BD FACS™ Sample Prep Assistant III

Instructions For Use

23-20278-01
1/2021

IVD

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Trademarks

This product includes software developed by the Apache Software Foundation (apache.org).

Regulatory Information

For In Vitro Diagnostic Use.

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.

This Class A digital apparatus meets all requirements of the Canadian Interference-Causing Equipment Regulations. Cet appareil numérique de la classe A respecte toutes les exigences du Réglement sur le matériel brouilleur du Canada.

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
23-13406-00 Rev. 01	5/2011	Initial release
23-13406-01	9/2013	Addition of the BD FACSCalibur™ system to the intended use statement.
23-13406-02	4/2016	Updated Software version to 5.0 and welcome screen in Software Installation and Startup section
23-20278-00	9/2019	Updated new part number, Diva 9.0 and Windows 10 information.
23-20278-01	1/2021	Updated to include beveled probe.

Contents

1

Introduction

This chapter covers the following topics:

About this Document

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

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

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

Conventions

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

Symbol[a]	Meaning
	**CAUTION**: hazard or unsafe practice that could result in material damage, data loss, minor or severe injury, or death
	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.

Convention	Use
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 *P*.

Technical Assistance

For technical questions or assistance in solving a problem:

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

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

When contacting BD, have the following information available:

- Product name, part number, and serial number, and details of recent system performance.
- Error messages, if any.

For support, go to bdbiosciences.com.

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

Intended Use

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

Indications for Use

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

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

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

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

- BD Tritest™ CD3/CD19/CD45 with or without BD Trucount™ Tubes
- BD Tritest™ CD3/CD4/CD45 with or without BD Trucount™ Tubes
- BD Tritest™ CD3/CD8/CD45 with or without Trucount™ Tubes
- BD Tritest™ CD4/CD8/CD3 with BD Trucount™ Tubes

For in vitro diagnostic use.

Validated Sample Types

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

- Human peripheral whole blood in K_2 or K_3 EDTA
- BD Multi-Check™ control
- BD Multi-Check™ CD4 low control

2

System Overview

This chapter covers the following topics:

Introduction

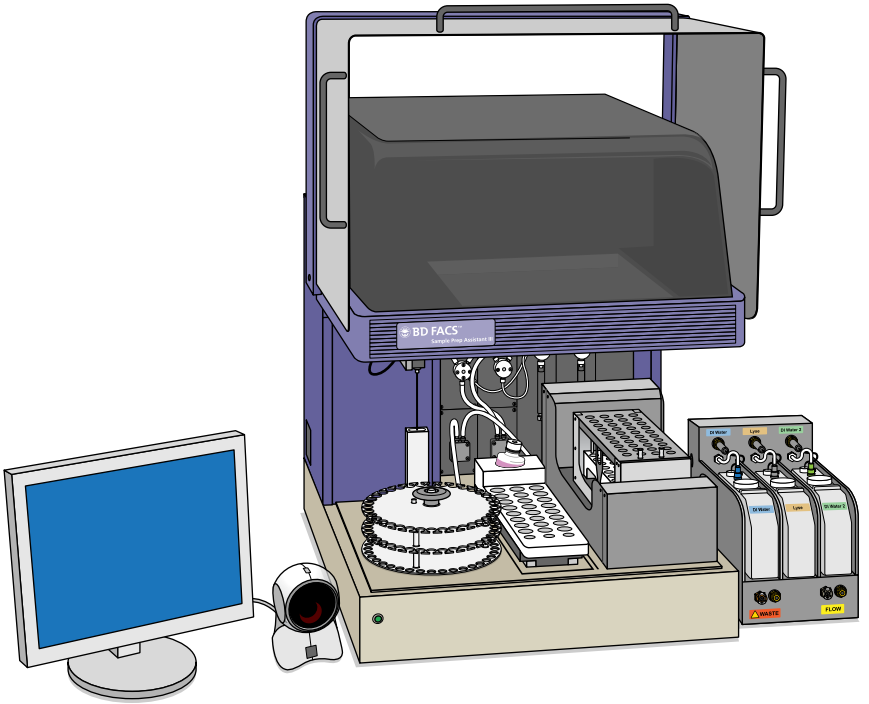
The SPA III instrument:

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

The workstation:

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

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


BD FACS
Sample Prep Assistant
DI Water
Lyse
DI Water 2
WASTE
FLOW

System Components

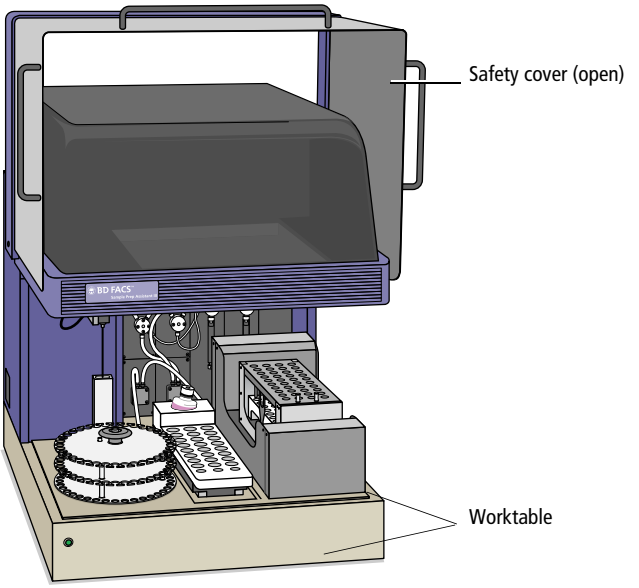
The SPA III consists of a sample preparation unit connected to an external computer workstation via an RS232 cable. A barcode reader enhances system functionality. The instrument ships with an accessory kit

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

Component	Section
Instrument	See Instrument on page 21.
Primary tube rack (with tube adapters)	See Primary Tube Rack on page 23.
Reagent rack	See Reagent Rack on page 25.
Carousel rack	See Loader Carousel Rack on page 27.
Fluidics tower	See Fluidics on page 28.
Barcode reader	See Barcode Scanner on page 29.
PC workstation	See your PC workstation documentation.

Instrument

Safety Cover and Worktable

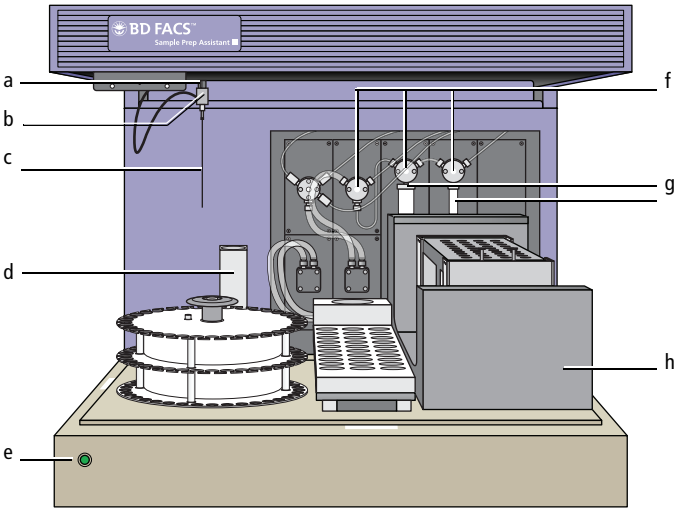


The SPA III *safety cover* protects the operator from moving parts and biological hazards.

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.

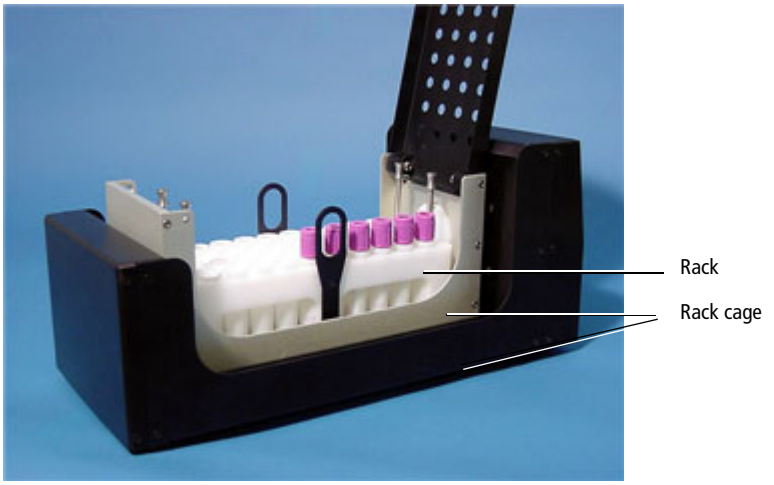
Instrument Components

Figure 2-1 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.
c	Probe	Dispenses samples, reagents, and bulk fluids. Pierces the primary sample tube caps.
d	Wash station	Washes the probe and disposes of waste.
e	On/Off light	Signals on/off status (of the instrument only).
f	Valves	Function as part of the fluidics system.
g	Syringe pumps	Move 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 24).
  - 11-mm diameter tubes using black adaptors
- One 13-mm tube rack that holds 13-mm diameter tubes.

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

Tube Adapters

Figure 2-2 White tube adapters for primary tube rack

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

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

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

Reagent Rack

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

Figure 2-3 Reagent rack

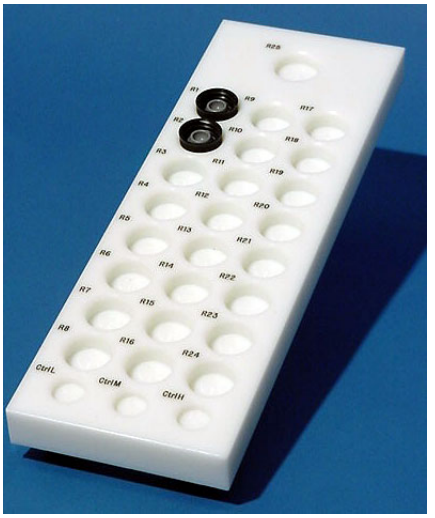
Each reagent rack position is numbered. The reagent vials sit in the rack at a slight angle to maximize reagent usage.

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

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

Reagent Cap Holder

Figure 2-4 Reagent cap holder



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

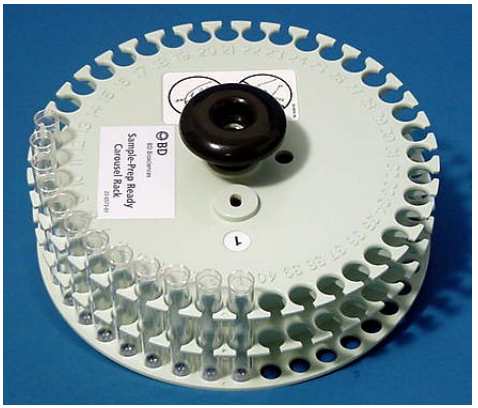
Loader Carousel Rack

The carousel rack (Figure 2-5) holds up to 40 uncapped 12 x 75-mm polystyrene secondary tubes or BD Trucount™ Tubes and is compatible with the BD 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 2-5 Carousel rack



Tube Labels

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

Fluidics

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

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

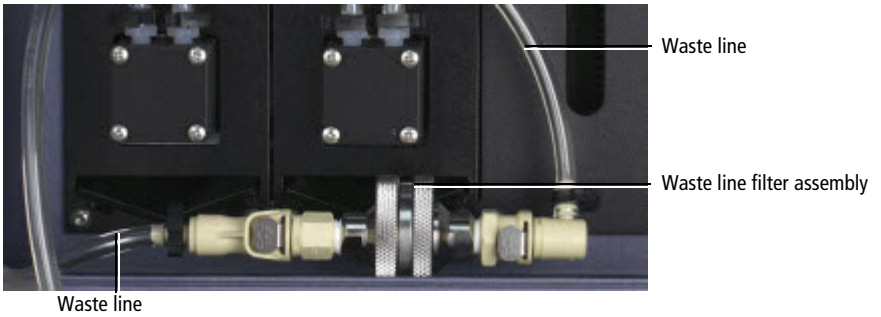
Level sensors indicate when the tanks and sheath fluid need service. Remove the tanks from the tower before emptying and refilling them.

See Fluid and Waste Tank Installation on page 38 and Emptying the Waste Tank on page 45.

Waste In-Line Filter Assembly

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

The waste in-line filter is serviced each time the instrument probe is replaced. See Monthly Servicing of Waste In-Line Filters on page 139 and Replacing the Probe on page 152.



Barcode Scanner

The Orbit® Presentation Laser Scanner supports many different barcode standards, and comes pre-programmed to read the ISBT 128 standard. The scanning window tilts up to 30° for easier scanning.

LEDs and audio signals indicate when the scanner is on and when a barcode has been successfully scanned.

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.
	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.

3

Instrument Requirements, Installation, and Startup

This chapter covers the following topics:

Required Equipment

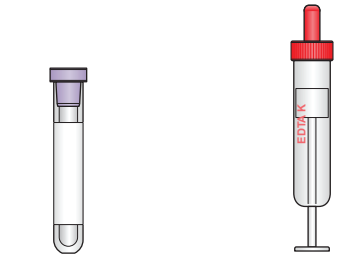
The tubes in Table 3-1 have been validated for use on the SPA III. See Validated Sample Types on page 16.

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

Table 3-1 Supported primary tubes

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	
Sarstedt S-Monovette®	2.7 mL, 11 x 66 mm	16 mm with black adapters
	2.6 mL, 13 x 65 mm	13 mm or 16 mm with white adapters
	3.4 mL, 13 x 65 mm	
	4.9 mL, 13 x 90 mm	
	4.0 mL, 15 x 75 mm	16 mm
	5.5 mL, 15 x 75 mm	

Figure 3-1 Examples of supported tube types



BD Vacutainer

Sarstedt
S-Monovette

The primary tube racks support up to 40 capped tubes.

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

Table 3-2 Required materials

Material	Details
Secondary tube types (up to 40 uncapped secondary tubes)	Corning 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

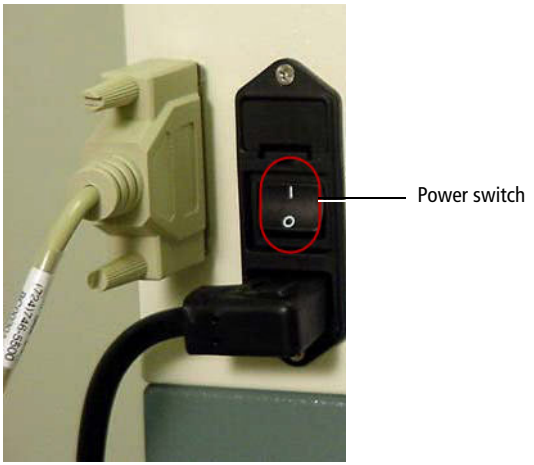
A BD service representative will initially install your SPA III. The following instructions are included if you need to reattach system components.

To install the SPA III:

1 Plug the following into their ports in the instrument.

- RS232 cable (connects to computer workstation)
- Power cord

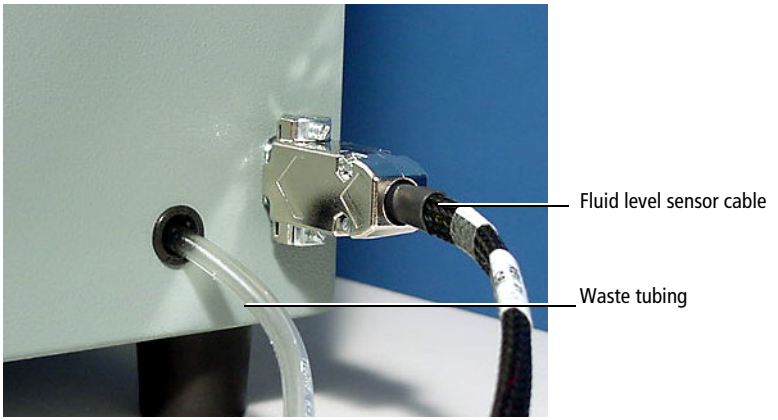
Figure 3-2 Power switch on instrument left side panel



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

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

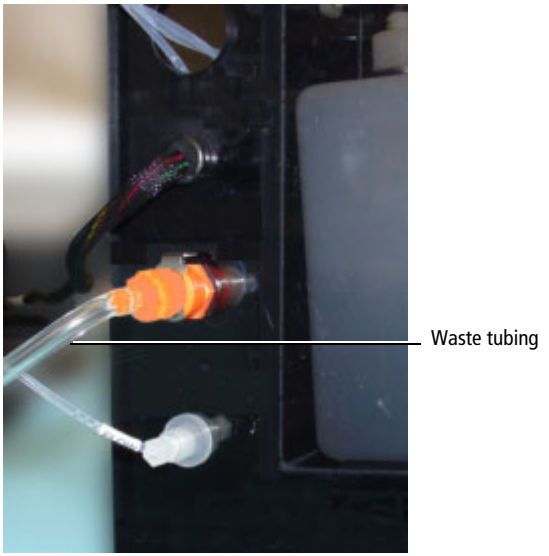
Figure 3-3 Instrument, right side panel



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

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

Figure 3-4 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 3-2 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 3-3 lists the bulk fluids required for the SPA III, including compatible fluids and capacities.

