:

1. Place the carousel rack in the instrument.
   The guide pins on the instrument fit should into the alignment holes on top of the carousel rack.

2. Ensure that the rack is completely seated on the instrument worktable.
   The pins are visible when the rack is seated properly.

Reagent Rack Setup

To run BD Multitest and BD Tritest panels in the same worklist, you must create a new reagent rack. See Creating a New Reagent Rack on page 89.

If you are using BD Trucount controls with this carousel rack, select a reagent rack ID with controls, such as BD Multitest control or BD Tritest control.

To set up a reagent rack:

1. Verify that the reagents are at room temperature.
2. Select the reagent rack from the **Reagent Rack ID** menu.

   ![Reagent Rack ID Menu]

   The reagent rack map in the worktable display is updated.

3. Uncap the reagent vials and store the caps in the numbered reagent cap rack in their corresponding positions, to avoid contaminating reagents.

   ![Reagent Rack Map]

   **Verify that all reagent vials are uncapped to prevent damage to the probe.**

4. Verify that there is sufficient reagent in each vial to complete the run, and that there are no bubbles in the vials.
5 If needed, mount the reagent rack on the worktable (see Replacing the Reagent Rack on page 115).

6 Load the required reagents into the reagent rack. Use one or more of the following methods to obtain rack position information.

⚠️ For accurate results, load the reagent bottles in the correct locations matching the Rack Map Editor. If using the multi-rack option, make sure to run the racks in the correct order.

- Use the Rack Map Editor to Display the rack map for your reagent rack. See Creating a New Reagent Rack on page 89 for instructions on displaying the rack map editor.

- Position your cursor over a reagent rack position in the worktable display to view the reagent assigned to that position (this works only after you select your reagent rack from the Reagent Rack ID menu).

- See Table 5-4 on page 86 for a list of the pre-programmed reagent racks and their reagent and control positions in the rack.

If your lab prefers another reagent configuration, you can create a new reagent rack. See Creating a New Reagent Rack on page 89.

⚠️ Use only the 60-mL vial in the R25 position, or a vial with a similar diameter (38.9 mm). Vials with a smaller diameter can move in the well and cause problems with the system, including damage to the probe.
Removing and Replacing the Reagent Rack

Removing the Reagent Rack

⚠️ Avoid cross-contamination of reagents by capping any reagent vials in the reagent rack before removing the rack.

To remove the reagent rack:

1. Locate the indentation on the underside of the reagent rack.

2. Slip your fingers into the indentation and slide the reagent rack forward all the way.

3. Grasp the tray with both hands to remove the reagent rack from the worktable.
Replacing the Reagent Rack

⚠️ Avoid cross-contamination of reagents by capping any reagent vials in the reagent rack before mounting the rack.

1. Place the reagent rack at the edge of the instrument worktable, aligning it within the guidelines.

2. With both hands, slide the reagent rack all the way onto the instrument worktable until it snaps into place.
Entering Reagent Lot IDs

You can enter the lot IDs for all reagents used within your reagent racks. You can also enter BD Trucount bead and control lot IDs, beads counts, and standard deviations. Entering lot IDs and bead counts is optional.

If you re-run a worklist that has already been completed, the software verifies that lot IDs and bead information in the worklist are the same as those entered for the current run. If the information is different, a dialog prompts you to proceed or cancel. If you proceed, the information for the run will be updated with the current ID information. If you cancel, the run will stop.

NOTE You cannot enter or change Lot IDs while a reagent is being run.

To enter reagent lot IDs:

1. Select Setup > Lot IDs from the main menu in the Prep Worklist window.

   The Lot IDs dialog opens. The list of reagents in this dialog is the same as the list of reagents found in the Reagent List.

2. Select a reagent name and type the lot number in the corresponding Lot ID field.

   This information will be saved in the worklist.

3. To enter BD Trucount lot IDs, bead counts, and standard deviations:
   a. Click the TruCOUNT Bead tab (Figure 6-9).

      This information is saved in the worklist and is displayed in the worklist printout only if the worklist contains the appropriate Trucount beads and/or control.

      NOTE You cannot enter or change bead information while the beads are being run.
b Type the TruCount bead lot ID in the Lot ID field (up to 10 characters).

c Type the Bead/Pellet value in the Bead/Pellet field (five numerical characters—00000 or 45000–55000).

d In the table, type the bead controls lot IDs in the Lot ID field (up to 10 characters) for each control (Low, Medium, and High).

e Type the bead/µL count for each control in the Bead/µL field.
  • Low: 0.00 or 40–60 with two digits after the decimal
  • Medium: 0.00 or 200–300 with two digits after the decimal
  • High: 0.00 or 900–1,100 with two digits after the decimal

f Type the standard deviation for each control (0 to 70).

4 Click OK.
Offline Incubation

The SPA III allows you to perform the lyse incubation offline by selecting the checkbox above the carousel map.

The sequence of events for offline incubation are as follows:

1. The operation progresses as usual until the lyse incubation.
2. A dialog appears indicating that the sample prep is complete and you can remove the carousel.

⚠️ CAUTION: You must keep track of the incubation time. To ensure proper results, do not run the worklist until the incubation is complete.

3. A new worklist opens and it can be created as usual.
Sample Processing

⚠️ For your protection, the safety cover locks in the closed position during instrument operation. Do not attempt to open it while a run is in progress.

⚠️ To protect data integrity, do not remove any tubes, the carousel rack, or the primary tube rack from the SPA III until the run is finished.

⚠️ If a run is interrupted due to loss of power to either the instrument or the computer workstation, do not continue the run if power is restored. To safely resume processing, see Resuming a Run After a Power Interruption on page 186.

When a run is interrupted due to loss of power, and power cannot be quickly restored, it may be possible to remove reagent vials and sample tubes from the instrument. See When Power Cannot Be Quickly Restored on page 187 for further instructions.

**NOTE** You cannot enter or delete information on a currently running worklist.

To process a sample:

1. Close the safety cover.

2. Click Run to begin the worklist.

   A dialog opens asking you to verify that key components of the Run have been properly prepared. It also specifies the volume of the reagents needed for the run.

3. After you verify preparation for the run, click **OK**.

4. Click **OK** again to save the worklist.

   Choose a location to save the worklist. Saved worklists can be opened from the **File** menu in the **Prep Worklist** window and re-used.
Observe the **Status** field while the instrument processes samples.

The **Status** field describes each action of the instrument.

After the instrument finishes scanning the carousel rack, the probe pierces a primary tube and aspirates sample. The instrument aliquots the sample into secondary tubes. The probe washes between dispenses.

When all of the samples and reagents have been dispensed into the secondary tubes, an incubation timer is displayed in the status field.

The timer is displayed again during the lyse incubation.

When the lyse incubation completes, the following dialog opens if you are running controls.
6 If you are running BD Trucount controls:
   
a  Click **OK**.

b  Open the safety cover.

c  Remove the BD Trucount controls from the reagent rack.

d  Cap and vortex each vial.

e  Uncap and replace each vial into its original rack location (place the caps into the reagent rack cap holder).

f  Close the safety cover and click **Resume**.

The instrument dispenses control beads into the designated tubes.

**LWA Information**

If you have entered LWA information and selected the **Remind to proceed to LWA at the end of sample prepping** option, an LWA confirmation dialog opens after sample prepping completes.
1 Click Yes.

An abbreviated LWA Info dialog opens.

2 Confirm the serial number or user name or select a different serial number or user name from the menus and click OK to add LWA information to the worklist.

The LWA information is included on worklist printouts.

Ending a Run

When all the tubes in a rack have been processed:

- A persistent, audible beep is produced.
- The End Prep Worklist execution dialog opens.

1 Click an ending option.

  - Print. Opens the Print dialog. Click OK to print the worklist.
  - Close. Return to the Prep Worklist window.

2 Remove the carousel rack from the instrument.

  Push on the center spindle with your thumb and pull the handle up.
3 Visually inspect all tubes for the correct preparation. All tubes should contain sample and reagent.

4 Analyze the prepared samples on a flow cytometer or store them as directed in the reagent package insert.

5 Remove the tubes from the primary tube rack and store or discard them according to your laboratory’s normal procedure.

6 Remove and recap the reagents, if necessary, and return them to the refrigerator.

After the last run of the day, follow the procedures described in Daily Shutdown on page 128.
Pausing and Resuming a Run

1. To pause the instrument during a run, click **Pause**.

   The probe returns to the wash station and the safety cover unlocks.

   ! To protect data integrity, do not remove any tubes, the carousel rack, or the primary tube rack from the SPA III until the run is finished.

   ! If you pause while the incubation timer displays, the instrument will not continue with the incubation countdown when you click Resume. It will begin the next processing step. To protect data integrity, track the incubation time if you pause while the timer displays.

2. To resume the run, click **Resume**.

   The instrument resumes processing after you close the safety cover.
Abort a Run

To end a run in mid-process:

1. Click Abort.

2. Choose one of the following options.
   - Click Yes if you want the instrument to wash and prime the probe.
   - Click No to end the run without priming.
   - Click Cancel to resume the run.
This chapter covers the following topics:

- Daily Shutdown on page 128
- Scheduled Maintenance on page 131
- Monthly Servicing of Waste In-Line Filters on page 141
- Unscheduled Maintenance on page 146
- Special Conditions on page 179
Daily Shutdown

Required Materials

- BD FACSClean solution or 10% bleach solution
- 60-mL BD FACSClean vial (in accessory kit)

Performing Daily Cleaning

Cleaning the instrument fluidics system every day prevents clogs. Daily cleaning and decontamination is essential for optimal instrument performance. This procedure takes approximately 10 minutes.

1. Select File > Shutdown from the main menu in the Prep Worklist window.

   The Sample Prep Assistant shutdown dialog opens.

2. Select the Daily Clean checkbox.
If you already performed the Daily Cleaning procedure, you can leave **Daily Clean** unchecked and proceed straight to Shutting Down the Instrument on page 130.

3  Click **OK**.

A second dialog opens.

![BD Sample Prep Assistant](image)

4  Open the safety cover.

5  Place a large vial containing 60-mL of BD FACSClean solution or 10% bleach solution in well R25 on the reagent rack.

6  Fill the DI water and DI water 2 tanks.

7  Empty the waste tank and add 1 L of bleach and 500 µL of Sigma Antifoam A Concentrate to the tank.

8  Close the safety cover.

9  Click **Yes**.

The probe, the wash station, and the syringes will be cleaned with BD FACSClean solution or 10% bleach. The system will also prime with BD FACSFlow solution and DI water.

When the daily cleaning procedure is complete, a dialog opens indicating that it is safe to power off the SPA III.
Shutting Down the Instrument

To shut down the instrument:

1. Open the safety cover.
2. Dispose of the waste and used sample tubes in the appropriate containers according to your laboratory procedures.
3. Remove and cap the BD FACSClean vial and store it at room temperature.
4. Cap and return the reagents to the refrigerator.
5. Remove any remaining secondary tubes from the carousel rack.
6. Close the safety cover.
7. Turn off the power to the instrument.
8. Turn off the computer.

Do not turn off the power before the dialog opens indicating that it is safe to power off. This is to prevent the overflow of potentially biohazardous fluid from the wash station.

Click OK.

SPA software quits.
Scheduled Maintenance

The SPA III requires minimal maintenance. However, basic preventive maintenance procedures are required to preserve the precision, accuracy, and reliability of the instrument.

Perform the following procedures as indicated:

- Daily Inspection on page 98
- Weekly Cleaning in next section
- Calibrating the Probe Reference Point on page 134
- Monthly Servicing of Waste In-Line Filters on page 141
- Monthly Replacement of the Waste Tank Cap on page 145

⚠️ Instrument hardware could be contaminated with biohazardous material. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Wear protective clothing, eyewear, and gloves.

Weekly Cleaning

The weekly cleaning procedure:

- Requires approximately 10 minutes.
- Decontaminates and cleans the fluidics system and Z-rack.
- Keeps the syringes and the valves free of clogs.
- Ensures optimal performance of the Z-rack, the probe, and the arm.
Required Materials

- BD FACSClean solution or 10% bleach solution
- 70% ethanol
- Large vial
- Clean, lint-free cloths or disposable wipes

Performing Weekly Cleaning

To perform the weekly cleaning procedure:

1. From the main menu in the Prep Worklist window, select Instrument > Clean > Weekly Clean.

A dialog opens.

2. Open the safety cover and place a large vial containing 60 mL of BD FACSClean solution or 10% bleach solution in well R25 on the reagent rack.

3. Fill the DI water and DI water 2 tanks.

4. Empty the waste tank according to your laboratory procedures, and then add 1 L of bleach and 500 µL of Sigma Antifoam A Concentrate to the tank.
5 Close the safety cover and click Yes to continue.

The fluidics system will be cleaned with BD FACSClean solution or 10% bleach solution for a total of 10 minutes. The system will then prime with BD FACSFlow solution and DI water.

After the weekly cleaning finishes, the probe will move to the front of the instrument and down for easy access, and a dialog opens.

6 Click OK.

7 Open the safety cover and wipe the Z-rack with a cloth dampened with 70% ethanol.

8 Wipe the Z-rack with a clean, dry cloth.
Look for residue, liquid, and cracks at the insulation block located above the probe. If found, call your BD Biosciences service representative.

9 Close the safety cover.

10 Select Instrument > Home from the main menu.

This returns the probe to the wash station and completes the procedure.

**Calibrating the Probe Reference Point**

The probe reference point is preset by BD Biosciences. You should verify the probe reference point weekly to ensure that the system will continue to perform accurately. You should also verify the probe reference point any time you change the probe or move the instrument to a new location in your lab.

**NOTE** Once you start the calibration procedure, make sure to complete the entire procedure. If you quit the procedure before it is done, the system may not function properly because the probe position data could be inaccurate.

To verify the reference point:

1 Open the safety cover and remove the reagent rack and the carousel rack.

   See Removing the Reagent Rack on page 114.

2 Select Instrument > Setup and Calibrate from the main menu in the Prep Worklist window.

   The Setup Reference Point dialog opens. (If you are performing the Probe Replacement Procedure, this dialog is already open.)
3 Click Verify Reference.

⚠ To prevent damage to the probe, do not adjust other settings such as Edit Instrument Property or Setup Reference.

The probe moves to the back of the work area and the SetupServices dialog opens.

