♡ BD FACS™ Sample **Prep Assistant III**

Instructions For Use

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FCC information

WARNING: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

NOTICE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, can cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense.

Shielded cables must be used with this unit to ensure compliance with the Class A FCC limits.

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Notice

BD delivers software and workstations that are intended for running the instruments supplied by BD. 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, it is the buyer/ user's responsibility to install and maintain up-to-date virus protection software. BD does not make any warranty with respect to the workstation remaining virus-free after installation. BD is not liable for any claims related to or resulting from buyer/user's failure to install and maintain virus protection.

History

Revision	Date	Change made
646540 Rev. A	6/09	Initial release for BD FACS™ SPA III
23-10990-00 Rev. A	12/09	For CE release of BD FACS™ SPA III: added CE mark, specified location of translated files, and updated information on error recovery.
23-10990-01	5/17	Updated for Benex contact information changes and patent and trademark changes.
23-20276-00	9/2019	Updated new part number, Diva 9.0 and Windows 10 information.
23-20276-01	1/2021	Updated to include beveled probe.

Contents

Preface	xi
Conventions	xii
Technical Assistance	xiii
Translated Documentation	xiii
Chapter 1: Introduction	15
Intended Use	16
Validated Sample Types	16
Overview	17
System Components	18
Instrument	19
Primary Tube Rack	21
Reagent Rack	23
Loader Carousel Rack	26
Fluidics	27
Barcode Scanner	29
Chapter 2: Instrument Requirements, Installation, and Startup	31
Required Equipment	32
Instrument Installation	34
Instrument Startup	36
Bulk Fluid Preparation	37
Fluid and Waste Tank Installation	38
Fluidics Tower	39

Flow Container and Waste Tank	42
Priming the Fluid Lines	43
Maintaining the Fluid and Waste Tanks	45
Before Beginning a Run	45
During a Run	45
Emptying the Waste Tank	46
Chapter 3: Software Installation and Startup	49
Software Requirements	50
Software Installation	51
Uninstalling the Software	54
Starting SPA Software	55
Administrative Login	56
Logging in to the Software	56
Prep Worklist Window	57
Worktable	60
Worklist	61
User Accounts	69
Passwords	69
Managing User Accounts	70
Entering LWA Information	73
Log Files	
Copying Log Files	75
Viewing and Printing Log Files	
Deleting Log Files	75
Shutting Down the Software	76
Chapter 4: Panels and Reagent Racks	77
Pre-Programmed Panels and Reagent Racks	78
BD Tritest TM Panels	79
PD MultitestTM Panels	01

	BD Accuracy and Precision Panel	3
	Pre-Programmed Reagent Racks 8	3
	Tube Protocols	5
	User-Defined Panel Types	5
	Creating a User-Defined Panel	6
	Creating a Panel Category8	7
	Creating a Panel	9
	Adding Secondary Tubes9	1
	Changing Incubation Times	3
	Specifying Tube Protocols9	4
	Printing the Panel Definition9	9
	Creating a Custom Reagent Rack	0
	Creating Multi-Rack Reagent Racks	4
	Adding Controls to a Custom Reagent Rack	5
		6
	Combining and Modifying BD Pre-programmed Panels	O
	Combining and Modifying BD Pre-programmed Panels	
Ch	, , ,	6
Ch	Combining Panels	6 3
Ch	Combining Panels	6 3 4
Ch	Combining Panels	6 3 4 8
Ch	Combining Panels	6 3 4 8
Ch	Combining Panels	6 4 8 9
Ch	Combining Panels	6 4 8 9 8
Ch	Combining Panels	6 3 4 8 9 8 9
Ch	Combining Panels	6 4 8 8 9 8 9
Ch	Combining Panels	6 4 8 8 9 8 9 0 2
Ch	Combining Panels	6 3 4 8 8 9 8 9 0 2 5
Ch	Combining Panels	6 3 4 8 8 9 8 9 0 2 5 7
Ch	Combining Panels	6 3 4 8 8 9 8 9 0 2 5 7 9

Ending a Run	144
Pausing and Resuming a Run	146
Aborting a Run	147
Chapter 6: Shutdown and Maintenance	149
Daily Shutdown	150
Required Materials	150
Performing Daily Cleaning	150
Shutting Down the Instrument	152
Scheduled Maintenance	153
Weekly Cleaning	153
Calibrating the Probe Reference Point	156
Monthly Servicing of Waste In-Line Filters	163
Monthly Replacement of the Waste Tank Cap	167
Periodic Maintenance	168
Internal and External Surface Cleaning and Decontamination	169
Extended Periods of Non-Use	170
Startup Following Extended Non-Use	173
Replacing the Probe	175
Replacing a Syringe	190
Fuse Replacement	199
Special Conditions	203
Homing the Probe	203
Initializing the Instrument	203
Recovering from an X, Y, or Z-Axis Error	204
Recovering from a Reinitialization Error	205
Manually Replacing the Probe	207
Recovering from a Power Interruption	210

Chapter 7: Troubleshooting	213
Appendix A: Accuracy and Precision	243
Method to Ensure Dispense Accuracy	244
Appendix B: Technical Specifications	251
Instrument	252
Environment	252
Sample Loading	253
Tube Compatibility	253
Pre-Programmed Dispense Volumes	254
Pre-Programmed Incubation Times	254
User-Definable Ranges	255
Optimizing Reagent Use	256
Minimum Sample Volumes	258
Performance	260
BD Multitest/BD Tritest/Absolute Count Panels	260
Other Panels	261
Fluidics Tower	261
Barcode Reader	261
Appendix C: Supplies and Replacement Parts	263
Contact Information	264
Accessories	264
Accessory Options	266
Replacement Parts	267
Consumables	270
Index	273

Preface

This Instructions For Use describes how to operate the BD FACSTM Sample Prep Assistant III (SPA III) instrument using BD FACSTM Sample Prep Assistant software (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 FACS*TM *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 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 1 lists symbols used either in this Instructions For Use or on safety labels to alert you to a potential hazard. Text and keyboard conventions are shown in Table 2.

Table 1 Hazard symbols^a

CAUTION: hazard or unsafe practice that could result in maddata loss, minor or severe injury, or death	erial damage,
Electrical danger	
Biological risk	

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 2 Text and keyboard conventions

Convention	Use
☑ Tip	Highlights features or hints that can save time and prevent difficulties.
NOTE	Provides information that supplements the topic material.
Italics	Italics are used to highlight book titles and new or unfamiliar terms on their first appearance in the text.
>	The arrow indicates a menu choice. For example, "select File > Print" means to select Print from the File menu.
Ctrl+X	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 <i>P</i> .

Technical Assistance

For technical questions or assistance in solving a problem:

- Read the section of the Instructions For Use specific to the operation you are performing.
- See Troubleshooting on page 213.

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

When contacting BD, have the following information available:

- Product name, part number, and serial number
- Any error messages
- Details of recent instrument performance

For instrument support from within the US and Canada, call (877) 232-8995.

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

Translated Documentation

You can access PDF files of translated versions of this Instructions For Use and the ReadMe file at this location:

C:\Program Files\BD FACS SPA Software\Instructions For Use Translations



1

Introduction

This chapter covers the following topics:

- Intended Use on page 16
- Overview on page 17
- System Components on page 18

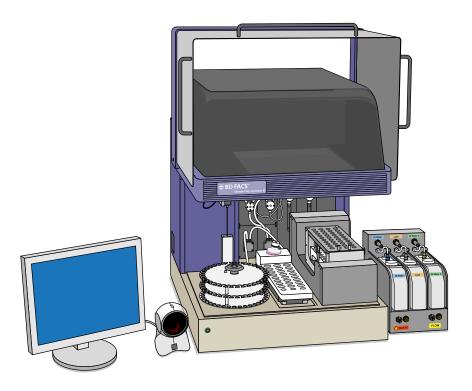
Intended Use

The intended use for the BD FACSTM Sample Prep Assistant III (SPA III) is to prepare human whole blood for flow cytometry.

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-CheckTM control
- BD Multi-CheckTM CD4 low control



Overview

The SPA III instrument and workstation:

- 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.
- 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, or you can create user-defined panels.

System Components

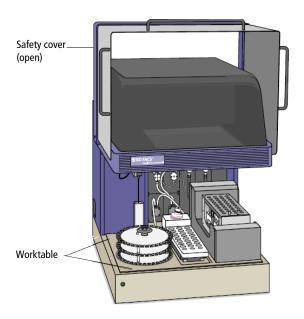
The SPA III consists of a sample preparation unit connected to an external computer workstation via an RS232 cable. A barcode reader enhances system functionality. The instrument ships with an accessory kit (see Accessories on page 264 for a list of accessory kit contents.)

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

Component	Section
Instrument	See Instrument on page 19.
Primary tube rack (with tube adapters and open tube port)	See Primary Tube Rack on page 21.
Reagent rack	See Reagent Rack on page 23.
Carousel rack	See Loader Carousel Rack on page 26.
Fluidics tower	See Fluidics on page 27.
Barcode reader	See Barcode Scanner on page 29.
PC workstation	See your PC workstation documentation.

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 1-1 SPA III instrument components

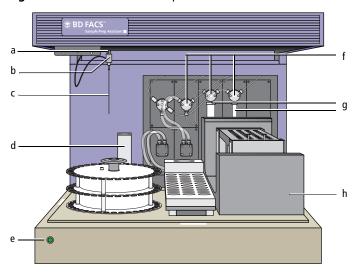
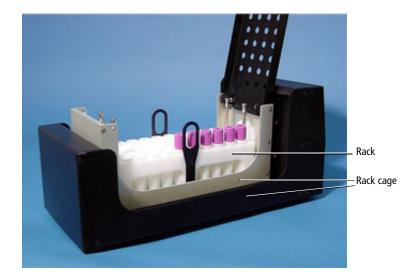


Table 1-1 SPA III instrument components

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

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 22).
 - 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 1-2 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 (22.9 mm in diameter), three BD TrucountTM control vials (15.9 mm in diameter), and one large bottle or vial (38.9 mm in diameter) for a total of 28 uncapped vials.



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

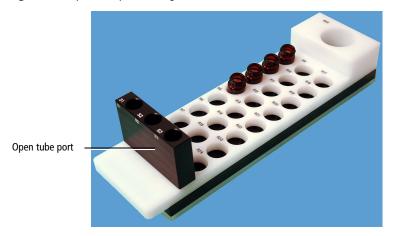
The R25 position on the reagent rack holds a 60-mL vial, which is included in the accessory kit. The 60-mL vial in R25 can be used for reagents during normal operations or for bleach during cleaning.



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.

Open Tube Port

Figure 1-3 Open tube port on reagent rack



An open tube port, which fits into the BD TrucountTM control vial locations on the reagent rack, allows you to add up to three uncapped, 15-mL conical (primary sample) tubes. The open tube port also accommodates these tubes:

- 11-mm diameter tubes with black adapters
- 13-mm diameter tubes with white adapters
- 15 or 16- mm diameter tubes, no adapters required

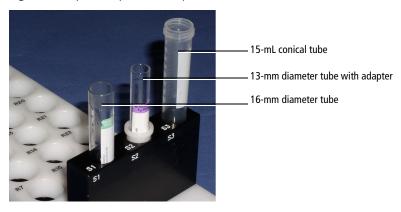
See Figure 1-4 on page 25.



Do not use Sarstedt tubes in the open tube port on the reagent rack due to possible damage to the probe.

Open primary tubes can be used when you create a custom panel and select Other Panel as the panel type. See User-Defined Panel Types on page 85, and Creating a Panel on page 89.

Figure 1-4 Open tube port with sample tubes



Reagent Cap Holder

Figure 1-5 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 1-6) holds up to 40 uncapped 12 x 75-mm polystyrene secondary tubes and is compatible with the BD FACS™ 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 1-6 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 FACSFlowTM cubitainer.

Figure 1-7 Tanks in 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 46.

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 replaced each time the instrument probe is replaced. See Monthly Servicing of Waste In-Line Filters on page 163 and Replacing the Probe on page 175.



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.



been successfully scanned.

LEDs and audio signals indicate when the scanner is on and when a barcode has

Status	Description	Condition
	Steady blue light, no white light	Ready to scan.
	Steady blue light and single white flash accompanied by a beep	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.
	Steady white light, steady blue light	Communicating with the PC.

Status	Description	Condition
	Alternating blue and white lights	Scanner is in configuration mode. A buzzing tone indicates that an invalid barcode has been scanned in this mode.
	No white or blue light	No power to scanner.

For more information about the barcode scanner, including how to program it for other standards, see the manufacturer's documentation.

Instrument Requirements, Installation, and Startup

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 45

Required Equipment

The tubes in Table 2-1 have been validated for use on the SPA III. See Validated Sample Types on page 16.

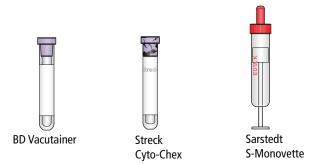


Do not use Terumo $^{\circledR}$ blood collection tubes (primary tubes). They are not compatible with the SPA III.

Table 2-1 Supported primary tubes

Tube Type	Tube Size	Primary Tube Rack
BD Vacutainer®	13 x 75 mm	13 mm or 16 mm with white adapters
	13 x 100 mm	
	16 x 75 mm	- 16 mm
	16 x 100 mm	16 111111
Sarstedt S-Monovette®	2.7 mL, 11 x 66 mm	16 mm with black adapters
	2.6 mL, 13 x 65 mm	
	3.4 mL, 13 x 65 mm	13 mm or 16 mm with white adapters
	4.9 mL, 13 x 90 mm	
	4.0 mL, 15 x 75 mm	- 16 mm
	5.5 mL, 15 x 75 mm	16 111111
Streck Cyto-Chex [®] BCT TM	5.0 mL, 13 x 75 mm	13 mm or 16 mm with white adapters

Figure 2-1 Examples of supported tube types



The primary tube racks support up to 40 capped tubes. Different tube types can be used on the same rack.

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

Table 2-2 Required materials

Material	Details
Secondary tube types (up to 40 uncapped secondary tubes)	Falcon® polystyrene tubes, 12 x 75-mm
	BD Trucount Tubes, 12 x 75-mm
Bulk fluids	BD FACS™ lysing solution
	BD FACSFlow™ solution
	DI water
Other fluids	BD FACSClean™ solution or 10% bleach
	70% ethanol
	Sigma Antifoam A Concentrate

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

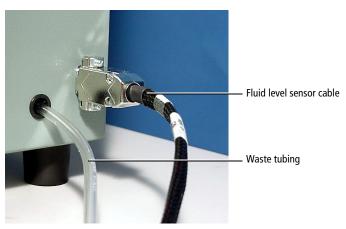
Figure 2-2 Power switch on instrument left side panel



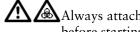
Leave sufficient room on the side of the instrument so you can easily access the power switch, which is located directly above the power cord.

2 Connect the fluid level sensor cable to the instrument right side panel.



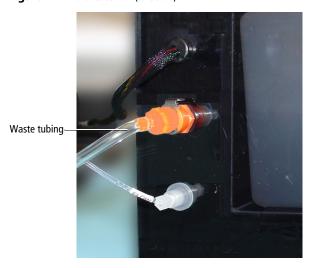


3 Attach the waste tubing to the left side of the fluidics tower (Figure 2-4).



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

Figure 2-4 Fluidics tower (left side)



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 2-2 on page 34).

- **5** Turn on the computer and monitor.
- **6** Start the software as described in Starting SPA Software on page 55.

Bulk Fluid Preparation

Table 2-3 lists the bulk fluids required for the SPA III, including compatible fluids and capacities.

Table 2-3 Bulk fluids required for the SPA III

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

Fluid and Waste Tank Installation



For accurate results, fill fluid tanks with **only** the indicated bulk fluid (see Table 2-3 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.





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.

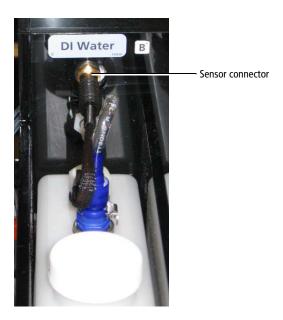
The following table lists the tanks and their corresponding label and quick-release connector colors.

Tank	Label Color	Connector Color
DI water (B)	Blue	Blue
Lyse (C)	Tan	Black
DI water 2 (D)	Green	Green

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



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.

Tank	Label Color	Connector Color
Flow	Yellow	White
Waste	Orange	Orange

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.



MYou 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 45.

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 FACSFlowTM solution
- LYSE: BD FACSTM 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 46 for more information.

Figure 2-6 Tank indicators



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 FACSFlowTM solution is low. It does not resume processing until you service the flow or waste tanks.

NOTE When you change the BD FACSFlowTM 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.



M 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 (BD part no. 338347), contact your local BD Biosciences representative.

To empty the waste tank:

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

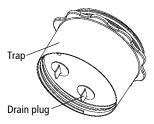


The waste tank can become pressurized when the SPA III is running. Always disconnect the tank from the fluidics tower before you empty it. Wait at least 1 minute for pressure to dissipate before you remove the waste cap or sensor.

- 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.



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



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

- Add approximately 1 L of undiluted household bleach to the empty tank. 5
- 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 (BD part no. 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 51
- Starting SPA Software on page 55
- Prep Worklist Window on page 57
- User Accounts on page 69
- Entering LWA Information on page 73
- Log Files on page 75
- Shutting Down the Software on page 76

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)
- 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® 10 operating system
- Adobe[®] Acrobat[®] Reader[®] DC v2019.012.20035 or later to use electronic documentation provided on the SPA software USB

Software Compatibility Issues

You must quit all other applications before running SPA software.

Do not change the screen saver settings or the power mode settings. They could interfere with the performance of the software.

Software Installation

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

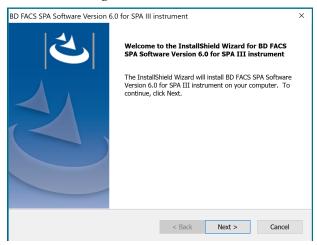
Before you begin, close any open programs.