Table 3-3 Bulk fluids required for the SPA III

Item	Compatible Fluid or Type(s)	Capacity	Details
DI water tank	Deionized (DI) water	1 L	N/A
Lyse tank	BD FACS™ lysing solution (1X)	1 L	Dilute 10X solution. See the *BD FACS™ Lysing Solution* 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™ solution	20 L	BD FACSFlow™ 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 3-3 on page 37). Dilute BD FACS™ lysing solution as described in the package insert before filling the lyse tank.

To fill the fluid and waste tanks:

1 Fill the DI water and lyse tanks completely.

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

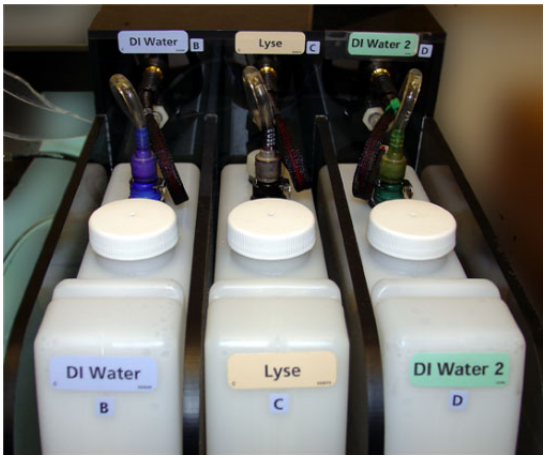
3 Add 500 µL of Sigma Antifoam A Concentrate to the waste tank to prevent foaming of potentially biohazardous waste around the cap.

4 Replace the caps on each tank and hand tighten.

Fluidics Tower

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

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



To install the tanks:

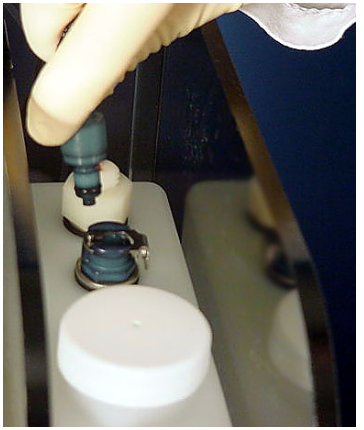
1 Place the tanks in their appropriate locations in the fluidics tower.

⚠ To ensure the appropriate solutions are dispensed, do not switch tank positions.

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

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.



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

Flow Container and Waste Tank

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

To install the flow container and waste tank:

1 Install the fluid sensors in the flow container and waste tank.

2 Replace each cap and hand-tighten.

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

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

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.

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

Priming the Fluid Lines

The SPA III will automatically self-prime:

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

Manually Priming the Instrument

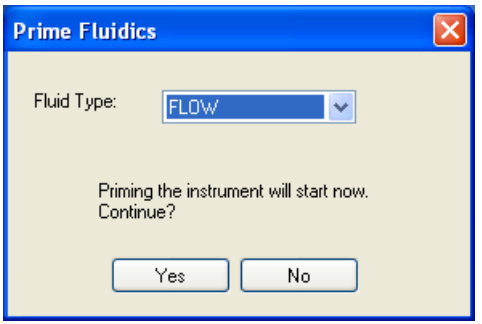
NOTE You must manually prime the instrument if you have to change the BD FACSFlow™ cubitainer during a run. See During a Run on page 44.

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

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

The **Prime Fluidics** dialog opens.

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



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

3 Click **Yes**.

The instrument prime begins.

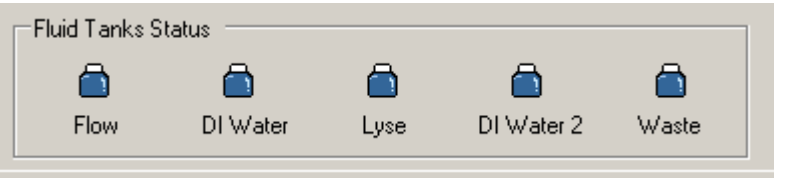
Maintaining the Fluid and Waste Tanks

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

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

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

Figure 3-6 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 FACSFlow™ solution is low. It does not resume processing until you service the flow or waste tanks.

NOTE When you change the BD FACSFlow™ cubitainer during a run, you **must** perform a manual prime with Flow before resuming operation, to clear any bubbles in the fluid line. See Manually Priming the Instrument on page 43.

If the SPA III senses that the lyse or either DI water tank fluid level is low during a run, processing continues. The indicators turn on, a message is displayed, and an alarm sounds. To turn off the alarm, you must acknowledge the message.

The tank indicators will clear after you service the tanks.

Emptying the Waste Tank

Empty the waste tank:

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

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

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

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

To empty the waste tank:

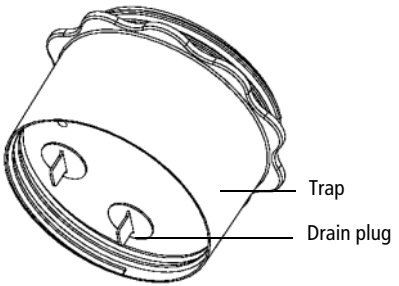
1 Disconnect the waste fluid and waste sensor lines from the instrument.

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

2 Take the waste tank to the appropriate location for emptying.

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

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



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

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

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

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

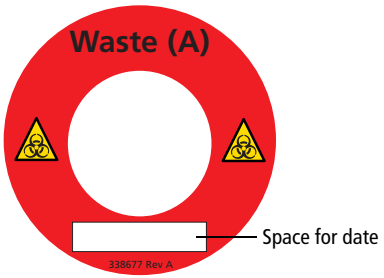
7 Replace the trap and attached filter cap.

To prevent over-pressurization during fluidics startup, do not overtighten the waste tank baffle or attached filter cap. Tighten each component only until it is hand-tight. Do not use sealants such as Teflon® tape or other adhesives.

If one month has passed since you last changed the cap, replace the filter cap with a new one.

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

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4

Software Installation and Startup

This chapter covers the following topics:

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 v8.1 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.

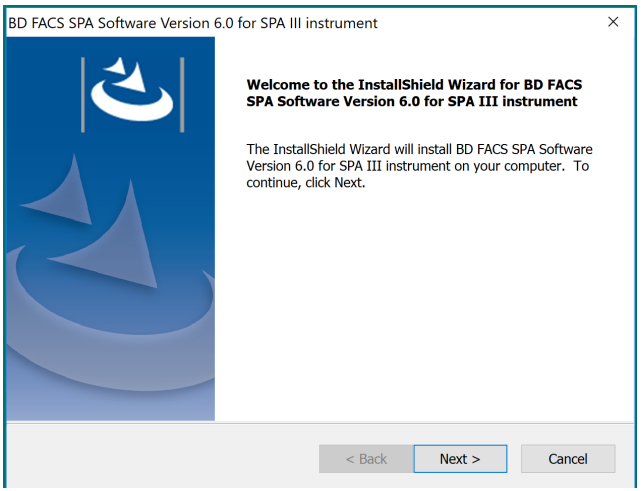
Before you begin, close any open programs.

To install the software:

1 Insert the SPA software version 6.0 or later installation USB into the USB drive.

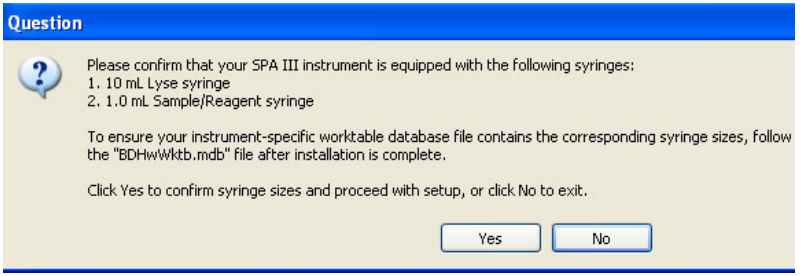
The installer should start up automatically. If it doesn't, use Windows Explorer to view the USB contents, and double-click the **Setup.exe** icon.

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 (x86)\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:\ProgramData\BD\FACS SPA\DataFiles**.** The file name is BD-HwWktb.mdb. Under normal circumstances, you do not have to do anything related to this file.

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

Table 4-1

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**.

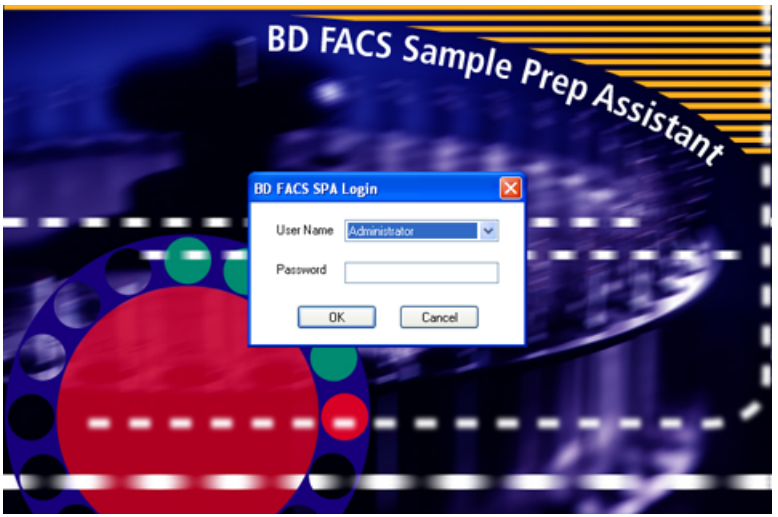
3 Select *BD FACS™ SPA Software Version 6.0 for SPA III instrument* from the list, right-click and then select **Uninstall**.

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 4-2 provide a brief orientation to the Prep Worklist window and its features.

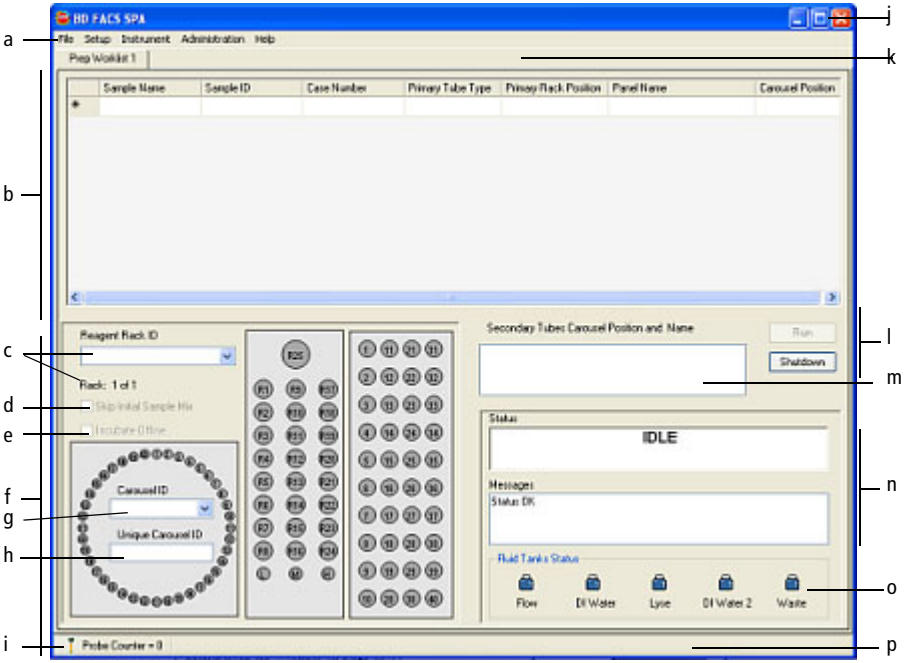


Table 4-2 Prep Worklist window

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).
c	Reagent Rack ID Multi-rack number	Select the reagent rack for a panel from the menu. The current rack is indicated below the menu.

Table 4-2 Prep Worklist window

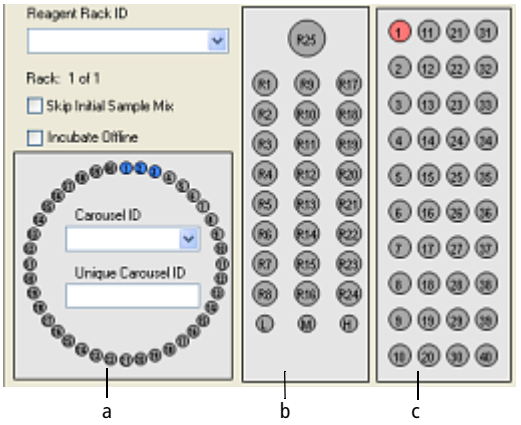
Component		Function
d	Skip Initial Sample Mix checkbox	Skips the initial 5-minute mix of samples in the primary tube rack. The checkbox is cleared by default.
e	Incubate Offline checkbox	Enables the option of performing the final incubation offline. The checkbox is cleared by default.
f	Worktable	Graphic 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 to 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.
l	Function buttons	Control instrument functions. The available function buttons depend on processing context, and include: • **Run.** Start processing a rack. • **Pause.** Pause processing. • **Abort.** Cancel a rack. • **Shutdown.** Exit the software and end a run. • **Close.** Close the selected worklist.
m	Secondary Tubes Carousel Position and Name field	Shows the secondary tubes associated with each panel for a selected Sample ID.

Table 4-2 Prep Worklist window

Component		Function
n	Status and Messages fields	Display current instrument processing and diagnostic messages.
o	Fluid Tanks Status	Indicates when the waste and fluid tanks need filling or emptying (See Fluid Tanks Status on page 68.)
p	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.



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

Worklist

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

	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:

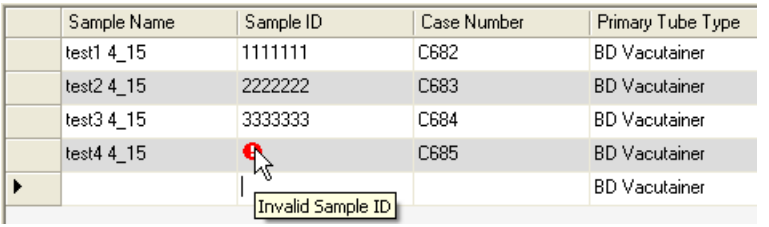
- **Saved and re-used.** See Initializing a Worklist on page 98 and Ending a Run on page 120.
- **Printed.** See Printing a Worklist on page 65.
- **Exported.** See Using SPA III Worklists with Other BD Software on page 67.

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 99.

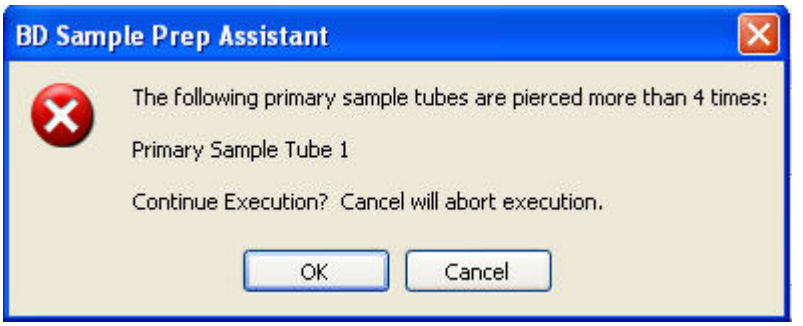
As you enter information into the worklist, symbols are displayed to indicate the entry status:

Symbol	Meaning
	Indicates which line is selected.
	Indicates the line being edited.
	Indicates the next line for which there are no current entries
	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 *Invalid Sample ID* indicates that the ID is missing. 

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.

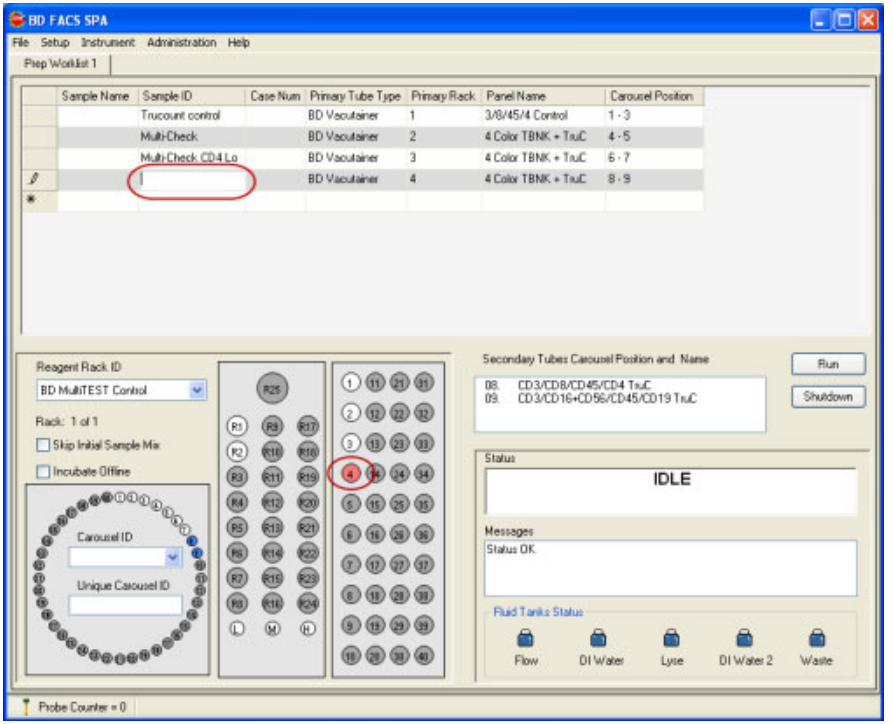


Entering the Last Line of a Worklist

⚠ 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.