⚠ Do not click Yes at this point.

**Figure 7-1** SetupServices dialog
Verify that the carousel and reagent racks are removed from the instrument worktable.

Place the reference point extension on the instrument worktable as shown in Figure 7-2 and Figure 7-3.

**Figure 7-2** Location of peg for use with reference point extension

**Figure 7-3** Reference point extension on peg

Close the safety cover.

It is important to close the safety cover at this time because the probe moves to the reference point in the next step.

Click Yes in the SetupServices dialog to continue (Figure 7-1 on page 135).
The probe moves to the reference point and the **Left Arm Configuration** dialog opens.

8. Open the safety cover.

9. Adjust the probe tip alignment, if necessary.

The probe tip should align directly above and in the center of the reference point extension (Figure 7-4 and Figure 7-5), and should not touch it.
Figure 7-4 Proper probe tip alignment

(a) Place a sheet of clean, white paper behind the calibration extension to help you see the probe tip more easily.

(b) To adjust the alignment, click the Move Arm buttons with your mouse.

Figure 7-5 Side (left) and top (right) view of proper probe tip alignment
Left, Right, Rear, and Front move the probe from side to side and from front to back on the horizontal plane.

Up and Down move the probe on the vertical plane.

c. Place the white paper on the extension peg and move the probe down until it just touches the paper. You should be able to slide the paper out easily.

10. When you are finished, click Next.

The Arm Configuration Summary is displayed.

11. Close the safety cover.

The probe moves to the back of the work area in the next step.

12. Click Finish to save the new settings or click Cancel to revert to the previous settings.

The Setup Reference Point dialog opens.

13. Click Print or Close.

• Clicking Print provides a Setup Report.
Clicking Close ends the probe reference point calibration.

14 Open the safety cover.

15 Remove the paper and the reference point extension.

16 Reinstall the reagent and carousel racks (see Replacing the Reagent Rack on page 115 and Mounting the Carousel Rack on page 111).

17 Close the safety cover.

**NOTE** If you are performing the calibration as part of the probe replacement procedure, return to Completing the Probe Replacement Procedure on page 166.
Monthly Servicing of Waste In-Line Filters

Service the waste in-line filters monthly and whenever you replace the probe. You should also service the filters if there is evidence of clogging.

Required Materials

- Gloves
- Paper towels
- Replacement fine filter, if needed
- BD FACSClean solution or 10% bleach solution
- DI water

⚠️ The waste in-line filter assembly may contain biohazardous material that can drip during filter replacement. Follow your standard laboratory procedures for biological hazards and use universal precautions when performing this procedure. Wear protective clothing, eyewear, and gloves.

Preparing the Instrument

If you are servicing the waste in-line filters in conjunction with replacing the probe, skip this section and proceed to the next section: Removing the Waste In-Line Filter Assembly.

1. If the probe is not in the wash station position, use the procedure Homing the Probe on page 179 to move the probe to the wash station position.

2. Open the safety cover.

3. Remove the reagent and carousel racks.
Removing the Waste In-Line Filter Assembly

To remove the waste in-line filter:

1. Lift the waste in-line filter assembly and place paper towels under it.

2. Disconnect the assembly from the tubing at both ends by pressing the quick-release tabs.

3. Hold the filter assembly with the printed face up and twist the locking rings in opposite directions to open the assembly.
4 Place the upper portion of the assembly on a dry surface.

⚠️ The locking ring with printed face should not be exposed to fluids.
5. Remove the fine-screen filter and the coarse filter from the lower portion of the assembly. You may need to gently tap the assembly on a benchtop to dislodge the filters.

6. Inspect the fine-screen filter for any clogging or damage. Replace the fine-screen filter if it is damaged.

7. Place the fine-screen and coarse filters in a beaker with DI water and rinse thoroughly.

8. Examine the curvature of the coarse filter, and return the coarse screen to its position in the assembly so that it is concave down.

9. Place the fine-screen filter on top of the coarse filter.

If the filters are oriented correctly, they will touch toward their centers and be separated at the edges.
10 Reassemble the locking rings.

11 Reattach the assembly to the instrument with the quick-release connectors.

The connectors allow the assembly to be attached only in the correct orientation (so that waste flows through the coarse filter first).

Completing the Waste In-Line Filter Service

1 Replace the reagent rack and carousel (see Replacing the Reagent Rack on page 115 and Mounting the Carousel Rack on page 111).

2 Perform the procedure in Initializing the Instrument on page 179.

3 Prime the instrument with DI water. Verify that the probe, waste in-line filter assembly, and hose connections do not leak.

Monthly Replacement of the Waste Tank Cap

Replace the waste tank cap (large-sized cap) monthly. See Emptying the Waste Tank on page 45 for instructions.
Unscheduled Maintenance

Perform the following procedures as needed:

- Internal and External Surface Cleaning and Decontamination on page 147
- Extended Periods of Non-Use on page 148
- Startup Following Extended Non-Use on page 151
- Replacing the Probe on page 154
- Replacing a Syringe on page 166
- Fuse Replacement on page 175

⚠️ Instrument hardware could be contaminated with biohazardous material. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Wear protective clothing, eyewear, and gloves.
Internal and External Surface Cleaning and Decontamination

Clean the instrument surfaces whenever needed.

⚠️ To avoid potential shock, always switch off the power and unplug the AC power cord before you begin external cleaning.

Required Materials

- BD FACSClean solution, 10% bleach solution, or 70% ethanol
- DI water
- Clean, lint-free cloths or disposable wipes

Cleaning the Instrument

To clean the instrument:

1. Switch off the instrument power and unplug the AC power cord.
2. Open the safety cover.
3. Wipe all accessible surfaces and racks with one of the cleaning solutions listed in Required Materials.
4. Wipe all exposed surfaces using a cloth dampened with DI water.
5. Wipe all exposed surfaces with a clean, dry cloth.

⚠️ Be sure to remove the bleach with DI water, and then dry the instrument surfaces to prevent corrosion.

6. Close the safety cover.
7. Repeat steps 3 to 5 on the instrument exterior.
**Extended Periods of Non-Use**

If you do not plan to use the instrument for an extended period (one week or longer), you must clean the system prior to shutdown. Perform the weekly cleaning procedure first.

**Required Materials**

- BD FACSClean solution or 10% bleach solution
- 60-mL BD FACSClean vial
- Clean, lint-free cloths or disposable wipes

**Performing Weekly Cleaning**

Before proceeding with the next section, perform the weekly cleaning procedure. See Weekly Cleaning on page 131.

**Prime the Lines with Water**

1. Empty all the tanks in the fluidics tower.
   
   Expose the waste container contents to bleach (10% of total volume) for a minimum of 30 minutes. Dispose of the waste using proper precautions in accordance with local health and safety regulations. Wear suitable protective clothing, eyewear, and gloves.

2. Fill all tanks in the fluidics tower with DI water. Reconnect all of the fluidics and fluid-sensing connectors.

3. Remove the fluid sensor from the BD FACSFlow cubitainer and place the sensor in a clean, suitable container filled with at least 0.5 L of DI water.

   The container should be an adequate size so that it is stable when the fluid sensor is placed in it.
4 Select Instrument > Prime from the main menu in the Prep Worklist window.

The Prime Fluidics dialog opens.

5 Select DI WATER from the Fluid Type menu and click Yes.

6 Repeat steps 3 and 4 with the following fluid types:
   - LYSE
   - FLOW
   - DI WATER 2

7 Empty all the tanks.
Completing the Non-Use Shutdown Procedure

To complete the procedure:

1. Select File > Shutdown from the main menu in the Prep Worklist window. Leave the Daily Clean checkbox cleared.

2. Click OK.

   The software quits.

3. Open the safety cover.

4. Dispose of the waste and used sample tubes in the appropriate containers according to your laboratory procedures.

5. Cap and return the reagents to the refrigerator (you can remove the entire rack).

6. Cap and remove the BD FACSClean vial and store it at room temperature.

   See Removing the Reagent Rack on page 114.

7. Remove any remaining secondary tubes from the carousel rack.
8 Close the safety cover.

9 Turn off the power to the instrument and the computer.

### Startup Following Extended Non-Use

Following the extended non-use of the instrument, you must prime the fluidics system with all of the appropriate bulk fluids. This will eliminate DI water from the fluidics system and ensure optimal instrument performance.

1 Ensure that the BD FACSFlow cubitainer has sufficient fluid.

2 Fill all tanks in the fluidics tower with their respective fluids.

   Add 1 L of bleach and 500 µL Sigma Antifoam A Concentrate to the waste tank.

3 Place all tanks back into the fluidics tower and reconnect all fluid lines and sensor connectors.

4 Turn on the instrument and the computer power.

   You must turn on the instrument before starting the software.

5 Start SPA software.

   The software will initialize the instrument.

6 Prime the instrument four times with DI WATER 2, four times with LYSE, four times with FLOW, and four times with DI WATER (a total of 16 primes).

   For each prime:

   a Select **Instrument > Prime**, from the main menu in the **Prep Worklist** window.

   The **Prime Fluidics** dialog opens.
Select a Fluid Type from the menu and click Yes.

The dialog closes and the fluidics system primes.

Check the fluidics tubing and syringes on the rear panel (Figure 7-6 on page 153) and the tubing on the instrument side (Figure 7-7 on page 153).

- If you see air bubbles, prime all fluid lines one more time, in the order listed in step 6.
- If you see any fluid leakage, see Troubleshooting on page 189 for tips on tightening valve fittings.
Figure 7-6  Fluid tubing and syringes on rear panel

Figure 7-7  Fluidics tubing on instrument side
Replacing the Probe

SPA software tracks the number of cap piercings since the last probe replacement. At 2,000 piercings, a software message recommends that you replace the probe. See Probe Replacement Guidelines for more details. After 2,500 piercings, the probe must be replaced before additional samples can be processed.

Before you begin, review the BD FACS SPA III Safety and Limitations Guide.

⚠️ The probe and tubing may contain biohazardous material that can drip during probe replacement. Follow your standard laboratory procedures for biological hazards and use universal precautions when performing this procedure. Wear protective clothing, eyewear, and gloves.

⚠️ The probe is sharp. Use caution when handling.

Probe Replacement Guidelines

The 2,500 piercings counter for probe replacement is a guideline. There are times when the probe should be replaced sooner than that. The probe should be inspected daily and replaced when any of the following conditions are observed.

<table>
<thead>
<tr>
<th>Action</th>
<th>Condition Requiring Replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual inspection of the Teflon-coated probe tip</td>
<td>The probe is considered damaged if there are signs of wear on the Teflon coating on the sides of the probe. See Figure 7-8 on page 155. Slight wear from the end view of the probe is acceptable.</td>
</tr>
<tr>
<td>Liquid level detection capability</td>
<td>The probe is considered damaged if at any point during operation, the liquid detection functionality fails. A liquid detection failure will be detected by the software or will be observed by the operator.</td>
</tr>
<tr>
<td>Cap-piercing ability of the probe</td>
<td>The probe is considered damaged if at any point during operation the probe fails to pierce a cap.</td>
</tr>
</tbody>
</table>
See Figure 7-8 for an example of a probe with extreme wear of the Teflon coating that would require replacement immediately.

**Figure 7-8** Probe with badly worn coating

See Figure 7-9 for examples of probe wear that are considered acceptable and do not require replacement.

**Figure 7-9** Examples of acceptable probe wear

---

**Table 7-1** Probe replacement guidelines (continued)

<table>
<thead>
<tr>
<th>Action</th>
<th>Condition Requiring Replacement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy and precision of the dispense</td>
<td>The probe is considered damaged if the accuracy and precision of the dispense changes. The accuracy and precision of the dispense of one probe should remain the same throughout operation.</td>
</tr>
</tbody>
</table>
**Required Materials**

The waste in-line filters need to be cleaned whenever you replace the probe, so also have at hand the items listed in Monthly Servicing of Waste In-Line Filters and Required Materials on page 141.

- Gloves
- Paper towels
- Kimwipes® wipes
- Flathead screwdriver
- Metric hex wrench (2 mm)
- Needle nose pliers
- Probe replacement kit (3-pack or 6-pack)
  - Probe 3-pack with BD Vacutainer tubes (13 x 100 mm, Part Number 647768)
  - Probe 6-pack with BD Vacutainer tubes (13 x 100 mm, Part Number 647769)

**Procedure Summary**

Perform all steps of the probe replacement procedure in the following order.

1. Preparing to Replace the Probe on page 157.
2. Removing the Old Probe on page 159.
3. Attaching the New Probe on page 162.
4. Calibrating the Reference Point on page 165.
5. Completing the Probe Replacement Procedure on page 166.
Whenever you replace the probe, you should also service the waste in-line filters. Follow the procedure in Monthly Servicing of Waste In-Line Filters on page 141.

**Preparing to Replace the Probe**

1. Select **Instrument > Replace Probe** from the main menu in the **Prep Worklist** window.

   A dialog opens.

   **BD Sample Prep Assistant**

   Please place a vial containing BD FACSClean in position R25 of the Reagent Rack for probe cleaning.

   Please place an empty 13×100mm BD Vacutainer tube with cap in carousel position 1 to encapsulate the probe during probe change.

   Once the Probe Replacement has been started, it can not be cancelled.

   See the SPA III User's Guide for details

   **Continue?**

   [Yes] [No]

2. Place the 60-mL BD FACSClean vial into position R25 of the reagent rack.

3. Place an empty 13 x 100-mm BD Vacutainer tube with cap in carousel position 1 to encapsulate the probe during the probe change.
4 Close the safety cover.

5 Click Yes to start the cleaning process.

The instrument flushes the probe with BD FACSClean solution. This takes approximately 10 minutes.

After the probe is cleaned, the SPA III inserts the probe into the empty Vacutainer tube and moves the probe forward for easier access.

The **Replace Probe** dialog opens.

**Figure 7-10** Replace Probe dialog

Replace Probe

Probe Counter: 0

To replace the probe, follow the steps in the User's Guide:

User Guide

☐ Follow the steps as listed in the user's guide link provided above. Check this box when you are finished.

Next >
6 Leave the Replace Probe dialog open.

**Removing the Old Probe**

Do not turn off the instrument during this procedure.

1 Open the safety cover.

2 Loosen the setscrew on the insulator block with a 2-mm hex wrench.

3 Slide the insulation block off of the Z-rack. The probe is attached to the fluidics tubing inside the Z-rack.
4 Pull the fluidics tubing off of the top of the probe.