To install the software:

1 Insert the SPA software installation USB version 6.0 or later for Windows® 10.

The installer should start up automatically. If it doesn't, use Windows Explorer to view the USB contents, and double-click the **Setup.exe** icon.

2 Click **Next** to begin the installation.



- **3** Enter the license key located on the USB.
- 4 Confirm that the SPA III system has the correct syringes installed as shown in the next dialog.



- 5 Click Yes to begin the installation.
- **6** 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.

7 Click Next.

A confirmation dialog opens.

8 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.

9 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 USB. 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 3-1 for the various instructions about the worktable database file.

Table 3-1

Situation	Action
Upgrading SPA software to a new version.	No action. The installer keeps the existing worktable file.
Re-installing SPA software because the previous version is not functioning.	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.
Re-installing SPA software on a new computer or a computer with a new hard drive.	Load the existing worktable file if a backup copy is available. If not, contact BD Customer Support.

Uninstalling the Software

- 1 In the search box on the taskbar, type Control Panel, and then select Control Panel.
- 2 Select Uninstall a program from the Programs.
- 3 Select $BD FACS^{TM} SPA Software Version 6.0 for SPA III instrument from the list, right-click and then select$ **Uninstall**.

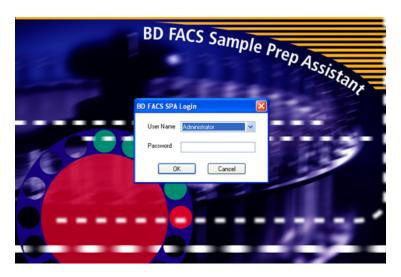
Starting SPA Software

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 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 69.

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 3-2 provide a brief orientation to the Prep Worklist window and its features.

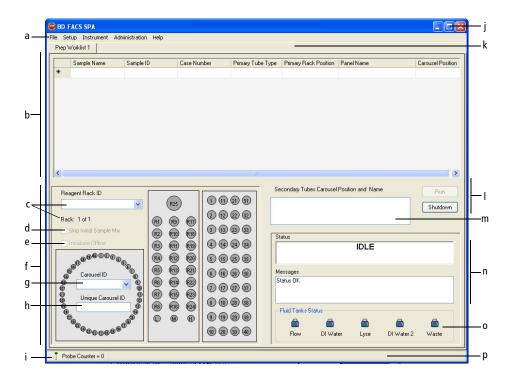


Table 3-2 Prep Worklist window

Component	Function
a Main menu	File, Setup, Instrument, Administration, and Help menus.
b Worklist	Enter primary tube identification, tube type, position, panel, and secondary tube positions here (for a full description, see Worklist on page 61).

 Table 3-2
 Prep Worklist window (continued)

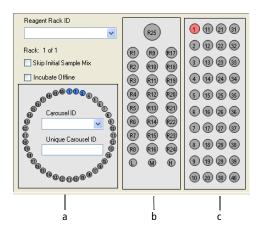
Co	mponent	Function
С	Reagent Rack ID Multi-rack number	Select the reagent rack for a panel from the menu. The current rack is indicated below the menu.
d	Skip Initial Sample Mix checkbox	Skips the initial 5-minute mix of samples in the primary tube rack. The checkbox is cleared by default.
е	Incubate Offline checkbox	Enables the option of performing the final incubation offline. The checkbox is cleared by default.
f	Worktable	Graphic representation of the placement of all primary and secondary tubes and reagent vials.
g	Carousel ID	Type an ID (serial number) for the carousel. Use a number from 1 through 16.
h	Unique Carousel ID	Scan the carousel barcode or type the unique carousel ID in this field. Limited to a maximum of 22 characters.
i	Probe Counter	Tracks the total number of cap piercings.
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.
I	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.

 Table 3-2
 Prep Worklist window (continued)

Co	mponent	Function
m	Secondary Tubes Carousel Position and Name field	Shows the secondary tubes associated with each panel for a selected Sample ID.
n	Status and Messages fields	Display current instrument processing and diagnostic messages.
0	Fluid Tanks Status	Indicates when the waste and fluid tanks need filling or emptying (See Fluid Tanks Status on page 68.)
р	Status bar	Displays the percentage of completion when processes are running.

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.



Co	mponent	Function		
а	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		

Co	omponent	Function
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.
С	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.

	Sample Name	Sample ID	Case Num	Primary Tube Type	Primary Rack	Panel Name	Carousel Position
		Trucount control		BD Vacutainer	1	3/8/45/4 Control	1 - 3
		Multi-Check		BD Vacutainer	2	4 Color TBNK + TruC	4-5
		Multi-Check CD4 Lo		BD Vacutainer	3	4 Color TBNK + TruC	6 - 7
)		Patient #1		BD Vacutainer	4	4 Color TBNK + TruC	8-9
*							

Worklists can be:

- Saved and re-used. See Initializing a Worklist on page 118 and Ending a Run on page 144
- Printed. See Printing a Worklist on page 65
- Exported. See Using SPA III Worklists with Other BD Software on page 67

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 119.

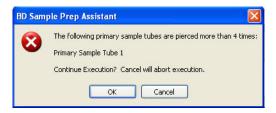
As you enter information into the worklist, symbols are displayed to indicate the entry status:

Symbol	Meanin	g							
•	Indicat	Indicates which line is selected.							
.0	Indicat	tes the line be	eing edited.						
*	Indicat	tes the next li	ne for which	there are no cui	rent entries				
•	Place y	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 <i>Invalid Sample ID</i> indicates that the ID is missing.							
		Sample Name	Sample ID	Case Number	Primary Tube Type				
		test1 4_15	1111111	C682	BD Vacutainer				
		test2 4_15	2222222	C683	BD Vacutainer				
		test3 4_15 3333333 C684 BD Vacutainer							
		test4 4_15							
	•		Invalid Sample	ID	BD Vacutainer				

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.



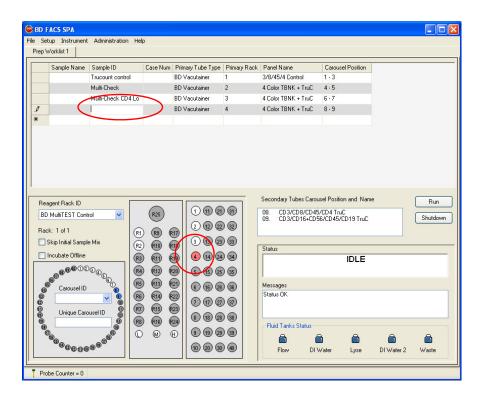
Entering the Last Line of a Worklist



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.



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 3-1.
- **2** Press the Delete key.

Figure 3-1 Deleting a line of information

	Sample Name	Sample ID	Case Num	Primary Tube Type	Primary Rack
		Trucount control		BD Vacutainer	1
(N		Multi-Check		BD Vacutainer	2
M		Multi-Check CD4 Lo		BD Vacutainer	3
		Patient #1		BD Vacutainer	4
*					

Printing a Worklist

To print the currently open worklist:

- 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.

Us Init Inc Pre Pre Fin	Process Date: Worklist: User: Initial Mix: Incubate Offline: Prep Start Time: Prep End Time: Final Incubation End Time: CD3/CD16+56/CD4		5/18/2009 1:59:05 PM C:\Documents and Settings\ My Documents\SPA III worklists\ 0905181229_SPA_Worklist.xml Administrator On Incubate Lyse Offline 5/18/2009 1:59:05 PM 5/18/2009 2:14:57 PM Lot ID 15/CD4/CD19/CD8 12345			Print Date: Status: Carousel #: Carousel ID:	5/18/2009 Complete 1 Training	
	ample ame	Sample ID	Case Number	Primary Tube Type	Primary Rack Position	Panel Name		Carousel Position
		Control Patient 1 Patient 2 Patient 3		BD Vacutainer Sarstedt Streck Cyto-Chex BD Vacutainer	1 2 3 4	6 Color TBNK 6 Color TBNK 6 Color TBNK 6 Color TBNK		1 2 3 4

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:

Icon	Description
No icon	The sample has not yet been processed
√	The sample was successfully processed
×	The sample processing was incomplete because of an error or because the Prep Worklist was aborted

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[®] WorklistManager 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 (MultiSETTM or CELLQuestTM). Additional information required by BD[®] Worklist Manager 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.

Tank	Fluid Level Indicator Trigger	
Waste	80% full (at 8 L)	
Bulk fluids	20% (of full) remaining	

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

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

2 Click Apply.

The account name is added to the current list of user names.

- **3** 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 71 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 FACSTM 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.





- 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 143).

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 date time code.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.

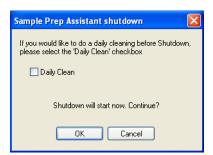
Shutting Down the Software

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 Select the Daily Clean checkbox.
- 3 Click OK.

Panels and Reagent Racks

This chapter includes the following topics:

- Pre-Programmed Panels and Reagent Racks on page 78
- Tube Protocols on page 85
- User-Defined Panel Types on page 85
- Creating a User-Defined Panel on page 86
- Creating a Custom Reagent Rack on page 100
- Creating Multi-Rack Reagent Racks on page 104
- Adding Controls to a Custom Reagent Rack on page 105
- Combining and Modifying BD Pre-programmed Panels on page 106

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 85).

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

- BD TritestTM
- BD MultitestTM
- BD Accuracy and Precision

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.

NOTE You can define custom panels containing any reagent combinations—your own reagents and/or BD reagents. You can also define a custom reagent rack for your new panels. See Creating a User-Defined Panel on page 86 and Creating a Custom Reagent Rack on page 100.

Table 4-1, Table 4-2, and Table 4-3 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 132 for specific reagents associated with each rack.

Table 4-4 lists the pre-programmed reagent racks and their reagents and controls.

BD Tritest™ Panels

Table 4-1 BD Tritest™ panels

Panel	Secondary Tube	Reagents	Reagent Rack ID Choices
MultiReagent Control (BD Trucount TM Tubes)	Tube 1	CD4/CD8/CD3 TruC Ctrl L	BD MultiReagent Control
	Tube 2	CD3/CD19/CD45 TruC Ctrl M	
	Tube 3	CD3/CD16+CD56/CD45 TruC Ctrl H	
4/8/3 Control (BD Trucount TM Tubes)	Tube 1	CD4/CD8/CD3 TruC Ctrl	3-Tube T cell Control
(BD Trucount Tubes)	Tube 2	CD4/CD8/CD3 TruC Ctrl M	
	Tube 3	CD4/CD8/CD3 TruC Ctrl H	
3/4/45 Control (BD Trucount TM Tubes)	Tube 1	CD3/CD4/CD45 TruC Ctrl L	BD Tritest TM Control
(SE Tracount Tusso)	Tube 2	CD3/CD4/CD45 TruC Ctrl M	or 3-Tube T cell Control
	Tube 3	CD3/CD4/CD45 TruC Ctrl H	Control
4-4-8 Control (BD Trucount TM Tubes)	Tube 1	CD3/CD4/CD45 TruC Ctrl L	BD Tritest™ Control
(== = 14000)	Tube 2	CD3/CD4/CD45 TruC Ctrl M	or 3-Tube T cell Control
	Tube 3	CD3/CD8/CD45 TruC Ctrl H	

Table 4-1 BD Tritest™ panels (continued)

Panel	Secondary Tube	Reagents	Reagent Rack ID Choices
8-4-8 Control	Tube 1	CD3/CD8/CD45 TruC Ctrl L	BD Tritest TM
(BD Trucount™ Tubes)	Tube 2	CD3/CD4/CD45 TruC Ctrl M	Control or
	Tube 3	CD3/CD8/CD45 TruC Ctrl H	3-Tube T cell Control
3 Color TBNK	Tube 1	CD3/CD4/CD45	DD T :TM
	Tube 2	CD3/CD8/CD45	BD Tritest TM or
	Tube 3	CD3/CD19/CD45	BD Tritest TM Control
	Tube 4	CD3/CD16+CD56/CD45	
3 Color TBNK TruC	Tube 1	CD3/CD4/CD45	DD ToiseastM
(BD Trucount TM Tubes)	Tube 2	CD3/CD8/CD45	BD Tritest TM or
	Tube 3	CD3/CD19/CD45	BD Tritest TM
	Tube 4	CD3/CD16+CD56/CD45	Control
2 4/8/3 and 3/4/45	Tube 1	CD4/CD8/CD3	
	Tube 2	CD3/CD4/CD45	BD Tritest TM 3-Tube T cell
	Tube 3	CD3/CD8/CD45	
2 3/4/45	Tube 1	CD3/CD4/CD45	
	Tube 2	CD3/CD8/CD45	BD Tritest TM 3-Tube T cell
	Tube 3	CD4/CD8/CD3	

BD Multitest™ Panels

Table 4-2 BD Multitest™ panels

Panel	Secondary Tube	Reagents	Reagent Rack ID Choices
3/8/45/4 Control (BD Trucount™ Tubes)	Tube 1	CD3/CD8/CD45/CD4 TruC Ctrl L	BD Multitest TM Control
	Tube 2	CD3/CD8/CD45/CD4 TruC Ctrl M	
	Tube 3	CD3/CD8/CD45/CD4 TruC Ctrl H	
3/16+56/45/19 Control (BD Trucount TM Tubes)	Tube 1	CD3/CD16+CD56/CD45/ CD19 TruC Ctrl L	BD Multitest TM Control
(SE Tracount Tubes)	Tube 2	CD3/CD16+CD56/CD45/ CD19 TruC Ctrl M	
	Tube 3	CD3/CD16+CD56/CD45/ CD19 TruC Ctrl H	
4 Color TBNK	Tube 1	CD3/CD8/CD45/CD4	BD Multitest TM
	Tube 2	CD3/CD16+CD56/CD45/ CD19	or BD Multitest TM Control
4 Color TBNK + TruC	Tube 1	CD3/CD8/CD45/CD4	BD Multitest TM
(BD Trucount™ Tubes)	Tube 2	CD3/CD16+CD56/CD45/ CD19	or BD Multitest TM Control
6 Color TBNK + TruC	Tube 1	CD3/CD16+CD56/CD45/	BD 6 Color
(BD Trucount TM Tubes)		CD4/CD19/CD8	
6 Color TBNK	Tube 1	CD3/CD16+CD56/CD45/ CD4/CD19/CD8	BD 6 Color
3/8/45/4	Tube 1	CD3/CD8/CD45/CD4	BD Multitest TM or BD Multitest TM Control

 Table 4-2
 BD Multitest™ panels (continued)

Panel	Secondary Tube	Reagents	Reagent Rack ID Choices
3/16+56/45/19	Tube 1	CD3/CD16+CD56/CD45/ CD19	BD Multitest TM or BD Multitest TM Control
3/8/45/4 + TruC	Tube 1	CD3/CD8/CD45/CD4 TruC	BD Multitest TM or BD Multitest TM Control
3/16+56/45/19 + TruC	Tube 1	CD3/CD16+CD56/CD45/ CD19 TruC	BD Multitest TM or BD Multitest TM Control
4-19-4 Control (BD Trucount™ Tubes)	Tube 1	CD3/CD8/CD45/CD4 TruC Ctrl L	BD Multitest TM Control
(BB Tracount Tubes)	Tube 2	CD3/CD16+CD56/CD45/ CD19 TruC Ctrl M	
	Tube 3	CD3/CD8/CD45/CD4 TruC Ctrl H	
19-4-19 (BD Trucount™ Tubes)	Tube 1	CD3/CD16+CD56/CD45/ CD19 TruC Ctrl L	BD Multitest TM Control
(== 11000000 1 0000)	Tube 2	CD3/CD8/CD45/CD4 TruC Ctrl M	
	Tube 3	CD3/CD16+CD56/CD45/ CD19 TruC Ctrl H	

BD Accuracy and Precision Panel

Table 4-3 BD Accuracy and Precision panel

Panel	Secondary Tube	Reagents	Reagent Rack ID Choices
A & P Dispense	Tube 1	N/A	N/A
	Tube 2		
	Tube 3		
	Tube 4		
	Tube 5		

Pre-Programmed Reagent Racks

 Table 4-4
 Pre-programmed reagent racks

Reagent Rack ID	Reagents	Reagent Rack Position
BD Multitest TM	CD3/CD8/CD45/CD4	R1
	CD3/CD16+CD56/CD45/CD19	R2
BD Multitest TM	CD3/CD8/CD45/CD4	R1
Control	CD3/CD16+CD56/CD45/CD19	R2
	BD Trucount Controls	
	• Ctrl L	L
	• Ctrl M	M
	Ctrl H	Н
BD Tritest TM	CD3/CD4/CD45	R1
	CD3/CD8/CD45	R2
	CD3/CD19/CD45	R3
	CD3/CD16+CD56/CD45	R4

Table 4-4 Pre-programmed reagent racks (continued)

Reagent Rack ID	Reagents	Reagent Rack Position
BD Tritest TM Control	CD3/CD4/CD45	R1
	CD3/CD8/CD45	R2
	CD3/CD19/CD45	R3
	CD3/CD16+CD56/CD45	R4
	BD Trucount Controls	
	Ctrl L	L
	• Ctrl M	M
	Ctrl H	Н
BD Tritest TM	CD4/CD8/CD3	R1
3-Tube T cell	CD3/CD4/CD45	R2
	CD3/CD8/CD45	R3
BD Tritest TM	CD4/CD8/CD3	R1
3-Tube T cell Controls	CD3/CD4/CD45	R2
Controls	CD3/CD8/CD45	R3
	BD Trucount Controls	
	Ctrl L	L
	Ctrl M	M
	Ctrl H	Н
BD MultiReagent	CD4/CD8/CD3	R1
Control	CD3/CD19/CD45	R2
	CD3/CD16+CD56/CD45	R3
	BD Trucount Controls	
	Ctrl L	L
	Ctrl M	M
	Ctrl H	Н
BD 6 Color	CD3/CD16+CD56/CD45/CD4/CD19/ CD8	R1

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 preprogrammed panel and the tube protocol settings associated with its secondary tubes.