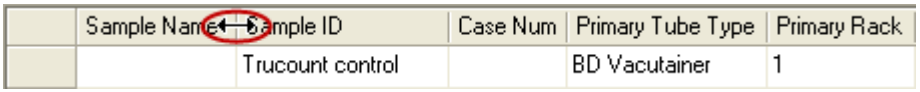
To assure traceability of samples processed by the SPA III, save and print the worklist before running samples.

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.



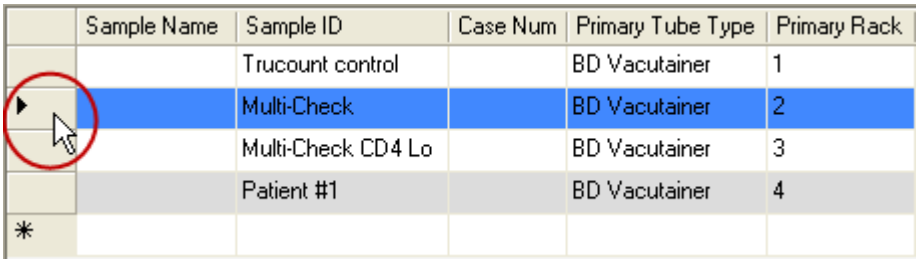
Deleting Samples from a Worklist

To delete a sample line from the worklist:

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

2 Press the **Delete** key.

Figure 4-1 Deleting a line of information



Printing a Worklist

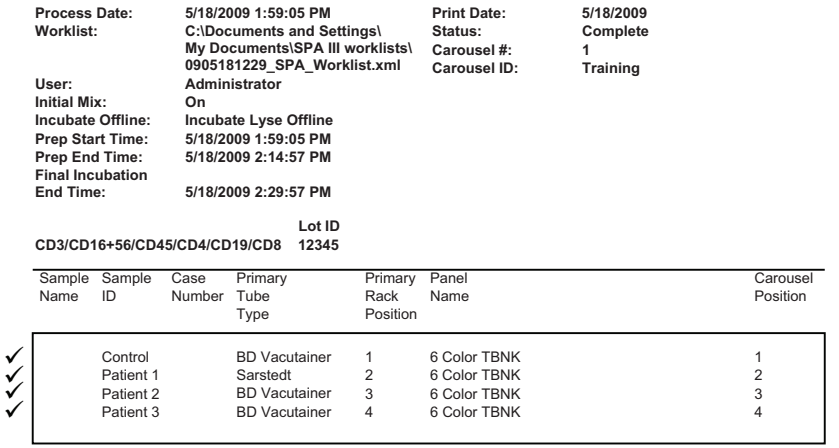
To print the currently open worklist:

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

The **Print** dialog opens.

2 Click **OK.**

A print version of the open worklist is sent to the printer.



Process Date:	**5/18/2009 1:59:05 PM**			**Print Date:**	**5/18/2009**		
Worklist:	**C:\Documents and Settings\ My Documents\SPA III worklists\ 0905181229_SPA_Worklist.xml**			**Status:**	**Complete**		
				Carousel #:	**1**		
User:	**Administrator**			**Carousel ID:**	**Training**		
Initial Mix:	**On**						
Incubate Offline:	**Incubate Lyse Offline**						
Prep Start Time:	**5/18/2009 1:59:05 PM**						
Prep End Time:	**5/18/2009 2:14:57 PM**						
Final Incubation End Time:	**5/18/2009 2:29:57 PM**						
			Lot ID				
CD3/CD16+56/CD45/CD4/CD19/CD8			**12345**				

	Sample Name	Sample ID	Case Number	Primary Tube Type	Primary Rack Position	Panel Name	Carousel Position
✓		Control		BD Vacutainer	1	6 Color TBNK	1
✓		Patient 1		Sarstedt	2	6 Color TBNK	2
✓		Patient 2		BD Vacutainer	3	6 Color TBNK	3
✓		Patient 3		BD Vacutainer	4	6 Color TBNK	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® Worklist Manager on a system running Mac OS® 9, you must export your SPA III worklist to make it available for use.

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

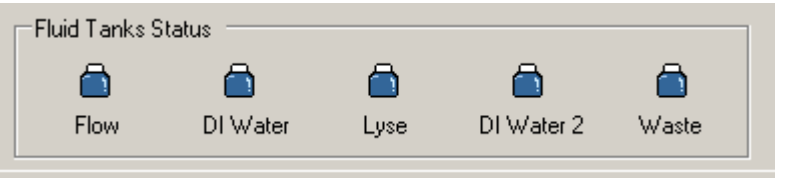
2 Locate and select the worklist file to export.

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.

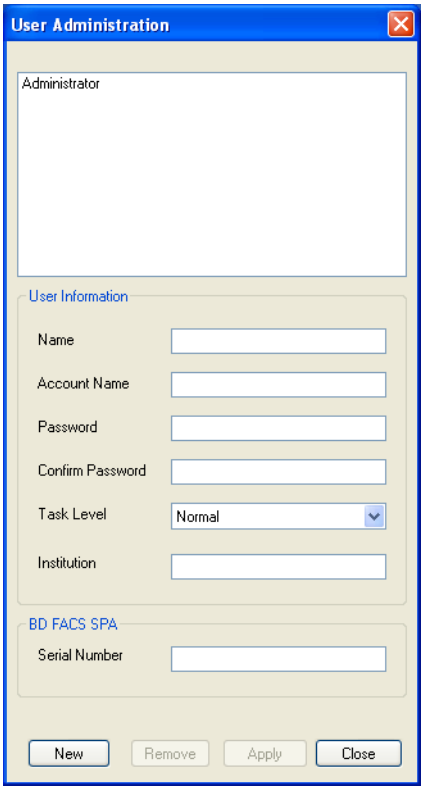
4 Click **OK**.

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.

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

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.

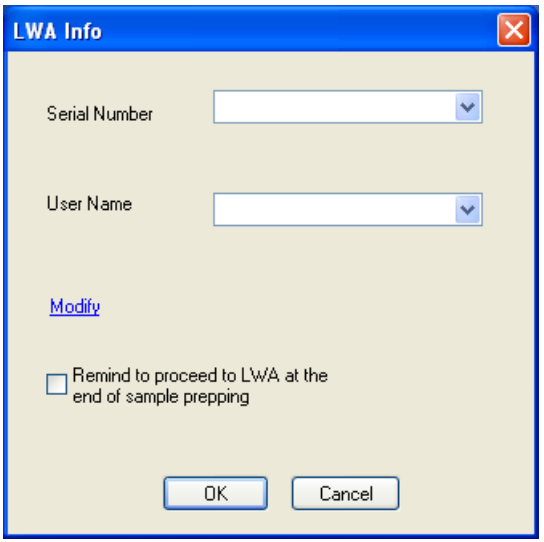
Entering LWA Information

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

1 Select **Administration > LWA Info** from the main menu.

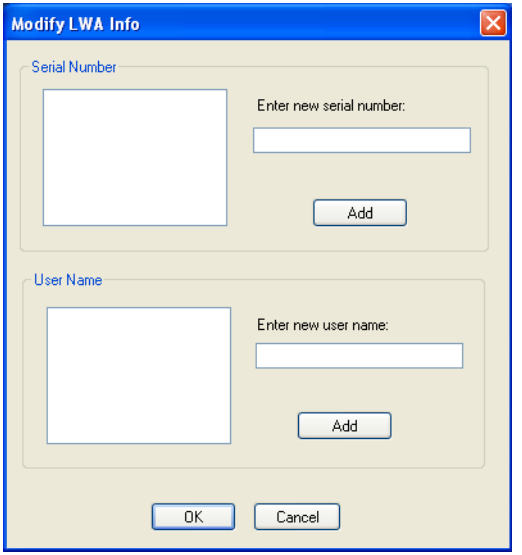
The **LWA Info** dialog opens.

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



3 (Optional) Click the **Modify** link to add serial numbers or user names to the lists.

The **Modify LWA Info** dialog opens.



- To add a new serial number or user name, type the new item in the corresponding text field and click **Add**.
- To delete an existing serial number or user name, select the item and press the **Delete** key.

4 (Optional) Select the **Remind to proceed to LWA at the end of sample prepping** checkbox.

When this option is enabled, a confirmation dialog opens after all materials have been dispensed during a run (see LWA Information on page 119).

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 C:\ProgramData\BD\FACS SPA\DataFiles folder.

- **Log files.** An instrument log file (instrument*datetimecode*.log*)* is created each time you start the software and log in. The files are stored in the C:\BD\FACS SPA\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 C: BD\FACS SPA folder. The error message file is overwritten in each session that an error occurs.

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

Copying Log Files

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

Viewing and Printing Log Files

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

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

Deleting Log Files

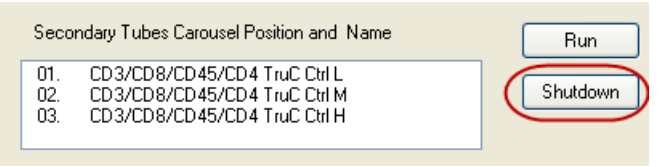
Use the standard Windows file system functions to delete a log file.

Shutting Down the Software

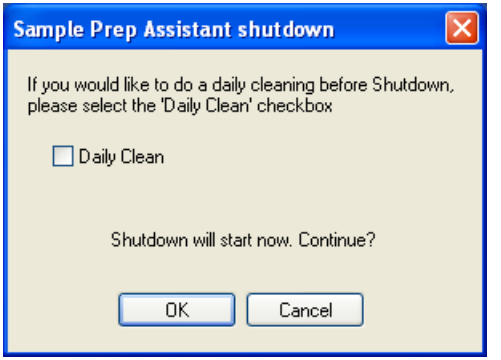
To quit the software:

1 Do one of the following:

- Select **File > Shutdown.**
- Click the **Shutdown** button on the right side of the application window.



The shutdown dialog opens.



2 Leave the **Daily Clean** checkbox cleared.

3 Click **OK**.

5

Panels and Reagent Racks

This chapter includes the following topics:

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 86).

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

- BD Tritest
- BD Multitest™
- BD Accuracy and Precision

To view the pre-programmed panels:

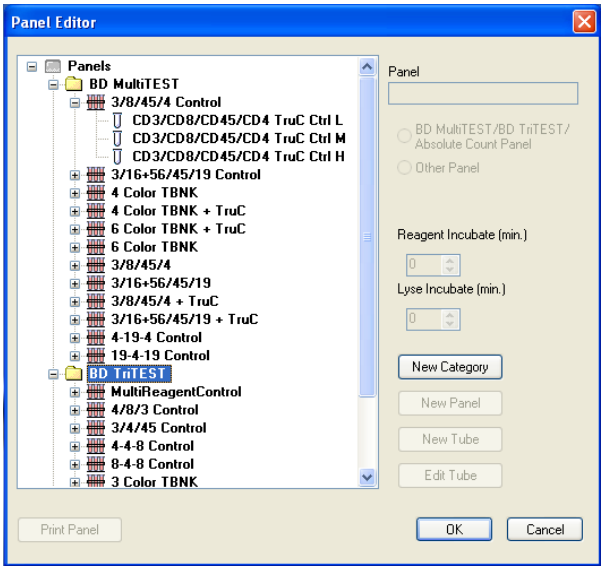
1 Select **Setup** > **Panel Editor** from the main menu in the Prep Worklist window.

The Panel Editor opens (Figure 5-1).

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

2 Expand the categories and panels by clicking the plus sign next to each item.

Figure 5-1 Panel Editor



NOTE Pre-programmed categories, panels and tubes appear in bold black text.

A reagent rack is a set of reagents and their positions in a rack (rack map). SPA software also includes pre-programmed reagent racks that correspond to the reagents and controls required by the pre-programmed panels.

Table 5-1, Table 5-2, and Table 5-3 list the pre-programmed panels and their secondary tube reagents, as well as the corresponding pre-programmed reagent racks. See Reagent Rack Setup on page 109 for specific reagents associated with each rack.

Table 5-4 lists the pre-programmed reagent racks and their reagents and controls.

BD Tritest Panels

Table 5-1 BD Tritest panels

Panel	Secondary Tube	Reagents	Reagent Rack ID Choices
MultiReagentControl (BD Trucount™ 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™ Tubes)	Tube 1	CD4/CD8/CD3 TruC Ctrl L	3-Tube T cell Control
	Tube 2	CD4/CD8/CD3 TruC Ctrl M	
	Tube 3	CD4/CD8/CD3 TruC Ctrl H	
3/4/45 Control (BD Trucount™ Tubes)	Tube 1	CD3/CD4/CD45 TruC Ctrl L	BD Tritest™ Control or 3-Tube T cell Control
	Tube 2	CD3/CD4/CD45 TruC Ctrl M	
	Tube 3	CD3/CD4/CD45 TruC Ctrl H	
4-4-8 Control (BD Trucount™ Tubes)	Tube 1	CD3/CD4/CD45 TruC Ctrl L	BD Tritest™ Control or 3-Tube T cell Control
	Tube 2	CD3/CD4/CD45 TruC Ctrl M	
	Tube 3	CD3/CD8/CD45 TruC Ctrl H	

Table 5-1 BD Tritest panels

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

BD Multitest™ Panels

Table 5-2 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™ 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™ Tubes)	Tube 1	CD3/CD16+CD56/CD45/ CD19 TruC Ctrl L	BD Multitest™ Control
	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™ or BD Multitest™ Control
	Tube 2	CD3/CD16+CD56/CD45/ CD19	
4 Color TBNK + TruC (BD Trucount™ Tubes)	Tube 1	CD3/CD8/CD45/CD4	BD Multitest™ or BD Multitest™ Control
	Tube 2	CD3/CD16+CD56/CD45/ CD19	
6 Color TBNK + TruC (BD Trucount™ Tubes)	Tube 1	CD3/CD16+CD56/CD45/ CD4/CD19/CD8	BD 6 Color
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™ or BD Multitest™ Control

Table 5-2 BD Multitest panels

Panel	Secondary Tube	Reagents	Reagent Rack ID Choices
3/16+56/45/19	Tube 1	CD3/CD16+CD56/CD45/ CD19	BD Multitest™ or BD Multitest™ Control
3/8/45/4 + TruC	Tube 1	CD3/CD8/CD45/CD4 TruC	BD Multitest™ or BD Multitest™ Control
3/16+56/45/19 + TruC	Tube 1	CD3/CD16+CD56/CD45/ CD19 TruC	BD Multitest™ or BD Multitest™ Control
4-19-4 Control (BD Trucount™ Tubes)	Tube 1	CD3/CD8/CD45/CD4 TruC Ctrl L	BD Multitest™ Control
	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™ Control
	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 5-3 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 5-4 Pre-programmed reagent racks

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

Table 5-4 Pre-programmed reagent racks (continued)

Reagent Rack ID	Reagents	Reagent Rack Position
BD Tritest™ 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	H
BD Tritest™ 3-Tube T cell	CD4/CD8/CD3	R1
	CD3/CD4/CD45	R2
	CD3/CD8/CD45	R3
BD Tritest™ 3-Tube T cell Controls	CD4/CD8/CD3	R1
	CD3/CD4/CD45	R2
	CD3/CD8/CD45	R3
	BD Trucount™ Controls	
	• Ctrl L	L
	• Ctrl M	M
	• Ctrl H	H
BD MultiReagent Control	CD4/CD8/CD3	R1
	CD3/CD19/CD45	R2
	CD3/CD16+CD56/CD45	R3
	BD Trucount™ Controls	
	• Ctrl L	L
	• Ctrl M	M
	• Ctrl H	H
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 pre-programmed panel and the tube protocol settings associated with its secondary tubes.

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

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

Maximum Number of Secondary Tubes Per Panel

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

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

BD MultiTEST/BD TriTEST/Absolute Count Panels

From a single draw, a maximum of 200 µL of sample can be dispensed for BD MultiTEST/BD TriTEST/Absolute Count panels. With a four cap-pierce

limit, a maximum of 800 µL of sample can be dispensed to the secondary tubes of these panels.

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

NOTE The carousel rack holds up to 40 secondary tubes.

Creating a New Reagent Rack

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

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

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

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

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

NOTE In this section, you use the Reagent Rack Editor to create a new reagent rack. The Reagent Rack Editor can also be used to copy and edit existing reagent racks (see Adding Controls to a New Reagent Rack on page 90).

To create a new reagent rack:

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

The Reagent Rack Editor opens.

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

The default name *New Category* is assigned.

3 Type a name for the new panel category.

a Right-click the new category and select **Rename**.

b Type the new name.

4 Select a new reagent rack category, and click **New Rack**.

The default name *New Rack* is assigned.

5 Type a name for the new reagent rack.

a Right-click the new rack and select **Rename**.

b Type the new name.

6 Use one of the following methods to open the Rack Map Editor:

- Select the new rack and click the **Edit Rack** button.
- Right-click the new rack and select **Properties**.
- Double-click the new rack.