5 Loosen the lower screw on the insulation block with the flat-head screwdriver by turning it counterclockwise.

6 Pull the probe and tube down and off of the insulation block.

If the probe is difficult to remove, use a pair of needle-nose pliers to pull down and loosen the probe.
Keep the probe inside of the tube at all times. Dispose of the entire tube and probe into a sharps container.

⚠️ Do not remove the probe from the tube.

The probe is sharp. Handle it with caution. After use, discard the tube and probe in an approved sharps container in accordance with applicable regulations and institutional policy.

7 Pull the tubing through the Z-rack until it extends about 3 inches (7.6 cm), beyond the tip.

If the tubing is not accessible from below, you can push the tubing down from the top of the Z-rack.

8 Inspect the insulation block. If it is wet or has a buildup of salt crystals, clean with DI water and dry it with a Kimwipes delicate task wiper or a similar low-lint wipe.
Attaching the New Probe

1. Remove the probe from the packaging by opening the end with the black cap.
   
The black cap covers the blunt tip of the probe.

2. Insert the silver, blunt end of the new probe into the insulation block until it is completely seated against the block.

3. Turn the lower screw with the flat-head screwdriver clockwise to tighten it.

4. Attach the tubing to the new probe through the insulation block, and press firmly until it stops (Figure 7-11 and Figure 7-12).
Figure 7-11  Point of attachment

⚠️ Do not bend or kink the tubing.
Bending or kinking the tubing will reduce instrument performance.

Figure 7-12  Tubing attached to probe through insulation block

5  Reattach the insulation block (with the new probe) to the Z-rack.

a  Make sure that the screw plate on the insulation block faces the same direction as the grooves of the Z-rack. See Figure 7-13
**Figure 7-13** Correct alignment of insulation block.

- Use the hex wrench to tighten the set screw (clockwise) and lock the insulation block in place. The set screw must sit against the flat portion of the Z-rack.

6. Return to the **Replace Probe** dialog (Figure 7-10 on page 158), and click in the checkbox to continue the process.
Calibrating the Reference Point

1. Click **Next** in the **Replace Probe** dialog.

   The **Setup Reference Point** dialog opens.

   ![Setup Reference Point dialog]

   The probe reference point is preset by BD Biosciences. Any time you service the probe, you should verify the probe reference point. Verifying the calibration or recalibrating the reference point for the probe ensures accurate instrument performance.

2. Perform the procedure in Calibrating the Probe Reference Point on page 134.
Completing the Probe Replacement Procedure

1. Click Finish in the Replace Probe Complete dialog to reset the probe counter.

2. Perform the procedure in Initializing the Instrument on page 179.

3. Prime the instrument with DI water. Verify that the probe, waste in-line filter assembly, and hose connections do not leak.

4. To complete the probe replacement procedure, you must perform all steps in Method to Ensure Dispense Accuracy on page 218.

Replacing a Syringe

Replace the syringe on one of the two syringe pumps whenever you observe it leaking or dripping. Also, replace the syringe if you observe crystals inside or around the fittings.

⚠️ Instrument hardware could be contaminated with biohazardous material. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Wear protective clothing, eyewear, and gloves.
Required Materials

- Gloves
- Replacement syringe

Syringe Replacement Procedure

1. Select Instrument > Replace Syringe from the main menu in the Prep Worklist window.
   
   The Syringe Replacement Procedure dialog opens.

2. Select a syringe and click Next.

   ![Syringe Replacement Procedure dialog](image)

   A second dialog is displayed.
3 Click OK.

- The instrument primes twice with DI water.
- The syringe plunger for the selected syringe moves to a halfway position.
• The software is shut down.

4 Turn off the power to the instrument and unplug the power cord.

Always turn off the instrument and unplug the power cord before performing this procedure to prevent electrical shock or injury.

5 Open the safety cover.

6 Remove the reagent and the primary tube racks from the instrument.

7 Slide the primary tube rack cage forward, then lift it up to disengage it from the guide pegs until you can fit your hands behind it.

Do not remove the rack cage, disconnect the cable, or place any tension on the cable.
Removing the Syringe

To remove the syringe:

1. With your fingers, loosen the plunger screw at the bottom of the syringe by manually turning it counterclockwise.

2. Remove the screw.

   Place the screw nearby where it is easily accessible, since it will be used for placement of the new syringe.

3. Turn the syringe barrel in the direction shown in the following figure until it loosens, then remove it.
4 Discard the syringes into a sharps container.

**Replacing the Syringe**

To replace the syringe:

1 Prepare the replacement syringe by depressing the plunger almost all the way.

   Inspect the new syringe for defects before using it.

2 Fit the hole in the bottom of the plunger over the pin on the instrument panel.

   Ensure that the syringe is aligned straight up.
3 Attach the screw to the pin by turning it clockwise.

4 Pull up on the syringe’s barrel toward the valve.

   Ensure that the syringe is pointed straight up.

5 Attach the syringe to the valve by rotating it in the direction shown in the following figure until it is finger-tight.
NOTE  If you twist the syringe a quarter turn in the opposite direction, and then in the direction shown, it will help seat the threads.

Replacing the Racks

To replace the racks:

1  Slide the primary tube rack cage all the way back until it locks into position.

   Follow the alignment guides on the base of the instrument.

! Make sure the primary tube rack cage snaps into position to prevent damage to the probe.

2  Replace any racks you removed from the instrument.

   See Replacing the Reagent Rack on page 115.
3 Close the safety cover.

4 Plug in the power cord and turn on the instrument power.

5 Start the software.
   A prime with flow occurs, and the instrument initializes.

6 Open the safety cover.

7 Examine the valve and the syringe for any leaks or drips.
   If you observe leaking, repeat the syringe replacement procedure with the
   same syringe. Make sure the syringe fits snugly to the valve and is correctly
   aligned. Reseat it if needed. When the software starts and the system
   primes, examine it again for leaks.

Performing an Accuracy and Precision Check

NOTE If you replaced the 1-mL syringe, go to Accuracy and Precision on
page 217 and perform all the steps listed prior to proceeding.

If you replaced the 10-mL syringe, you are not required to perform the accuracy
and precision test.

Fuse Replacement

The SPA III contains two fuses, located on the right panel above the power
switch.

Replace these fuses whenever necessary.
**Required Materials**

- Small standard screwdriver
- Two replacement fuses: rating 5 A, 250 V

⚠️ For protection against risk of fire, replace fuses only with those of the specified type and rating.

**Removing Fuses**

⚠️ To protect against shock, always turn off the instrument and unplug the power cord before performing this procedure.

To remove a fuse:

1. Select **File > Shutdown**, from the main menu in the Prep Worklist window.
2. Turn off the instrument and unplug the power cord from the wall outlet.
3. Disconnect the cord from the side of the instrument.

This allows easy access to the fuse drawer.
4 Place a small screwdriver into the slit at the bottom of the fuse drawer and gently pry out the drawer.

![Image of a screwdriver being inserted into a fuse drawer]

⚠️ If you apply too much pressure, you might break the clip.

5 Remove the fuse drawer.

![Image of a fuse drawer being removed]

6 Remove and dispose of the old fuses.
NOTE We recommend that you replace both fuses at the same time.

Installing New Fuses

To install new fuses:

1. Replace both fuses.

2. Slide the drawer back into the instrument, as shown in the following figure.

3. Push gently on the drawer until it snaps into place.
4 Reconnect the power cord to the instrument, then plug the cord into the wall outlet.

5 Turn on the instrument power.

6 Restart the software.

**Special Conditions**

This section gives instructions for tasks that address special conditions that may arise during instrument operation.

**Homing the Probe**

To home the probe and return it to the wash station:

1 Close the safety cover.

2 Select Instrument > Home from the main menu in the Prep Worklist window.

**Initializing the Instrument**

To initialize the instrument:

1 Close and lock the primary rack cage.

2 Close the safety cover.

3 Select Instrument > Initialize from the main menu in the Prep Worklist window.
Recovering from an X, Y, or Z-Axis Error

If you encounter a step loss error on the x, y, or z-axis, follow the procedure in this section to correct the problem. These types of errors can happen, especially on the z-axis, if the wrong primary tube type is selected in the worklist. If a BD Vacutainer tube is selected but a Sarstedt tube is placed in the tube rack, then the probe extends down too far and hits the bottom of the tube, and can cause this type of error.

The following dialog appears when this error occurs.

To recover from a step loss error:

1. Click OK to close the dialog.
2. Exit SPA software and turn off power to the instrument.
3. Inspect all racks to make sure they are seated correctly and that nothing is blocking the path of the probe.
4. Discard all secondary tubes from the run that was in process when the error occurred and check all volumes (reagents and primary tubes).
5. Power up the instrument.
6 Start SPA software and log in.

7 Calibrate the probe reference point. See Calibrating the Probe Reference Point on page 134.
   a When the probe moves to the reference point extension at step 9 on page 137, inspect the probe to see if it is bent or damaged.
      If the probe is bent or damaged, cancel the calibration procedure and replace the probe by performing the standard probe replacement procedure. See Replacing the Probe on page 154.
   b If the probe does not require replacement, complete the calibration procedure.

8 Open the worklist, verify that all primary tube type selections are correct, and restart the run from the beginning.

Recovering from a Reinitialization Error

If you encounter a reinitialization error, follow the procedure in this section to correct the problem. These types of errors can happen if the probe becomes stuck in a tube and cannot return to the home position.

The following dialog appears when this error occurs.

![BD Sample Prep Assistant dialog](image)

To recover from a reinitialization error:
1 Click OK to close the dialog.

2 Turn off power to the instrument.

3 Quit SPA software by pressing Ctrl+Alt+Delete and using the Task Manager in Windows to end the task.

4 Inspect all racks to make sure they are seated correctly and that nothing is blocking the path of the probe.

5 Discard all secondary tubes from the run that was in process when the error occurred and check all volumes (reagents and primary tubes).

6 Power up the instrument.

7 Start SPA software and log in.

8 Calibrate the probe reference point. See Calibrating the Probe Reference Point on page 134.

   a When the probe moves to the reference point extension at step 9 on page 137, inspect the probe to see if it is bent or damaged.

      If the probe is bent or damaged, cancel the calibration procedure and replace the probe by performing the standard probe replacement procedure. See Replacing the Probe on page 154.

   b If the probe does not require replacement, complete the calibration procedure.

9 Open the worklist, verify that all primary tube type selections are correct, and restart the run from the beginning.
Manually Replacing the Probe

If an error situation results in the probe being damaged or bent and the standard replacement procedure cannot be used, this section shows how to safely replace the probe manually. You should contact BD Customer Support to confirm that this procedure is the recommended action in this situation.

⚠️ Instrument hardware could be contaminated with biohazardous material. Use universal precautions. Wear protective clothing, eyewear, and two pairs of gloves.

1. Turn off power to the SPA III instrument.
2. Exit SPA software.
   If the software is frozen, use the Windows Task Manager to quit the application.
3. Open the safety cover and perform the safety decontamination procedures recommended in your lab for dealing with biohazardous materials.
4. Load an empty carousel onto the worktable.
5. Place an empty 13 x 100-mm BD Vacutainer tube in position 1 in the carousel.
6. Grasp the probe arm at the insulation block and move the probe arm over to the carousel and position it above the empty tube in position 1. See Figure 7-14 on page 184.

⚠️ Be careful to avoid contact with the probe tip while moving the probe arm. Do not attempt to insert the probe into the empty tube unless the tube is loaded onto the carousel.
7 While holding at the insulation block, pull the probe arm down until the probe tip pierces the cap of the empty tube and continue to pull down until the probe is fully enclosed inside the tube. See Figure 7-15.

Figure 7-15 Pulling the probe arm down into the empty tube

8 Pull the probe arm up to clear the carousel, then pull it forward so you will have clear access to replace the probe.

9 Replace the probe by performing the following two steps in the main probe replacement procedure.
a Removing the Old Probe on page 159.

b Attaching the New Probe on page 162.

10 Once the new probe is installed, grasp the probe arm at the insulation block and move it to the back of the work area. See Figure 7-16 on page 185.

Figure 7-16 Moving the probe arm to the back of the work area

11 Close the safety cover and turn on power to the SPA III.

12 Start SPA software and log in.

The system initializes and is ready to run.

13 Reset the probe counter by doing a modified version of Replacing the Probe on page 154, as directed in the steps below.

a Perform Preparing to Replace the Probe on page 157, except substitute an uncapped, empty 12 x 75-mm tube in position 1 in the carousel.

b Skip the following sections:

- Removing the Old Probe on page 159
• Attaching the New Probe on page 162

Perform the next section: Calibrating the Reference Point on page 165.

Make sure to complete the entire procedure. Do not abort it once it is started.

Perform the final section: Completing the Probe Replacement Procedure on page 166.

This section is where you reset the probe counter and finish the remaining tasks.

Recovering from a Power Interruption

⚠️ When a run is interrupted due to loss of power to either the instrument or the computer workstation, do not attempt to continue the run if power is restored. Read the next two sections to determine how to proceed.

Resuming a Run After a Power Interruption

To safely resume processing:

1. If SPA software is still running, abort the run, save the worklist, and exit the software.

2. Restore power to the instrument and workstation. Restart the instrument and start the software.

   The instrument initializes and the probe arm returns to the wash station.

3. Open the safety cover and check for hazardous fluid spills.

4. Restore the worklist (open a saved worklist, or recreate the worklist).

5. Discard any secondary tubes that received dispensed fluids, and replace the tubes.
6 Verify that the reagent vials and primary tubes have sufficient volume.

7 Restart the run.

**When Power Cannot Be Quickly Restored**

⚠️ Do NOT attempt to move the probe while the power is off.

If the SPA III stops with the probe position preventing removal of materials, contact your local BD Biosciences technical support representative for recovery instructions.

⚠️ Turn off the power switch to the instrument before you open the safety cover.

⚠️ Instrument hardware could be contaminated with biohazardous material. Use universal precautions. Wear protective clothing, eyewear, and gloves.

When a run is interrupted due to loss of power, and power cannot be quickly restored, you can remove reagent vials and sample tubes from the instrument if the probe position is not preventing removal.
The tips in this chapter are designed to help you troubleshoot any problems with the instrument or software. If additional assistance is required, contact your local BD Biosciences technical support representative. Refer to our website, bdbiosciences.com, for up-to-date contact information.