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

Sample Amount	50 μL
Reagent Amount	20 μL
Post-Reagent Dispense Mix	At end of dispense
Reagent Incubation	15 minutes
Lyse Amount	450 μL
Post-Lyse Dispense Mix	At end of dispense
Lyse Incubation	15 minutes
BD Trucount Controls	50 μL

User-Defined Panel Types

There are two types of user-defined panels:

- BD MultiTEST/BD TriTEST/Absolute Count Panels
- Other Panels

If you create a BD MultiTEST/BD TriTEST/Absolute Count panel, the tube variables (for example, sample and reagent volume) initially have the same values as those in pre-programmed BD MultiTEST/BD TriTEST/Absolute Count Panels. However, you can modify these values.

Other Panels allow you to choose from a wider range of tube variable values. Also, you can use the open tube port. Refer to Table 4-6 on page 94 for a list of tube variables and ranges for each user-defined panel type.

NOTE A worklist cannot contain panels from both BD MultitestTM/BD TritestTM/Absolute Count Panels and Other Panels.

Creating a User-Defined Panel

This section provides instructions for creating a user-defined panel, organized into the following tasks:

- Creating a Panel Category on page 87
- Creating a Panel on page 89
- Adding Secondary Tubes on page 91
- Changing Incubation Times on page 93
- Specifying Tube Protocols on page 94
- Printing the Panel Definition on page 99

Before you use your new panel, you may need to create a custom reagent rack (see Creating a Custom Reagent Rack on page 100).

NOTE To create user-defined panels or custom reagent rack maps, you must have administrator privileges. If your account does not have administrative privileges, ask your lab manager or system administrator to set up such an account for you.

Creating a Panel Category

To create a new panel:

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

The Panel Editor opens (Figure 4-1).

Panel category is at the top level of the Panel Editor browser hierarchy (for example: BD MultitestTM, BD TritestTM, 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.

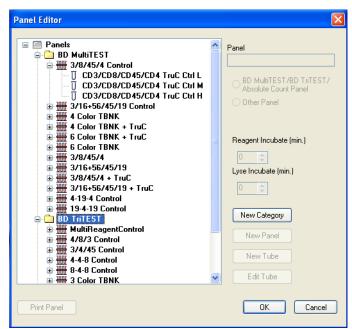


Figure 4-1 Panel Editor

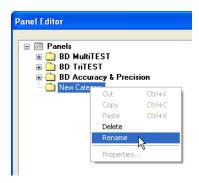
NOTE The icon indicates that the panel and its tube protocols are available for editing. When a panel is being used in a running worklist, it is displayed in the Panel Editor with a icon, to indicate that its information is currently read-only.

Example: H Panel

3 Click the New Category button to create a new panel category.

The default name New Category is assigned.

- **4** Type a name for the new panel category.
 - **a** Right-click the new category and select **Rename**.



b Type the new name.

NOTE Custom category, panel, and tube names are displayed blue. Pre-programmed (non-editable) categories, panels, and tubes are black.

Creating a Panel

Panel Editor

Use the Panel Editor to:

- Create and specify a user-defined (UD) panel (described in this section).
- Copy and edit pre-programmed or existing custom panels (see Combining and Modifying BD Pre-programmed Panels on page 106).

To create a user-defined (UD) panel:

- **1** Use one of the following methods:
 - Select a new panel category, then click the **New Panel** button to create a panel in the selected category.
 - Right-click an existing panel and select **Copy**, then right-click a new panel category and select **Paste**.
 - Drag and drop an existing panel onto a new panel category.

A new panel is displayed in the panel browser. The default name depends on how the panel was created.

Method	Default Name
Click New Panel	Untitled-Panel
Copy/Paste (or Drag/Drop) a BD panel	<bd panel=""> UD</bd>
Copy/Paste (or Drag/Drop) a UD panel	Copy of <ud panel=""></ud>

- **2** Enter a name for the new panel.
 - a Right-click the new panel and select Rename.
 - **b** Type the new name.
- 3 Select a type (either BD MultiTEST/BD TriTEST/Absolute Count Panel, or Other Panel). See User-Defined Panel Types on page 85.



4 Click Close to close the dialog.

NOTE When **Other Panel** is selected, BD Trucount control vial locations (L, M, H) on the Reagent Rack Map switch to S1, S2, S3. These provide open sample tube placements in the reagent rack. Use the open tube port to place up to three samples at the front of the reagent rack.

See Open Tube Port on page 24 for more information.

Adding Secondary Tubes

To add secondary tubes for your new panel:

- 1 Use one or more of the following methods
 - Select the new panel, then click the **New Tube** button.
 - Right-click an existing secondary tube and select Copy. Then right-click the new panel and select Paste.
 - Drag and drop an existing secondary tube onto the new panel.

A new secondary tube is displayed in the panel browser. The default name depends on how the tube was created:

Method	Default Name
Click New Tube	Untitled-Tube
Copy/Paste (or Drag/Drop) a tube	Copy of <ud panel=""></ud>

- 2 Name the new secondary tube:
 - a Right-click the new secondary tube and select Rename.
 - **b** Type the new name.
- **3** Continue to create tubes for your panel.
- 4 Click Close to exit the dialog.

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 $50~\mu L$ sample dispense, a maximum of 16 secondary tubes can be used.

Other Panels

From a single draw, a maximum of $300 \,\mu\text{L}$ of sample can be dispensed for Other Panels. With a four cap-pierce limit, a maximum of $1,200 \,\mu\text{L}$ of sample can be dispensed from capped primary tubes to the secondary tubes of these panels.

If uncapped tubes are used with the open tube port, sample dispense is limited only by the available volume of the primary tube (see Minimum Sample Volumes on page 259).

The maximum number of secondary tubes that can be used in Other Panels with capped primary tubes depends on the amount of sample dispensed per secondary tube. Table 4-5 gives the limits for several choices of a uniform secondary tube sample dispense.

☑ **Tip** The carousel rack holds up to 40 secondary tubes.

Table 4-5 Secondary tube limits for Other Panels with capped primary tubes

Sample Dispense per Secondary Tube	Maximum Number of Secondary Tubes for Other Panels with Capped Primary Tubes
30 μL	40
50 μL	24
100 μL	12

Changing Incubation Times

In a user-defined panel, the default incubation times are 15 minutes (for both lyse and reagent). You can adjust each incubation time between 0 and 60 minutes (5-minute increments).

To change an incubation time:

1 Select the new panel.

The panel name is displayed in the **Panel** field on the right of the Panel Editor.



2 Use the up and down arrows to adjust the Reagent Incubate time.

3 Use the up and down arrows to adjust the Lyse Incubate time.

Specifying Tube Protocols

Tube protocols define which reagents are included, and how much sample, reagent, and lyse are used. Specify tube protocols with the Tube Editor.

Table 4-6 describes the tube protocol values that can be specified with the Tube Editor.

Table 4-6 Tube protocol value ranges

Field	BD Multitest TM /BD Tritest TM / Absolute Count Panels Value Range	Other Panels Value Range
Sample Volume	0 or 50 μL	0–100 μL (5-μL increments)
Reagent	Up to 2 different reagents	Up to 9 different reagents
Reagent Volume	0, 5, 10, 20 μL	0–400 μL (5-μL increments)
Lyse Volume	0–450 μL (25-μL increments)	0–2,000 μL (25-μL increments)
BD Trucount TM Tubes	Yes or No	Yes or No
Controls	(volume preset to 50 µL)	Not available
• BD Trucount TM Control	Yes or No	
• Type	Ctrl L, Ctrl M, Ctrl H	



Accuracy and precision for lyse dispense have been validated for volumes of 0 $\mu L,\,450~\mu L,\,1,000~\mu L,$ and 2,000 μL only. Other lyse dispense volumes must be validated in your laboratory.

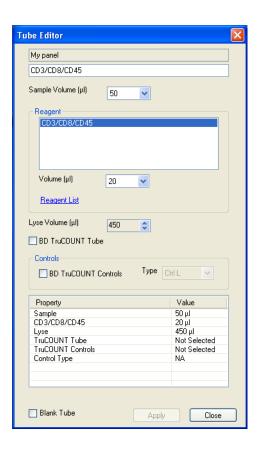
Based on the tube protocol value ranges of Table 4-6:

- The maximum total volume for a BD MultiTEST/BD TriTEST/Absolute Count Panels secondary tube is 590 µL.
- The maximum total volume for an Other Panels secondary tube is 3,000 μL .

Perform this procedure with each tube of the new panel.

To specify tube protocols for a tube:

- 1 Use one of the following methods to open the Tube Editor:
 - Select the tube and click the Edit Tube button.
 - Right-click the tube and select **Properties**.
 - Double-click the tube name.



NOTE In a user-defined panel, you have the option of declaring a tube as a blank tube by selecting the checkbox at the bottom of the Tube Editor. Once that option is selected, the tube will be ignored by the system. (At least one tube in a rack has to be defined. They cannot all be blank.) This allows you to manually prep part of a panel while letting the system perform the remainder of the preparation. Blank tubes have all values reduced to zero when you click **Apply**.

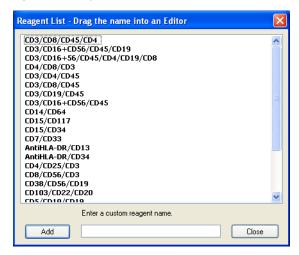
2 Select a sample volume from the menu.

3 Click the Reagent List link.



The Reagent List is displayed. The Reagent List contains all of the preprogrammed reagents and any custom reagents already added. More reagents can be added. The Tube Editor remains open. Any reagent name from the Reagent List can be dragged to the **Reagent** field in the Tube Editor.

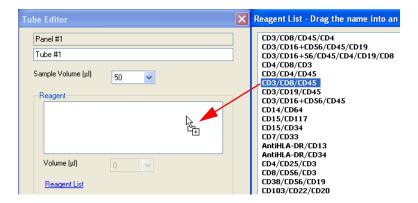
Figure 4-2 Reagent List



4 To add a new reagent to the list, type a reagent name into the field at the bottom of the dialog, then click **Add**.

The new reagent is added to the list.

5 Drag a reagent from the Reagent List to the Reagent field in the Tube Editor.



- **6** Select a volume for the new reagent from the menu.
- 7 As needed, add more reagents to the tube and specify volumes for each.
 - **Tip** When more than one reagent has been added to the tube, select a reagent in the Tube Editor reagent list to set its volume.
- **8** Use the up and down arrows to specify lyse volume for the tube.
- 9 If you are using a BD Trucount Tube, select the BD Trucount™ Tube checkbox.
- **10** If you are using BD Trucount controls, select the **BD Trucount[™] Controls** checkbox, and then select the control Type (Ctrl L, Ctrl M, or Ctrl H) from the menu.

11 Click Apply.

The tube protocol property values are now displayed in the Tube Editor Property/Value table.

Property	Value
Sample	100 ul
CD8 FITC/CD56 PE/ CD3 PerCP-Cy5.5	20 ul
CD45 APC	5 ul
Lyse	2000 ul
TruCOUNT Tube	Not Selected
TruCOUNT Controls	Not Selected
Control Type	NA

- **12** Verify the new tube protocol.
- 13 Click Close to exit the Tube Editor.



Closing the Tube Editor without applying changes results in the loss of all changes to the tube.

The Reagent List also closes.

Printing the Panel Definition

When you finish specifying tube protocols for the tubes of your panel, the panel definition is complete.

- 1 Click **Print** in the Panel Editor for a printed copy of your panel and tube protocol settings.
- 2 Click OK to return to the Prep Worklist window.
- 3 Create a sample entry in the worklist and verify that your new panel is available in the **Panel Name** menu.

Creating a Custom Reagent Rack

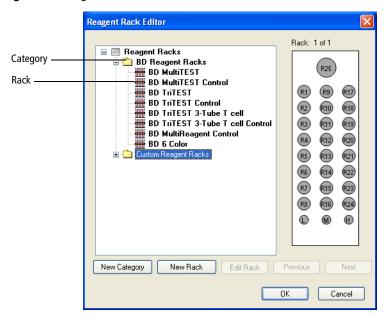
There are two pre-programmed reagent rack categories:

- BD Reagent Racks. All pre-programmed reagent racks are contained in this category.
- Custom Reagent Racks. This category is provided for your custom reagent racks.

To create a custom 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. See Figure 4-3. You can create additional reagent rack categories to organize your custom reagent racks.

Figure 4-3 Reagent Rack Editor



User-defined (custom) reagent racks and categories for user-defined reagent racks are displayed in blue. Pre-programmed reagent racks and the BD Reagent Racks category are black.

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.

Example: - My Custom Rack

NOTE In this section, you use the Reagent Rack Editor to create a new custom reagent rack. The Reagent Rack Editor can also be used to copy and edit preprogrammed or existing custom reagent racks (see Adding Controls to a Custom Reagent Rack on page 105).

To create a custom reagent rack:

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

The Reagent Rack Editor opens (Figure 4-3 on page 100).

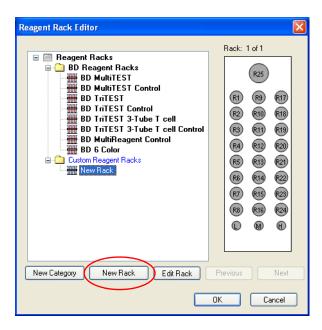
2 Click the New Category button to create a new reagent rack category.

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 custom reagent rack category, and click New Rack.

The default name New Rack is assigned.



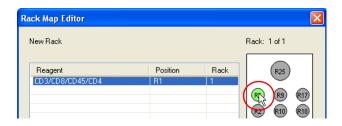
- **5** Type a name for the new reagent rack.
 - a Right-click the new rack and select Rename.
 - **b** Type the new name.
- **6** Use one of the following methods to open the Rack Map Editor:
 - Select the new rack and click the Edit Rack button.
 - Right-click the new rack and select Properties.
 - Double-click the new rack.

7 Click the Reagent List link.

The Reagent List window is displayed. See Figure 4-2 on page 97. The Reagent List contains all of the BD pre-programmed reagents and any custom reagents previously created. New reagents can be added.

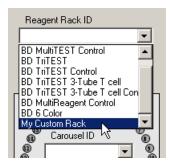
The Rack Map Editor remains open. Any reagent name in the Reagent List can be dragged to the **Reagent/Position** table in the Rack Map Editor.

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



- 10 Continue to add reagents and assign their positions until your custom reagent rack is complete.
- 11 Click **OK** to save the custom 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.

Verify that your new custom 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.
- Add reagents to the added racks as described in Creating a Custom Reagent Rack on page 100.

Adding Controls to a Custom Reagent Rack

To add BD Trucount controls to a custom reagent rack:

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

The Reagent Rack Editor opens.

2 Select a custom reagent rack without BD TrucountTM controls.

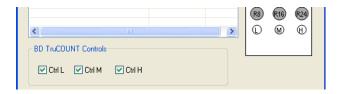
If none exists, create one using the instructions of Creating a Custom Reagent Rack on page 100.



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.

Combining and Modifying BD Pre-programmed Panels

Combining Panels

If your protocol requires that a sample is used with more than one BD pre-programmed panel, the panels must be combined into a single user-defined panel.

This section contains an example of creating a user-defined panel that combines the following BD panels:

- 3/8/45/4 Control
- 4 Color TBNK + TruC

To combine panels:

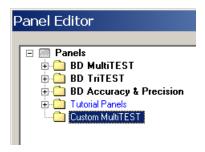
- 1 Start SPA software, if needed.
- 2 Select Setup > Panel Editor.

The Panel Editor opens.

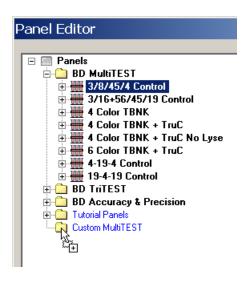
3 Click New Category.

A **New Category** folder is listed below the existing panel category folders in the browser pane.

4 Rename of the new category to *Custom MultiTEST*.



- 5 Click the plus sign next to BD MultiTEST to expand the folder.
- 6 Select the 3/8/45/4 Control panel and drag it onto the folder icon of the Custom MultiTEST category.



A copy of the BD pre-programmed panel opens in the *Custom MultiTEST* category. The name of the copy has UD (user-defined) appended to it.

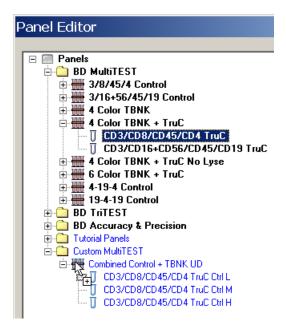


- **7** Rename the 3/8/45/4 Control UD panel to Combined Control + TBNK UD.
- 8 Click the plus sign next to the 4 *Color TBNK* + *TruC* panel.

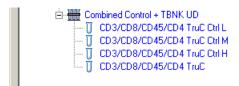
Reagent tubes for that panel are displayed.

- CD3/CD8/CD45/CD4 TruC
- CD3/CD16+CD56/CD45/CD19 TruC

9 Select the *CD3/CD8/CD45/CD4 TruC* tube and drag it onto the panel icon of the *Combined Control* + *TBNK UD* panel.



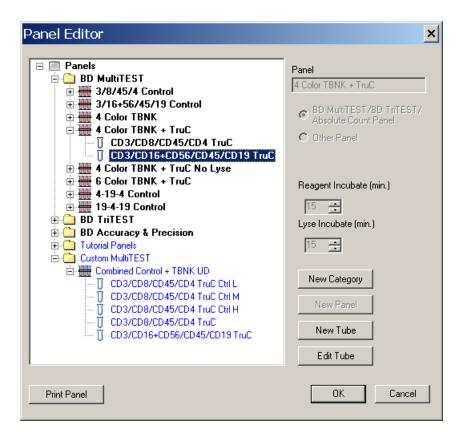
The CD3/CD8/CD45/CD4 TruC tube is added as the fourth tube in the Combined Control + TBNK UD panel.



10 Repeat step 9 with the *CD3/CD16+CD56/CD45/CD19 TruC* tube (to add it to the *Combined Control + TBNK UD* panel).

The completed Combined Control + TBNK UD panel:

- Contains five tubes
- Combines two BD pre-programmed panels into a single user-defined panel.



11 Click **OK** to close the Panel Editor and preserve the *Combined Control* + *TBNK UD* panel.

The BD MultitestTM Control Reagent Rack can be used with the *Combined Control* + $TBNK\ UD$ panel.