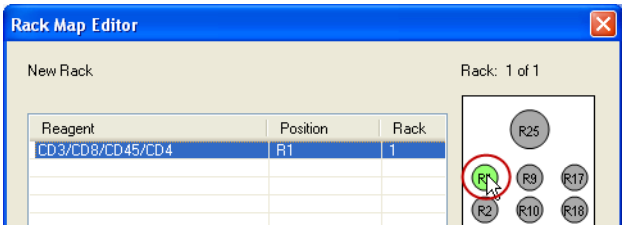
7 Click the **Reagent List** link.

The Reagent List window is displayed. The Reagent List contains all of the BD pre-programmed reagents.

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

8 Drag a reagent from the **Reagent List** to the **Reagent/Position** table in the Rack Map Editor.

9 Assign a rack position for the reagent by selecting the reagent in the Rack Map Editor, then clicking on a map position.



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

11 Click **OK** to save the reagent rack.

12 The Rack Map Editor and the Reagent List close.

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

14 Click **OK** to close the Reagent Rack Editor.

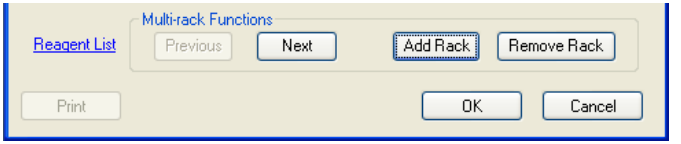
15 Verify that your new reagent rack is available in the **Reagent Rack ID** menu of the **Prep Worklist** window.

Creating Multi-Rack Reagent Racks

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

To create a multi-rack reagent rack:

1 In the Rack Map Editor, click **Add Rack** under **Multi-rack Functions**.



A new rack is created.

2 Move between racks with the **Previous** and **Next** buttons.

3 Add reagents to the added racks as described in Creating a New Reagent Rack on page 87.

Adding Controls to a New Reagent Rack

To add BD Trucount™ controls to a new reagent rack:

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

The Reagent Rack Editor opens.

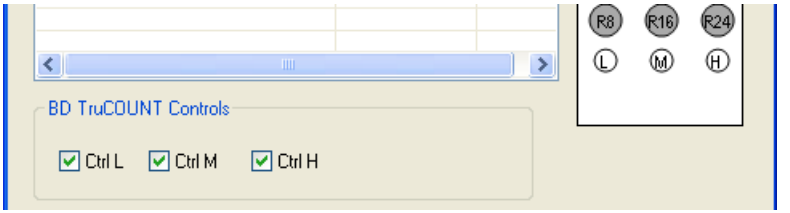
2 Select a new reagent rack without BD Trucount™ controls.

If none exists, create one using the instructions of Creating a New Reagent Rack on page 87.

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.

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6

Sample Preparation

This chapter covers the following topics:

Daily Startup

Perform the daily startup procedure each day before processing samples.

1. Remove reagents from the refrigerator to allow them to warm up to room temperature.
2. Open the safety cover.
3. Close and lock the primary tube rack cage.
4. Close the safety cover.
5. Turn on the instrument power and the computer power.

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

Do NOT start SPA software at this time.

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

6. Empty the waste tank, and then add 1 L of undiluted household bleach and 500 µL of Sigma Antifoam A Concentrate to the tank. See Emptying the Waste Tank on page 45.
7. Ensure that the waste tank fluid and sensor connectors are properly attached to the fluidics tower (front and side connections).
8. Empty any DI water from the previous day.

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

To service a bulk fluid container:

a. Detach the quick-release connector on the fluid tank by pressing the metal clip.

b Disconnect the wire connector from the fluidics tower.

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

See Fluid and Waste Tank Installation on page 38.

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

11 Start the software and log in.

The **Prep Worklist** window opens and the instrument initializes.

12 Open the safety cover.

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 189 for help with identifying and solving the problem.

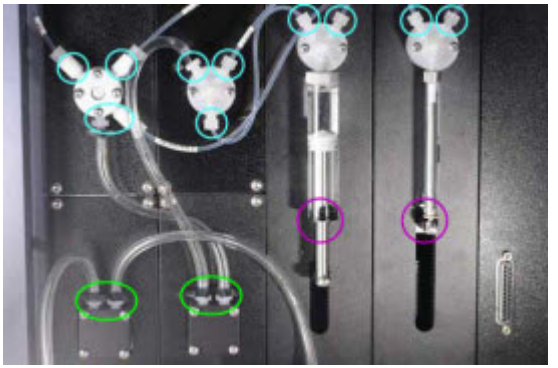
Instrument hardware could be contaminated with biohazardous material. Follow your standard laboratory procedures for biological hazards during all cleaning and maintenance procedures. Wear protective clothing, eyewear, and gloves.

Check for the following:

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

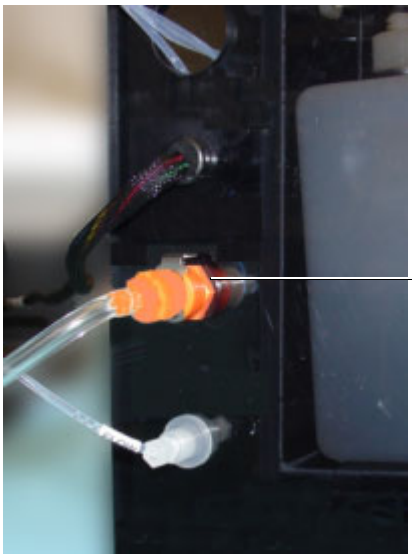
  In Figure 6-1, the valve fittings are circled in blue, the pump fittings are circled in green, and the syringe seals are circled in magenta.

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

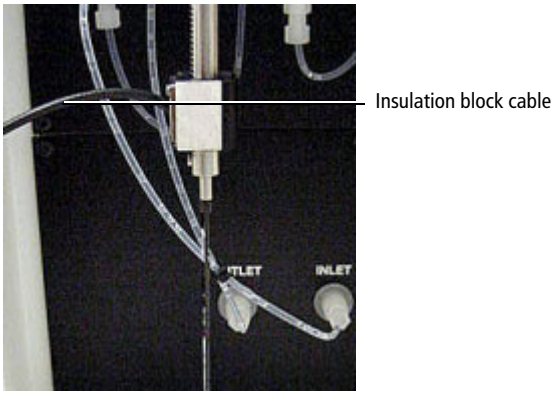
  

- Crimps in the fluidics tubing
- Bubbles in the fluidics tubing. If there are bubbles, see Manually Priming the Instrument on page 43.

- Leaks or mineral deposits around the waste port (left side of fluidics tower)



- Kinks or twists in the insulation block cable



- Signs of probe wear (the probe is no longer straight or the Teflon coating is gone). See Figure 7-8 on page 153.

Setting Up for a Processing Run

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

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.

	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
*							

Preparing Primary Tubes

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

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

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

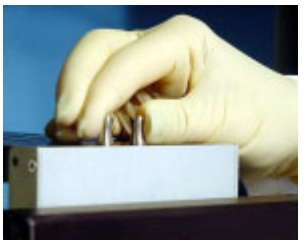
You can mix different tube types in a rack using the tube adapters provided.

If you are running BD Multi-Check controls, see Running BD Multi-Check Controls on page 104.

Loading the Primary Tube Rack

1 Unlock the primary tube rack cage and remove the primary tube rack.

Squeeze the lock mechanism together to unlock and open the cage.



2 Prepare samples for loading into a primary tube rack.

See Minimum Sample Volumes on page 232, to determine the minimum volume required in the capped primary tubes.

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

Sample Entry

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

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

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

To enter sample information:

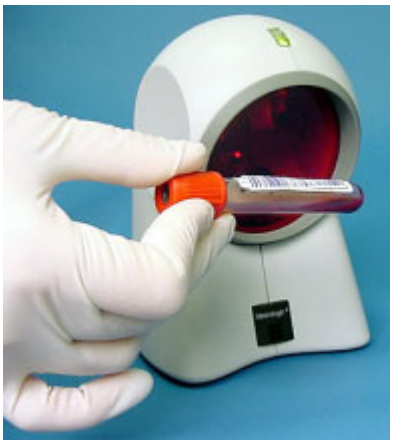
1 (Optional) Type a Sample Name.

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

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

Each tube must have a unique Sample ID.

- To enter the sample identification using the barcode scanner, place the cursor in the appropriate **Sample ID** field (see Figure 6-2) and then swipe the label past the scanning window.



The white indicator light on top will flash once and a beep will sound when a barcode has been successfully scanned.

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

Figure 6-2 Sample ID field

	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
*							

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.

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

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

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

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

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

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

6 Load the primary tube into the primary rack position assigned in the worklist (each position in the primary tube rack has a unique map number).

Figure 6-3 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.

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

To override the software-assigned position:

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

	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
*							

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

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

NOTE To advance to the next sample entry, click in any field in the next row.

Running BD Multi-Check Controls

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

Depending on how often you run controls, you might reach the cap-piercings limit (4) when using BD Multi-Check™ controls in the primary sample rack. See Maximum Number of Secondary Tubes Per Panel on page 86. If this occurs, we recommend replacing the BD Multi-Check™ controls tube cap. Obtain identical caps from BD Vacutainer Plus® plastic serum tubes (Part Number 367812).

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

To replace the cap on a BD Vacutainer® tube:

1. Place the BD Vacutainer® tube cap on the BD Multi-Check™ control tube.
2. Discard the BD Vacutainer® tube.
3. Follow the procedure in Venting Primary Sample Tubes on page 104 before running the control on the SPA III.

Re-capping Primary Sample Tubes

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

If running re-capped primary sample tubes is standard operating procedure in your lab, vent the tubes prior to loading them on the SPA III. We recommend using a device such as the BD Vacutainer® blood transfer device (Part Number 364880). See Figure 6-4 on page 105.

Venting Primary Sample Tubes

Under a biosafety hood, insert the internal needle of the blood transfer device into the tube cap just far enough to break the septum. The pressure above the

sample is released within the biosafety hood. The tube is now ready for loading on the SPA III.

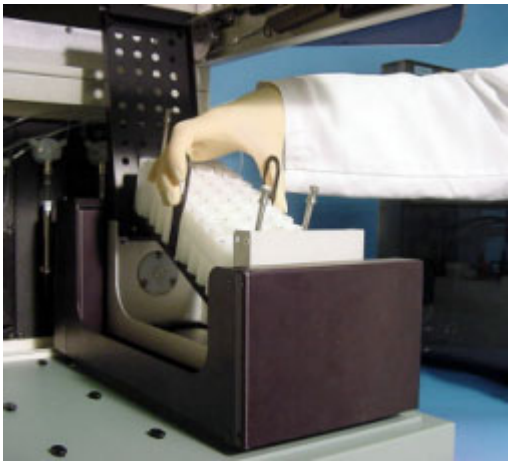
Figure 6-4 BD Vacutainer® blood transfer device

Mounting the Primary Tube Rack

To mount the primary tube rack:

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

Figure 6-5 Placing the primary tube rack in the primary tube rack cage



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

2 Close and lock the primary tube rack cage.

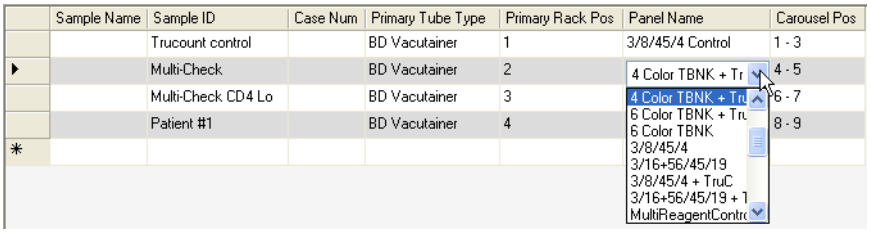
Pinch the latch to lock it.

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

Panel Selection

1 Select a panel from the **Panel Name** menu.

Figure 6-6 Panel Name menu



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

These changes reflect the new panel choice.

Carousel Rack Setup

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

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

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

To set up a carousel rack:

1 (Optional) Enter a Carousel ID number (1 to 16) in the **Carousel ID** field in the center of the carousel rack map (Figure 6-7).

2 (Optional) Scan the carousel barcode or type a unique carousel ID in the **Unique Carousel ID** field.

The ID is limited to a maximum of 22 characters.

3 Label the uncapped secondary tubes.

4 (Optional) Print the worklist and use it for help in loading the carousel rack. See Printing a Worklist on page 65.

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

Figure 6-7 Secondary tube position in carousel rack

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

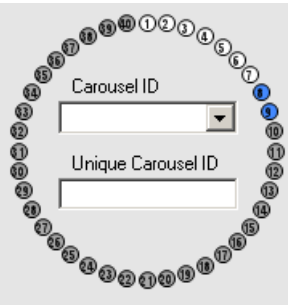
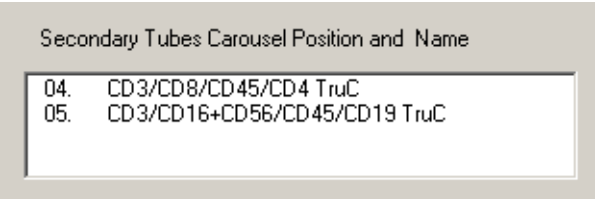


Figure 6-8 Secondary Tubes Carousel Position and Name field



Mounting the Carousel Rack

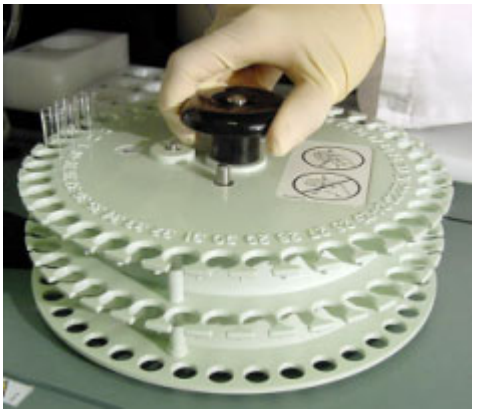
To mount the carousel rack:

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

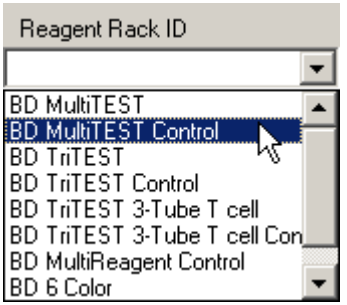
To run BD Multitest™ and BD Tritest™ panels in the same worklist, you must create a new reagent rack. See Creating a New Reagent Rack on page 87.

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

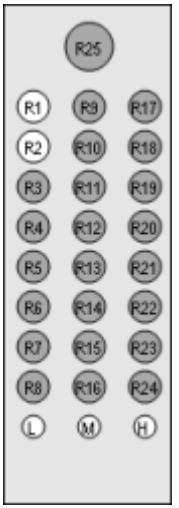
To set up a reagent rack:

1 Verify that the reagents are at room temperature.

2 Select the reagent rack from the **Reagent Rack ID** menu.



The reagent rack map in the worktable display is updated.



Reagent rack map for BD Multitest™/BD Tritest™/Absolute Count

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.

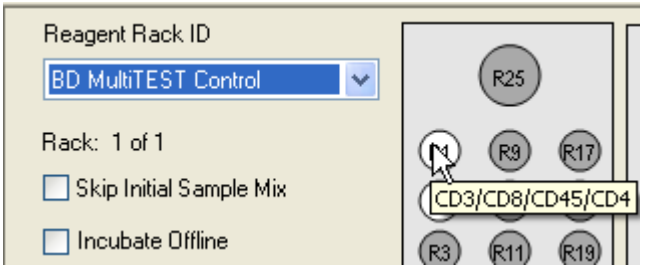
5 If needed, mount the reagent rack on the worktable (see Replacing the Reagent Rack on page 113).

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

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

- Use the Rack Map Editor to Display the rack map for your reagent rack. See Creating a New Reagent Rack on page 87 for instructions on displaying the rack map editor.

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



- See Table 5-4 on page 84 for a list of the pre-programmed reagent racks and their reagent and control positions in the rack.

  If your lab prefers another reagent configuration, you can create a new reagent rack. See Creating a New Reagent Rack on page 87.

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

Removing and Replacing the Reagent Rack

Removing the Reagent Rack

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

To remove the reagent rack:

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

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

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

Replacing the Reagent Rack

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

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

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

Entering Reagent Lot IDs

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

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

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

To enter reagent lot IDs:

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

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

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

This information will be saved in the worklist.

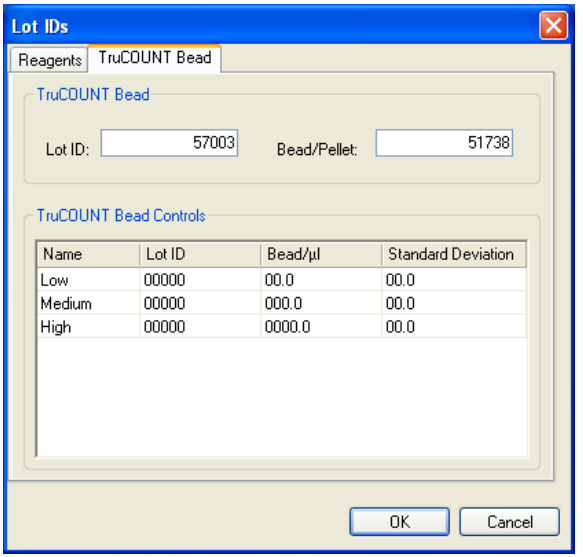
3 To enter BD Trucount™ lot IDs, bead counts, and standard deviations:

a Click the **TruCOUNT Bead** tab (Figure 6-9).