You can find troubleshooting suggestions under the following topics:

- Instrument Troubleshooting on page 190
- Software Troubleshooting on page 204
- Software Error Messages on page 205
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy and precision results not meeting</td>
<td>Tubes not weighed properly</td>
<td>Make sure</td>
</tr>
<tr>
<td>specifications</td>
<td></td>
<td>• you follow the procedure for Accuracy and Precision exactly.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• you do not touch the tubes with your hands or gloved hands.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• the balance stabilizes before recording the weight.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• you weigh the tubes immediately after dispense.</td>
</tr>
<tr>
<td>Insufficient water in tubes</td>
<td></td>
<td>Verify volume of water in primary tubes is correct.</td>
</tr>
<tr>
<td>One or more fluidics tanks disconnected</td>
<td></td>
<td>Connect the tank(s).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perform the Startup Following Extended Non-Use on page 151.</td>
</tr>
<tr>
<td>Loose valve or fittings</td>
<td></td>
<td>Check all fittings and hand-tighten them, if necessary.</td>
</tr>
<tr>
<td>Probe clogged</td>
<td></td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td>Calculations incorrect</td>
<td></td>
<td>Double check your calculations and spreadsheet setup.</td>
</tr>
<tr>
<td>Probe improperly installed</td>
<td></td>
<td>Make sure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• the probe was installed in the correct orientation. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• the fluid connection to the probe is secure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• the probe is tightly connected to the Z-rack.</td>
</tr>
</tbody>
</table>
### Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy and precision results not meeting specifications (continued)</td>
<td>Worklist incorrectly set up</td>
<td>Make sure you choose the worklist specified in the procedure and an adequate number of primary and secondary tubes.</td>
</tr>
<tr>
<td></td>
<td>Other hardware problem</td>
<td>Make sure all tanks are filled with bulk fluids and connected.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Check valves; hand-tighten all fittings.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Check syringe fittings to make sure they are snug.</td>
</tr>
<tr>
<td></td>
<td>Air in tubing and syringes</td>
<td>Connect the tank(s).</td>
</tr>
<tr>
<td></td>
<td>One or more fluidics tanks disconnected</td>
<td>Perform the Startup Following Extended Non-Use on page 151.</td>
</tr>
<tr>
<td></td>
<td>Loose valve or pump fittings</td>
<td>Check all fittings and hand-tighten them, if necessary.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>See Figure 6-1 on page 98 for the locations of the valves.</td>
</tr>
<tr>
<td>Flow cytometry results inaccurate</td>
<td><strong>Incomplete lysing</strong></td>
<td>Prepare a new batch of BD FACS lysing solution.</td>
</tr>
<tr>
<td></td>
<td>• BD FACS lysing solution at incorrect concentration</td>
<td>Allow the full lyse incubation time.</td>
</tr>
<tr>
<td></td>
<td>• Offline Incubation enabled, but did not finish</td>
<td></td>
</tr>
<tr>
<td>Problem with bulk fluids</td>
<td><strong>Inspect fluids for clarity. If suspect, prepare a new batch of BD bulk fluids.</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Bulk fluids contaminated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Insufficient dispensing of bulk fluids: dispense probe tip worn, damaged, or clogged</td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cytometry results inaccurate (continued)</td>
<td>• Insufficient dispensing of bulk fluids: supply tank empty</td>
<td>Check the bulk fluid tanks. If empty, refill.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>If tanks are full, check valves—hand-tighten all fittings. See Figure 6-1 on page 98 for the locations of the valves.</td>
</tr>
<tr>
<td></td>
<td>• Wrong solution dispensed</td>
<td>Ensure that the bulk fluid tubing connections are correct and that tanks are filled with the correct fluids (see Bulk Fluid Preparation on page 37 and Fluid and Waste Tank Installation on page 38).</td>
</tr>
<tr>
<td></td>
<td>• Insufficient dispensing of bulk fluids: intake tubing not completely submersed in bulk fluids</td>
<td>Inspect the bulk fluid containers. Make sure the bulk fluids intake tubing is completely submersed and the cap is completely sealed.</td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cytometry results inaccurate (continued)</td>
<td>Insufficient sample</td>
<td></td>
</tr>
<tr>
<td>• Primary sample tube in wrong position or no tube in position</td>
<td>Make sure the tube is in the correct position in the primary tube rack. Rerun tube if necessary.</td>
<td></td>
</tr>
<tr>
<td>• Primary sample tube received no sample dispense</td>
<td>See solutions for probe clogged, then rerun sample.</td>
<td></td>
</tr>
<tr>
<td>• Probe clogged</td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154. Primary tube caps should be pierced no more than four times. With additional piercings, particles from the rubber cap are more likely to clog the probe, resulting in blocked or inaccurate sample dispense.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>See Maximum Number of Secondary Tubes Per Panel on page 88.</td>
<td></td>
</tr>
<tr>
<td>• Recapped tubes</td>
<td>Vent tubes as described in Running BD Multi-Check Controls on page 106, and rerun.</td>
<td></td>
</tr>
<tr>
<td>• Clotted blood sample</td>
<td>Do not run clotted samples on the SPA III.</td>
<td></td>
</tr>
<tr>
<td>Insufficient reagent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Wrong reagent or no reagent in rack</td>
<td>Make sure reagent vial is in correct position in reagent rack. Rerun tube if necessary.</td>
<td></td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow cytometry results inaccurate (continued)</td>
<td><strong>Insufficient BD Trucount Control</strong></td>
<td>• Wrong BD Trucount control or no BD Trucount control Make sure BD Trucount controls are in correct positions in the reagent rack; rerun tube if necessary.</td>
</tr>
<tr>
<td></td>
<td><strong>Worn probe</strong></td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td>Fluidics tubing creased, crimped, or bent</td>
<td>Fluidics tower too close to wall or instrument</td>
<td>Call BD Biosciences service representative.</td>
</tr>
<tr>
<td></td>
<td><strong>Tubing incorrectly replaced onto probe</strong></td>
<td>Call BD Biosciences service representative. Review probe replacement procedure for next run. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td>Incubation timer disappearing</td>
<td>Screen saver interference with incubation timer display</td>
<td>Turn off all screen savers. See Software Compatibility Issues on page 50.</td>
</tr>
<tr>
<td>Instrument not responding to software</td>
<td>Cable between instrument and PC disconnected</td>
<td>• Ensure the instrument power is on.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure the cable is connected to both instrument and PC.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Shut down software. Restart application.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Call BD Biosciences.</td>
</tr>
<tr>
<td></td>
<td>Cable to PC not connected to Com Port 1</td>
<td>Ensure the cable is connected to Com Port 1, and not Com Port 2. See the documentation that came with your PC.</td>
</tr>
</tbody>
</table>
# Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaks or mineral deposits at syringe seals</td>
<td>Syringe not correctly or fully attached</td>
<td>Check that the syringe fitting is finger-tight and attached correctly. See Replacing a Syringe on page 166.</td>
</tr>
<tr>
<td>Insufficient cleaning and maintenance of instrument</td>
<td>Make sure you perform daily and weekly cleaning. See Daily Shutdown on page 128 and Weekly Cleaning on page 131.</td>
<td></td>
</tr>
<tr>
<td>Fluids left in instrument for extended time periods</td>
<td>Make sure you perform extended non-use procedure if you intend to leave the instrument unused for long periods. See Extended Periods of Non-Use on page 148.</td>
<td></td>
</tr>
<tr>
<td>Break or crack in syringe</td>
<td>Perform syringe replacement procedure. See Replacing a Syringe on page 166.</td>
<td></td>
</tr>
<tr>
<td>Break or crack in valve</td>
<td>Call BD Biosciences.</td>
<td></td>
</tr>
<tr>
<td>Leaks or mineral deposits at valve and pump fittings</td>
<td>Syringe not correctly or fully attached</td>
<td>Check that the syringe fitting is finger-tight and attached correctly.</td>
</tr>
<tr>
<td>Improper cleaning and maintenance of instrument</td>
<td>Make sure you perform daily and weekly cleaning. See Daily Shutdown on page 128 and Weekly Cleaning on page 131.</td>
<td></td>
</tr>
<tr>
<td>Leave any fluids in instrument for extended time periods</td>
<td>Make sure you perform extended non-use procedure if you intend to leave the instrument unused for long periods. See Extended Periods of Non-Use on page 148.</td>
<td></td>
</tr>
<tr>
<td>Break or crack in valve</td>
<td>Call BD Biosciences.</td>
<td></td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaks or mineral deposits at waste port</td>
<td>Disconnected waste line</td>
<td>Reconnect the waste line. See Emptying the Waste Tank on page 45.</td>
</tr>
<tr>
<td></td>
<td>Damaged quick-release connector</td>
<td>Call BD Biosciences.</td>
</tr>
<tr>
<td>Leaks or mineral deposits at fittings and caps of all bulk fluid tanks and waste tank</td>
<td>Cap not on securely</td>
<td>Tighten the cap.</td>
</tr>
<tr>
<td></td>
<td>Cracked tank</td>
<td>Order a new tank.</td>
</tr>
<tr>
<td></td>
<td>Loose or worn fittings</td>
<td>Call BD Biosciences.</td>
</tr>
<tr>
<td>Mixing not occurring, primary tube rack</td>
<td>Cable to primary rack cage disconnected or partially disconnected</td>
<td>Make sure the cable from the instrument to the primary rack cage is completely connected.</td>
</tr>
<tr>
<td>Mixing not occurring, carousel rack</td>
<td>Hardware problem</td>
<td>Call BD Biosciences.</td>
</tr>
<tr>
<td></td>
<td>Carousel rack not properly seated on pins</td>
<td>Make sure the carousel rack is completely seated on carousel pins.</td>
</tr>
<tr>
<td>Monitor inactive, PC not responding</td>
<td>Power cord not plugged in</td>
<td>Plug in the power cord.</td>
</tr>
<tr>
<td></td>
<td>Monitor/PC power not turned on</td>
<td>Turn on the monitor/PC power.</td>
</tr>
<tr>
<td></td>
<td>Cable not connected between PC and monitor</td>
<td>Reconnect cable. Refer to PC manufacturer's documentation.</td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inadequate fluid dispensed</td>
<td>Clogged probe</td>
<td>Perform the probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td>Air leak</td>
<td></td>
<td>Check all fittings and hand-tighten them, if necessary.</td>
</tr>
<tr>
<td>Loose valve or pump fittings</td>
<td></td>
<td>Check all fittings and hand-tighten them, if necessary.</td>
</tr>
<tr>
<td>Fluidics tubing cramped,</td>
<td>Call BD Biosciences service</td>
<td>Call BD Biosciences service representative.</td>
</tr>
<tr>
<td>creased, or bent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Empty fluidics tanks</td>
<td></td>
<td>Fill the tanks.</td>
</tr>
<tr>
<td>Disconnected fluids lines to</td>
<td></td>
<td>Reconnect the tanks.</td>
</tr>
<tr>
<td>tanks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>No reagent aspirated</td>
<td>Probe clogged</td>
<td>Perform the probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary tube caps should be pierced no more than four times.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With additional piercings, particles from the rubber cap are more likely to clog the</td>
</tr>
<tr>
<td></td>
<td></td>
<td>probe, resulting in blocked or inaccurate sample dispense.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>See Maximum Number of Secondary Tubes Per Panel on page 88.</td>
</tr>
<tr>
<td>No reagent in vial, or no vial</td>
<td>Verify that your worklist matches your reagent rack map.</td>
<td>Make sure you have a reagent vial with sufficient volume to complete the run.</td>
</tr>
<tr>
<td>in reagent rack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bubbles in vial</td>
<td>Remove bubbles before running.</td>
<td></td>
</tr>
<tr>
<td>False trigger of conductivity</td>
<td>Call BD Biosciences.</td>
<td></td>
</tr>
<tr>
<td>Power indicator not lighting;</td>
<td>Power cord not plugged in</td>
<td>Plug in the power cord.</td>
</tr>
<tr>
<td>instrument not activating</td>
<td>Instrument power not turned on</td>
<td>Turn on the instrument power.</td>
</tr>
<tr>
<td></td>
<td>Blown fuse</td>
<td>Replace the fuse as described in Fuse Replacement on page 175.</td>
</tr>
<tr>
<td></td>
<td>Internal power failure</td>
<td>Contact your BD Biosciences service representative.</td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe bent or damaged</td>
<td>Non-supported primary tubes</td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perform probe calibration. See Calibrating the Probe Reference Point on page 134.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Make sure you use the appropriate supported tubes as listed in Required Equipment on page 32.</td>
</tr>
<tr>
<td>Used 11-mm or 13-mm tube in 16-mm rack without a tube adapter</td>
<td>Use a tube adapter.</td>
<td></td>
</tr>
<tr>
<td>Caps on reagent vials</td>
<td></td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perform probe calibration. See Calibrating the Probe Reference Point on page 134.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Make sure you take the caps off the reagent vials and place them in reagent cap holder as described Reagent Rack Setup on page 111.</td>
</tr>
<tr>
<td>Used vial other than 60-mL vial in position R25 in reagent rack</td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
<td></td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe contacting instrument worktable</td>
<td>Racks not in proper place</td>
<td>• Click Abort; turn off the power.</td>
</tr>
<tr>
<td>components</td>
<td></td>
<td>• Ensure all racks are aligned and seated properly (reagent, carousel, primary tube racks).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Inspect the probe. Make sure it's straight. If it's bent, replace the probe. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Perform probe calibration. See Calibrating the Probe Reference Point on page 134.</td>
</tr>
<tr>
<td>Software installed without instrument-specific worktable database</td>
<td>Install the instrument-specific worktable.</td>
<td></td>
</tr>
<tr>
<td>Probe tip realignment not correctly done</td>
<td></td>
<td>• Click Abort; turn off the power.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Ensure all racks are aligned and seated properly (reagent, carousel, primary tube racks).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Perform probe calibration. See Calibrating the Probe Reference Point on page 134.</td>
</tr>
<tr>
<td>Cable from primary tube rack cage to instrument loose or not completely connected</td>
<td>1 Make sure cable is connected to both the instrument and the primary tube rack cage.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 Initialize the software as described in Initializing the Instrument on page 179.</td>
<td></td>
</tr>
<tr>
<td>Hardware problem</td>
<td>Call BD Biosciences.</td>
<td></td>
</tr>
<tr>
<td>Probe not piercing sample tubes</td>
<td>Probe worn or bent and not able to pierce tube caps</td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tube rack cage not stationary while sample is aspirated</td>
<td>Primary tube rack cage cable loose or disconnected</td>
<td>Shut down the instrument. Push the cable in all the way, then engage the fastener.</td>
</tr>
<tr>
<td></td>
<td>Primary tube rack cage not seated properly</td>
<td>Ensure that the primary tube rack cage is properly seated on its guide pins.</td>
</tr>
<tr>
<td></td>
<td>Hardware problem</td>
<td>Call your BD Biosciences service representatives.</td>
</tr>
<tr>
<td>Spraying of fluid or sample from probe</td>
<td>Clogged probe</td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary tube caps should be pierced no more than four times. With additional piercings, particles from the rubber cap are more likely to clog the probe, resulting in blocked or inaccurate sample dispense.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>See Maximum Number of Secondary Tubes Per Panel on page 88.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In the future, perform Daily Cleaning and Weekly Cleaning as described in Shutdown and Maintenance on page 127.</td>
</tr>
<tr>
<td>Tubes, secondary, (12 x 75-mm) not fitting into carousel rack</td>
<td>Incompatible secondary tubes</td>
<td>Discard tubes and begin again, using compatible tube. See Required Equipment on page 32.</td>
</tr>
<tr>
<td></td>
<td>Too many labels on tubes</td>
<td>Remove excess labels. Do not force tubes into carousel rack.</td>
</tr>
<tr>
<td></td>
<td>Labels too thick</td>
<td>Use labels &lt;5 mils thick (127 microns).</td>
</tr>
</tbody>
</table>
## Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubes, primary, not fitting into primary tube rack</td>
<td>Incompatible primary tubes</td>
<td>Use compatible tube. See Required Equipment on page 32.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use compatible rack or use tube adapters.</td>
</tr>
<tr>
<td></td>
<td>Too many labels on tubes</td>
<td>Remove excess labeling. Do not force tubes into sample rack.</td>
</tr>
<tr>
<td>Tubing disconnected from probe</td>
<td>Probe clogged</td>
<td>Perform probe replacement procedure. See Replacing the Probe on page 154.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Primary tube caps should be pierced no more than four times. With additional piercings,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>particles from the rubber cap are more likely to clog the probe, resulting in blocked</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or inaccurate sample dispense.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>See Maximum Number of Secondary Tubes Per Panel on page 88.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In the future, perform Daily Cleaning and Weekly Cleaning as described in Shutdown and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maintenance on page 127.</td>
</tr>
<tr>
<td>Tubing not properly reconnected onto probe during replacement procedure</td>
<td>Perform the probe replacement procedure again, this time making sure the tubing is pushed down onto the probe until it is fully seated. See step 4 on page 162.</td>
<td></td>
</tr>
</tbody>
</table>
# Instrument Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wash station overflowing</td>
<td>Quick-release connectors on waste tank not connected</td>
<td>Connect the quick-release connectors for the waste tank.</td>
</tr>
<tr>
<td></td>
<td>Waste in-line filter clogged</td>
<td>Replace waste in-line filter.</td>
</tr>
<tr>
<td></td>
<td>Quick-release connectors on waste in-line filter not connected</td>
<td>Connect the quick-release connectors for the waste in-line filter.</td>
</tr>
<tr>
<td></td>
<td>Waste tank cap vent obstructed</td>
<td>Replace the waste tank cap.</td>
</tr>
<tr>
<td></td>
<td>Power to instrument turned off before shutdown cycle completed</td>
<td>Do not turn the instrument power off prior to the appearance of the following dialog:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- If the power is off, perform the Daily Startup procedure.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- If the power is on, perform the Daily Shutdown procedure.</td>
</tr>
<tr>
<td></td>
<td>Waste tank sensor malfunction, no software indicator</td>
<td>Call BD Biosciences.</td>
</tr>
<tr>
<td></td>
<td>Waste pump failure</td>
<td>Call BD Biosciences.</td>
</tr>
</tbody>
</table>
## Software Troubleshooting