Tip When combining or modifying pre-programmed panels, verify that the reagent rack contains all of the required reagents. You may need to create a custom reagent rack.

Modifying a Panel

This section shows an example of modifying a panel by creating a copy of the BD TritestTM 3 Color TBNK panel, and then modifying it to obtain a panel for analyzing only T cells.

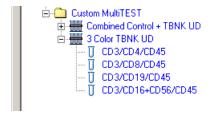
To modify a panel:

1 Select Setup > Panel Editor.

The Panel Editor is opens.

- **2** Click on the plus sign next to **BD TriTEST** to expand the folder.
- 3 Select the *3 Color TBNK* panel, and drag it onto the folder icon of the *Custom MultiTEST* category.

A copy of the BD pre-programmed panel opens in the *Custom MultiTEST* category. The name of the copy has UD (user-defined) appended to it.



- **4** Rename of the copied panel to 3 *Color T cell UD*.
- **5** Right-click *CD3/CD19/CD45* and select **Delete** from the menu.

The CD3/CD19/CD45 tube is removed from the 3 Color T cell UD panel.

6 Delete *CD3/CD16+CD56/CD45* from the 3 *Color T cell UD* panel.

The 3 Color T cell UD panel now consists of two tubes useful for the analysis of T cells.



7 Click **OK** to close the Panel Editor and preserve the 3 Color T cell UD panel.

Sample Preparation

This chapter covers the following topics:

- Daily Startup on page 114
- Setting Up for a Processing Run on page 118
- Offline Incubation on page 139
- Sample Processing on page 140

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.



Mon 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 46.
- 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.
- **13** 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 213 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 5-1, the valve fittings are circled in blue, the pump fittings are circled in green, and the syringe seals are circled in magenta.

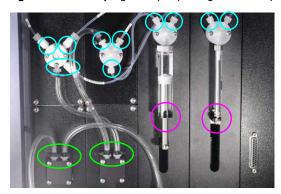
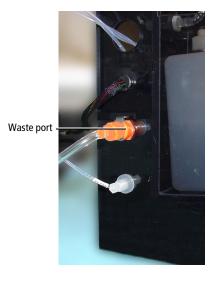


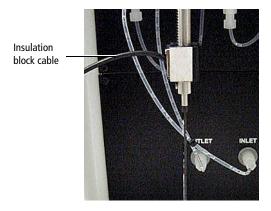
Figure 5-1 Valve, syringe, and pump fittings on fluidics panel

- 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 6-8 on page 177.

Setting Up for a Processing Run

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

- Initializing a Worklist on this page
- Preparing Primary Tubes on page 119
- Sample Entry on page 120
- Panel Selection on page 129
- Carousel Rack Setup on page 130
- Reagent Rack Setup on page 132

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.

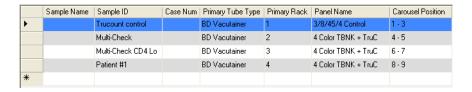
To open a previously saved worklist:

1 Select File > Open Prep Worklist.

The Open Prep Worklist dialog opens.

2 Select a worklist and click **Open**.

The selected worklist opens.



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 and brands, 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.

See Intended Use on page 16, and the appropriate reagent package insert for acceptable sample types.

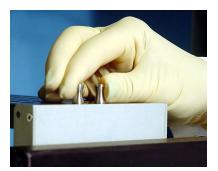
You can mix different tube types in a rack using the tube adapters provided.

If you are using the open tube port only, go to Using the Open Tube Port on page 124.

If you are running BD Multi-Check controls, see Running BD Multi-Check™ Controls on page 126.

Loading the Primary Tube Rack

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 259, to determine the minimum volume required in the capped primary tubes.



1 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.

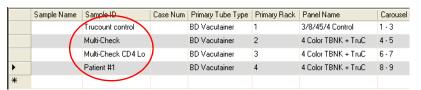
To enter the sample identification using the barcode scanner, place the cursor in the appropriate Sample ID field (see Figure 5-2) and then swipe the label past the scanning window.



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 5-2). The **Sample ID** field accepts up to 22 alphanumeric characters.

Figure 5-2 Sample ID field



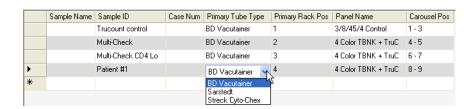
When you first click in a row, default values for the following fields are displayed: Primary Rack Position, Panel Name, and Carousel Position. These fields are editable.

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.

3 (Optional) Type a case number.

Select a tube type from the Primary Tube Type menu. The tube types are BD Vacutainer, Sarstedt, and Streck Cyto-Chex. See Table 2-1 on page 32 for more details on supported tube types.

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 BD Vacutainer or Streck Cyto-Chex tube type when actually using a Sarstedt tube 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).

Figure 5-3 Primary tube rack map



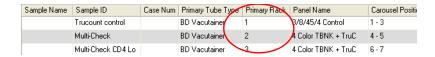
Warning: Verify that the correct primary tube type is selected in the worklist for each tube in the rack. A wrong tube type can cause incorrect results and/or damage to the probe.

You can override the software-assigned primary rack position and load a sample into any available primary rack or open tube port position.

Tip 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.



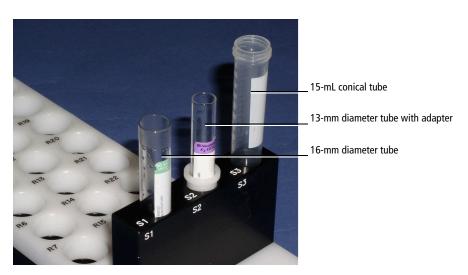
- **b** Ensure that you load the sample tube into the corresponding position.
- **c** Repeat steps 1 through 6 until all required samples have been specified and loaded.
- **☑ Tip** To advance to the next sample entry, click in any field in the next row.

Using the Open Tube Port

If you are using a panel in the Other Panel category that requires open tubes, place the open tube port in the CtrlL, CtrlM, and CtrlH positions of the reagent rack. These open port positions are referred to as S1, S2, and S3 in the panel.



You can place up to three uncapped primary tubes in positions S1, S2, and S3. They must contain at least 150 μL of sample plus the volume of sample to be dispensed. Keep the tubes capped until you are ready to start the run. Mix the tubes and then remove the caps just before you begin the run.



NOTE 15-mL conical tubes are used only with the open tube port. Select BD Vacutainer as the primary tube type.



Do not use Sarstedt tubes in the open tube port on the reagent rack. If you have Sarstedt selected as the primary tube type, and you enter S1, S2, or S3 as the primary rack position, SPA software displays a warning.



Sample Settling with the Open Tube Port



Tubes in the open tube port are not mixed during processing. Sample preparation protocols using the open tube port must be validated by your laboratory.

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-CheckTM controls in the primary sample rack. See Maximum Number of Secondary Tubes Per Panel on page 92. If this occurs, we recommend replacing the BD Multi-CheckTM controls tube cap. Obtain identical caps from BD Vacutainer Plus plastic serum tubes (BD P/N 367812).



Do not transfer the contents of the BD Multi-CheckTM 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.
- Follow the procedure in Venting Primary Sample Tubes on page 127 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 (BD P/N 364880). See Figure 5-4 on page 127.

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.

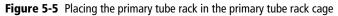
Figure 5-4 BD Vacutainer blood transfer device



Mounting the Primary Tube Rack

To mount the primary tube rack:

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





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



You cannot combine BD Panels and Other Panels in the same worklist.

1 Select a panel from the Panel Name menu.

Figure 5-6 Panel Name menu

	Sample Name	Sample ID	Case Num	Primary Tube Type	Primary Rack Pos	Panel Name	Carousel Pos
		Trucount control		BD Vacutainer	1	3/8/45/4 Control	1 - 3
>		Multi-Check		BD Vacutainer	2	4 Color TBNK + Tr	4-5
		Multi-Check CD4 Lo		BD Vacutainer	3	TOOLOG TOTAL	6 - 7
		Patient #1		BD Vacutainer	4	6 Color TBNK + Tru	8-9
*						3/8/45/4 3/16+56/45/19	
						3/16+36/43/13 3/8/45/4 + TruC 3/16+56/45/19 + 1 MultiReagentContro	



When there are multiple panels of the same type within a worklist, the software will default to the panel with the longest incubation time. For sample preparation process integrity, group panels in a carousel rack to avoid excessive incubation.

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 FACSTM 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 Falcon polystyrene tubes or 12 x 75-mm BD TrucountTM 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 5-7).
- 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 65.
- 5 Load the labeled secondary tubes into the positions indicated by the Carousel Position field (Figure 5-7), and the Secondary Tubes Carousel Position and Name field (Figure 5-8).

Figure 5-7 Secondary tube position in carousel rack

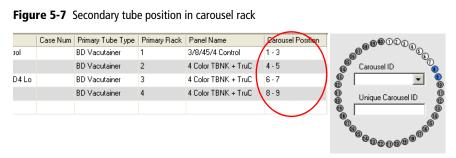


Figure 5-8 Secondary Tubes Carousel Position and Name field



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.



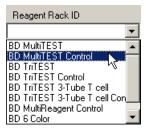
Reagent Rack Setup

To run BD MultitestTM and BD TritestTM panels in the same worklist, you must create a custom reagent rack. See Creating a Custom Reagent Rack on page 100.

If you are using BD TrucountTM controls with this carousel rack, select a reagent rack ID with controls, such as BD MultitestTM control or BD TritestTM 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.



The reagent rack map in the worktable display is updated.







Reagent rack map for Other Panel

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



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.
- If needed, mount the reagent rack on the worktable (see Replacing the Reagent Rack on page 136).

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 Custom Reagent Rack on page 100 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 4-4 on page 83 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 userdefined reagent rack. See Creating a Custom Reagent Rack on page 100.



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.

- Place the reagent rack at the edge of the instrument worktable, aligning it 1 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 (Figure 5-9). The list of reagents in this dialog is the same as the list of reagents found in the Reagent List (see Figure 4-2 on page 97).

Lot IDs Reagents TruCOUNT Bead Reagent Name Lot ID CD3/CD8/CD45/CD4 CD3/CD16+CD56/CD45/CD19 CD3/CD16+56/CD45/CD4/CD19/CD8 CD4/CD8/CD3 CD3/CD4/CD45 CD3/CD8/CD45 CD3/CD19/CD45 CD3/CD16+CD56/CD45 nnnnn CD14/CD64 00000 CD15/CD117 00000 CD15/CD34 00000 CD7/CD33 00000 AntiHLA-DR/CD13 00000 AntiHLA-DR/CD34 nnnnn CD4/CD25/CD3 00000 > OΚ Cancel

Figure 5-9 Lot IDs dialog

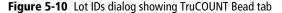
Select a reagent name and type the lot number in the corresponding Lot ID field.

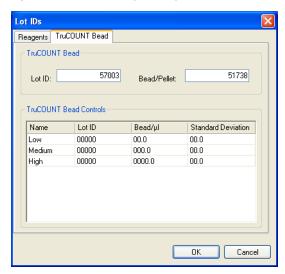
This information will be saved in the worklist.

- **3** To enter BD Trucount[™] lot IDs, bead counts, and standard deviations:
 - a Click the TruCOUNT Bead tab (Figure 5-10).

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 900–1100 with two digits after the decimal
- **f** Type the standard deviation for each control (0 to 70).
- 4 Click OK.

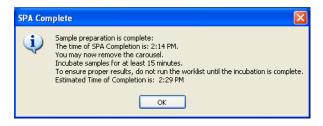
Offline Incubation

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.
- 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 210.

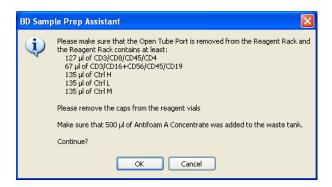
When a run is interrupted due to loss of power, and power cannot be quickly restored, it may be possible to remove reagent vials and sample tubes from the instrument. See When Power Cannot Be Quickly Restored on page 211 for further instructions.

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.

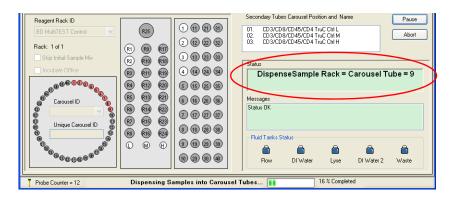
A dialog opens asking you to verify that key components of the Run have been properly prepared. It also specifies the volume of the reagents needed for the run.



- 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.

5 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.

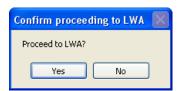


- **6** If you are running BD TrucountTM controls:
 - a Click OK.
 - **b** Open the safety cover.
 - **c** Remove the BD TrucountTM controls from the reagent rack.
 - **d** Cap and vortex each vial.
 - **e** Uncap and replace each vial into its original rack location (place the caps into the reagent 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.



Click Yes.

An abbreviated LWA Info dialog opens.

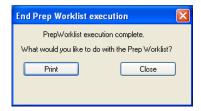
2 Confirm the serial number or user name or select a different serial number or user name from the menus and click OK to add LWA information to the worklist.

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.

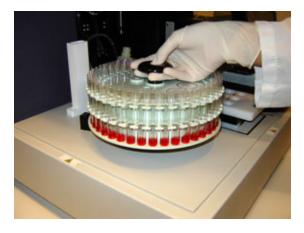


- **1** Click an ending option.
 - Print. Opens the Print dialog. Click OK to print the worklist.
 - Close. Return to the Prep Worklist window.
- **2** Remove the carousel rack from the instrument.

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, unless a tube was specified as a blank tube.



- 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 150.

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.



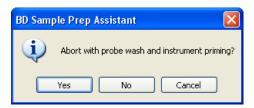
2 To resume the run, click Resume.

The instrument resumes processing after you close the safety cover.

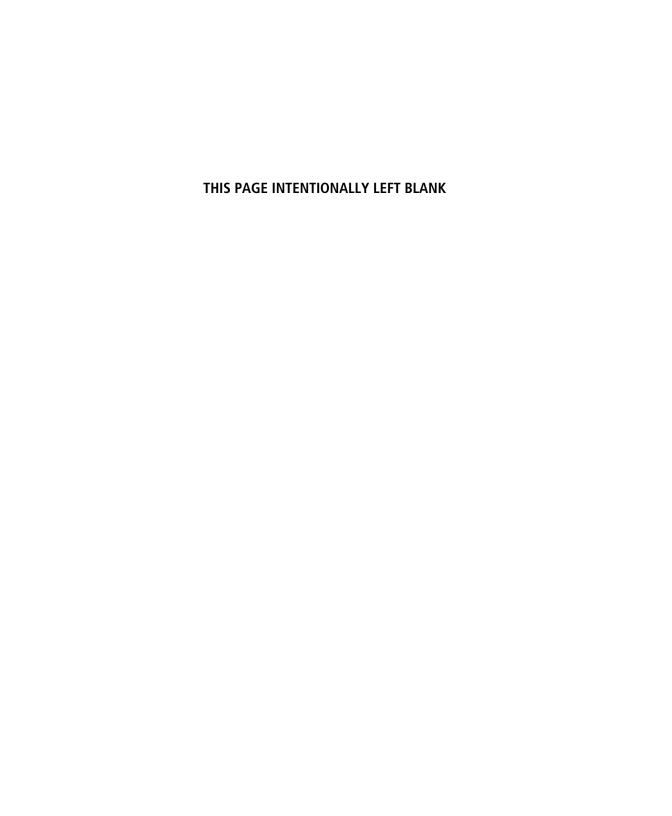
Aborting a Run

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.



Shutdown and Maintenance

This chapter covers the following topics:

- Daily Shutdown on page 150
- Scheduled Maintenance on page 153
- Monthly Servicing of Waste In-Line Filters on page 163
- Periodic Maintenance on page 168
- Special Conditions on page 203

Daily Shutdown

Required Materials

- BD FACSClean™ solution or 10% bleach solution
- 60-mL BD FACSCleanTM 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.

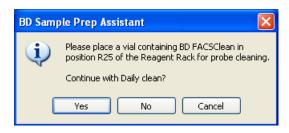
2 Select the Daily Clean checkbox.



If you already performed the Daily Cleaning procedure, you can leave Daily Clean unchecked and proceed straight to Shutting Down the Instrument on page 152.

3 Click OK.

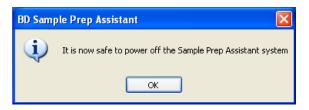
A second dialog opens.



- 4 Open the safety cover.
- 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 μ L 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 FACSCleanTM solution or 10% bleach. The system will also prime with BD FACSFlowTM 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.



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.

10 Click OK.

SPA software quits.

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.

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 116
- Weekly Cleaning in next section
- Calibrating the Probe Reference Point on page 156
- Monthly Servicing of Waste In-Line Filters on page 163
- Monthly Replacement of the Waste Tank Cap on page 167



Mail 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

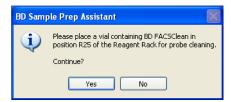
- BD FACSClean™ solution or 10% bleach solution
- 70% ethanol
- Large vial
- Clean, lint-free cloths or disposable wipes

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.

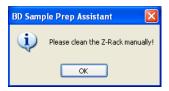


- **2** Open the safety cover and place a large vial containing 60 mL of BD FACSCleanTM solution or 10% bleach solution in well R25 on the reagent rack.
- **3** Fill the DI water and DI water 2 tanks.
- Empty the waste tank according to your laboratory procedures, and then add 1 L of bleach and 500 μ L of Sigma Antifoam A Concentrate to the tank.

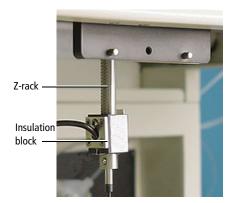
5 Close the safety cover and click **Yes** to continue.

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 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

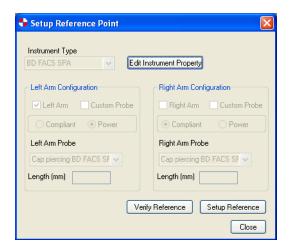
The probe reference point is preset by BD. You should verify the probe reference point weekly to ensure that the system will continue to perform accurately. You should also verify the probe reference point any time you change the probe or move the instrument to a new location in your lab.