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

NOTE You cannot enter or change bead information while the beads are being run.

Figure 6-9 Lot IDs dialog showing TruCOUNT Bead tab



b Type the Trucount bead lot ID in the **Lot ID** field (up to 10 characters).

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

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

e Type the bead/µL count for each control in the **Bead/µL** field.

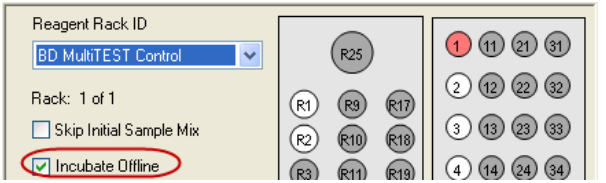
- Low: 0.00 or 40–60 with two digits after the decimal
- Medium: 0.00 or 200–300 with two digits after the decimal
- High: 0.00 or 900–1,100 with two digits after the decimal

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

4 Click **OK**.

Offline Incubation

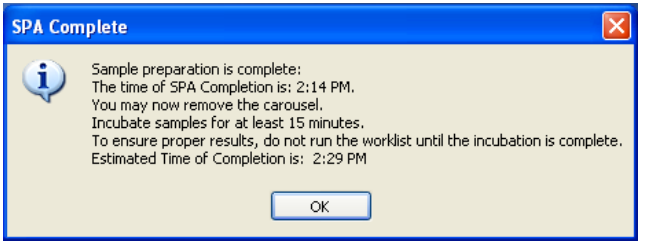
The SPA III allows you to perform the lyse incubation offline by selecting the checkbox above the carousel map.



The sequence of events for offline incubation are as follows:

1 The operation progresses as usual until the lyse incubation.

2 A dialog appears indicating that the sample prep is complete and you can remove the carousel.



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

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

Sample Processing

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

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

If a run is interrupted due to loss of power to either the instrument or the computer workstation, do not continue the run if power is restored. To safely resume processing, see Resuming a Run After a Power Interruption on page 187.

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 188 for further instructions.

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

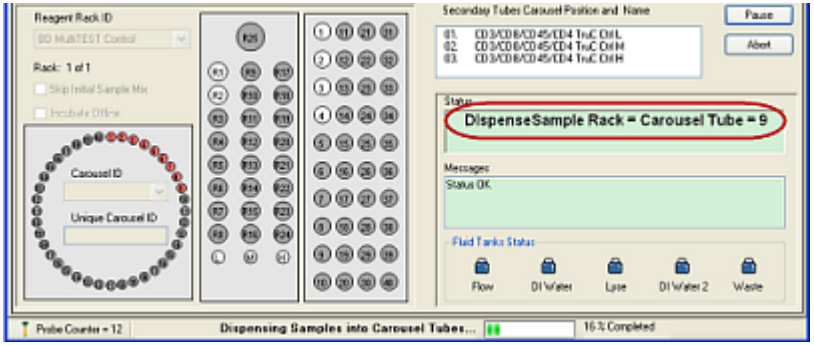
To process a sample:

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.

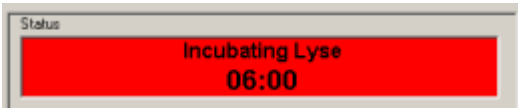


The **Status** field describes each action of the instrument.

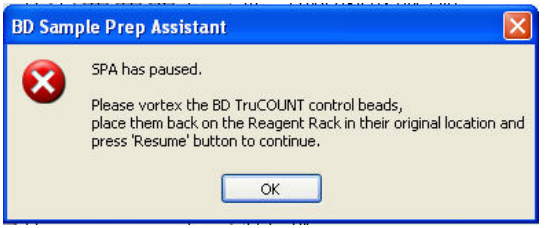
After the instrument finishes scanning the carousel rack, the probe pierces a primary tube and aspirates sample. The instrument aliquots the sample into secondary tubes. The probe washes between dispenses.

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 Trucount™ controls:

a Click **OK**.

b Open the safety cover.

c Remove the BD Trucount™ controls from the reagent rack.

d Cap and vortex each vial.

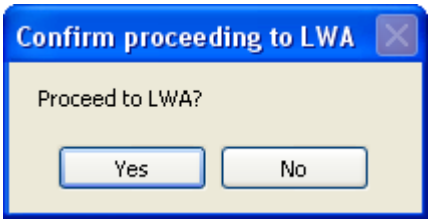
e Uncap and replace each vial into its original rack location (place the caps into the reagent rack cap holder).

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

The instrument dispenses control beads into the designated tubes.

LWA Information

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



1 Click **Yes**.

An abbreviated **LWA Info** dialog opens.

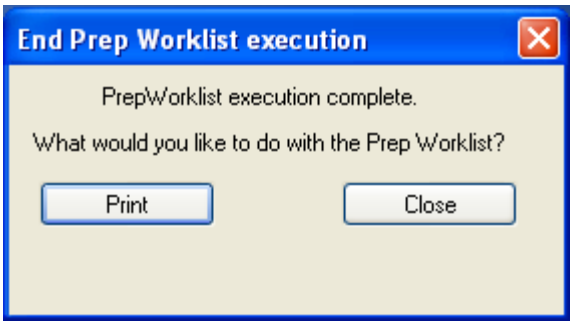
2 Confirm the serial number or user name or select a different serial number or user name from the menus and click **OK** to add LWA information to the worklist.

The LWA information is included on worklist printouts.

Ending a Run

When all the tubes in a rack have been processed:

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



1 Click an ending option.

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

2 Remove the carousel rack from the instrument.

Push on the center spindle with your thumb and pull the handle up.

3 Visually inspect all tubes for the correct preparation.

All tubes should contain sample and reagent.

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

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

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

After the last run of the day, follow the procedures described in Daily Shutdown on page 126.

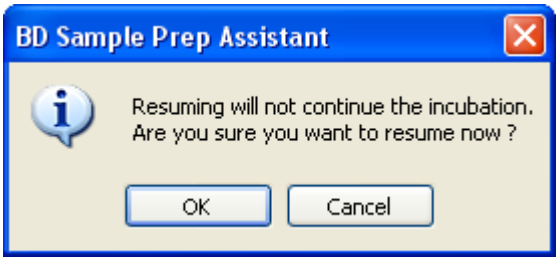
Pausing and Resuming a Run

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.



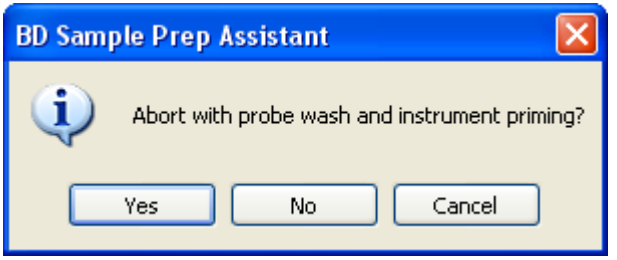
2 To resume the run, click **Resume.**

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.

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7

Shutdown and Maintenance

This chapter covers the following topics:

Daily Shutdown

Required Materials

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

Performing Daily Cleaning

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

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

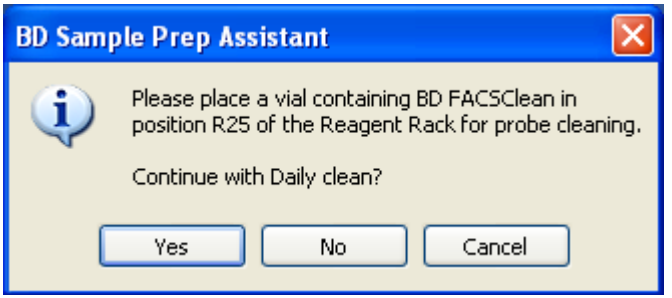
The **Sample Prep Assistant shutdown** dialog opens.

2 Select the **Daily Clean** checkbox.

If you already performed the Daily Cleaning procedure, you can leave **Daily Clean** unchecked and proceed straight to Shutting Down the Instrument on page 128.

3 Click **OK**.

A second dialog opens.



4 Open the safety cover.

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

6 Fill the DI water and DI water 2 tanks.

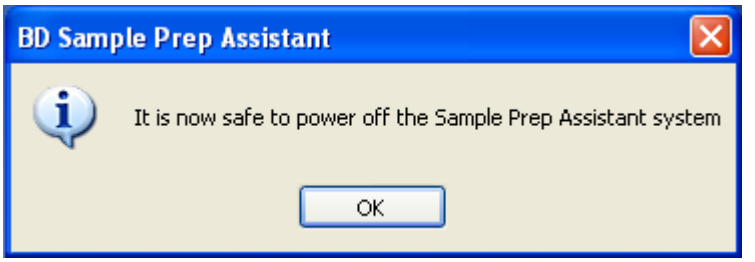
7 Empty the waste tank and add 1 L of bleach and 500 µL of Sigma Antifoam A Concentrate to the tank.

8 Close the safety cover.

9 Click **Yes**.

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

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



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 96
- Weekly Cleaning in next section
- Calibrating the Probe Reference Point on page 132
- Monthly Servicing of Waste In-Line Filters on page 139
- Monthly Replacement of the Waste Tank Cap on page 143

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

Weekly Cleaning

The weekly cleaning procedure:

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

Required Materials

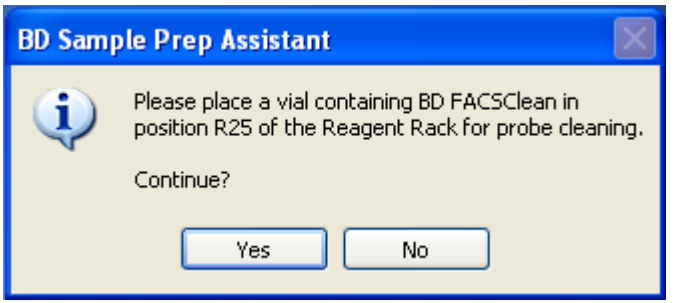
- BD FACSClean™ solution or 10% bleach solution
- 70% ethanol
- Large vial
- Clean, lint-free cloths or disposable wipes

Performing Weekly Cleaning

To perform the weekly cleaning procedure:

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

A dialog opens.



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

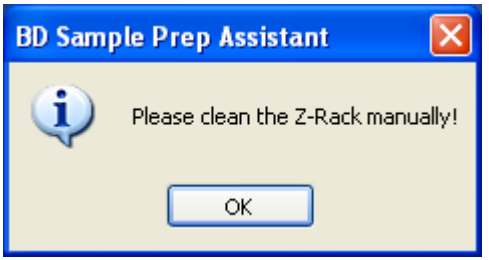
3 Fill the DI water and DI water 2 tanks.

4 Empty the waste tank according to your laboratory procedures, and then add 1 L of bleach and 500 µL of Sigma Antifoam A Concentrate to the tank.

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

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

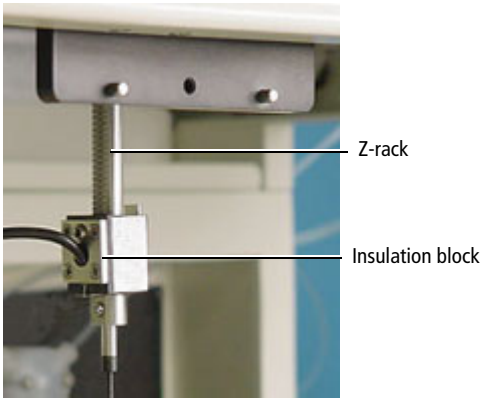
After the weekly cleaning finishes, the probe will move to the front of the instrument and down for easy access, and a dialog opens.



6 Click **OK**.

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

8 Wipe the Z-rack with a clean, dry cloth.



Look for residue, liquid, and cracks at the insulation block located above the probe. If found, call your BD 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.

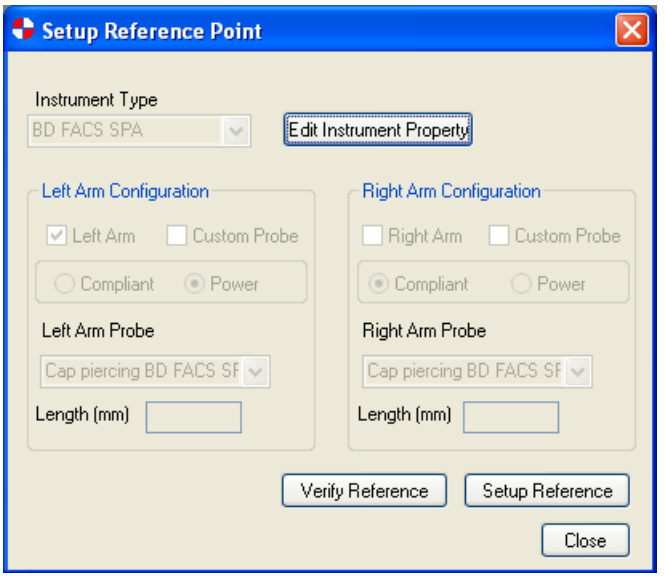
To verify the reference point:

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

See Removing the Reagent Rack on page 112.

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

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



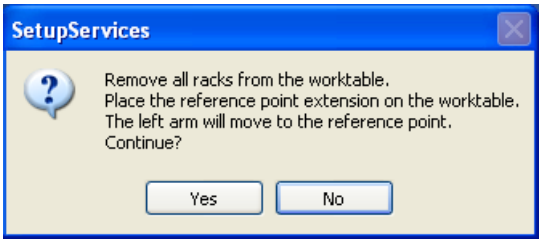
3 Click **Verify Reference**.

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

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

Do not click Yes at this point.

Figure 7-1 SetupServices dialog



4 Verify that the carousel and reagent racks are removed from the instrument worktable.

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

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

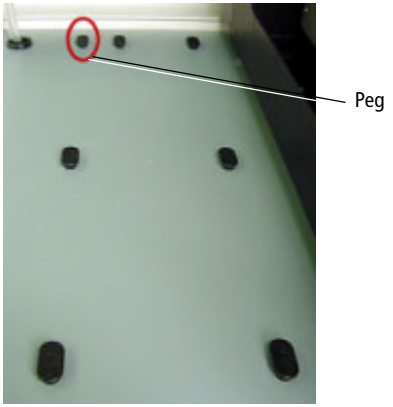


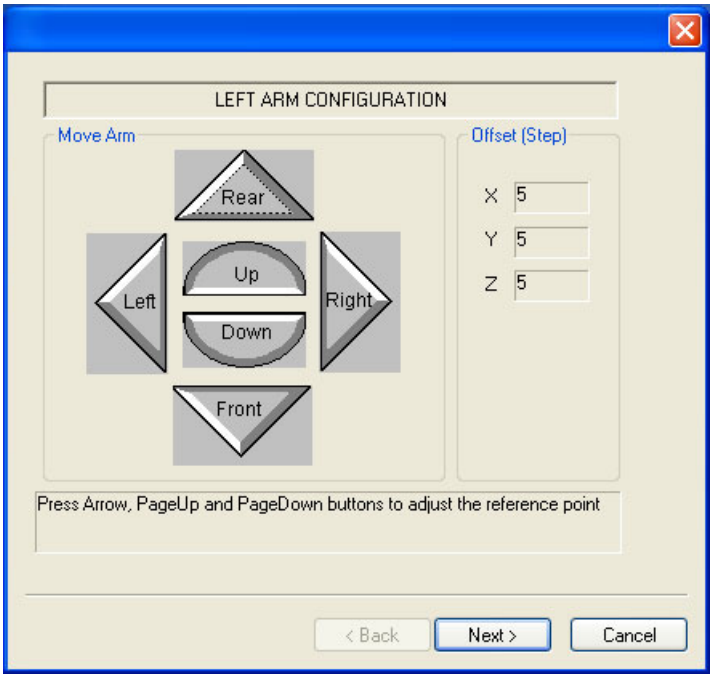
Figure 7-3 Reference point extension on peg

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 7-1 on page 133).

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



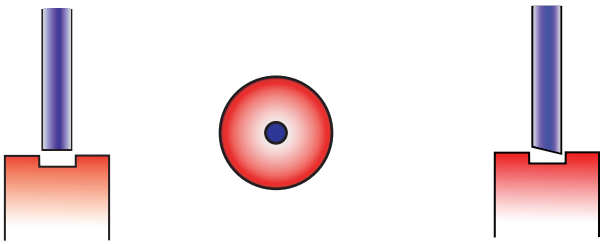
8 Open the safety cover.