<table>
<thead>
<tr>
<th>Observation</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Software not starting</td>
<td>File path to software application corrupted</td>
<td>1 Uninstall the software.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Reinstall the software and the instrument-specific worktable database.</td>
</tr>
<tr>
<td>Software application renamed or moved</td>
<td></td>
<td>1 Uninstall the software.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Reinstall the software and the instrument-specific worktable.</td>
</tr>
<tr>
<td>Instance of software already running</td>
<td></td>
<td>1 Check Windows taskbar to see if software is running.</td>
</tr>
<tr>
<td></td>
<td>running but minimized.</td>
<td>2 If not, reboot computer.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 If problem persists, call Customer Support.</td>
</tr>
<tr>
<td>Software not responding</td>
<td>Software frozen</td>
<td>1 Press <code>Ctrl+Alt+Delete</code> to open the Windows Task Manager.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Click the <strong>Applications</strong> tab. Select BD FACS SPA in the Windows Task Manager, and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>click <strong>End Task</strong>.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>![Alert] Unsaved data will be lost.</td>
</tr>
<tr>
<td>No keyboard/mouse response</td>
<td>Keyboard or mouse not connected to computer</td>
<td>Connect keyboard or mouse to computer.</td>
</tr>
<tr>
<td></td>
<td>Keyboard and mouse connections switched</td>
<td>Make sure keyboard is connected to keyboard port and mouse connected to mouse port.</td>
</tr>
<tr>
<td>Password not working</td>
<td>Caps Lock or Num Lock on</td>
<td>Disable Caps Lock and Num Lock and try again.</td>
</tr>
<tr>
<td>During software installation, a</td>
<td>The installer needs to reconfigure or update the</td>
<td>Proceed with the installation and the SPA software will install and function correctly.</td>
</tr>
<tr>
<td>message displays asking to reboot</td>
<td>installer engine.</td>
<td></td>
</tr>
<tr>
<td>the computer.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

204  *BD FACS Sample Prep Assistant III Instructions For Use*
# Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
</table>
| The DI WATER (or DI WATER 2) tank liquid level is too low. Please fill the tank. | DI water or DI water 2 tank empty | 1 Fill the appropriate DI water tank.  
2 Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run. |
| The FACS FLOW tank liquid level is too low. Please replace the tank. | Flow tank empty | 1 Replace BD FACSFlow cubitainer.  
2 Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run. |
| Fluid tank sensor not plugged in | | 1 Connect tank sensor connector to fluidics tower bulkhead.  
2 Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run. |
| Faulty flow sensor | | Call BD Biosciences. |
| The FACS LYSE tank liquid level is too low. Please fill the tank. | Lyse tank empty | 1 Fill the FACS LYSE tank.  
2 Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run. |
## Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Reagent Rack does not contain all the reagents required to perform the Prep Worklist.</td>
<td>Reagent rack missing required reagents</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td>The following reagents are missing:</td>
<td></td>
<td>2 Place the required reagents in the reagent rack according to the Reagent Rack Map for the panel.</td>
</tr>
<tr>
<td>&lt;missing reagent list&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>The sample prep cannot run because the worklist has errors.</td>
<td>Incorrect data entered into the worklist</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Place your mouse on the red error icon with the exclamation point and correct the displayed error.</td>
</tr>
<tr>
<td>Sample prep has paused.</td>
<td>Reagent level too low</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td>Please press 'OK' button add the following reagent: &lt;reagent name&gt; and press 'Resume' button to continue</td>
<td></td>
<td>2 Add specified reagent to reagent vial.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Click Resume.</td>
</tr>
<tr>
<td>Sample prep is pausing because the WASTE tank is full.</td>
<td>Waste tank is full</td>
<td>1 Empty the Waste tank.</td>
</tr>
<tr>
<td>Please wait for the probe wash to complete. Then empty the WASTE tank, make sure to add 500 µL of Sigma Antifoam A Concentrate and press 'Resume' button to continue!</td>
<td></td>
<td>2 Add 500 µL of Sigma Antifoam A Concentrate.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Click Resume.</td>
</tr>
</tbody>
</table>
Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
</table>
| Sample prep is pausing because [DI WATER/FACS FLOW/DI WATER 2/LYSE]    | Specified tank liquid level is too low.                                       | 1 Wait for the probe wash to complete.  
| tank liquid level is too low.                                           |                                                                                | 2 Fill the tank.  
|                                                                         |                                                                                | 3 Click Resume.     |
| Sample rack is disconnected.                                            | Primary tube rack cage cable loose or disconnected                           | 1 Shut down the instrument.  
|                                                                         |                                                                                | 2 Tighten or connect the cable, then engage the fastener.  
|                                                                         |                                                                                | 3 Click Retry.       |
| The Carousel rack is not loaded.                                        | Carousel rack not loaded or not fully seated on pins                         | 1 Load the carousel rack.  
|                                                                         |                                                                                | 2 Verify that it is fully seated on the spindle and both pins. |
| The Secondary tubes are not loaded at their defined locations.          | Missing tube                                                                  | Verify that the carousel matches your worklist and your panel. Compare the worktable display to the carousel. |
| <misplaced tubes>                                                       |                                                                                |                   |
|                                                                         |                                                                                |                   |
## Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Secondary tubes are not loaded at their defined locations.</td>
<td>Missing tube</td>
<td>Verify that the carousel matches your worklist and your panel. Compare the worktable display to the carousel.</td>
</tr>
<tr>
<td>The Carousel could not be scanned due to an Instrument error.</td>
<td>Hardware problem</td>
<td>Call BD Biosciences.</td>
</tr>
<tr>
<td>Please load all secondary tubes at their defined locations and press 'Retry' to continue.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least one tank needs to be filled or emptied.</td>
<td>Tank full or empty</td>
<td>Compare tanks to display, and fill or empty tanks.</td>
</tr>
<tr>
<td>Please adjust liquids level and press 'Retry' to continue.</td>
<td>Fluid tank sensor connector not plugged in</td>
<td>1 Connect tank sensor connector to fluidics tower bulkhead.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Click Retry to continue. If you click Cancel, you will have to reinitialize the instrument prior to the next run.</td>
</tr>
<tr>
<td>&lt;Tank Name&gt; tank needs to be filled</td>
<td>The specified tank fluid level is too low</td>
<td>1 Add fluid to the specified tank.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Click Retry.</td>
</tr>
<tr>
<td>Sample prep is paused because the Safety Cover is open.</td>
<td>Safety cover open</td>
<td>Close the safety cover and press Resume.</td>
</tr>
<tr>
<td>Please wait for the probe wash to complete. Then close the Safety Cover and press 'Resume' button to continue!</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample prep is paused because the Safety Cover could not be locked. Please wait for the probe wash to complete. Then close the cover and press 'Resume' button to continue!</td>
<td>Safety cover not locked</td>
<td>Close the safety cover and press Resume.</td>
</tr>
<tr>
<td>The correct help file could not be found.</td>
<td>File path to help file corrupted</td>
<td>1 Uninstall the software. 2 Reinstall the software and the instrument-specific worktable database.</td>
</tr>
<tr>
<td>The software cannot give a worklist the same name as an open worklist. Type a different name for the worklist you want to save.</td>
<td>Attempt to name new worklist with same name as running worklist.</td>
<td>Type a different name for the worklist you want to save.</td>
</tr>
<tr>
<td>Selected file &lt;file name&gt; is not a valid Prep Worklist.</td>
<td>Non-worklist file opened (not an XML file or not a worklist XML file)</td>
<td>Choose only worklist XML files.</td>
</tr>
<tr>
<td>File corrupted</td>
<td>Discard old worklist, and create a new worklist</td>
<td></td>
</tr>
<tr>
<td>The selected Reagent Rack contains controls that are not available on the rack: &lt;missing control list&gt;</td>
<td>Reagent rack for selected panel is missing required controls</td>
<td>1 Click OK. 2 Place the required controls in the reagent rack according to the reagent rack map.</td>
</tr>
</tbody>
</table>
## Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample cap-piercing Probe Counter exceeded 2000.</td>
<td>Probe needs replacement soon</td>
<td>Be aware that the probe will need to be replaced after the next 500 pieces.</td>
</tr>
<tr>
<td>The Probe needs to be replaced soon.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample cap-piercing Probe Counter exceeded 2500.</td>
<td>Probe needs replacement now</td>
<td>Perform probe replacement procedure.</td>
</tr>
<tr>
<td>Immediate Probe replacement is required!</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unable to lock because the Safety Cover is open</td>
<td>Safety cover open</td>
<td>1 Close the safety cover.</td>
</tr>
<tr>
<td>Please close the cover and press 'Retry' key</td>
<td></td>
<td>2 Click Retry.</td>
</tr>
<tr>
<td>Unable to lock the Safety Cover!</td>
<td>Safety cover not locked</td>
<td>1 Inspect the lock mechanism.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Remove any obstruction.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Attempt to close the safety cover.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 Click Retry.</td>
</tr>
<tr>
<td>Unable to unlock the Safety Cover!</td>
<td>Safety cover not unlocking</td>
<td>Click Retry.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Call BD Biosciences.</td>
</tr>
<tr>
<td>Current procedure was canceled because the Safety Cover was not closed.</td>
<td>Safety cover was not closed</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Restart the procedure.</td>
</tr>
<tr>
<td>Current procedure was canceled because the Safety Cover was not closed.</td>
<td>Safety cover was not locked</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Restart the procedure.</td>
</tr>
</tbody>
</table>
## Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current procedure was canceled because <code>{Tank Name}</code> tank was not filled.</td>
<td>The specified tank fluid level is too low</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Fill the specified tank.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Restart the procedure.</td>
</tr>
<tr>
<td>Current procedure was canceled because Waste tank was not emptied.</td>
<td>Waste tank was not emptied</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Empty the waste tank.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Restart the procedure.</td>
</tr>
<tr>
<td>Current procedure was canceled because an error occurred on the Instrument.</td>
<td>Hardware error detected</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Visually inspect the instrument worktable. Make sure all racks are seated correctly and that there are no moving parts obstructed. Make sure all cables are connected.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Reinitialize the instrument.</td>
</tr>
<tr>
<td>Please abort the sample prep or wait for the current Instrument operation to finish before Shutdown!</td>
<td>Pressed <code>Esc</code> button while sample prep running</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Then do one of the following:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Use the <code> Esc</code> control to minimize and hide the display.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Click <code>Abort</code> to stop the run.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Wait for the instrument operation to finish before shutdown.</td>
</tr>
</tbody>
</table>
## Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unable to load Reagent Racks file &lt;File Name&gt;</td>
<td>File not in expected location (moved or renamed)</td>
<td>Return file to original location.</td>
</tr>
<tr>
<td>&lt;additional Windows OS message&gt;</td>
<td></td>
<td>1 Uninstall the software.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Reinstall the software.</td>
</tr>
<tr>
<td>Sample prep cannot continue the menu selection because: The WASTE tank is full. Please empty the WASTE tank and make sure to add 500 µl of Sigma Antifoam A Concentrate. Please try again after fixing the problem!</td>
<td>Waste tank is full at the beginning of the instrument process.</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Empty the waste tank.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Retry the menu selection.</td>
</tr>
<tr>
<td>Sample prep cannot continue the menu selection because: The [DI WATER/FACS FLOW/DI WATER 2/ FACS LYSE] tank liquid level is too low. Please fill the tank. Please try again after fixing the problem!</td>
<td>Specified tank fluid level is too low at the beginning of the instrument process.</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Fill the specified tank.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Retry the menu selection.</td>
</tr>
<tr>
<td>Unique Carousel ID cannot be longer than 22 characters. Please re-type the Unique Carousel ID.</td>
<td>Name entered for Carousel ID is longer than 22 characters.</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Re-type name of 22 characters or less for Carousel ID.</td>
</tr>
<tr>
<td>Cannot save the Worklist because it has errors.</td>
<td>The worklist has entry errors.</td>
<td>1 Click OK.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Fix the worklist errors.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Save the worklist.</td>
</tr>
</tbody>
</table>
### Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
</table>
| Couldn't print the worklist to the selected printing device. | Selected printer is not available  
Not connected to selected printer | 1 Click OK.  
2 Select a new printer.  
3 Restart the print.  
Alternatively,  
1 Click OK.  
2 Connect the printer.  
3 Restart the print. |