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 135.
- 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.



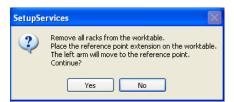
To prevent damage to the probe, do not adjust other settings such as Edit Instrument Property or Setup Reference.

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



Do not click Yes at this point.

Figure 6-1 SetupServices dialog



- **4** 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 6-2 and Figure 6-3.

Figure 6-2 Location of peg for use with reference point extension

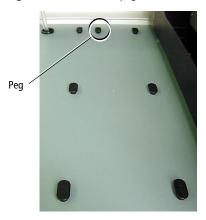


Figure 6-3 Reference point extension on peg

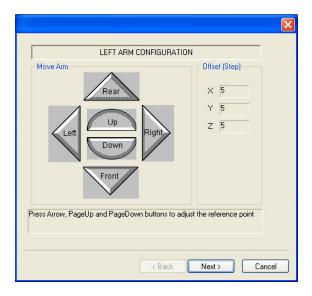


6 Close the safety cover.

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

7 Click Yes in the SetupServices dialog to continue (Figure 6-1 on page 157).

The probe moves to the reference point and the Left Arm Configuration dialog opens.



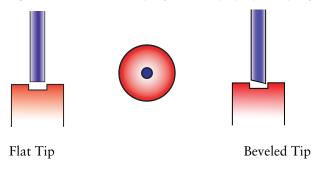
- **8** Open the safety cover.
- **9** Adjust the probe tip alignment, if necessary.

The probe tip should align directly above and in the center of the reference point extension (Figure 6-4 and Figure 6-5), and should not touch it.

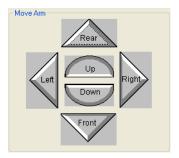
Figure 6-4 Proper probe tip alignment



Figure 6-5 Side (left) and top (right) view of 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.



- 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.

```
*** CALIBRATION OUTPUT SECTION ***
______
[Current Definition at 17:51:38, 04/15/09]
______
Firmware Version: Simulation-RSP9000-Vn.nn: 04/15/09
Machine Range -Left: X: Y: Z:
  1745 2109 3400
Step Size -Left: X: Y: Z:
1.422400 9.817500
Instrument type: , Compliant left \operatorname{\mathsf{arm}}
Left probe type: Cap piercing BD FACS SPA (length = 114)
EEPROM Value: Left Arm: -22398 ( 0xffffa882 )
_____
[ *** Setup result *** ]
_____
Left arm: Changed
 Previous settings: X = 5, Y = 5, Z = 20
 Current settings: X = 5, Y = 6, Z = 5
```

- Clicking Close ends the probe reference point calibration.
- **14** Open the safety cover.
- **15** Remove the paper and the reference point extension.
- Reinstall the reagent and carousel racks (see Replacing the Reagent Rack on page 136 and Mounting the Carousel Rack on page 131).
- 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 189.

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.

What You Need

- Gloves
- Paper towels
- Replacement fine filter, if needed
- BD FACSCleanTM 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 203 to move the probe to the wash station position.
- **2** Open the safety cover.
- **3** Remove the reagent and carousel racks.

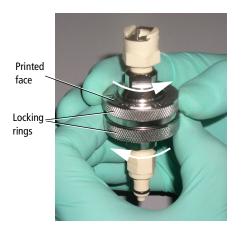
Removing the Waste In-Line Filter Assembly

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.



Hold the filter assembly with the printed face up and twist the locking rings in opposite directions to open the assembly.



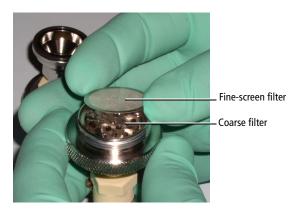


4 Place the upper portion of the assembly on a dry surface.



The locking ring with printed face should not be exposed to fluids.

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 136 and Mounting the Carousel Rack on page 131).
- **2** Perform the procedure in Initializing the Instrument on page 203.
- 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 46 for instructions.

Periodic Maintenance

Perform the following procedures as needed:

- Internal and External Surface Cleaning and Decontamination on page 169
- Extended Periods of Non-Use on page 170
- Startup Following Extended Non-Use on page 173
- Replacing the Probe on page 175
- Replacing a Syringe on page 190
- Fuse Replacement on page 199

Most of the parts described in this section are provided in the accessory kit. For information on reordering parts, see Accessories on page 264.



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 FACSCleanTM 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.
- Wipe all accessible surfaces and racks with one of the cleaning solutions 3 listed in Required Materials.
- Wipe all exposed surfaces using a cloth dampened with DI water. 4
- Wipe all exposed surfaces with a clean, dry cloth. 5



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 through 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 FACSCleanTM 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 153.

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.
- 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.

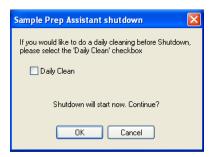


- **6** Repeat steps 3 and 4 with the following fluid types:
 - LYSE
 - FLOW
 - DI WATER 2
- **7** Empty all the tanks.

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 FACSCleanTM vial and store it at room temperature.

See Removing the Reagent Rack on page 135.

- **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 FACSFlowTM 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.

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.

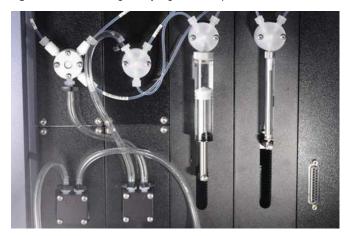
b Select a Fluid Type from the menu and click Yes.



The dialog closes and the fluidics system primes.

- 7 Check the fluidics tubing and syringes on the rear panel (Figure 6-6 on page 174) and the tubing on the instrument side (Figure 6-7 on page 175).
 - 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 213 for tips on tightening valve fittings.

Figure 6-6 Fluid tubing and syringes on rear panel



174

Figure 6-7 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 FACSTM 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.

The following guidelines and instructions are applicable to both the Teflon-coated flat tip probe and the uncoated beveled probe.

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 6-1 Probe replacement guidelines

Action	Condition Requiring Replacement
Visual inspection of the Teflon-coated probe tip	The probe is considered damaged if there are signs of wear on the Teflon coating on the sides of the probe. See Figure 6-8 on page 177. Slight wear from the end view of the probe is acceptable.
Liquid level detection capability	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.
Cap-piercing ability of the probe	The probe is considered damaged if at any point during operation the probe fails to pierce a cap.
Accuracy and precision of the dispense	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.

See Figure 6-8 for an example of a probe with extreme wear of the Teflon coating that would require replacement immediately.

Figure 6-8 Probe with badly worn coating



See Figure 6-9 for examples of probe wear that are considered acceptable and do not require replacement.

Figure 6-9 Examples of acceptable probe wear







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, What You Need on page 163.

- Gloves
- Paper towels
- Kimwipes[®] wipes

- Flathead screwdriver
- Metric hex wrench (2 mm)
- Needle nose pliers
- BD FACSTM SPA III Software Configurator v1.0
- Probe replacement kit (3-pack or 6-pack)
 - 1 Probe (uncoated beveled probe), Part Number 665016
 - Teflon-coated flat tip probe 3-pack with BD Vacutainer tubes (13 x 100 mm, Part Number 647768)
 - Teflon-coated flat tip 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 178.
- **2** Removing the Old Probe on page 183.
- **3** Attaching the New Probe on page 186.
- **4** Calibrating the Reference Point on page 188.
- **5** Completing the Probe Replacement Procedure on page 189.

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 163.

Preparing to Replace the Probe

With the SPA software already installed, the existing Liquid Class database file will need to be updated in order to accommodate updated pipetting parameters

for the beveled tip probe. The updated Liquid Classes file is compatible for both the Teflon-coated flat tip probe and the uncoated beveled tip probe and does not affect existing sample preparation workflow.

NOTE The updated Liquid Class database file and the steps for the patch installation outlined below are mandatory for the uncoated beveled tip probe. The Teflon-coated probe does not require this update.

NOTE The patch installation is a one-time installation to be performed prior to the first time use of the beveled tip probe. The user can confirm if the patch is installed already by performing steps 4 – 6. If the patch is installed, skip to step 7 to start the probe replacement procedure.

NOTE With the patch installed, the user can still switch to the Teflon-coated flat tip probe with no additional updates needed.

NOTE There is no specific orientation required for installing the Beveled tip probe.

If problem persists or installation fails, call Customer Support.

Before you begin, close any open programs and ensure the FACS™ SPA III instrument is turned off.

To install the new liquid class file:

1 Insert the BD FACSTM SPA III Software Configurator v1.0 or later installation USB into the USB drive.

The installer should start up automatically. If it does not, use Windows Explorer to view the USB contents, and double-click the SPA SW Configurator icon.

2 Click **Install** to begin the installation.



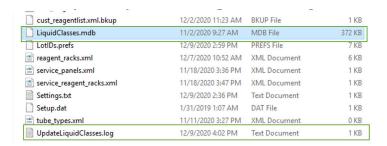
The configurator will automatically load the updated LiquidClasses database file. The dialog refreshes when the installation is complete.

3 Click Close to exit the installer.



- 4 Open Windows File Explorer and navigate to the following file path: C:\ProgramData\BD\FACS SPA\DataFiles.
- 5 Confirm LiquidClasses.mdb file has an updated Date Modified timestamp.

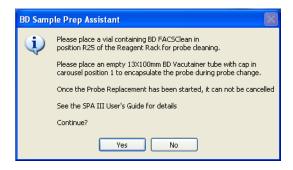
6 Confirm UpdateLiquidClasses.log file has been generated.



The UpdateLiquidClasses.log records the date and time of the completed installation for the updated LiquidClasses database file.

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

A dialog opens.



- **8** Place the 60-mL BD FACSClean[™] vial into position R25 of the reagent rack.
- 9 Place an empty 13 x 100-mm BD Vacutainer® tube with cap in carousel position 1 to encapsulate the probe during the probe change.



- **10** Close the safety cover.
- 11 Click Yes to start the cleaning process.

The instrument flushes the probe with BD FACSClean $^{\text{TM}}$ 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 6-10 Replace Probe dialog



12 Leave the **Replace Probe** dialog open.

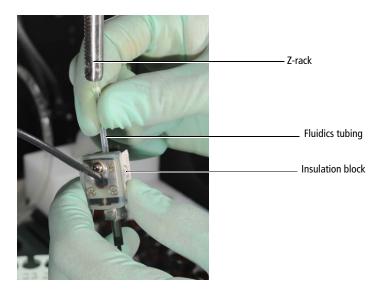
Removing the Old Probe

Do not turn off the instrument during this procedure.

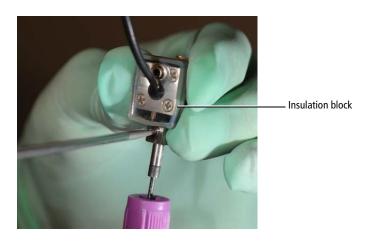
- 1 Open the safety cover.
- **2** Loosen the setscrew on the insulator block with a 2-mm hex wrench.



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.

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.

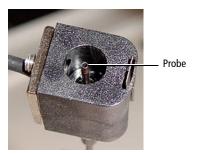


- 2 Insert the silver, blunt end of the new probe into the insulation block until it is completely seated against the block.
- **3** Turn the lower screw with the flat-head screwdriver clockwise to tighten it.



4 Attach the tubing to the new probe through the insulation block, and press firmly until it stops (Figure 6-11 and Figure 6-12).

Figure 6-11 Point of attachment





Do not bend or kink the tubing.

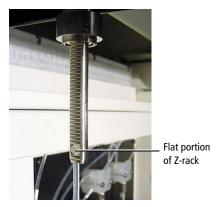
Bending or kinking the tubing will reduce instrument performance.

Figure 6-12 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 6-13.

Figure 6-13 Correct alignment of insulation block



b Use the Allen 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.

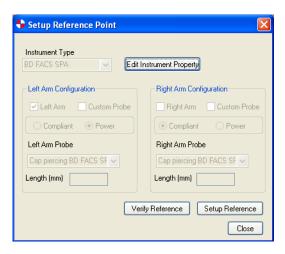


Return to the Replace Probe dialog (Figure 6-10 on page 182), and click in the checkbox to continue the process.

Calibrating the Reference Point

1 Click Next in the Replace Probe dialog.

The Setup Reference Point dialog opens.



The probe reference point is preset by BD. 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 156.

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 203.

- 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 244.

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.



A lnstrument 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

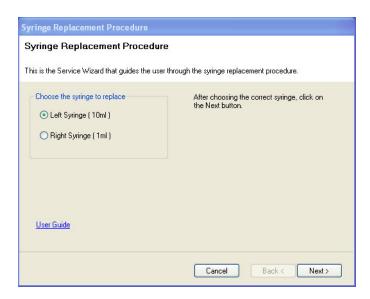
- Gloves
- Replacement syringe

Syringe Replacement Procedure

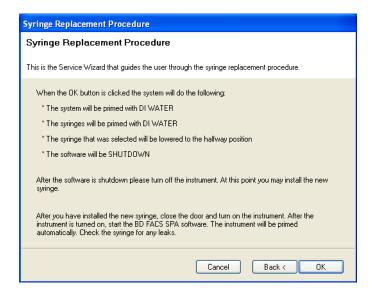
Select Instrument > Replace Syringe from the main menu in the Prep 1 Worklist window.

The Syringe Replacement Procedure dialog opens.

2 Select a syringe and click Next.

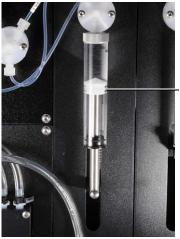


A second dialog is displayed.



3 Click OK.

- The instrument primes twice with DI water.
- The syringe plunger for the selected syringe moves to a halfway position.



Syringe in halfway position

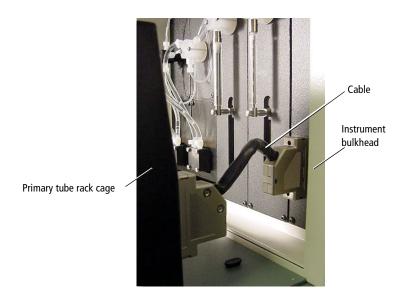
- The software is shut down.
- **4** Turn off the power to the instrument and unplug the power cord.

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.

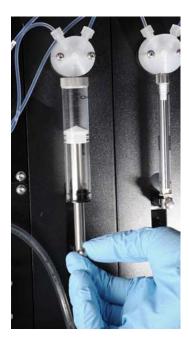
Do not remove the rack cage, disconnect the cable, or place any tension on the cable.



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

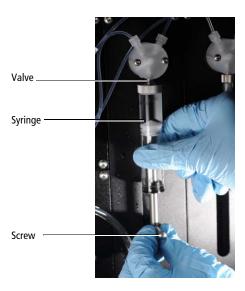
To replace the syringe:

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.



Tip 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 136.

- **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 243 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.





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

5 Remove the fuse drawer.

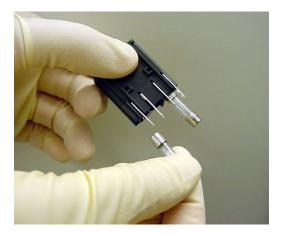


- **6** Remove and dispose of the old fuses.
 - **Tip** It's a good idea to replace both fuses at the same time.

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

Initializing the Instrument

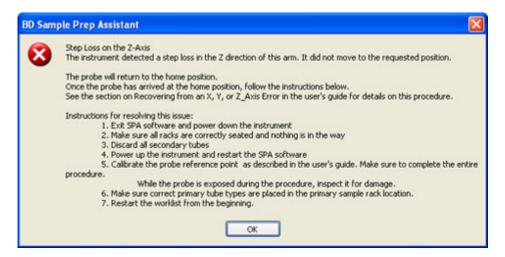
To initialize the instrument:

- 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.
- 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.

2.04

- **6** Start SPA software and log in.
- 7 Calibrate the probe reference point. See Calibrating the Probe Reference Point on page 156.
 - **a** When the probe moves to the reference point extension at step 9 on page 159, 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 175.
 - **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.



To recover from a reinitialization error:

- 1 Click **OK** to close the dialog.
- **2** Turn off power to the instrument.
- Quit SPA software by pressing Crtl+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.
- 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 156.
 - **a** When the probe moves to the reference point extension at step 9 on page 159, 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 175.
 - **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.

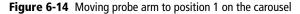
Instrument hardware could be contaminated with biohazardous material.

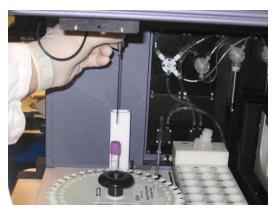
Use universal precautions. Wear protective clothing, eyewear, and two pairs of gloves.

- **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.
- 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 6-14 on page 208.

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.





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 6-15.

Figure 6-15 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 183.

- **b** Attaching the New Probe on page 186.
- 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 6-16 on page 209.

Figure 6-16 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.

- Reset the probe counter by doing a modified version of Replacing the Probe on page 175, as directed in the steps below.
 - **a** Perform Preparing to Replace the Probe on page 178, 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 183
 - Attaching the New Probe on page 186
 - **c** Perform the next section: Calibrating the Reference Point on page 188.

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

d Perform the final section: Completing the Probe Replacement Procedure on page 189.

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

Recovering from a Power Interruption



Mhen 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 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.