9 Adjust the probe tip alignment, if necessary.

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

Figure 7-4 Proper probe tip alignment

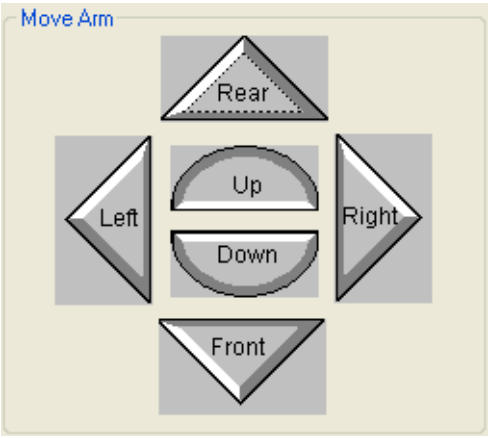
Figure 7-5 Side (left) and top (right) view of proper probe tip alignment



Flat Tip Beveled Tip

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.

```
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 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.

16 Reinstall the reagent and carousel racks (see Replacing the Reagent Rack on page 113 and Mounting the Carousel Rack on page 109).

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 167.

Monthly Servicing of Waste In-Line Filters

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

Required Materials

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

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

Preparing the Instrument

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

1 If the probe is not in the wash station position, use the procedure Homing the Probe on page 180 to move the probe to the wash station position.

2 Open the safety cover.

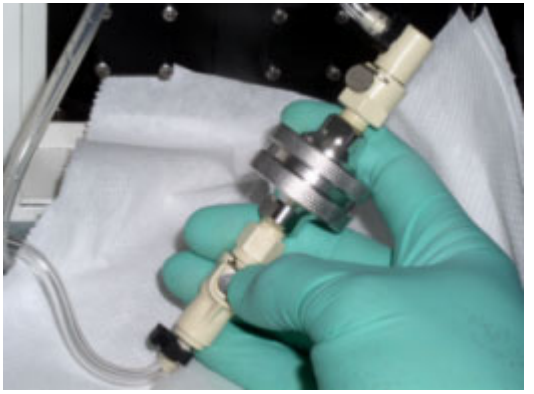
3 Remove the reagent and carousel racks.

Removing the Waste In-Line Filter Assembly

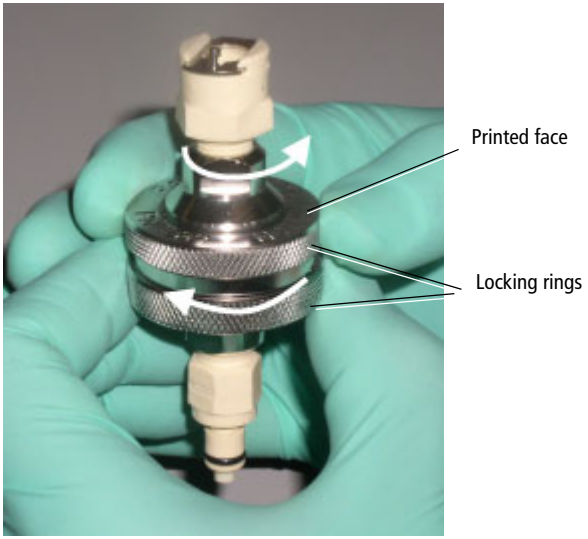
To remove the waste in-line filter:

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

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



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



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

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

5 Remove the fine-screen filter and the coarse filter from the lower portion of the assembly. You may need to gently tap the assembly on a benchtop to dislodge the filters.

6 Inspect the fine-screen filter for any clogging or damage.

Replace the fine-screen filter if it is damaged.

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

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

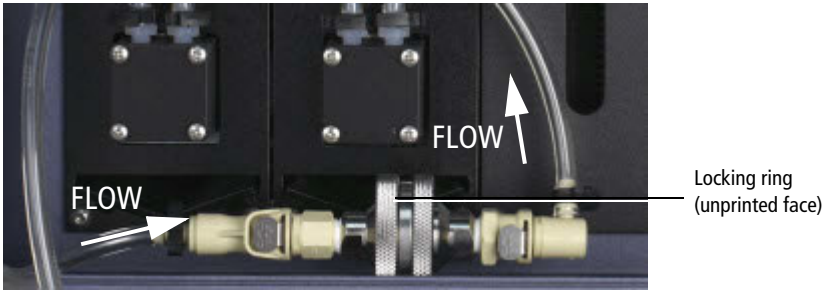
9 Place the fine-screen filter on top of the coarse filter.

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

10 Reassemble the locking rings.

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

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



Completing the Waste In-Line Filter Service

1 Replace the reagent rack and carousel (see Replacing the Reagent Rack on page 113 and Mounting the Carousel Rack on page 109).

2 Perform the procedure in Initializing the Instrument on page 180.

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

Monthly Replacement of the Waste Tank Cap

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

Periodic Maintenance

Perform the following procedures as needed:

- Internal and External Surface Cleaning and Decontamination on page 145
- Extended Periods of Non-Use on page 146
- Startup Following Extended Non-Use on page 149
- Replacing the Probe on page 152
- Replacing a Syringe on page 167
- Fuse Replacement on page 176

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

Internal and External Surface Cleaning and Decontamination

Clean the instrument surfaces whenever needed.

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

Required Materials

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

Cleaning the Instrument

To clean the instrument:

1 Switch off the instrument power and unplug the AC power cord.

2 Open the safety cover.

3 Wipe all accessible surfaces and racks with one of the cleaning solutions listed in Required Materials.

4 Wipe all exposed surfaces using a cloth dampened with DI water.

5 Wipe all exposed surfaces with a clean, dry cloth.

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

6 Close the safety cover.

7 Repeat steps 3 to 5 on the instrument exterior.

Extended Periods of Non-Use

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

Required Materials

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

Performing Weekly Cleaning

Before proceeding with the next section, perform the weekly cleaning procedure. See Weekly Cleaning on page 129.

Prime the Lines with Water

1 Empty all the tanks in the fluidics tower.

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

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

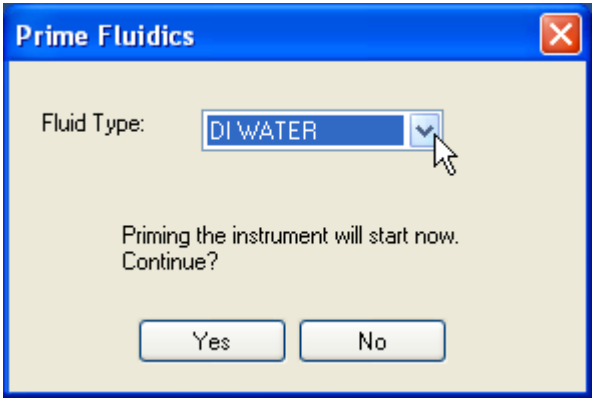
3 Remove the fluid sensor from the BD FACSFlow™ cubitainer and place the sensor in a clean, suitable container filled with at least 0.5 L of DI water.

The container should be an adequate size so that it is stable when the fluid sensor is placed in it.

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

The **Prime Fluidics** dialog opens.

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



6 Repeat steps 3 and 4 with the following fluid types:

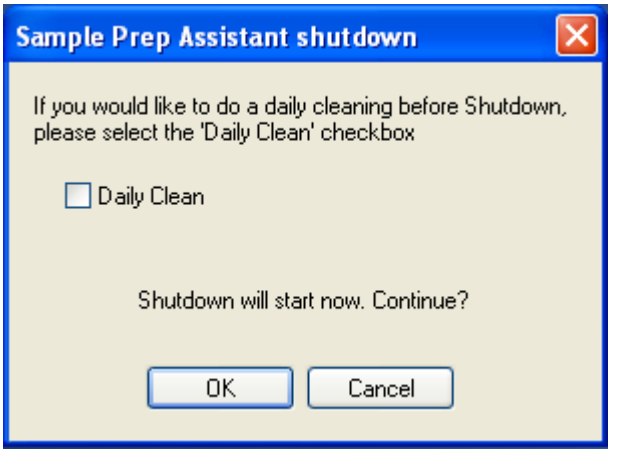
- LYSE
- FLOW
- DI WATER 2

7 Empty all the tanks.

Completing the Non-Use Shutdown Procedure

To complete the procedure:

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



Leave the **Daily Clean** checkbox cleared.

2 Click **OK**.

The software quits.

3 Open the safety cover.

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

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

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

See Removing the Reagent Rack on page 112.

7 Remove any remaining secondary tubes from the carousel rack.

8 Close the safety cover.

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

Startup Following Extended Non-Use

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

1 Ensure that the BD FACSFlow™ cubitainer has sufficient fluid.

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

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

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

4 Turn on the instrument and the computer power.

You must turn on the instrument before starting the software.

5 Start SPA software.

The software will initialize the instrument.

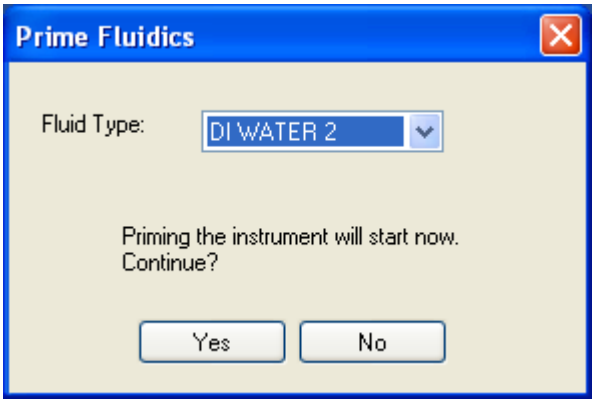
6 Prime the instrument four times with DI WATER 2, four times with LYSE, four times with FLOW, and four times with DI WATER (a total of 16 primes).

For each prime:

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

The **Prime Fluidics** dialog opens.

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 7-6 on page 151) and the tubing on the instrument side (Figure 7-7 on page 151).

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

Figure 7-6 Fluid tubing and syringes on rear panel

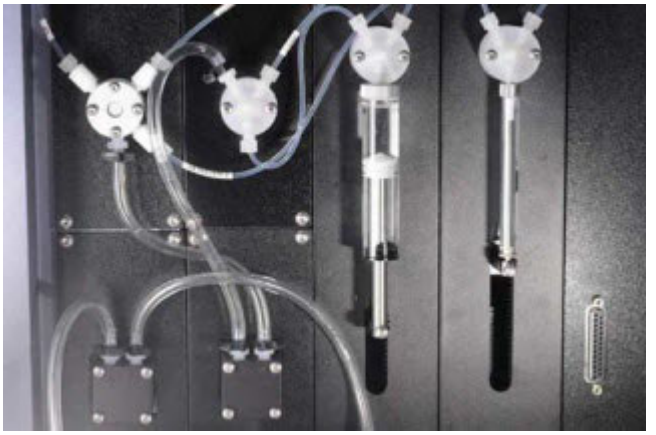


Figure 7-7 Fluidics tubing on instrument side

Replacing the Probe

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

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

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

The probe is sharp. Use caution when handling.

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 7-1 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 7-8 on page 153. 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.

Table 7-1 Probe replacement guidelines (continued)

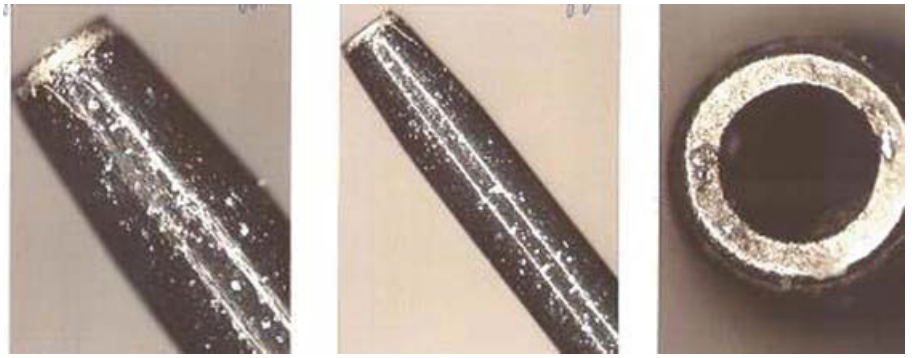
Action	Condition Requiring Replacement
Cap-piercing ability of the probe	The probe is considered damaged if at any point during operation the probe fails to pierce a cap.
Accuracy and precision of the dispense	The probe is considered damaged if the accuracy and precision of the dispense changes. The accuracy and precision of the dispense of one probe should remain the same throughout operation.

See Figure 7-8 for an example of a probe with extreme wear of the Teflon coating that would require replacement immediately.

Figure 7-8 Probe with badly worn coating

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

Figure 7-9 Examples of acceptable probe wear



Required Materials

The waste in-line filters need to be cleaned whenever you replace the probe, so also have at hand the items listed in Monthly Servicing of Waste In-Line Filters and Required Materials on page 139.

- Gloves
- Paper towels
- Kimwipes® wipes
- Flathead screwdriver
- Metric hex wrench (2 mm)
- Needle nose pliers
- BD FACS™ 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 155.

2 Removing the Old Probe on page 160.

3 Attaching the New Probe on page 163.

4 Calibrating the Reference Point on page 166.

5 Completing the Probe Replacement Procedure on page 167.

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 139.

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 FACS™ 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.

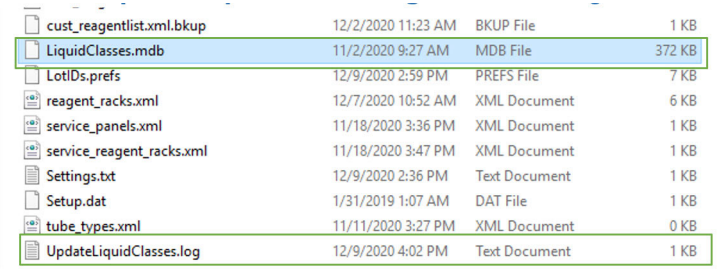
The configurator will automatically load the updated LiquidClasses database file. The dialog refreshes when the installation is complete.

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.

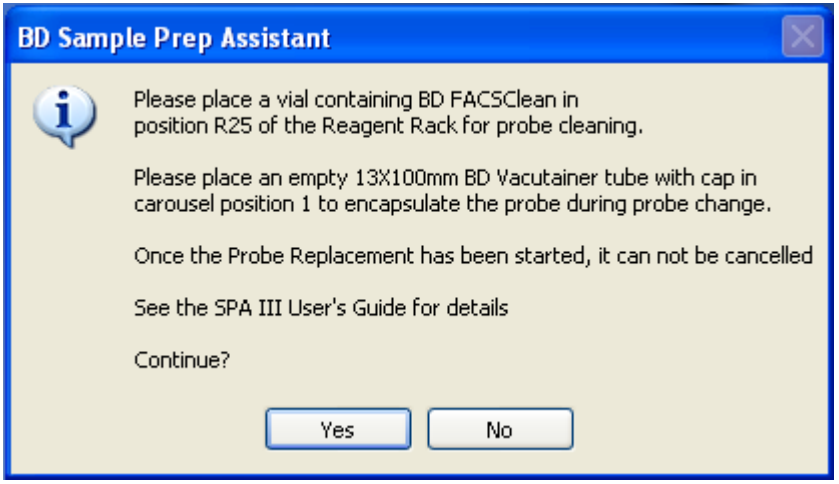
6 Confirm UpdateLiquidClasses.log file has been generated.



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

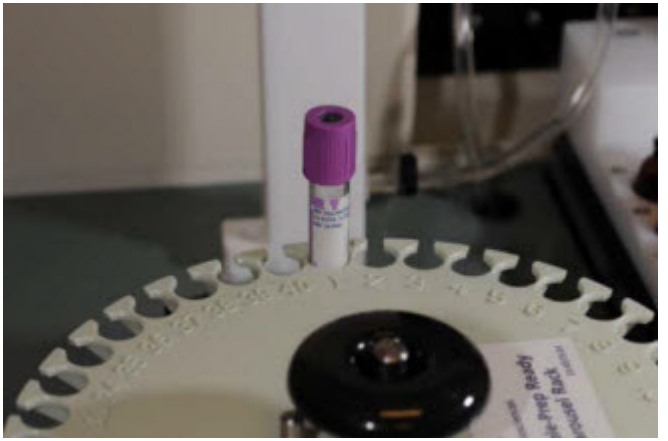
7 Select **Instrument > Replace Probe** from the main menu in the **Prep Worklist** window.

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.

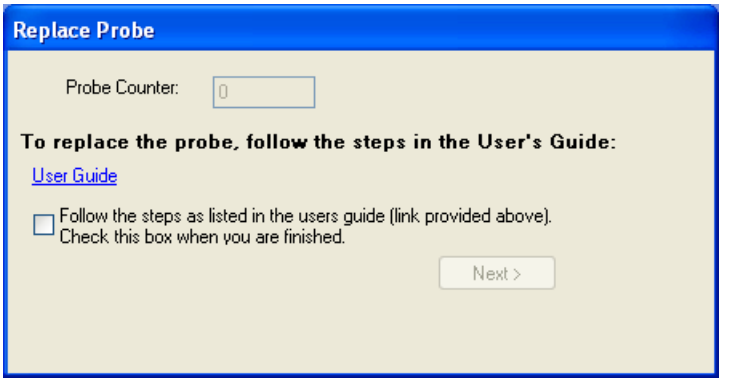
11 Click **Yes** to start the cleaning process.

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

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

The Replace Probe dialog opens.

Figure 7-10 Replace Probe dialog



12 Leave the Replace Probe dialog open.

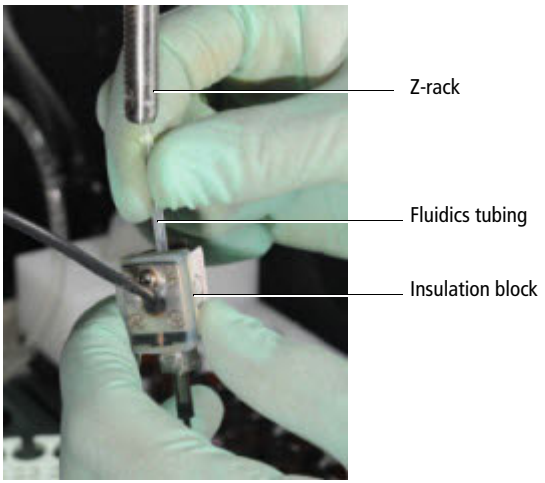
Removing the Old Probe

Do not turn off the instrument during this procedure.