The worklist you are about to run contains Lot IDs and/or TruCOUNT bead information that is different from the information defined in the software.

If you choose to proceed the information in the worklist will be replaced with the information from the software. The following information will be replaced:

<list of items to replace>

Would you like to proceed?

You are attempting to run a previously prepped worklist after modifying the Lot ID information.

Click Yes to replace specified items and proceed with run.  
The Lot ID information will be updated based on the latest information entered in the Lot ID dialog.  
Click No to cancel the run.
## Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
</table>
| Step loss on the x, y, or z-axis | Probe crash due to unexpected object on worktable restricting range of motion, or probe not calibrated correctly | 1 Click **OK**.  
2 Perform the procedure in Recovering from an X, Y, or Z-Axis Error on page 180.  
3 If the instrument continues to experience step loss, or if you are unable to calibrate the probe to the reference point, call BD Biosciences. |
| Instrument on unstable surface | | 1 Click **OK**.  
2 Place the instrument on a stable, level surface.  
3 Perform the procedure in Recovering from an X, Y, or Z-Axis Error on page 180. |
| No liquid detected. | No reagent or not enough reagent in vial | 1 Press **Pause**.  
2 Add a reagent vial with sufficient volume to the reagent rack. |
| Reagents are cold | | 1 Press **Pause**.  
2 Allow reagents to come to room temperature. |
| The WASTE tank is full. Please empty the WASTE tank, and make sure to add 500 µL of Antifoam A Concentrate. | Waste tank is full | 1 Empty the waste tank and add 1 L bleach and 500 µL Antifoam A Concentrate.  
2 Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run. |
### Software Error Messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause</th>
<th>Possible Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication Error</td>
<td>The instrument did not respond within the expected time.</td>
<td>1 Exit SPA software normally and power down the instrument.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 Make sure all racks are correctly seated and nothing is in the way.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 Check cables between computer and instrument.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 Discard all secondary tubes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 Power up the instrument and restart SPA software.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 Calibrate the probe reference point. See Calibrating the Probe Reference Point on page 134. While the probe is exposed, inspect it for damage.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7 Restart the worklist from the beginning.</td>
</tr>
</tbody>
</table>

| Failure to Reinitialize the arm              | The instrument has failed to reinitialize the arm.            | 1 Click OK.                                                                     |
|                                              |                                                               | 2 Perform the procedure in Recovering from a Reinitialization Error on page 181. |

| The instrument detected a {error type} error on the {device type} device. | Various causes | See the solution for Timeout Error above.                                       |
Following either a probe change or a replacement of the 1-mL syringe, you must verify that the SPA III is delivering an accurate and precise volume of fluid.

You can use your own laboratory method for qualifying pipettors. If you do not have a validated method, use Method to Ensure Dispense Accuracy on page 218.
Method to Ensure Dispense Accuracy

Materials

- Calibrated pan balance (approved for weighing materials from 0.5 g to 5 g, with a resolution of 0.0001 g)
- Four capped primary tubes (any size) containing 3 mL of DI water (must be supported tubes)

  For a list of supported tubes, see Required Equipment on page 32.
- 20 uncapped 12 x 75-mm secondary test tubes
- Forceps or tongs
- Beaker

Pan Balance Setup

⚠️ Do not touch tubes with your hands as this could change the tube’s weight. Handle with forceps only.

1 Using forceps, place the secondary tubes in a carousel rack in positions 1–20 and label them according to their position.

2 Place a beaker on the balance.

3 Tare the balance.

4 Using forceps, place empty secondary tube #1 in the beaker and record the weight.

5 Remove the tube, place it back in the carousel rack, and tare the balance again.

6 Repeat steps 3 to 5 until all 20 tubes have been weighed.
Use the following worksheet to record your weights.

<table>
<thead>
<tr>
<th>Secondary Tube No.</th>
<th>Weight (g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Empty</td>
<td>Full</td>
<td>Fluid = (Full – Empty)</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
</table>

Chapter 9: Accuracy and Precision  219
Fluid Dispense

1. Turn on the instrument, and then the computer.
2. Start the software and log in.
3. Fill all bulk fluid tanks.
4. Empty the waste tank, then add 1 L of bleach and 500 µL Sigma Antifoam A Concentrate to the waste tank.
5. Create four sample entries in a new worklist.

Specify the Sample ID and Primary Tube Type. Use the default Primary Rack Position 1, 2, 3, and 4.

For information on creating a worklist, see Preparing Primary Tubes on page 101, Panel Selection on page 109, and Carousel Rack Setup on page 109.

6. Select A & P Dispense panel from the Panel Name menu for each primary tube.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Case Number</th>
<th>Primary Tube Type</th>
<th>Primary Rack Position</th>
<th>Panel Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP 1</td>
<td></td>
<td>BD Vacutainer</td>
<td>1</td>
<td>A &amp; P Dispense</td>
</tr>
<tr>
<td>AP 2</td>
<td></td>
<td>BD Vacutainer</td>
<td>2</td>
<td>A &amp; P Dispense</td>
</tr>
<tr>
<td>AP 3</td>
<td></td>
<td>BD Vacutainer</td>
<td>3</td>
<td>A &amp; P Dispense</td>
</tr>
<tr>
<td>AP 4</td>
<td></td>
<td>BD Vacutainer</td>
<td>4</td>
<td>A &amp; P Dispense</td>
</tr>
</tbody>
</table>

7. Place the primary tubes containing 3 mL of DI water in primary tube rack positions 1–4.
8 Close the safety cover and click Run.

9 Save the worklist when prompted.

The probe dispenses the water to all 20 tubes.

10 Immediately weigh each of the 20 tubes, individually.

   IMPORTANT: Use forceps to handle the secondary tubes.

   a Place a beaker on the balance.

   b Tare the balance before each tube.

   c Using forceps, place each secondary tube individually in the beaker and record the weight.

11 Click Close to end the process.
Analysis

Determine the weight of the fluid dispensed for each tube by subtracting the weight of the empty tube from that of the same tube containing fluid.

Use the formulas provided to calculate the mean (X), the standard deviation (SD), and percent coefficient of variation (%CV) of the fluid weight.

Calculate the mean:

\[
\bar{X} = \frac{\sum X}{N} \quad \text{where } X \text{ represents each data point (fluid weight) and } N \text{ represents the sample size (number of tubes).}
\]

Convert the fluid weight mean to fluid volume:

\[
\text{fluid volume (µL)} = \frac{\text{fluid weight (g)}}{\text{fluid density (g/mL)}} \times 1000 \text{ (µL/mL)}
\]

Example:

\[
X = 0.0495 \text{ g; density of water} = 1 \text{ g/mL}
\]

\[
\text{fluid volume} = \frac{0.0495 \text{ g}}{1 \text{ g/mL}} \times 1000 \text{ µL/mL}
\]

\[
\text{fluid volume} = 49.5 \text{ µL}
\]

Calculate the standard deviation:

\[
\text{SD} = \sqrt{\frac{\sum X^2 - \left(\frac{\sum X}{N}\right)^2}{N - 1}}
\]

Use the following formula to calculate the %CV:

\[
\%CV = \frac{\text{SD}}{\bar{X}} \times 100
\]
You can use a spreadsheet to do these calculations.

Configure the spreadsheet like the following.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Empty</td>
<td>Full</td>
<td>Fluid</td>
<td>(Full – Empty)</td>
</tr>
<tr>
<td>6</td>
<td>Tube No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>=D7-C7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>=D8-C8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>=D9-C9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>=D10-C10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>=D11-C11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>=D12-C12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>7</td>
<td>=D13-C13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>=D14-C14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>=D15-C15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>10</td>
<td>=D16-C16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>11</td>
<td>=D17-C17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>12</td>
<td>=D18-C18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>13</td>
<td>=D19-C19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>14</td>
<td>=D20-C20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>15</td>
<td>=D21-C21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>16</td>
<td>=D22-C22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>17</td>
<td>=D23-C23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>18</td>
<td>=D24-C24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>19</td>
<td>=D25-C25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>20</td>
<td>=D26-C26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>Mean =AVERAGE(E7:E26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>SD =STDEV(E7:E26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>%CV =E25/E27*100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do not multiply the standard deviation or %CV by 1,000 or your results will be incorrect.
Acceptance Criteria

The 20 replicates must pass the following specifications.

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Type</td>
<td>Water</td>
</tr>
<tr>
<td>Volume Dispensed</td>
<td>50 µL</td>
</tr>
<tr>
<td>n</td>
<td>20</td>
</tr>
<tr>
<td>Mean Value</td>
<td>48.5 to 51.5</td>
</tr>
<tr>
<td>%CV</td>
<td>≤3% CV</td>
</tr>
</tbody>
</table>

For values that fall outside these specifications, see Troubleshooting on page 189.
Technical Specifications

This chapter covers the following topics:

- Instrument on page 226
- Performance on page 233
- Fluidics Tower on page 239
- Barcode Reader on page 239
Instrument

Dimensions
- Height: 76.2 cm (30 in.)
- Width: 63.5 cm (25 in.)
- Width (with fluidics tower): 88 cm (35 in.)
- Depth: 66 cm (26 in.)

Required workspace dimensions
- Height (with safety cover open): 94 cm (37 in.)
- Unit designed to fit lab bench: 76 cm (30 in.) depth

Weight
- 64 kg (≤140 lb)—instrument only, excluding computer
- 100 kg (≤220 lb)—including packaging

Power requirements
- 100–240 VAC (50–60 Hz)

Power consumption
- 150 W

Fuses (2)
- Type T 5.0 Amp–250 V

Environment

Storage temperature
- –20°C to 50°C

Operating temperature
- 18°C to 28°C (64°F to 82°F)

Operating relative humidity
- 20–80% (non-condensing)

Noise level
- ≤60 dBA (idle mode)
- ≤75 dBA (run mode)

Facilities
- No special room requirements
Sample Loading

Primary tube rack compatibility
- 13-mm rack
- 16-mm rack

Reagent rack
Standard

Carousel rack compatibility
Sample-prep ready BD FACS™ Loader carousel racks, numbers 1–16

Tube Compatibility

Carousel rack
Accommodates up to 40 uncapped 12 x 75-mm tubes
- Corning Falcon® polystyrene test tubes
- BD Trucount tubes

Primary tube rack
Accommodates up to 40 tubes in the following sizes:
BD Vacutainer
- 13 x 75 mm
- 13 x 100 mm
- 16 x 75 mm
- 16 x 100 mm

Use BD Hemogard™ closures or standard rubber stoppers.

Sarstedt S-Monovette
- 2.7 mL, 11 x 66 mm
- 2.6 mL, 13 x 65 mm
- 3.4 mL, 13 x 65 mm
- 4.9 mL, 13 x 90 mm
- 4.0 mL, 15 x 75 mm
- 5.5 mL, 15 x 75 mm
Reagent rack  Accommodates up to
  • 24 standard BD Biosciences reagent vials (22.9 mm in
diameter), uncapped
  • Three BD Trucount Control vials (15.9 mm in diameter),
   uncapped
  • One 60-mL BD FACSClean vial (38.9 mm in diameter),
   uncapped
Tube adapters  Allows use of 11-mm and 13-mm tube in a primary tube rack
Labels  ≤5 mils (127 microns) thick

**Pre-Programmed Dispense Volumes**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>50 µL</td>
</tr>
<tr>
<td>Reagent</td>
<td>20 µL</td>
</tr>
<tr>
<td>Lyse</td>
<td>450 µL</td>
</tr>
<tr>
<td>BD Trucount Control</td>
<td>50 µL</td>
</tr>
</tbody>
</table>

**Pre-Programmed Incubation Times**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyse</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Reagent</td>
<td>15 minutes</td>
</tr>
</tbody>
</table>
Optimizing Reagent Use

Use the information in this section to optimize reagent use when processing samples with the SPA III.