Troubleshooting

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 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 214
- Software Troubleshooting on page 228
- Software Error Messages on page 230

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Accuracy and precision results not meeting specifications	Tubes not weighed properly	Make sure
		 you follow the procedure for Accuracy and Precision exactly.
		 you do not touch the tubes with your hands or gloved hands.
		• the balance stabilizes before recording the weight.
		 you weigh the tubes immediately after dispense.
	Insufficient water in tubes	Verify volume of water in primary tubes is correct.
	One or more fluidics tanks disconnected	Connect the tank(s).
		Perform the Startup Following Extended Non-Use on page 173.
	Loose valve or fittings	Check all fittings and hand-tighten them, if necessary.
	Probe clogged	Perform probe replacement procedure. See Replacing the Probe on page 175.
	Calculations incorrect	Double check your calculations and spreadsheet setup.
	Probe improperly installed	Make sure
		• the probe was installed in the correct orientation. See Replacing the Probe on page 175.
		• the fluid connection to the probe is secure.
		• the probe is tightly connected to the Z-rack.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Accuracy and precision results not meeting specifications (continued)	Worklist incorrectly set up	Make sure you choose the worklist specified in the procedure and an adequate number of primary and secondary tubes.
	Other hardware problem	Make sure all tanks are filled with bulk fluids and connected.
		Check valves; hand-tighten all fittings.
		Check syringe fittings to make sure they are snug.
Air in tubing and syringes	One or more fluidics tanks disconnected	Connect the tank(s).
		Perform the Startup Following Extended Non-Use on page 173.
	Loose valve or pump fittings	Check all fittings and hand-tighten them, if necessary.
		See Figure 5-1 on page 116 for the locations of the valves.
Flow cytometry results	Incomplete lysing	
inaccurate	BD FACS TM lysing solution at incorrect concentration	Prepare a new batch of BD FACS™ lysing solution.
	Insufficient lyse incubation time	Check to ensure that the appropriate lyse incubation time is programmed into the user-defined panel. See Adding Secondary Tubes on page 91.
	Offline Incubation enabled, but did not finish	Allow the full lyse incubation time.
	Volume of specimen and reagent too large	Reduce specimen or reagent volume in custom tube protocol. See Adding Secondary Tubes on page 91.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Flow cytometry results inaccurate (continued)	• Insufficient dispensing of BD FACS TM lysing solution: inadequate volume programmed into custom protocol	Check to ensure that the appropriate lyse volume is programmed into the custom protocol. See Adding Secondary Tubes on page 91.
	Problem with bulk fluids	
	Bulk fluids contaminated	Inspect fluids for clarity. If suspect, prepare a new batch of BD bulk fluids.
	Insufficient dispensing of bulk fluids: dispense probe tip worn, damaged, or clogged	Perform probe replacement procedure. See Replacing the Probe on page 175.
	Insufficient dispensing of bulk fluids: supply tank empty	Check the bulk fluid tanks. If empty, refill.
		If tanks are full, check valves—hand-tighten all fittings. See Figure 5-1 on page 116 for the locations of the valves.
	Wrong solution dispensed	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).
	 Insufficient dispensing of bulk fluids: intake tubing not completely submersed in bulk fluids 	Inspect the bulk fluid containers. Make sure the bulk fluids intake tubing is completely submersed and the cap is completely sealed.

Observation	Possible Causes	Recommended Solutions
Flow cytometry results inaccurate (continued)	Insufficient sample	
	Primary sample tube in wrong position or no tube in position	Make sure the tube is in the correct position in the primary tube rack. Rerun tube if necessary.
	Primary sample tube received no sample dispense	See solutions for probe clogged, then rerun sample.
	Probe clogged	Perform probe replacement procedure. See Replacing the Probe on page 175.
		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.
		See Maximum Number of Secondary Tubes Per Panel on page 92.
	Recapped tubes	Vent tubes as described in Running BD Multi-Check™ Controls on page 126, and rerun.
	Clotted blood sample	Do not run clotted samples on the SPA III.
	Insufficient reagent	
	Wrong reagent or no reagent in rack	Make sure reagent vial is in correct position in reagent rack. Rerun tube if necessary.
	User-defined panel with incorrect amount of reagent	Check user-defined panel reagent amounts.

Observation	Possible Causes	Recommended Solutions
Flow cytometry results inaccurate (continued)	Insufficient BD Trucount™ Control	
	Wrong BD Trucount TM control or no BD Trucount TM control	Make sure BD Trucount™ controls are in correct positions in the reagent rack; rerun tube if necessary.
	User-defined panel with incorrect amount of BD Trucount™ control	Check user-defined BD Trucount™ control amounts.
	Worn probe	Perform probe replacement procedure. See Replacing the Probe on page 175.
Fluidics tubing creased, crimped, or bent	Fluidics tower too close to wall or instrument	Call BD service representative.
	Tubing incorrectly replaced	Call BD service representative.
	onto probe	Review probe replacement procedure for next run. See Replacing the Probe on page 175.
Incubation timer showing too much/too little time on it	Incubation time incorrect	Adjust incubation time in custom panel. See Adding Secondary Tubes on page 91.
Incubation timer disappearing	Screen saver interference with incubation timer display	Turn off all screen savers. See Software Compatibility Issues on page 50.

Observation	Possible Causes	Recommended Solutions
Instrument not responding to software	Cable between instrument and PC disconnected	 Ensure the instrument power is on. Ensure the cable is connected to both instrument and PC. Shut down software. Restart application. Call BD Biosciences.
	Cable to PC not connected to Com Port 1	Ensure the cable is connected to Com Port 1, and not Com Port 2. See the documentation that came with your PC.
Leaks or mineral deposits at syringe seals	Syringe not correctly or fully attached	Check that the syringe fitting is finger-tight and attached correctly. See Replacing a Syringe on page 190.
	Insufficient cleaning and maintenance of instrument	Make sure you perform daily and weekly cleaning. See Daily Shutdown on page 150 and Weekly Cleaning on page 153.
	Fluids left in instrument for extended time periods	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 170.
	Break or crack in syringe	Perform syringe replacement procedure. See Replacing a Syringe on page 190.
	Break or crack in valve	Call BD Biosciences.

Observation	Possible Causes	Recommended Solutions
Leaks or mineral deposits at valve and pump fittings	Syringe not correctly or fully attached	Check that the syringe fitting is finger-tight and attached correctly.
	Improper cleaning and maintenance of instrument	Make sure you perform daily and weekly cleaning. See Daily Shutdown on page 150 and Weekly Cleaning on page 153.
	Leave any fluids in instrument for extended time periods	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 170.
	Break or crack in valve	Call BD Biosciences.
Leaks or mineral deposits at waste port	Disconnected waste line	Reconnect the waste line. See Emptying the Waste Tank on page 46.
	Damaged quick-release connector	Call BD Biosciences.
Leaks or mineral	Cap not on securely	Tighten the cap.
deposits at fittings and caps of all bulk fluid	Cracked tank	Order a new tank.
tanks and waste tank	Loose or worn fittings	Call BD Biosciences.
Mixing not occurring, primary tube rack	Cable to primary rack cage disconnected or partially disconnected	Make sure the cable from the instrument to the primary rack cage is completely connected.
	Hardware problem	Call BD Biosciences.
Mixing not occurring, carousel rack	Hardware problem	Call BD Biosciences.
	Carousel rack not properly seated on pins	Make sure the carousel rack is completely seated on carousel pins.

Observation	Possible Causes	Recommended Solutions
Monitor inactive, PC not	Power cord not plugged in	Plug in the power cord.
responding	Monitor/PC power not turned on	Turn on the monitor/PC power.
	Cable not connected between PC and monitor	Reconnect cable. Refer to PC manufacturer's documentation.
Inadequate fluid dispensed	Clogged probe	Perform the probe replacement procedure. See Replacing the Probe on page 175.
	Air leak	Check all fittings and hand-tighten them, if necessary.
	Loose valve or pump fittings	Check all fittings and hand-tighten them, if necessary.
	Fluidics tubing crimped, creased, or bent	Call BD Biosciences service representative.
	Empty fluidics tanks	Fill the tanks.
	Disconnected fluids lines to tanks	Reconnect the tanks.

Observation	Possible Causes	Recommended Solutions
No reagent aspirated	Probe clogged	Perform the probe replacement procedure. See Replacing the Probe on page 175.
		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.
		See Maximum Number of Secondary Tubes Per Panel on page 92.
	No reagent in vial, or no vial in reagent rack	Verify that your worklist matches your reagent rack map.
		Make sure you have a reagent vial with sufficient volume to complete the run.
	Bubbles in vial	Remove bubbles before running.
	False trigger of conductivity	Call BD Biosciences.
Power indicator not lighting; instrument not activating	Power cord not plugged in	Plug in the power cord.
	Instrument power not turned on	Turn on the instrument power.
	Blown fuse	Replace the fuse as described in Fuse Replacement on page 199.
	Internal power failure	Contact your BD Biosciences service representative.

Observation	Possible Causes	Recommended Solutions
Probe bent or damaged	Non-supported primary tubes	Perform probe replacement procedure. See Replacing the Probe on page 175.
		Perform probe calibration. See Calibrating the Probe Reference Point on page 156.
		Make sure you use the appropriate supported tubes as listed in Required Equipment on page 32.
	Used 11-mm or 13-mm tube in 16-mm rack without a tube adapter	Use a tube adapter.
	Caps on reagent vials	Perform probe replacement procedure. See Replacing the Probe on page 175.
		Perform probe calibration. See Calibrating the Probe Reference Point on page 156.
		Make sure you take the caps off the reagent vials and place them in reagent cap holder as described Reagent Rack Setup on page 132.
	Used vial other than 60-mL vial in position R25 in reagent rack	Perform probe replacement procedure. See Replacing the Probe on page 175.

Observation	Possible Causes	Recommended Solutions
Probe contacting instrument worktable components	Racks not in proper place	 Click Abort; turn off the power. Ensure all racks are aligned and seated properly (reagent, carousel, primary tube racks).
		• Inspect the probe. Make sure it's straight. If it's bent, replace the probe. See Replacing the Probe on page 175.
		• Perform probe calibration. See Calibrating the Probe Reference Point on page 156.
	Software installed without custom worktable database	Install the instrument-specific worktable.
	Probe tip realignment not correctly done Cable from primary tube rack cage to instrument loose or not completely connected	Click Abort; turn off the power.
		• Ensure all racks are aligned and seated properly (reagent, carousel, primary tube racks).
		• Perform probe calibration. See Calibrating the Probe Reference Point on page 156.
		1 Make sure cable is connected to both the instrument and the primary tube rack cage.
		2 Initialize the software as described in Initializing the Instrument on page 203.
	Hardware problem	Call BD Biosciences.
Probe not piercing sample tubes	Probe worn or bent and not able to pierce tube caps	Perform probe replacement procedure. See Replacing the Probe on page 175.

Observation	Possible Causes	Recommended Solutions
Primary tube rack cage not stationary while sample is aspirated	Primary tube rack cage cable loose or disconnected	Shut down the instrument. Push the cable in all the way, then engage the fastener.
	Primary tube rack cage not seated properly	Ensure that the primary tube rack cage is properly seated on its guide pins.
	Hardware problem	Call your BD Biosciences service representatives.
Spraying of fluid or sample from probe	Clogged probe	Perform probe replacement procedure. See Replacing the Probe on page 175.
		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.
		See Maximum Number of Secondary Tubes Per Panel on page 92.
		In the future, perform Daily Cleaning and Weekly Cleaning as described in Shutdown and Maintenance on page 149.
Tubes, secondary, (12 x 75-mm) not fitting into carousel rack	Incompatible secondary tubes	Discard tubes and begin again, using compatible tube. See Required Equipment on page 32.
	Too many labels on tubes	Remove excess labels. Do not force tubes into carousel rack.
	Labels too thick	Use labels <5 mils thick (127 microns).

Observation	Possible Causes	Recommended Solutions
Tubes, primary, not fitting into primary tube	Incompatible primary tubes	Use compatible tube. See Required Equipment on page 32.
rack		Use compatible rack or use tube adapters.
	Too many labels on tubes	Remove excess labeling. Do not force tubes into sample rack.
Tubing disconnected from probe	Probe clogged	Perform probe replacement procedure. See Replacing the Probe on page 175.
		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.
		See Maximum Number of Secondary Tubes Per Panel on page 92.
		In the future, perform Daily Cleaning and Weekly Cleaning as described in Shutdown and Maintenance on page 149.
	Tubing not properly reconnected onto probe during replacement procedure	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 187.

Observation	Possible Causes	Recommended Solutions
Wash station overflowing	Quick-release connectors on waste tank not connected	Connect the quick-release connectors for the waste tank.
	Waste in-line filter clogged	Replace waste in-line filter.
	Quick-release connectors on waste in-line filter not connected	Connect the quick-release connectors for the waste in-line filter.
	Waste tank cap vent obstructed	Replace the waste tank cap.
	Power to instrument turned off before shutdown cycle completed	Do not turn the instrument power off prior to the appearance of the following dialog:
		It is now safe to power off the Sample Prep Assistant system OK If the power is off, perform the Daily Startup procedure. If the power is on, perform the Daily Shutdown procedure.
	Waste tank sensor malfunction, no software indicator	Call BD.
	Waste pump failure	Call BD.

Software Troubleshooting

Observation	Possible Causes	Recommended Solutions
Software not starting	File path to software application corrupted	 Uninstall the software. Reinstall the software and the instrument-specific worktable database.
	Software application renamed or moved	 Uninstall the software. Reinstall the software and the instrument-specific worktable.
	Instance of software already running but minimized.	 Check Windows taskbar to see if software is running. If not, reboot computer.
		3 If problem persists, call Customer Support.
Software not responding	Software frozen	1 Press Ctrl+Alt+Delete to open the Windows Task Manager.
		2 Click the Applications tab. Select BD FACS SPA in the Windows Task Manager, and click End Task .
		⚠ Unsaved data will be lost.
No keyboard/mouse response	Keyboard or mouse not connected to computer	Connect keyboard or mouse to computer.
	Keyboard and mouse connections switched	Make sure keyboard is connected to keyboard port and mouse connected to mouse port.
Tubes created, panel not showing up in worklist	Did not access Tube Editor to define properties for tubes in panel	Recreate the panel, this time following the procedure outlined in Specifying Tube Protocols on page 94 for each tube.
Password not working	Caps Lock or Num Lock on	Disable Caps Lock and Num Lock and try again.

Software Troubleshooting

Observation	Possible Causes	Recommended Solutions
During software installation, a message displays asking to reboot the computer.	The installer needs to reconfigure or update the installer engine.	Proceed with the installation and the SPA software will install and function correctly.

Message	Possible Cause	Possible Solution
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	 Fill the appropriate DI water tank. 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	 Replace BD FACSFlowTM cubitainer. 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	 Connect tank sensor connector to fluidics tower bulkhead. 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.
The FACS LYSE tank liquid level is too low. Please fill the tank.	Lyse tank empty	 Fill the appropriate DI water tank. Click Retry to continue. If you click Cancel, you will have to reinitialize the instrument prior to the next run.

Message	Possible Cause	Possible Solution
The Prep Worklist cannot contain both BD Multitest/BD Tritest/ Absolute Count panels and Other panels. If you choose to switch to a new panel type the	You cannot combine BD Multitest/BD Tritest/Absolute Count panels and Other Panels in the same run.	 Click Yes to clear the specified fields. Select panels of the same type for all samples in the worklist.
following will be cleared in the worklist:		1 Click No.
Primary Tube Size, Primary Rack Position, Panel Name, Carousel Position.		2 Delete samples so that only one panel type remains.
Would you like to proceed?		
The Reagent Rack does	Reagent rack missing required	1 Click OK.
not contain all the reagents required to perform the Prep Worklist.	reagents	2 Place the required reagents in the reagent rack according to the Reagent Rack Map for the panel.
The following reagents are missing:		
<missing list="" reagent=""></missing>		
The sample prep cannot		1 Click OK.
run because the worklist has errors.		2 Place your mouse on the red error icon with the exclamation point and correct the displayed error.

Message	Possible Cause	Possible Solution
Sample prep has paused.	Reagent level too low	1 Click OK.
Please press 'OK' button add the following		2 Add specified reagent to reagent vial.
reagent: <reagent name=""> and press 'Resume' button to continue</reagent>		3 Click Resume.
Sample prep has paused.	Sample level in open tube(s) too	1 Click OK.
Please press 'OK' button, make sure that the Open Tube has enough Sample; then press 'Resume'	low	2 Ensure open tubes have a minimum of 150 μL of sample.
button to continue.		3 Click Resume.
Sample prep is pausing	Waste tank is full	1 Empty the Waste tank.
because the WASTE tank is full.		2 Add 500 μL of Sigma Antifoam A Concentrate.
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!		3 Click Resume.
Sample prep is pausing because [DI WATER/	Specified tank liquid level is too low	1 Wait for the probe wash to complete.
FACSFLOW/DIWATER		2 Fill the tank.
2/LYSE] tank liquid level is too low.		3 Click Resume.
Please wait for the probe wash to complete. Then fill the tank and press 'Resume' button to continue!		

Message	Possible Cause	Possible Solution
The Sample rack is disconnected.	Primary tube rack cage cable loose or disconnected	1 Shut down the instrument.
Please connect it and press 'Retry' to continue		2 Tighten or connect the cable, then engage the fastener.
		3 Click Retry .
The Carousel rack is not	Carousel rack not loaded or not	Load the carousel rack.
loaded. Please load it and press 'Retry' to continue	fully seated on pins	 Verify that it is fully seated on the spindle and both pins.
The Secondary tubes are not loaded at their defined locations. *misplaced tubes>	Missing tube	Verify that the carousel matches your worklist and your panel. Compare the worktable display to the carousel.
Please load all secondary tubes at their defined locations and press 'Retry' to continue		
The Secondary tubes are not loaded at their defined locations.	Missing tube Hardware problem	Verify that the carousel matches your worklist and your panel. Compare the
The Carousel could not be scanned due to an		worktable display to the carousel.
Instrument error.		Call BD Biosciences.
Please load all secondary tubes at their defined locations and press 'Retry' to continue		

Message	Possible Cause	Possible Solution
At least one tank needs to be filled or emptied.	Tank full or empty	Compare tanks to display, and fill or empty tanks.
Please adjust liquids level and press 'Retry' to continue.	Fluid tank sensor connector 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.
<tank name=""> tank needs to be filled</tank>	The specified tank fluid level is too low	1 Add fluid to the specified tank.
		2 Click Retry.
Sample prep is paused because the Safety Cover is open.	Safety cover open	Close the safety cover and press Resume .
Please wait for the probe wash to complete. Then close the Safety Cover and press 'Resume' button to continue!		
Sample prep is paused because the Safety Cover could not be locked.	Safety cover not locked	Close the safety cover and press Resume .
Please wait for the probe wash to complete. Then close the cover and press 'Resume' button to continue!		