1 Open the safety cover.

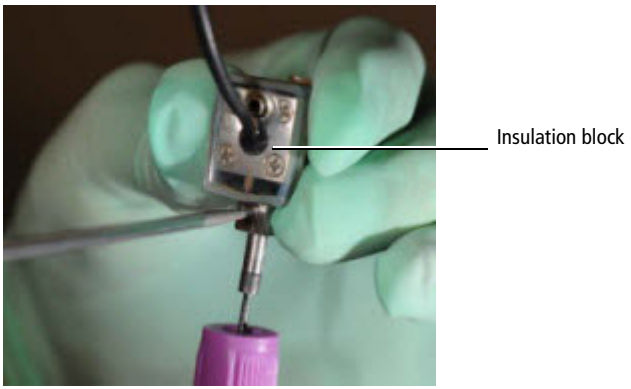
2 Loosen the setscrew on the insulator block with a 2-mm hex wrench.

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



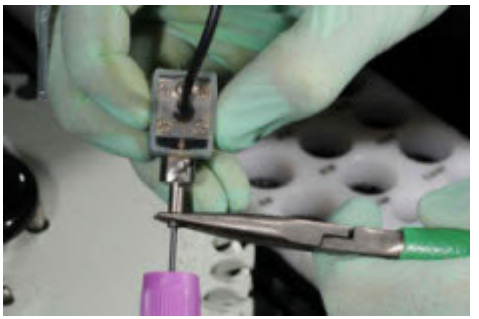
4 Pull the fluidics tubing off of the top of the probe.

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.

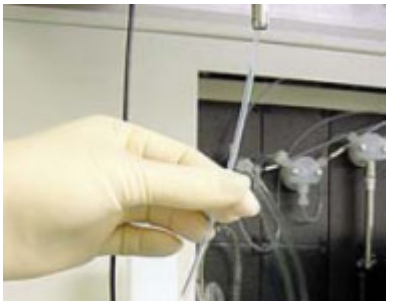


Keep the probe inside of the tube at all times. Dispose of the entire tube and probe into a sharps container.

⚠ 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.

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



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

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

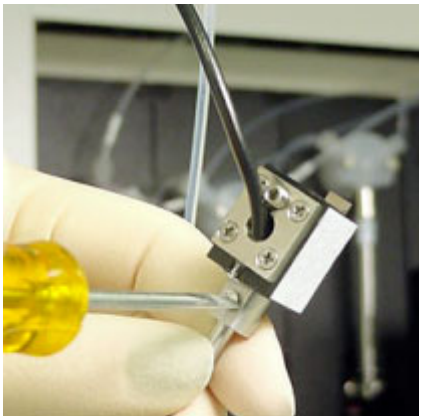
Attaching the New Probe

1 Remove the probe from the packaging by opening the end with the black cap.

The black cap covers the blunt tip of the probe.

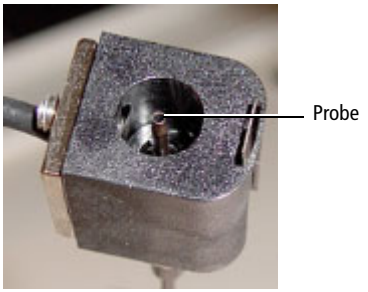
2 Insert the silver, blunt end of the new probe into the insulation block until it is completely seated against the block.

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



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

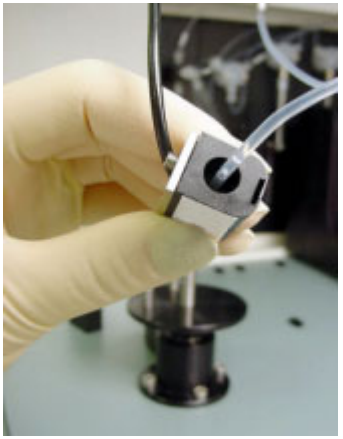
Figure 7-11 Point of attachment



⚠ Do not bend or kink the tubing.

Bending or kinking the tubing will reduce instrument performance.

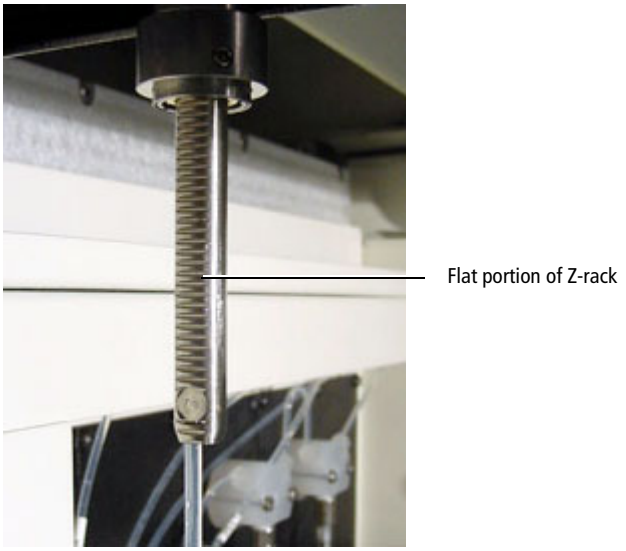
Figure 7-12 Tubing attached to probe through insulation block



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

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

Figure 7-13 Correct alignment of insulation block.



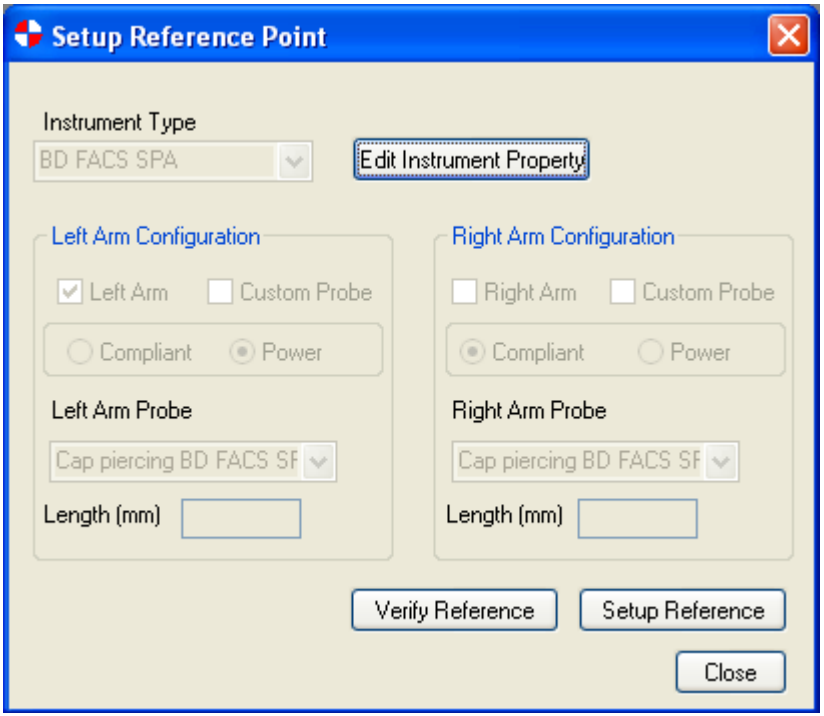
b Use the hex wrench to tighten the set screw (clockwise) and lock the insulation block in place. The set screw must sit against the flat portion of the Z-rack.

6 Return to the **Replace Probe** dialog (Figure 7-10 on page 159), 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 132.

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 180.

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

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

Replacing a Syringe

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

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

Required Materials

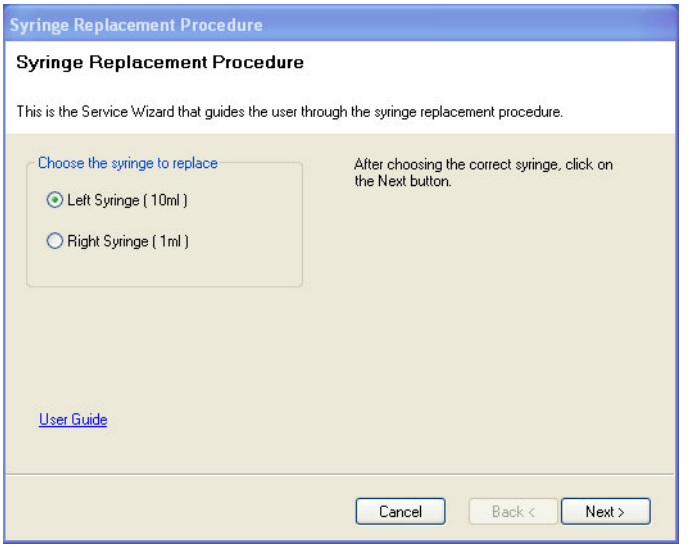
- Gloves
- Replacement syringe

Syringe Replacement Procedure

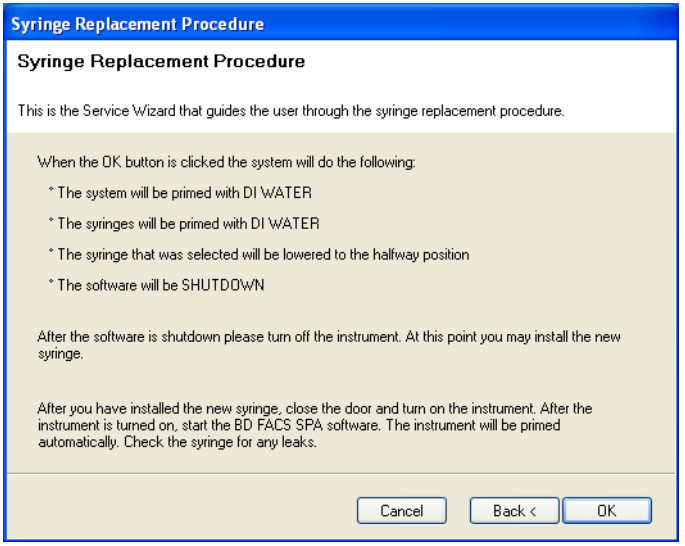
1 Select **Instrument > Replace Syringe** from the main menu in the **Prep Worklist** window.

The **Syringe Replacement Procedure** dialog opens.

2 Select a syringe and click **Next.**

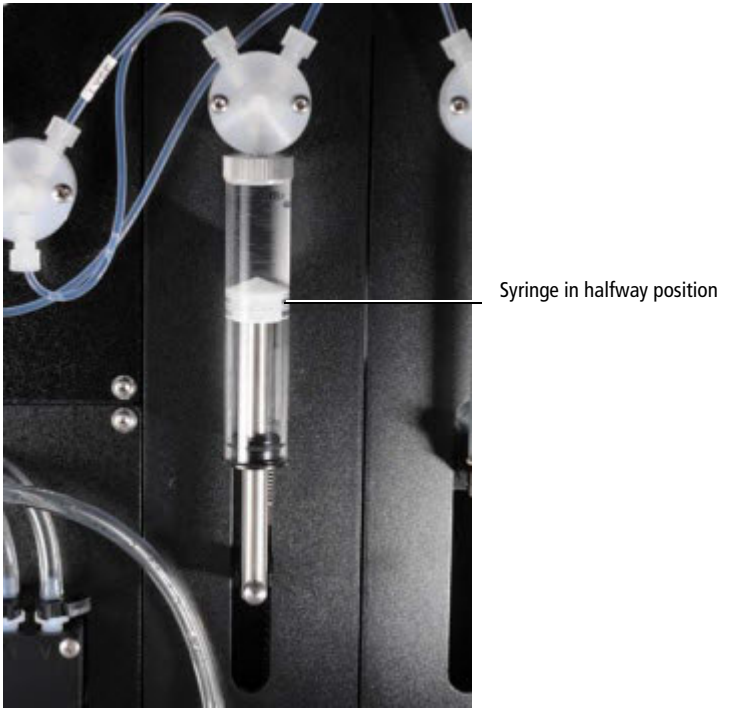


A second dialog is displayed.



3 Click **OK**.

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



- The software is shut down.

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

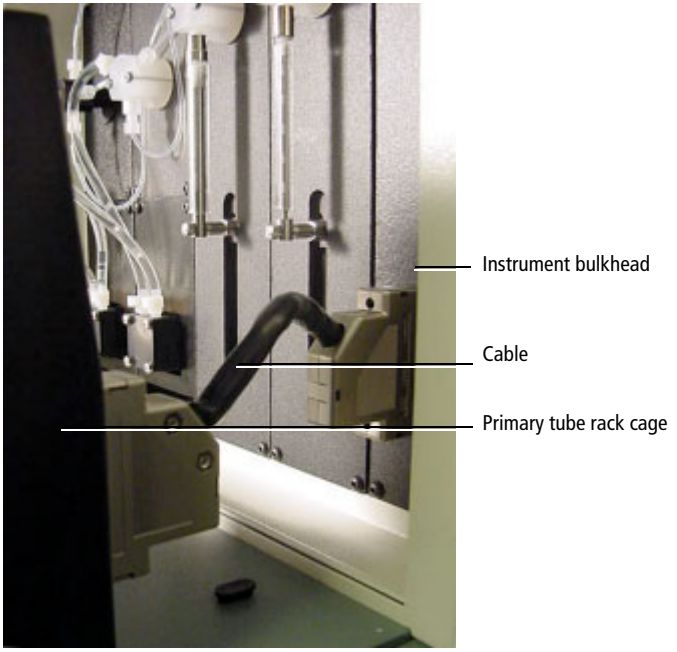
Always turn off the instrument and unplug the power cord before performing this procedure to prevent electrical shock or injury.

5 Open the safety cover.

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

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

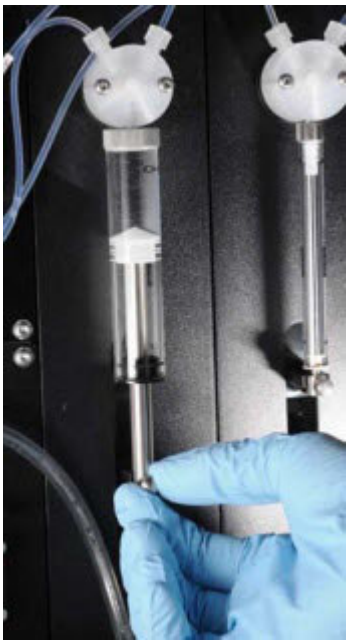
Do not remove the rack cage, disconnect the cable, or place any tension on the cable.


Instrument bulkhead
Cable
Primary tube rack cage

Removing the Syringe

To remove the syringe:

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



2 Remove the screw.

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

3 Turn the syringe barrel in the direction shown in the following figure until it loosens, then remove it.

4 Discard the syringes into a sharps container.

Replacing the Syringe

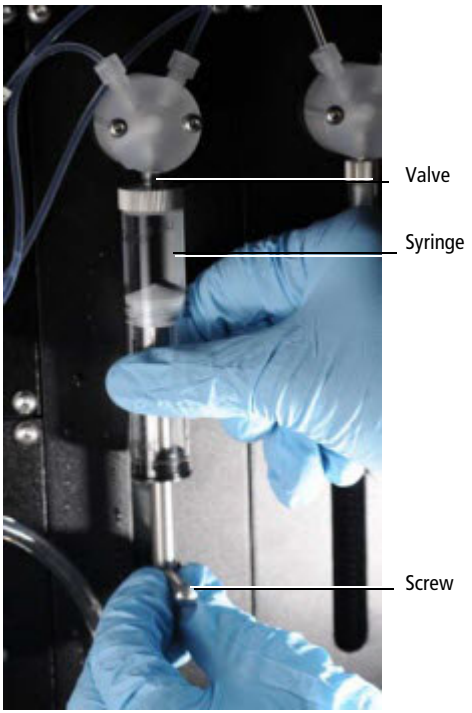
To replace the syringe:

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

Inspect the new syringe for defects before using it.

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

Ensure that the syringe is aligned straight up.



3 Attach the screw to the pin by turning it clockwise.

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

Ensure that the syringe is pointed straight up.

5 Attach the syringe to the valve by rotating it in the direction shown in the following figure until it is finger-tight.

NOTE If you twist the syringe a quarter turn in the opposite direction, and then in the direction shown, it will help seat the threads.

Replacing the Racks

To replace the racks:

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

Follow the alignment guides on the base of the instrument.

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

2 Replace any racks you removed from the instrument.

See Replacing the Reagent Rack on page 113.

3 Close the safety cover.

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

5 Start the software.

A prime with flow occurs, and the instrument initializes.

6 Open the safety cover.

7 Examine the valve and the syringe for any leaks or drips.

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

Performing an Accuracy and Precision Check

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

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

Fuse Replacement

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

Replace these fuses whenever necessary.

Required Materials

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

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

Removing Fuses

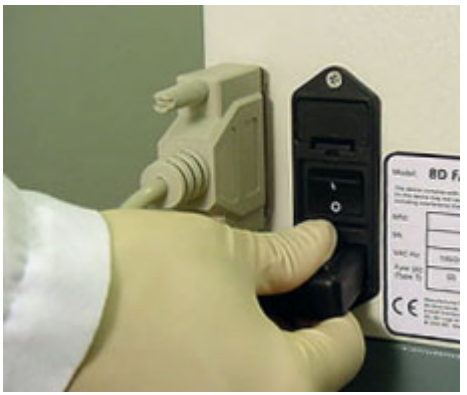
To protect against shock, always turn off the instrument and unplug the power cord before performing this procedure.

To remove a fuse:

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

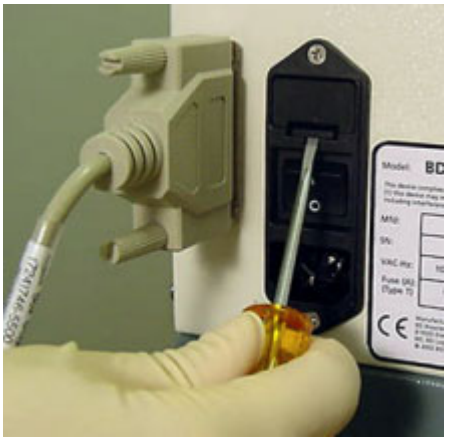
2 Turn off the instrument and unplug the power cord from the wall outlet.

3 Disconnect the cord from the side of the instrument.



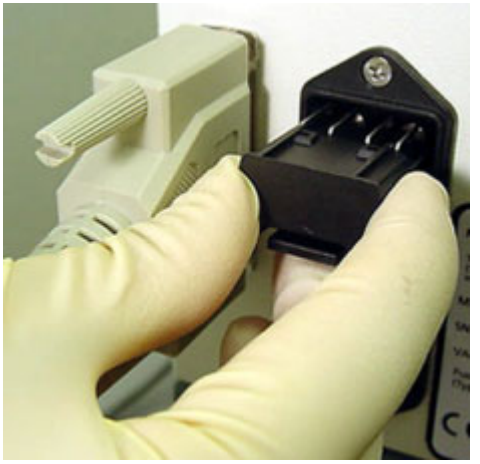
This allows easy access to the fuse drawer.

4 Place a small screwdriver into the slit at the bottom of the fuse drawer and gently pry out the drawer.



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

5 Remove the fuse drawer.



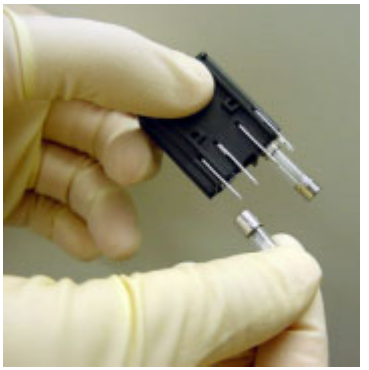
6 Remove and dispose of the old fuses.

NOTE We recommend that you replace both fuses at the same time.

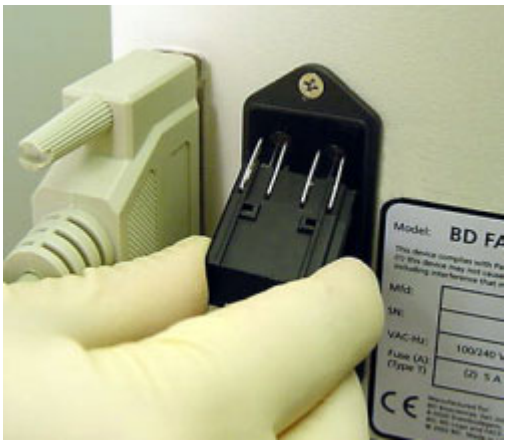
Installing New Fuses

To install new fuses:

1 Replace both fuses.



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



3 Push gently on the drawer until it snaps into place.

4 Reconnect the power cord to the instrument, then plug the cord into the wall outlet.

5 Turn on the instrument power.

6 Restart the software.

Special Conditions

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

Homing the Probe

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

1 Close the safety cover.

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

Initializing the Instrument

To initialize the instrument:

1 Close and lock the primary rack cage.

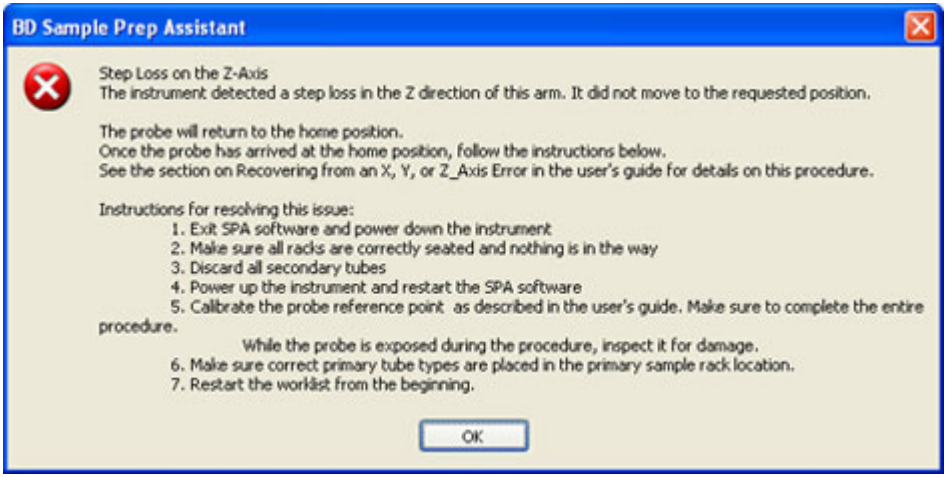
2 Close the safety cover.

3 Select **Instrument > Initialize** from the main menu in the **Prep Worklist** window.

Recovering from an X, Y, or Z-Axis Error

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

The following dialog appears when this error occurs.



To recover from a step loss error:

1 Click **OK** to close the dialog.

2 Exit SPA software and turn off power to the instrument.

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

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

5 Power up the instrument.

6 Start SPA software and log in.

7 Calibrate the probe reference point. See Calibrating the Probe Reference Point on page 132.

   a When the probe moves to the reference point extension at step 9 on page 135, 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 152.

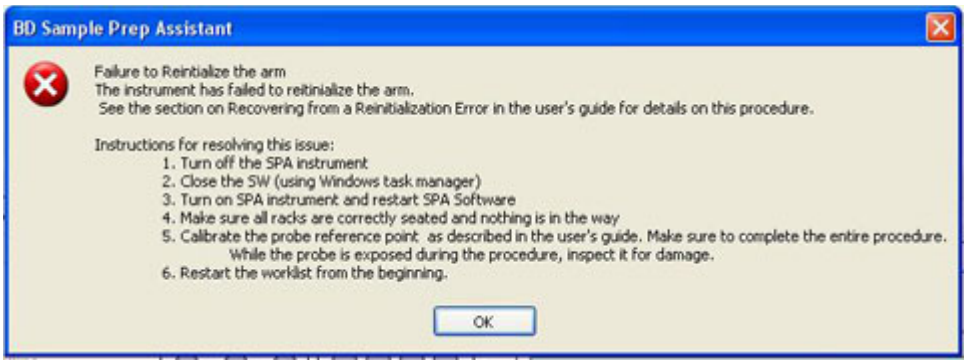
   b If the probe does not require replacement, complete the calibration procedure.

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.

3. 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.

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

6. Power up the instrument.

7. Start SPA software and log in.

8. Calibrate the probe reference point. See Calibrating the Probe Reference Point on page 132.

    a. When the probe moves to the reference point extension at step 9 on page 135, 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 152.

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

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

Manually Replacing the Probe

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

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

1 Turn off power to the SPA III instrument.

2 Exit SPA software.

If the software is frozen, use the Windows Task Manager to quit the application.

3 Open the safety cover and perform the safety decontamination procedures recommended in your lab for dealing with biohazardous materials.

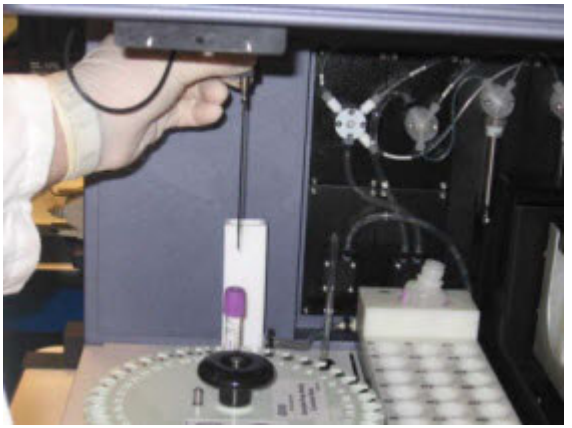
4 Load an empty carousel onto the worktable.

5 Place an empty 13 x 100-mm BD Vacutainer® tube in position 1 in the carousel.

6 Grasp the probe arm at the insulation block and move the probe arm over to the carousel and position it above the empty tube in position 1. See Figure 7-14 on page 185.

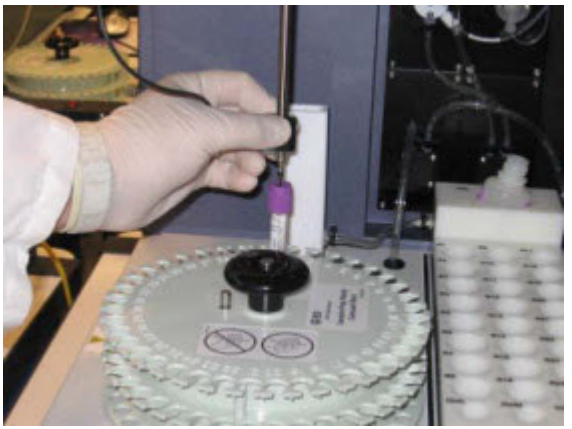
Be careful to avoid contact with the probe tip while moving the probe arm. Do not attempt to insert the probe into the empty tube unless the tube is loaded onto the carousel.

Figure 7-14 Moving probe arm to position 1 on the carousel