Reagent Volume Definitions

<table>
<thead>
<tr>
<th>Volume Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditioning volume</td>
<td>Necessary for coating the inside of the probe to ensure that the conditions for first sample volume resembles the others. This volume is dispensed back into the reagent vial.</td>
</tr>
<tr>
<td>Excess volume</td>
<td>Ensures that the last volume dispensed equals all others and does not contain sheath. This volume is discarded to waste.</td>
</tr>
<tr>
<td>Dispensed volume</td>
<td>Defined by BD in pre-defined panels or by the user in Other Panels.</td>
</tr>
<tr>
<td>Minimum volume</td>
<td>Conditioning Volume + Excess Volume + Dispensed Volume</td>
</tr>
</tbody>
</table>

**NOTE** Conditioning and excess volumes cannot be changed.

Using Multi-Dispense to Optimize Reagent Use

The SPA III is designed as a multi-dispense system. The probe is capable of aspirating enough reagent, in MultiTest mode, to dispense into six secondary tubes at a time. To optimize the amount of reagent used, it is a good practice to dispense reagents into multiples of six secondary tubes.

The following tables and examples demonstrate how using the multi-dispense feature results in more efficient use of reagents.
For reagent volumes of 20 µL:

<table>
<thead>
<tr>
<th>Secondary Tubes</th>
<th>Excess Volume</th>
<th>Conditioning Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 tube</td>
<td>4 µL</td>
<td>7 µL</td>
</tr>
<tr>
<td>2–6 tubes</td>
<td>7 µL</td>
<td>7 µL</td>
</tr>
</tbody>
</table>

Example 1

The minimum volume of reagent required for one secondary tube (single dispense) of a BD-defined panel equals:

7 µL conditioning volume + 4 µL excess volume + 20 µL dispensed volume = 31 µL minimum volume (7 µL returned to vial)

Example 2

The minimum volume of reagent required for two secondary tubes (multi-dispense) of a BD-defined panel equals:

7 µL conditioning volume + 7 µL excess volume + 2 (20 µL dispensed volume) = 54 µL minimum volume (7 µL returned to vial)

Conclusion

Based on these examples, if the two secondary tubes used in Example 2 had been run independently, then 62 µL of reagent would have been required as the minimum volume. By utilizing the multi-dispense function of the SPA III, 8 µL of reagent was saved.
Minimum Sample Volumes

This section provides the recommended minimum sample volumes required, depending on tube type and size, to ensure accurate sample dispensing with the SPA III using the primary tube rack. Note that the recommended volumes take into account required conditioning, excess, and dead volume, along with the sample volume to be dispensed.

Sample Volume Definitions

<table>
<thead>
<tr>
<th>Volume Type</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conditioning volume</td>
<td>Necessary for coating the inside of the probe to ensure that the conditions for first sample volume resemble the others. This volume is discarded to waste.</td>
</tr>
<tr>
<td>Excess volume</td>
<td>Ensures that the last volume dispensed equals all others and does not contain sheath. This volume is discarded to waste.</td>
</tr>
<tr>
<td>Dispensed volume</td>
<td>Defined by BD in pre-defined panels or by the user in Other Panels.</td>
</tr>
<tr>
<td>Dead volume</td>
<td>Tube-dependent volume that cannot be aspirated or dispensed.</td>
</tr>
<tr>
<td>Minimum volume</td>
<td>Conditioning volume + Excess volume + Dispensed volume + Dead volume</td>
</tr>
</tbody>
</table>

NOTE  Conditioning, excess, and dead volumes cannot be changed.
## Minimum Sample Volumes

### Table 10-1 For BD Vacutainer Tubes

<table>
<thead>
<tr>
<th>Sample Volume (µL)</th>
<th>Number of Secondary Tubes</th>
<th>Minimum Sample Volume (µL)</th>
<th>13 x 75-mm BD Vacutainer</th>
<th>16 x 100-mm BD Vacutainer</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1–2</td>
<td></td>
<td>400</td>
<td>700</td>
</tr>
<tr>
<td>50</td>
<td>3–4</td>
<td></td>
<td>500</td>
<td>700</td>
</tr>
<tr>
<td>50</td>
<td>5–6</td>
<td></td>
<td>700</td>
<td>900</td>
</tr>
<tr>
<td>50</td>
<td>7–8</td>
<td></td>
<td>900</td>
<td>1,000</td>
</tr>
</tbody>
</table>

### Table 10-2 For Sarstedt Tubes

<table>
<thead>
<tr>
<th>Sample Volume (µL)</th>
<th>Number of Secondary Tubes</th>
<th>Minimum Sample Volume (µL)</th>
<th>Sarstedt 2.6 mL</th>
<th>Sarstedt 2.7 mL</th>
<th>Sarstedt 3.4 mL</th>
<th>Sarstedt 4.0 mL</th>
<th>Sarstedt 4.9 mL</th>
<th>Sarstedt 5.5 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>1–2</td>
<td></td>
<td>600</td>
<td>400</td>
<td>600</td>
<td>700</td>
<td>600</td>
<td>700</td>
</tr>
<tr>
<td>50</td>
<td>3–4</td>
<td></td>
<td>800</td>
<td>600</td>
<td>800</td>
<td>900</td>
<td>800</td>
<td>900</td>
</tr>
<tr>
<td>50</td>
<td>5–6</td>
<td></td>
<td>1,000</td>
<td>700</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>50</td>
<td>7–8</td>
<td></td>
<td>1,100</td>
<td>800</td>
<td>1,100</td>
<td>1,200</td>
<td>1,100</td>
<td>1,200</td>
</tr>
</tbody>
</table>
Performance

BD Multitest/BD Tritest/Absolute Count Panels

| Accuracy (dispense) | Blood: 50 µL ±3% volume  
|                     | Reagent: 20 µL ±7% volume  
|                     | Lyse: 450 µL ±3% volume  |
| Precision (dispense) | Blood: 50-µL aliquots, CV ≤3%  
|                     | Reagent: 20-µL aliquots, CV ≤5%  
|                     | Lyse: 450-µL aliquots, CV ≤3%  |

In BD studies on a SPA II, an inadequate sample dispense occurred in 0.5% of 720 samplings. BD identified the following causes:

- Recapped tubes
- Clogged probe (from rubber particles from cap piercing or clotted blood)
### Table 10-3 Lymphocyte Subset Absolute Counts

<table>
<thead>
<tr>
<th>Site (n)</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
</tr>
<tr>
<td>Lymphocyte Subset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>0.5</td>
<td>(–1.3, 2.3)</td>
<td>0.9</td>
<td>(–0.1, 1.9)</td>
<td>1.2</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>–0.1</td>
<td>(–1.8, 1.7)</td>
<td>0.8</td>
<td>(–0.04, 1.7)</td>
<td>–0.01</td>
</tr>
<tr>
<td>Total CD3+</td>
<td>0.2</td>
<td>(–1.5, 1.9)</td>
<td>0.7</td>
<td>(0.04, 1.4)</td>
<td>–0.02</td>
</tr>
<tr>
<td>CD3–CD19+</td>
<td>0.7</td>
<td>(–1.4, 2.8)</td>
<td>3.0</td>
<td>(1.3, 4.7)</td>
<td>1.7</td>
</tr>
<tr>
<td>CD3–(CD16 + CD56)+</td>
<td>4.1</td>
<td>(1.9, 6.3)</td>
<td>3.8</td>
<td>(2, 5.6)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

### Table 10-4 Lymphocyte Subset Percent Positives

<table>
<thead>
<tr>
<th>Site (n)</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
</tr>
<tr>
<td>Lymphocyte Subset</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3+CD4+</td>
<td>0.02</td>
<td>(–0.1, 0.2)</td>
<td>±3</td>
<td>–0.5</td>
<td>±10%</td>
</tr>
<tr>
<td>CD3+CD8+</td>
<td>–0.5</td>
<td>(–0.9, –0.1)</td>
<td>±10%</td>
<td>–0.5</td>
<td>±10%</td>
</tr>
<tr>
<td>Total CD3+</td>
<td>–0.2</td>
<td>(–0.5, –0.0002)</td>
<td>±10%</td>
<td>–0.6</td>
<td>±10%</td>
</tr>
<tr>
<td>CD3–CD19+</td>
<td>–0.04</td>
<td>(–0.2, 0.1)</td>
<td>±3</td>
<td>0.1</td>
<td>(0.1, 0.2)</td>
</tr>
<tr>
<td>CD3–(CD16 + CD56)+</td>
<td>0.4</td>
<td>(0.2, 0.5)</td>
<td>±3</td>
<td>0.1</td>
<td>(0, 0.3)</td>
</tr>
</tbody>
</table>

* When the mean of the manual method is greater than 30%, the criteria is a relative difference of 10%. When the mean of the manual method is less than or equal to 30%, the criteria is the absolute difference of 3.
NOTE  Data were acquired using BD FACSCanto II.

**BD Multitest IMK Method Comparison**

**Table 10-5** Lymphocyte Subset Absolute Counts

<table>
<thead>
<tr>
<th>Tube Type (n)</th>
<th>Tube Type 1</th>
<th>Tube Type 2</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte Subset</td>
<td>Mean Bias</td>
<td>95% CI</td>
<td>Mean Bias</td>
</tr>
<tr>
<td>CD3⁺CD4⁺</td>
<td>-3.3 (-4.3, -2.3)</td>
<td>0.9 (-0.6, 2.4)</td>
<td>±10%</td>
</tr>
<tr>
<td>CD3⁺CD8⁺</td>
<td>-3.2 (-4.1, -2.2)</td>
<td>0.0 (-1.4, 1.4)</td>
<td>±10%</td>
</tr>
<tr>
<td>Total CD3⁺</td>
<td>-3.5 (-4.2, -2.7)</td>
<td>-1.0 (-1.9, -0.2)</td>
<td>±10%</td>
</tr>
<tr>
<td>CD3⁻CD19⁺</td>
<td>-3.7 (-5.6, -1.8)</td>
<td>-1.5 (-3.3, 0.3)</td>
<td>±20%</td>
</tr>
<tr>
<td>CD3⁺(CD16⁺+56⁺)</td>
<td>-1.5 (-2.9, -0.1)</td>
<td>1.8 (-0.6, 4.1)</td>
<td>±20%</td>
</tr>
</tbody>
</table>

**Table 10-6** Lymphocyte Subset Percent Positives

<table>
<thead>
<tr>
<th>Tube Type (n)</th>
<th>Tube Type 1</th>
<th>Tube Type 2</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte Subset</td>
<td>Mean Bias</td>
<td>95% CI</td>
<td>Mean Bias</td>
</tr>
<tr>
<td>CD3⁺CD4⁺</td>
<td>-0.03 (-0.2, 0.1)</td>
<td>0.1 (-0.1, 0.3)</td>
<td>±3</td>
</tr>
<tr>
<td>CD3⁺CD8⁺</td>
<td>0.3 (0, 0.7)</td>
<td>-0.5 (-1.1, 0.1)</td>
<td>±10%</td>
</tr>
<tr>
<td>Total CD3⁺</td>
<td>0.2 (0, 0.4)</td>
<td>-0.3 (-0.5, -0.1)</td>
<td>±10%</td>
</tr>
<tr>
<td>CD3⁻CD19⁺</td>
<td>-0.1 (-0.2, 0)</td>
<td>0 (-0.1, 0.1)</td>
<td>±3</td>
</tr>
<tr>
<td>CD3⁺(CD16⁺+56⁺)</td>
<td>0.1 (0, 0.2)</td>
<td>0.2 (0, 0.4)</td>
<td>±3</td>
</tr>
</tbody>
</table>

a. When the mean of the manual method is greater than 30%, the criteria is a relative difference of 10%. When the mean of the manual method is less than or equal to 30%, the criteria is the absolute difference of 3.

NOTE  Data were acquired using BD FACSCalibur.
**BD Multitest Assay Precision**

**Table 10-7** Precision of Lymphocyte Subset Absolute Counts

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>CDLa Upper CV</th>
<th>CDN b Upper CV</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within Run</td>
<td>Within Device</td>
<td>Within Run</td>
</tr>
<tr>
<td>CD3*CD4+</td>
<td>8.2</td>
<td>8.1</td>
<td>5.0</td>
</tr>
<tr>
<td>CD3*CD8+</td>
<td>5.1</td>
<td>5.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Total CD3+</td>
<td>5.0</td>
<td>5.1</td>
<td>4.1</td>
</tr>
<tr>
<td>CD3*CD19+</td>
<td>6.4</td>
<td>6.3</td>
<td>6.3</td>
</tr>
<tr>
<td>CD3*(CD16 + CD56)*</td>
<td>8.1</td>
<td>8.1</td>
<td>6.9</td>
</tr>
</tbody>
</table>

*a.* CD-Chex Low  
*b.* CD-Chex Normal

**Table 10-8** Precision of Lymphocyte Subset Percent Positives

<table>
<thead>
<tr>
<th>Lymphocyte Subset</th>
<th>CDLa Upper SD</th>
<th>CDN b Upper SD</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within Run</td>
<td>Within Device</td>
<td>Within Run</td>
</tr>
<tr>
<td>CD3*CD4+</td>
<td>0.78</td>
<td>0.74</td>
<td>1.08</td>
</tr>
<tr>
<td>CD3*CD8+</td>
<td>1.38</td>
<td>1.51</td>
<td>1.06</td>
</tr>
<tr>
<td>Total CD3+</td>
<td>1.24</td>
<td>1.38</td>
<td>1.03</td>
</tr>
<tr>
<td>CD3*CD19+</td>
<td>0.95</td>
<td>1.01</td>
<td>0.64</td>
</tr>
<tr>
<td>CD3*(CD16 + CD56)*</td>
<td>1.11</td>
<td>1.11</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*a.* CD-Chex Low  
*b.* CD-Chex Normal

**NOTE**  Data were acquired over 21 days, using three instruments.
Pipetting Accuracy and Precision

**Table 10-9** Pipetting Accuracy

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Acceptance criteria</th>
<th>Volume dispensed (µL)</th>
<th>Pass/Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SPA III A</td>
<td>SPA III B</td>
</tr>
<tr>
<td>Blood (single dispense) 50 µL</td>
<td>20</td>
<td>50 µL ±3%</td>
<td>50.42</td>
<td>50.55</td>
</tr>
<tr>
<td>Blood (multi-dispense) 50 µL</td>
<td>20</td>
<td>50 µL ±3%</td>
<td>49.98</td>
<td>49.87</td>
</tr>
<tr>
<td>Reagent 20 µL</td>
<td>20</td>
<td>20 µL ±7%</td>
<td>19.98</td>
<td>20.39</td>
</tr>
</tbody>
</table>

**Table 10-10** Pipetting Precision

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Acceptance criteria</th>
<th>90% CI of CV (upper limit)</th>
<th>Pass/Fail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SPA III A</td>
<td>SPA III B</td>
</tr>
<tr>
<td>Blood (single dispense) 50 µL</td>
<td>20</td>
<td>≤3% CV</td>
<td>1.58</td>
<td>0.4</td>
</tr>
<tr>
<td>Blood (multi-dispense) 50 µL</td>
<td>20</td>
<td>≤3% CV</td>
<td>0.74</td>
<td>0.75</td>
</tr>
<tr>
<td>Reagent 20 µL</td>
<td>20</td>
<td>≤5% CV</td>
<td>1.27</td>
<td>1.58</td>
</tr>
</tbody>
</table>

**Sample and Reagent Carryover**

Sample and reagent carryover were each tested with two equations:

- **Equation 1**: Carryover = \(|(\text{First Low} – \text{Third Low})/(\text{Third High} – \text{Third Low})| \times 100

• **Equation 2:** Carryover = \(\left(\frac{\text{First Low} - \text{Third Low}}{\text{Third High} - \text{First Low}}\right) \times 100\)

  - From CLSI (formerly known as NCCLS) Guideline H52-A Approved Guideline for Fetal Red Cell Detection.