Message	Possible Cause	Possible Solution
The correct help file could not be found.	File path to help file corrupted	 Uninstall the software. Reinstall the software and the instrument-specific worktable database.
	Help file renamed or moved	1 Uninstall the software.
		2 Reinstall the software and the instrument-specific worktable database.
The software cannot give a worklist the same name as an open worklist. Type a different name for the worklist you want to save.	Attempt to name new worklist with same name as running worklist.	Type a different name for the worklist you want to save.
Selected file <i><file name=""></file></i> is not a valid Prep Worklist.	Non-worklist file opened (not an XML file or not a worklist XML file)	Choose only worklist XML files.
	File corrupted	Discard old worklist, and create a new worklist
The selected Reagent	Reagent rack for selected panel is	1 Click OK.
Rack contains controls that are not available on the rack:	missing required controls	2 Place the required controls in the reagent rack according to the reagent
<missing control="" list=""></missing>		rack map.
Sample cap-piercing Probe Counter exceeded 2000.	Probe needs replacement soon	Be aware that the probe will need to be replaced after the next 500 pierces.
The Probe needs to be replaced soon.		

Message	Possible Cause	Possible Solution
Sample cap-piercing Probe Counter exceeded 2500.	Probe needs replacement now	Perform probe replacement procedure.
Immediate Probe replacement is required!		
Unable to lock because	Safety cover open	1 Close the safety cover.
the Safety Cover is open Please close the cover and press 'Retry' key		2 Click Retry.
Unable to lock the Safety Cover!	Safety cover not locked	1 Inspect the lock mechanism.
		2 Remove any obstruction.
		3 Attempt to close the safety cover.
		4 Click Retry.
Unable to unlock the Safety Cover!	Safety cover not unlocking	Click Retry.
		Call BD.
Current procedure was	Safety cover was not closed	1 Click OK.
canceled because the Safety Cover was not closed.		2 Restart the procedure.
Current procedure was	Safety cover was not locked	1 Click OK.
canceled because the Safety Cover was not closed.		2 Restart the procedure.
Current procedure was canceled because <i><tank name=""></tank></i> tank was not filled.	The specified tank fluid level is too low	1 Click OK.
		2 Fill the specified tank.
		3 Restart the procedure.

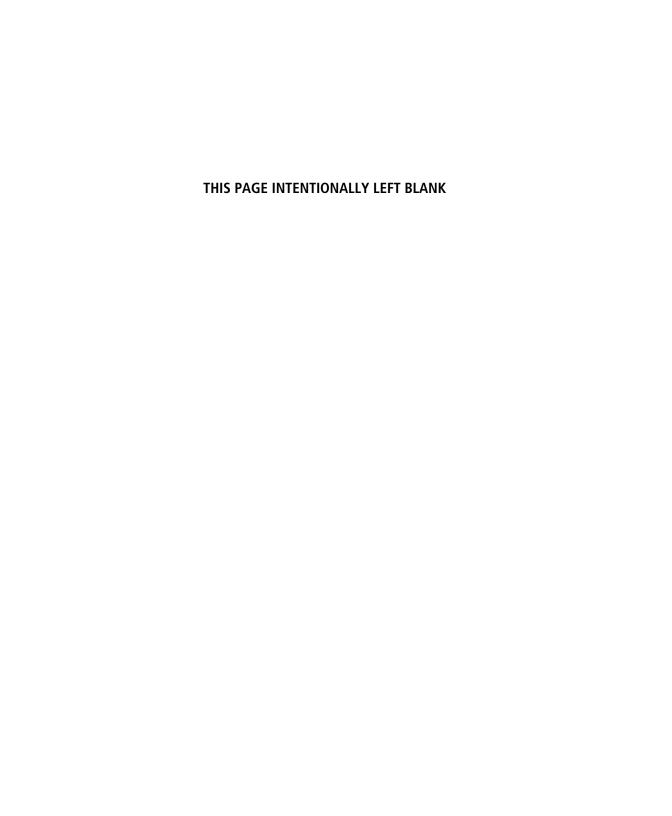
Message	Possible Cause	Possible Solution
Current procedure was canceled because Waste tank was not emptied.	Waste tank was not emptied	 Click OK. Empty the waste tank. Restart the procedure.
Current procedure was canceled because an error occurred on the Instrument.	Hardware error detected	 Click OK. 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.
Please abort the sample prep or wait for the current Instrument operation to finish before Shutdown!	Pressed button while sample prep running	 Reinitialize the instrument. Click OK. Then do one of the following: Use the control to minimize and hide the display. Click Abort to stop the run. Wait for the instrument operation to finish before shutdown.
Unable to load Reagent Racks file <file name=""> <additional windows<br="">OS message></additional></file>	File not in expected location (moved or renamed)	Return file to original location. 1 Uninstall the software. 2 Reinstall the software.

Message	Possible Cause	Possible Solution
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!	Waste tank is full at the beginning of the instrument process.	1 Click OK.2 Empty the waste tank.3 Retry the menu selection.
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!	Specified tank fluid level is too low at the beginning of the instrument process.	 Click OK. Fill the specified tank. Retry the menu selection.
Unique Carousel ID cannot be longer than 22 characters. Please re-type the Unique Carousel ID.	Name entered for Carousel ID is longer than 22 characters.	1 Click OK.2 Re-type name of 22 characters or less for Carousel ID.
Cannot save the Worklist because it has errors.	The worklist has entry errors.	 Click OK. Fix the worklist errors. Save the worklist.

Message	Possible Cause	Possible Solution
Couldn't print the worklist to the selected printing device.	Selected printer is not available Not connected to selected printer	 Click OK. Select a new printer. Restart the print. Alternatively, Click OK. Connect the printer. 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.	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.
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:		Chek 140 to cancer the run.
<pre><list items="" of="" replace="" to=""> Would you like to proceed?</list></pre>		

Message	Possible Cause	Possible Solution
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	 Click OK. Perform the procedure in Recovering from an X, Y, or Z-Axis Error on page 204. 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	 Click OK. Place the instrument on a stable, level surface. Perform the procedure in Recovering from an X, Y, or Z-Axis Error on page 204.
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	 Press Pause. 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	 Empty the waste tank and add 1 L bleach and 500 μL Antifoam A Concentrate. Click Retry to continue. If you click Cancel, you will have to reinitialize the instrument prior to the next run.

Message	Possible Cause	Possible Solution
Communication Error	The instrument did not respond within the expected time.	1 Exit SPA software normally and power down the instrument.
		2 Make sure all racks are correctly seated and nothing is in the way.
		3 Check cables between computer and instrument.
		4 Discard all secondary tubes.
		5 Power up the instrument and restart SPA software.
		6 Calibrate the probe reference point. See Calibrating the Probe Reference Point on page 156. While the probe is exposed, inspect it for damage.
		7 Restart the worklist from the beginning.
Failure to Reinitialize the	The instrument has failed to reinitialize the arm.	1 Click OK.
arm		2 Perform the procedure in Recovering from a Reinitialization Error on page 205.
The instrument detected a {error type} error on the {device type} device.	Various causes	See the solution for Timeout Error above.



Appendix A

Accuracy and Precision

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 244.

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 through 5 until all 20 tubes have been weighed.

Use the following worksheet to record your weights.

	Weight (g)		
Secondary	Empty	Full	Fluid =
Tube No.			(Full – Empty)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
		Mean	
		SD	
		%CV	

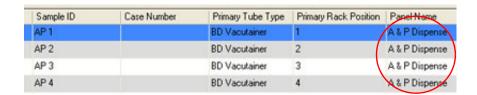
Fluid Dispense

- 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.

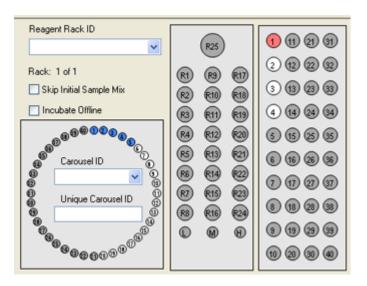
Specify the **Sample ID** and **Primary Tube Type**. Use the default Primary Rack Position 1, 2, 3, and 4.

For information on creating a worklist, see Preparing Primary Tubes on page 119, Panel Selection on page 129, and Carousel Rack Setup on page 130.

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



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 (\overline{X}) , the standard deviation (SD), and percent coefficient of variation (%CV) of the fluid weight.

Calculate the mean:

$$\overline{X} = \frac{\Sigma X}{N}$$
 where X represents each data point (fluid weight) and N represents the sample size (number of tubes).

Convert the fluid weight mean to fluid volume:

fluid volume (
$$\mu$$
L) = $\frac{\text{fluid weight (g)}}{\text{fluid density (g/mL)}} \times 1000 \text{ (}\mu\text{L/mL)}$

Example:

$$\overline{X}$$
 = 0.0495 g; density of water = 1 g/mL

fluid volume
$$= \frac{0.0495 \text{ g}}{1 \text{g/mL}} \times 1000 \text{ } \mu \text{L/mL}$$

fluid volume
$$= 49.5 \mu L$$

Calculate the standard deviation:

$$SD = \sqrt{\frac{\sum X^2 - \frac{(\sum X)^2}{N}}{N-1}}$$

Use the following formula to calculate the %CV:

$$\%CV = \frac{SD}{X} \times 100$$

You can use a spreadsheet to do these calculations.

Configure the spreadsheet like the following.

	Α	В	С	D	E
1					
2					
3				Weight (g)	
4					
5		Secondary	Empty	Full	Fluid (Full — Empty)
6		Tube No.			(Full — Empty)
7		1			=D7-C7
8		2			=D8-C8
9		3			=D9-C9
10		4			=D10-C10
11		5			=D11-C11
12		6			=D12-C12
13		7			=D13-C13
14		8			=D14-C14
15		9			=D15-C15
16		10			=D16-C16
17		11			=D17-C17
18		11 12			=D18-C18
19		113			=D19-C19
20		14			=D20-C20 =D21-C21
21		15			=D21-C21
22		16 17			=D22-C22
23		17			=D23-C23
21 22 23 24		18			!=D24-C24
25		19			=D25-C25 =D26-C26
26		20			=D26-C26
27				Mean	=AVERAGE(E7:E26)
28				SD	=STDEV(E7:E26)
29				%CV	=E28/E27*100

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.

Specification	Value	
Sample Type	Water	
Volume Dispensed	50 μL	
n	20	
Mean Value	48.5 to 51.5	
%CV	≤3%CV	

For values that fall outside these specifications, see Troubleshooting on page 213.

Appendix B

Technical Specifications

This appendix covers the following topics:

- Instrument on page 252
- Performance on page 260
- Fluidics Tower on page 261
- Barcode Reader on page 261

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

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

Operating relative humidity 15-80% (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 FACSTM Loader carousel racks,

numbers 1-16

Tube Compatibility

Carousel rack Accommodates up to 40 uncapped 12 x 75-mm tubes

Falcon polystyrene test tubes

• BD TrucountTM 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 HemogardTM 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

Streck Cyto-Chex BCT

• 5.0 mL, 13 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 FACSCleanTM vial (38.9 mm in diameter), uncapped

Tube adapters Allows use of 11-mm and 13-mm tube in a primary

tube rack and in the open tube port

Open tube port Allows the use of uncapped 13-mm, 16-mm, and

15-mL conical primary tubes. Holds three tubes.

Labels $\leq 5 \text{ mils (127 microns) thick}$

Pre-Programmed Dispense Volumes

 $Sample & 50 \ \mu L \\ Reagent & 20 \ \mu L \\ Lyse & 450 \ \mu L \\ BD \ Trucount^{TM} \ Control & 50 \ \mu L \\$

Pre-Programmed Incubation Times

Lyse 15 minutes
Reagent 15 minutes

User-Definable Ranges

BD Multitest/BD Tritest/Absolute Count Panels

Sample 0 or 50 µL

Reagent 0, 5, 10, or 20 μL

Lyse $0-450 \mu L$, in $25-\mu L^a$ increments

BD TrucountTM control 0 or 50 μL

Incubation times

Reagent 0–60 minutes, in 5-minute increments
 Lyse 0–60 minutes, in 5-minute increments

Number of reagents Up to two per tube

Maximum volume per tube 590 µL

Other Panels

Sample $0-100~\mu L$ in 5- μL increments

Reagent $0-400~\mu L$ in 5- μL increments

Lyse $0-2,000~\mu L$, in 25- μL increments

BD TrucountTM control Not available

Incubation times

Reagent 0–60 minutes, in 5-minute increments
 Lyse 0–60 minutes, in 5-minute increments

Number of reagents Up to nine per tube

Maximum volume per tube 3,000 μL

a. Lyse dispense for accuracy and precision has been validated for volumes of 0 $\mu L, 450~\mu L, 1,000~\mu L,$ and 2,000 μL only. Validate other lyse dispense volumes in your laboratory.

Optimizing Reagent Use

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

Reagent Volume Definitions

Volume Type	Definition
Conditioning volume	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.
Excess volume	Ensures that the last volume dispensed equals all others and does not contain sheath. This volume is discarded to waste.
Dispensed volume	Defined by BD in pre-defined panels or by the user in Other Panels.
Minimum volume	Conditioning Volume + Excess Volume + Dispensed Volume

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 ≤20 µL:

Secondary Tubes	Excess Volume	Conditioning Volume
1 tube	4 μL	7 μL
2–6 tubes	7 μL	7 μL

For reagent volumes >20 μL:

Secondary Tubes	Excess Volume	Conditioning Volume
1 tube	4 μL	7 μL
2–6 tubes	10 μL	7 μL

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 (multidispense) 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

Volume Type	Definition
Conditioning volume	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.
Excess volume	Ensures that the last volume dispensed equals all others and does not contain sheath. This volume is discarded to waste.
Dispensed volume	Defined by BD in pre-defined panels or by the user in Other Panels.
Dead volume	Tube-dependent volume that cannot be aspirated or dispensed.
Minimum volume	Conditioning volume + Excess volume + Dispensed volume + Dead volume

NOTE Conditioning, excess, and dead volumes cannot be changed.

Minimum Sample Volumes

Table B-1 For BD Vacutainer and Streck Cyto-Chex Tubes

Cample	Number of	Minimum Sample Volume (μL)			
Sample Volume (µL)	Secondary Tubes	13 x 75-mm BD Vacutainer	13 x 75-mm Streck Cyto-Chex	16 x 100-mm BD Vacutainer	
50	1–2	400	400	700	
50	3–4	500	500	700	
50	5-6a	700	700	900	
50	7–8 ^a	900	900	1,000	

a. Only applicable for Other Panels.

Table B-2 For Sarstedt Tubes

Sample	Number of	Minimum Sample Volume (μL)					
Volume (μL)	Secondary Tubes	Sarstedt 2.6 mL	Sarstedt 2.7 mL	Sarstedt 3.4 mL	Sarstedt 4.0 mL	Sarstedt 4.9 mL	Sarstedt 5.5 mL
50	1–2	600	400	600	700	600	700
50	3–4	800	600	800	900	800	900
50	5-6a	1,000	700	1,000	1,000	1,000	1,000
50	7-8a	1,100	800	1,100	1,200	1,100	1,200

a. Only applicable for Other Panels.

Performance

BD Multitest/BD Tritest/Absolute Count Panels

Accuracy (dispense) Blood: 50 µL ±3% volume

Reagent: $20 \mu L \pm 7\%$ volume Lyse: $450 \mu L \pm 3\%$ 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)

Other Panels

Accuracy (dispense) Blood: 20–45 µL ±10% volume

50–100 μL ±5% volume

Reagent: 5–15 μL ±20% volume

 $20-100 \mu L \pm 7\%$ volume

Lyse: $450-2,000 \mu L \pm 3\%$ volume

Precision (dispense) Blood: 20–100 μL aliquots, CV ≤5%

Reagent: 5–15 μL aliquots, CV ≤15%

20–100 μL aliquots, CV ≤5%

Lyse: $450-2,000 \mu L \text{ aliquots, CV} \leq 3\%$

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

DI Water 1.0 L

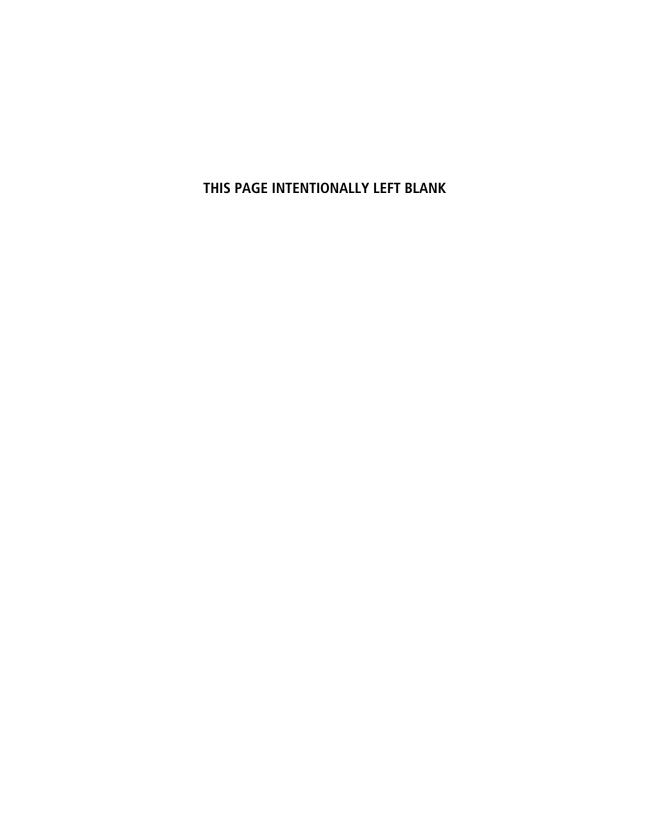
Lyse 1.0 L

DI Water 2 1.0 L

Waste 10.0 L

Barcode Reader

Compatibility ISBT 128 standard barcode labels



Appendix C

Supplies and Replacement Parts

This appendix covers the following topics:

- Contact Information on page 264
- Accessories on page 264
- Accessory Options on page 266
- Replacement Parts on page 267
- Consumables on page 270

Contact Information

The SPA III comes equipped with an accessory kit containing spare parts. To order spare parts and consumables, such as bulk fluids, from BD Biosciences within the US, call: (877) 237-8995, prompt 2-2-4.