### Sample Carryover

Sample carryover was tested using three donors (each tested twice) on three SPA III instruments.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Manual Preparation</th>
<th>SPA III</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>minimum</td>
<td>maximum</td>
<td>minimum</td>
</tr>
<tr>
<td>1</td>
<td>-0.47</td>
<td>0.08</td>
<td>-0.12</td>
</tr>
<tr>
<td>2</td>
<td>-0.47</td>
<td>0.08</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

### Reagent Carryover

Sample carryover was tested using three donors (each tested twice) on three SPA III instruments.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Manual Preparation</th>
<th>SPA III</th>
<th>Acceptance criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>minimum</td>
<td>maximum</td>
<td>minimum</td>
</tr>
<tr>
<td>1</td>
<td>-0.011</td>
<td>0.001</td>
<td>-0.021</td>
</tr>
<tr>
<td>2</td>
<td>-0.011</td>
<td>0.001</td>
<td>-0.021</td>
</tr>
</tbody>
</table>
### Fluidics Tower

**Dimensions**
- Height: 25.4 cm (10 in.)
- Width: 24.1 cm (9.5 in.)
- Depth: 29.2 cm (11.5 in.)

**Tank Capacities**

<table>
<thead>
<tr>
<th>Fluid Type</th>
<th>Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI Water</td>
<td>1.0 L</td>
</tr>
<tr>
<td>Lyse</td>
<td>1.0 L</td>
</tr>
<tr>
<td>DI Water 2</td>
<td>1.0 L</td>
</tr>
<tr>
<td>Waste</td>
<td>10.0 L</td>
</tr>
</tbody>
</table>

### Barcode Reader

**Compatibility**
- ISBT 128 standard barcode labels
Symbols
%CV, calculating for fluid weight 222

A
aborting a run 125
Account Name field 72
accuracy and precision
  acceptance criteria 224
  specifications 233
  spreadsheet setup 223
  troubleshooting 190, 191
  when to perform 217
Acrobat Reader 50
adapters, tube 24, 228
alcohol, for cleaning 33
assistance, technical 14

B
barcode scanner
  connecting 36
  described 29
  specifications 239
  using 62, 102
BD FACS lysing solution 33
BD FACS SPA Login dialog 56
BD FACSClean solution 33, 128
BD FACSFlow solution 33
BD Trucount controls 25, 88, 92
  lot IDs 116
BD Trucount tubes 33
BD Vacutainer tubes 32
biohazardous waste 45, 46
bleach 33
blood transfer device, for venting
tubes 107
bulk fluids
  refilling 38
  supported 33
  table of 37
tanks
  capacities 239
  troubleshooting 196
buttons
  Edit Tube 90
  Minimize, Maximize, Close 59
  Shutdown 77

C
cable
  fluid level sensor 34
  insulation block 99
  primary tube rack cage, shown 169
  RS232 34, 50
calculating %CV for fluid weight 222
calibration peg 136
cap holder, reagent 26
caps, bulk fluid tanks,
  troubleshooting 196
Carousel Position field 62, 110
carousel rack  
compatibility  
   Loader 27  
tubes 227  
discontinued 27  
empty positions 109  
ID 59  
inserting 111  
loading 110  
map 61  
removing 122  
troubleshooting 196  
unique ID 59  

case number 103  
Change Password dialog 70  
changing password 70  
cleaning  
   daily 128  
   instrument surface 147  
   solutions 33  
   weekly 131  
components, system 20  
computer, troubleshooting 196  
controls  
   BD Trucount 25, 88, 92  
   beads 120  

corporations  
   keyboard 13  
   text 13  
cord, power 34, 36  
Corning Falcon tubes 33  
counter, probe 59, 154, 166  
cover, safety 21  
creating user accounts 72  
cubitainer 28  

D  
daily  
   cleaning 128  

shutdowm 128  
startup 96  
decommissioning  
   daily 128  
   surface 147  
deleting sample from worklist 66  

deposits see mineral deposits  
DI water tanks 37  
dialogs  
   BD FACS SPA Login 56  
   Change Password 70  
   Left Arm Configuration 137  
   Open Worklist 100  
   Print 66  
   Reagent Rack Editor 89, 92  
   Replace Probe 158  
   Sample Prep Assistant shutdown 77  
   Setup Reference Point 134, 139, 165  
   Syringe Replacement Procedure 167  
   User Administration 71  
dispense  
   accuracy 218, 233  
   method to ensure 218  
   precision 233  
   volumes, preprogrammed 228  
door, instrument 21  

draining waste cap trap 46  

E  
emptying waste tank 45  
ending a run 122  
error messages  
   file 76  
   storage of 76  
ethanol 33  
extended non-use, instrument 148  
   startup following 151
F

FACS lysing solution 33
FACSClean solution 33, 128
FACSFlow solution 33
Falcon tubes 33
fields
  Account Name 72
  Carousel Position 62, 110
  Institution 72
  Messages 60
  Names 72
  Panel Name 62, 109
  Password 72
  Primary Rack Position 62, 105
  Primary Tube Type 62, 104
  Sample ID 103
  Secondary Tubes Carousel Position and Name 59, 110
  Status 60, 120
filter
  cap, waste tank 45, 47
  waste in-line 29
fittings
  pump 98
  syringe 98
flow
  container 41
    level sensor 41
  tank 37
fluid level sensor cable 34
Fluid Tanks Status indicators 60, 69
fluidics
system
  priming 42
  syringe pumps 22
  troubleshooting 197
  valves 22
tower 28
dimensions 239
  waste connection 35
  waste port 99
fluids
  bulk 33, 37
  level indicator 44
  refilling 38
fuses
  replacing 175
  type 226
H
holder, reagent cap 26
Home command 179
I
incubate offline
  description 59
  procedure 118
incubation time
  during sample prep 120
  lyse 88
  preprogrammed 228
  reagent 88
  troubleshooting 124, 194
indicator lights
  barcode scanner 29
  instrument, troubleshooting 198
indicators, tank levels 44, 69
in-line filter assembly, waste 29
installation
  software 52
  tanks 38
Instrument field 72
instrument
  aborting 125
  cleaning 147
  components 21

Index 243
initializing 179
log files 76
pausing 124
performance 233
power switch 34
priming 42
requirements 32
software requirements 50
specifications 226, 239
storage 148
supplies 32
troubleshooting 190–203
worktable 21
insulation block
cable 99
correct alignment 164
described 22
removing 159

K
keyboard
conventions 13
troubleshooting 204
wedge 50

L
label thickness 27, 228
laser scanner 29
leaks
at syringe seals 195
at valves 195
at waste port 196
Left Arm Configuration dialog 137
loading carousel rack 201
log files 76
log in
procedure 57
troubleshooting 204
login files, instrument 76
lot IDs
BD Trucount controls 116
reagents 116
LWA information 121
lyse
amount 88
incubation time 88
solution 33
tank 37
troubleshooting 191

M
mean, calculating for fluid weight 222
menu bar 61
menus
Panel Name 109
Primary Tube Type 104
Reagent Rack ID 58, 112, 113
Task Level 72
Messages field 60
mineral deposits
at syringe seals 195
at valves 195
at waste port 196
mixing
control beads 120
post-lyse 88
post-reagent 88
troubleshooting 196
monitor, troubleshooting 196, 198
mouse, troubleshooting 204
Multi-Check controls 16, 106
multiple panels, with one primary
sample 63
multi-rack reagent racks, creating 91

N
Names field 72
Index 245

O
offline incubation 118
on/off
light 22
switch 34
Open Worklist dialog 100
operating system 50

P
Panel
   Name menu 109
Panel Name field 62
panels
   preprogrammed 80
   splitting 109
password
   changing 70
   login 57
   troubleshooting 204
Password field 72
pausing instrument during run 124
peg, calibration 136
post-lyse mix 88
post-reagent mix 88
power
cord 34, 36
interruption
during a run 119
long-term 187
recoverying after 186
switch 34
precision, dispense 233
Prep Worklist
   main menu 58
   window 58
preprogrammed
dispense volumes 228
incubation time 228
panels 80
reagent racks 81
Primary Rack Position field 62, 105
primary sample, used with multiple
   panels 63
Primary Tube
   Rack Map 61
   Type field 62
   Type menu 104
primary tube rack
cage
   closing and locking 108
   described 22
   during syringe replacement 169
   loading 107
   locking into position 174
   troubleshooting 201
   unlocking 101
   troubleshooting 196, 202
tube compatibility 227
primary tubes
   maximum number of pierces 88
   re-capping 106
   supported 32
   venting 106
priming the instrument 42
Print dialog 66
printing
   log, txt, xml files 76
   worklist 66
probe
   accuracy and precision after
      changing 217
   attaching new 162
counter 59, 154, 166
described 22
homing 179
replacement 154, 156
signs of wear 99
pumps
inspecting 98
syringe 22, 98

Q
quitting the software 77

R
rack
carousel 110, 122, 227
primary tube 227
reagent 25, 114, 228
ReadMe file 53
Reagent
Rack Editor 89, 92
Rack ID choices 86
Rack ID menu 58, 112
Rack Map 61
reagent
cap holder 26
incubation time 88
lot IDs 116
preprogrammed, list of 84
rack 25
preprogrammed 81
removal 114
specifications 228
Reagent List 90
reagent use
optimizing 229
volume definitions 229
with multi-dispense 229
reference point extension 136
removing carousel rack 122
Replace Probe dialog 158
replacing
probe 154, 156
waste cap trap 46
waste tank cap 45, 47
results, troubleshooting 191
RS232 cable 34, 50

S
safety cover 21
sample
aborting run 125
amount dispensed 88
deleting from worklist 66
ending a run 122
rocker 22
traceability of 64
types 16, 101
Sample ID
field 103
invalid 63
required 64, 102
unique 102
Sample Prep Assistant shutdown
dialog 77
sample volumes
definitions 231
minimum 232
Sarstedt tubes 32
scanner, barcode 29
screen savers 50
seals, syringe 98
secondary tubes
label thickness 27
per panel 88
supported 33
Secondary Tubes Carousel Position and
Name field 59, 110
serial cable 34, 50
serial number 72
Setup
Reference Point dialog 134, 139, 165
report 139
sheath fluid 28
shutdown
  daily 128
  extended non-use 148
Shutdown button 77
Sigma Antifoam A Concentrate 33
Skip Initial Sample Mix checkbox 59
software
  compatibility 50
  files, log 76
  installation 52
  log in 57
  quitting 77
  requirements 50
  shortcut 53
  startup 56
  troubleshooting 189–??, 204–214
  uninstalling 55
  worktable 60
specifications 226, 239
standard deviation, calculating for fluid weight 222
startup
  daily 96
  software 56
Status field 60, 120
storage, instrument 148
switch, power 34
syringe pumps
  described 22
  inspecting 98
  troubleshooting 195
Syringe Replacement Procedure
  dialog 167
syringes
  installed for upgrades 50
  removing 171
  replacing 172, 217
system
  components 20
  requirements 32

T
  tanks
    capacities 37, 239
    compatible fluids 37
    connector colors 42
    installation 38
    label colors 40, 42
    level
      indicators 44, 69
      sensors 28
  Task Level menu 72
  technical assistance 14
  text conventions 13
tower, fluidics 28
traceability of samples 64
troubleshooting 189–215
  accuracy and precision 190, 191
  bulk fluids tanks 196
carousel rack 196
  computer 196
  fluidics system 197
  incubation time 124
  indicator lights 198
  instrument 190–203
  keyboard 204
  logging in 204
  lyse 191
  mixing 196
  monitor 196, 198
  mouse 204
  password 204
  primary tube rack 196, 202
    cage 201
  probe 198–200
  results 191
  software 204–214
    error messages 205
  syringe pumps 195
  Trucount controls 88
tubes 194, 201
valves 195
wash station 203
waste
  port 196
tank 196
worklist 63
Trucount controls 25, 88
Trucount tubes see BD Trucount tubes
tubes
  adapters 24, 228
  BD Vacutainer 32, 227
  racks 23
  Sarstedt 32
  secondary see secondary tubes
  troubleshooting 194, 201

U
uninstalling software 55
User
  Administration dialog 71
  Name 57
user accounts
  creating 72
  deleting 73

V
Vacutainer tubes 32
valves
  described 22
  shown 98, 173
  troubleshooting 195

W
wash station
  described 22
  troubleshooting 203
waste
  biohazardous 45, 46
cap trap
  draining 46
  replacing 46
in-line filter
  assembly 29
  removing 142
  replacing 141
  service 141
port
  location 99
  troubleshooting 196
tank 28, 41
  adding bleach 37
  capacity 37
  emptying 45, 148
  filter cap 45, 47
  level sensor 41
  replacing cap 45, 47
  troubleshooting 196
  wedge, keyboard 50
window
  Prep Worklist 58
  Reagent List 90
  worklist
    defined 58
    deleting information from 66
    entering information into 62
    last line of 64
    messages 44
    opening saved 100
    printing 66
    resizing fields 65
    troubleshooting 63
  worktable
    components 60
    defined 59
    instrument 21

X
XML files 68
Z
Z-rack
  described 22
  probe replacement and 159