For other countries, contact your local BD Biosciences customer service representative. Worldwide contact information can be found at bdbiosciences.com.

Accessories

The accessory kit that ships with the SPA III contains:

- Allen wrench kit, metric (1)
- BD FACSClean vial, 60 mL, for R25 position on reagent rack (1)
- BD FACS Loader carousel racks labeled Sample-Prep Ready (4)
- Cap (extra) for bulk fluid tanks (2)
- Caps (extra) for waste tank
- Documentation
 - Instructions For Use with BD FACSTM Sample Prep Assistant III Safety and Limitations booklet and BD FACSTM Sample Prep Assistant Software USB containing translations
- Fluidics tower (with tanks, connections, and caps)
 - DI water tank (1)
 - Lyse tank (1)
 - DI water 2 tank (1)

- Waste tank (1)Fuse kit (1)
- Instrument-specific worktable database USB (1)
- Power cords
 - US (1)
 - Europe (1)
- Power strip, US (1)
- Primary tube racks (2)
 - 13 mm (1)
 - 16 mm (1)
- Primary rack cage cable (1)
- Probes
 - Probes (3)
 - Vacutainers (3)
- Reagent rack (1)
- Reagent rack cap holder (1)
- Open tube port (1)
- Reference point extension (1)
- Screwdriver (1)
- Screw kit (1)

- Serial cable (1)
- Syringes
 - 1 mL (1)
 - 10 mL (1)
- Syringe plunger screws (5)
- Tube adapters (40 white and 40 black)
- Needle-nose pliers (1)

Accessory Options

Additional accessories, available as options, ship with some SPA III packages:

- Barcode reader, with accessories (1)
- PC workstation: includes computer, monitor, keyboard, mouse, and cables (1 of each)

Replacement Parts

Order replacements and replacement parts from BD Biosciences using the following part numbers.

Item	Part No.
Barcode reader	Contact your BD Biosciences representative. Part numbers could vary.
BD FACSClean TM vial, 60 mL (for R25 position on reagent rack)	334030
BD FACS Loader carousel rack labeled sample-prep ready	
• Racks 1–4	332727
• Racks 5–8	332728
• Racks 9–12	332729
• Racks 13–16	332730
Cap, with gasket for bulk fluid tanks (2)	334029
Documentation	
• BD FACS TM Sample Prep Assistant III Instructions For Use	23-20276-01
• BD FACS TM Sample Prep Assistant III Safety and Limitations booklet	23-18109-02
• BD FACS™ Sample Prep Assistant software USB	664115
PC workstation	Contact your BD Biosciences representative. Part numbers could vary.
Primary tube racks	
• 13 mm	333492
• 16 mm	333491

Item	Part No.
Probes	
• 3 pack with BD Vacutainer® tubes	647768
• 6 pack with BD Vacutainer® tubes	647769
Uncoated beveled probe kit	665016
Probe accessories	
• Reference point extension (1)	334037
• Screw kit	334038
• Worktable peg kit (5 worktable alignment pegs and 1 reference point extension peg)	334034
Reagent rack	333489
Reagent rack cap holder	334022
Syringes for T0246 and higher systems (XLP)	
• Kit with 1-mL and 10-mL syringes	647906
• Individual 1-mL syringe	647348
• Individual 10-mL syringe	647349
• Syringe plunger screws (5)	642436
Syringes for pre-T0246 systems (XL)	
• Kit with 1-mL and 10-mL syringes	647905
• Individual 1-mL syringe	647911
• Individual 10-mL syringe	647912
• Syringe plunger screws (5)	334033

Item	Part No.
Tank set	
• DI water 2 tank	337622
• Lyse tank	338031
• DI water tank	334027
• Waste tank	337625
• Fluid sensor (waste tank)	337628
• Fluid sensor (BD FACSFlow TM cubitainer)	337627
Open tube port	337620
Tube adapters, 13 mm, white (package of 10)	333493
Tube adapters, 11 mm, black (package of 10)	647914

Consumables

To order consumables, use the following product catalog numbers.

Item	Manufacturer and Product(s)	Catalog No.	
Blood collection tubes	BD Vacutainer [®] blood collection tubes (plastic or glass) with caps (BD Hemogard [™] closures or standard rubber caps)	Contact BD Vacutainer® Systems.	
	• 13 x 75 mm		
	• 16 x 75 mm		
	• 16 x 100 mm		
	• 13 x 100 mm		
	Sarstedt S-Monovette	Contact Sarstedt.	
	• 2.7 mL, 11 x 66 mm		
	• 2.6 mL, 13 x 65		
	• 3.4 mL, 13 x 65 mm		
	• 4.9 mL, 13 x 90mm		
	• 4.0 mL, 15 x 75 mm		
	• 5.5 mL, 15 x 75 mm		
	Streck Cyto-Chex BCT	Contact Streck.	
	• 5.0 mL, 13 x 75-mm		
BD Vacutainer® blood transfer device	BD	364880	
Disposable	Falcon polystyrene test tubes	Contact BD Biosciences.	
12 x 75-mm tubes	BD Trucount™ Tubes	BD Trucount TM Tubes are bundled with various reagent products. Order using the catalog number for the required reagent kit.	

Item (continued)	Manufacturer and Product(s)	Catalog No.
Fine-screen filter	Pall Corporation Support Screen	640527
Flow solution	BD FACSFlow™ solution	342003
Lyse tank solution	BD FACS TM lysing solution (10X)	349202
Waste tank solution	Sigma Antifoam A Concentrate	334887
	• Full-strength household bleach	N/A
Waste tank cap	• BD	338347
Bleach-based surface	• BD FACSClean TM solution	340345
cleaning agent	• 10% dilution of household bleach	N/A
Alcohol-based surface cleaning agent	70% ethanol	N/A

Index

Symbols	Panels 85			
%CV, calculating for fluid weight 248	BD Trucount controls 23, 85, 105 lot IDs 138			
A	BD Trucount tubes 33 BD Vacutainer tubes 32			
aborting a run 147 Account Name field 71 accuracy and precision acceptance criteria 250 specifications 260, 261 spreadsheet setup 249 troubleshooting 214, 215 when to perform 243 Acrobat Reader 50 adapters, tube 22, 254 alcohol, for cleaning 33 assistance, technical xiii	biohazardous waste 46, 47 blank tube, selecting 96 bleach 33 blood transfer device, for venting tubes 127 blue panels 101 bulk fluids refilling 38 supported 33 table of 37 tanks capacities 261 troubleshooting 220			
В	buttons Edit Tube 95, 102			
barcode scanner connecting 36 described 29 specifications 261 using 62, 120	Minimize, Maximize, Close 58 New Category 88 New Panel 89 New Tube 91 Shutdown 76			
BD FACS lysing solution 33 BD FACS SPA Login dialog 55	C			
BD FACSClean solution 33, 150 BD FACSFlow solution 33 BD Falcon tubes 33 BD Multitest/BD Tritest/Absolute Count	cable fluid level sensor 35 insulation block 117			

primary tube rack cage, <i>shown</i> RS232 34, 50	193	cord, power 34, 36 counter, probe 58, 175, 189
calculating % CV for fluid weight	248	cover, safety 19
calibration peg 158	270	creating user accounts 71
cap holder, reagent 25		cubitainer 27
caps, bulk fluid tanks,		Cubitaniei 27
troubleshooting 220		_
		D
Carousel Position field 62, 131		daily
carousel rack 26		cleaning 150
compatibility		shutdown 150
Loader 26		startup 114
tubes 253		decontamination
discontinued 26		daily 150
empty positions 130		surface 169
ID 58		default name
inserting 131		
loading 130		panel 89 tubes 91
map 60		
removing 144		deleting sample from worklist 65
troubleshooting 220		deposits see mineral deposits
unique ID 58		DI water tanks 37
case number 121		dialogs
Change Password dialog 69		BD FACS SPA Login 55
changing password 69		Change Password 69
cleaning		Left Arm Configuration 159
daily 150		Open Worklist 118
instrument surface 169		Panel Editor 87
solutions 33		Print 65
weekly 153		Reagent Rack Editor 101, 105
components, system 18		Replace Probe 182
computer, troubleshooting 221		Sample Prep Assistant shutdown 76
consumables		Setup Reference Point 157, 161, 183
ordering 264		Syringe Replacement Procedure 190
part numbers 270		User Administration 70
controls		dispense
BD Trucount 23, 85, 105		accuracy 244, 260, 261
beads 143		method to ensure 244
conventions		precision 260, 261
keyboard xii		volumes, preprogrammed 254
text xii		documentation, translated xiii

door, instrument 19	syringe 116
draining waste cap trap 47	flow
E Edit Tube button 95 emptying waste tank 46 ending a run 144 error messages file 75 storage of 75 ethanol 33 extended non-use, instrument 170 startup following 173	container 42 level sensor 42 tank 37 fluid level sensor cable 35 Fluid Tanks Status indicators 59, 68 fluidics system priming 43 syringe pumps 20 troubleshooting 221 valves 20 tower 27
F	dimensions 261 waste connection 35
FACS lysing solution 33 FACSClean solution 33, 150 FACSFlow solution 33 Falcon tubes 33 fields Account Name 71 Carousel Position 62, 131 Institution 71 Messages 59 Names 71 Panel 93 Panel Name 62, 129	waste port 117 fluids bulk 33, 37 level indicator 45 refilling 38 fuses replacing 199 type 252 H holder, reagent cap 25 Home command 203
Password 71 Primary Rack Position 62, 123 Primary Tube Type 62, 122 Sample ID 121 Secondary Tubes Carousel Position and Name 59, 131 Status 59, 141 filter cap, waste tank 46, 48 waste in-line 28 fittings pump 116	incubate offline description 58 procedure 139 incubation time changing 93 during sample prep 142 lyse 85 preprogrammed 254 reagent 85

troubleshooting 146, 218	L
indicator lights	1.1.1.1.1. 26.254
barcode scanner 29	label thickness 26, 254
instrument, troubleshooting 222	laser scanner 29
indicators, tank levels 45, 68	leaks
in-line filter assembly, waste 28	at syringe seals 219
installation	at valves 220
software 51	at waste port 220
tanks 38	Left Arm Configuration dialog 159
Institution field 71	Loader rack 26
instrument	loading carousel rack 225
aborting 147	log files 75
cleaning 169	log in
components 19	procedure 56
initializing 203	troubleshooting 228
log files 75	login files, instrument 75
pausing 146	lot IDs
performance 260, 261	BD Trucount controls 138
power switch 34	reagents 137
priming 43	LWA information 143
requirements 32	lyse
software requirements 50	amount 85
specifications 252, 261	dispense 94
storage 170	validated accuracy and
supplies 32	precision 94
troubleshooting 214–227	incubation time 85
worktable 19	solution 33
insulation block	tank 37
cable 117	troubleshooting 215
correct alignment 188	
described 20	M
removing 183	
removing 105	mean, calculating for fluid weight 248
V	menu bar 60
K	menus
keyboard	Panel Name 129
conventions xii	Primary Tube Type 122
troubleshooting 228	Reagent Rack ID 58, 132, 134
wedge 50	Task Level 71
<i>O</i>	Messages field 59
	mineral deposits

at syringe seals 219 at valves 220 at waste port 220 mixing control beads 143 post-lyse 85 post-reagent 85 troubleshooting 220 monitor, troubleshooting 221, 222 mouse, troubleshooting 228 Multi-Check controls 16, 126 multiple panels, with one primary sample 63 multi-rack reagent racks, creating 104	Panel Name field 62 panels blue vs black 101 categories, creating 87 preprogrammed 78 printing 99 splitting 130 troubleshooting 228 user-defined 85 password changing 69 login 56 troubleshooting 228 Password field 71
Names field 71 New Category button 88 New Panel button 89 New Tube button 91	pausing instrument during run 146 peg, calibration 158 post-lyse mix 85 post-reagent mix 85 power cord 34, 36 interruption during a run 140
offline incubation 139 on/off light 20 switch 34 open tube port 24, 90, 124 Open Worklist dialog 118 ordering spare parts 264, 267 Other Panels 85, 92 P Panel BD Multitest/BD Tritest/Absolute Count 85 Editor 87 field 93 Name menu 129	long-term 211 recovering after 210 switch 34 precision, dispense 260, 261 Prep Worklist main menu 57 window 57 preprogrammed dispense volumes 254 incubation time 254 panels 78 reagent racks 78 preprogrammed panels combining 106 modifying 111 Primary Rack Position field 62, 123 primary sample, used with multiple panels 63

Primary Tube	Q
Rack Map 61	
Type field 62	quitting the software 76
Type menu 122	_
primary tube rack	R
cage closing and locking 129 described 20 during syringe replacement 193 loading 128 locking into position 197 troubleshooting 225 unlocking 119 troubleshooting 220, 226 tube compatibility 253 primary tubes maximum number of pierces 92 maximum volume 95 re-capping 126 supported 32 venting 127 priming the instrument 43 Print dialog 65 printing log, txt, xml files 75 worklist 65 probe accuracy and precision after changing 243 attaching new 186 counter 58, 175, 189 described 20 homing 203 replacement 176, 178 signs of wear 117 troubleshooting 222–224 pumps inspecting 116 syringe 20, 116	rack carousel 26, 130, 144, 253 primary tube 253 reagent 23, 135, 254 ReadMe file 52 Reagent Rack Editor 101, 105 Rack ID choices 83 Rack ID menu 58, 132 Rack Map 61 reagent cap holder 25 incubation time 85 list, adding reagent 97 lot IDs 137 preprogrammed, list of 81 rack 23 custom 100, 101 preprogrammed 78 removal 135 specifications 254 Reagent List 97, 103 reagent use optimizing 256 volume definitions 256 with multi-dispense 256 reference point extension 158 removing carousel rack 144 Replace Probe dialog 182 replacing probe 176, 178 waste cap trap 47 waste tank cap 46, 48 results, troubleshooting 215 RS232 cable 34, 50

278

5	sneath fluid 2/
cafaty cover 10	shutdown
safety cover 19	daily 150
sample	extended non-use 170
aborting run 147	Shutdown button 76
amount dispensed 85	Sigma Antifoam A Concentrate 33
deleting from worklist 65	Skip Initial Sample Mix checkbox 58
ending a run 144	software
maximum volume per primary	compatibility 50
tube 95	files, log 75
rocker 20	installation 51
settling 125	log in 56
traceability of 63	quitting 76
types 16, 119	requirements 50
Sample ID	shortcut 52
field 121	startup 55
invalid 62	troubleshooting 228-240
required 63, 120	uninstalling 54
unique 120	worktable 60
Sample Prep Assistant shutdown	spare parts
dialog 76	ordering 264
sample volumes	part numbers 267
definitions 258	specifications 252, 261
minimum 259	standard deviation, calculating for fluid
Sarstedt tubes 24, 32, 253, 270	weight 248
scanner, barcode 29	startup
seals, syringe 116	daily 114
secondary tubes	software 55
adding 91	Status field 59, 141
defined 26	storage, instrument 170
label thickness 26	Streck Cyto-Chex tubes 32, 253, 270
per panel 92	switch, power 34
rack 26	syringe pumps
supported 33	described 20
Secondary Tubes Carousel Position and	inspecting 116
Name field 59, 131	troubleshooting 219
serial cable 34, 50	Syringe Replacement Procedure
serial number 71	dialog 190
Setup	syringes
Reference Point dialog 157, 161, 188	installed for upgrades 50
report 161	

removing 194	primary tube rack 220, 226
replacing 195, 243	cage 225
system	probe 222–224
components 18	results 215
requirements 32	software 228–240
-	error messages 230
Т	syringe pumps 219
	timer 218
tanks	Trucount controls 85
capacities 37, 261	tubes 218, 225
compatible fluids 37	valves 220
connector colors 42	wash station 227
installation 38	waste
label colors 40, 42	port 220
level	tank 220
indicators 45, 68	worklist 62, 228
sensors 27	Trucount controls 23, 85
Task Level menu 71	Trucount tubes see BD Trucount tubes
technical assistance xiii	tubes
text conventions xii	13-mm diameter 24
timer, troubleshooting 218	15-mL conical 24
tower, fluidics 27	16-mm diameter 24
traceability of samples 63	adapters 22, 254
translated documentation xiii	BD Vacutainer 32, 253
troubleshooting	blank 96
accuracy and precision 214, 215	compatibility 26
bulk fluids tanks 220	maximum volumes 95
carousel rack 220	port, open 24
computer 221	protocols
fluidics system 221	specifying 94
incubation time 146, 218	values 94
indicator lights 222	racks 21
instrument 214–227	Sarstedt 24, 32, 253, 270
keyboard 228	secondary see secondary tubes
logging in 228	Streck Cyto-Chex 32, 253, 270
lyse 215	troubleshooting 218, 225
mixing 220	,
monitor 221, 222	U
mouse 228	
panels 228	uninstalling software 54
password 228	User

Administration dialog 70	tank 27, 42
Name 56	adding bleach 37
user accounts	capacity 37
creating 71	emptying 46, 170
deleting 72	filter cap 46, 48
user-defined panels	level sensor 42
maximum volume per tube 95	replacing cap 46, 48
primary tube parameters 92	troubleshooting 220
tube variables 255	wedge, keyboard 50
types 85	window
71	Prep Worklist 57
V	Reagent List 97, 103
V	worklist
Vacutainer tubes 32	defined 57
valves	deleting information from 65
described 20	entering information into 61
shown 116, 196	last line of 63
troubleshooting 220	messages 45
-	opening saved 118
W	printing 65
••	resizing fields 64
wash station	troubleshooting 62, 228
described 20	worktable
troubleshooting 227	components 60
waste	defined 58
biohazardous 46, 47	instrument 19
cap trap	motiument 17
draining 47	V
replacing 47	X
in-line filter	XML files 67
assembly 28	
removing 164	Z
replacing 163	_
service 163	Z-rack
port	described 20
location 117	probe replacement and 183
troubleshooting 220	shown 155
C	

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