7 While holding at the insulation block, pull the probe arm down until the probe tip pierces the cap of the empty tube and continue to pull down until the probe is fully enclosed inside the tube. See Figure 7-15.

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



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

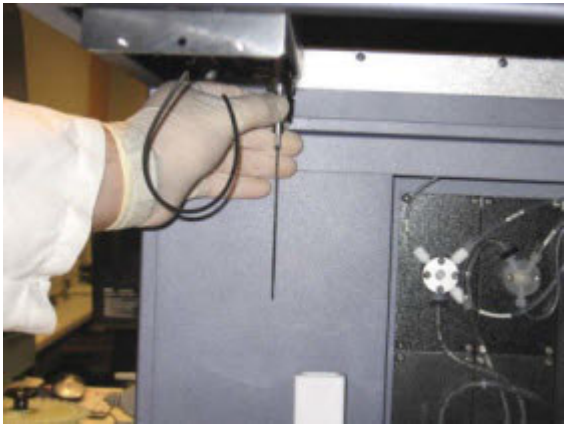
9 Replace the probe by performing the following two steps in the main probe replacement procedure.

a Removing the Old Probe on page 160.

b Attaching the New Probe on page 163.

10 Once the new probe is installed, grasp the probe arm at the insulation block and move it to the back of the work area. See Figure 7-16 on page 186.

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



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

12 Start SPA software and log in.

The system initializes and is ready to run.

13 Reset the probe counter by doing a modified version of Replacing the Probe on page 152, as directed in the steps below.

a Perform Preparing to Replace the Probe on page 155, 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 160

- Attaching the New Probe on page 163

c Perform the next section: Calibrating the Reference Point on page 166.

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 167.

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

Recovering from a Power Interruption

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

Resuming a Run After a Power Interruption

To safely resume processing:

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

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

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

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

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

5 Discard any secondary tubes that received dispensed fluids, and replace the tubes.

6 Verify that the reagent vials and primary tubes have sufficient volume.

7 Restart the run.

When Power Cannot Be Quickly Restored

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

If the SPA III stops with the probe position preventing removal of materials, contact your local BD 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.

8

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

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 149.
	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 152.
	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 152. • 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 149.
	Loose valve or pump fittings	Check all fittings and hand-tighten them, if necessary. See Figure 6-1 on page 96 for the locations of the valves.
Flow cytometry results inaccurate	**Incomplete lysing**	
	• BD FACS™ lysing solution at incorrect concentration	Prepare a new batch of BD FACS™ lysing solution.
	• Offline Incubation enabled, but did not finish	Allow the full lyse incubation time.
	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 152.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Flow cytometry results inaccurate (continued)	• 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 6-1 on page 96 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.

Instrument Troubleshooting

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 152. 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 86.
	• Recapped tubes	Vent tubes as described in Running BD Multi-Check Controls on page 104, 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.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Flow cytometry results inaccurate (continued)	**Insufficient** BD Trucount™ **Control**	
	• Wrong BD Trucount™ control or no BD Trucount™ control	Make sure BD Trucount™ controls are in correct positions in the reagent rack; rerun tube if necessary.
	• Worn probe	Perform probe replacement procedure. See Replacing the Probe on page 152.
Fluidics tubing creased, crimped, or bent	Fluidics tower too close to wall or instrument	Call BD service representative.
	Tubing incorrectly replaced onto probe	Call BD service representative. Review probe replacement procedure for next run. See Replacing the Probe on page 152.
Incubation timer disappearing	Screen saver interference with incubation timer display	Turn off all screen savers. See Software Compatibility Issues on page 50.
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.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
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 167.
	Insufficient cleaning and maintenance of instrument	Make sure you perform daily and weekly cleaning. See Daily Shutdown on page 126 and Weekly Cleaning on page 129.
	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 146.
	Break or crack in syringe	Perform syringe replacement procedure. See Replacing a Syringe on page 167.
	Break or crack in valve	Call BD Biosciences.
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 126 and Weekly Cleaning on page 129.
	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 146.
	Break or crack in valve	Call BD Biosciences.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Leaks or mineral deposits at waste port	Disconnected waste line	Reconnect the waste line. See Emptying the Waste Tank on page 45.
	Damaged quick-release connector	Call BD Biosciences.
Leaks or mineral deposits at fittings and caps of all bulk fluid tanks and waste tank	Cap not on securely	Tighten the cap.
	Cracked tank	Order a new 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.
Monitor inactive, PC not responding	Power cord not plugged in	Plug in the power cord.
	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.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Inadequate fluid dispensed	Clogged probe	Perform the probe replacement procedure. See Replacing the Probe on page 152.
	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.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
No reagent aspirated	Probe clogged	Perform the probe replacement procedure. See Replacing the Probe on page 152. 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 86.
	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 176.
	Internal power failure	Contact your BD Biosciences service representative.

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Probe bent or damaged	Non-supported primary tubes	Perform probe replacement procedure. See Replacing the Probe on page 152. Perform probe calibration. See Calibrating the Probe Reference Point on page 132. 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 152. Perform probe calibration. See Calibrating the Probe Reference Point on page 132. Make sure you take the caps off the reagent vials and place them in reagent cap holder as described Reagent Rack Setup on page 109.
	Used vial other than 60-mL vial in position R25 in reagent rack	Perform probe replacement procedure. See Replacing the Probe on page 152.

Instrument Troubleshooting

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 152. • Perform probe calibration. See Calibrating the Probe Reference Point on page 132.
	Software installed without instrument-specific worktable database	Install the instrument-specific worktable.
	Probe tip realignment not correctly done	• 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 132.
	Cable from primary tube rack cage to instrument loose or not completely connected	**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 180.
	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 152.

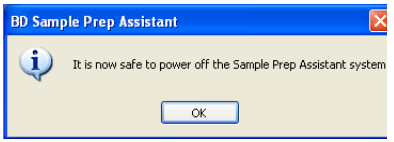
Instrument Troubleshooting

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 152. 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 86. In the future, perform Daily Cleaning and Weekly Cleaning as described in Shutdown and Maintenance on page 125.
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).

Instrument Troubleshooting

Observation	Possible Causes	Recommended Solutions
Tubes, primary, not fitting into primary tube rack	Incompatible primary tubes	Use compatible tube. See Required Equipment on page 32. 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 152. 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 86. In the future, perform Daily Cleaning and Weekly Cleaning as described in Shutdown and Maintenance on page 125.
	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 163.

Instrument Troubleshooting

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:  • 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	**1** Uninstall the software. **2** Reinstall the software and the instrument-specific worktable database.
	Software application renamed or moved	**1** Uninstall the software. **2** Reinstall the software and the instrument-specific worktable.
	Instance of software already running but minimized.	**1** Check Windows taskbar to see if software is running. **2** 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.
Password not working	Caps Lock or Num Lock on	Disable Caps Lock and Num Lock and try again.
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.

Software Error Messages

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	**1** Fill the appropriate DI water tank. **2** Click **Retry** to continue. If you click Cancel, you will have to reinitialize the instrument prior to the next run.
The FACS FLOW tank liquid level is too low. Please replace the tank.	Flow tank empty	**1** Replace BD FACSFlow™ cubitainer. **2** Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run.
	Fluid tank sensor not plugged in	**1** Connect tank sensor connector to fluidics tower bulkhead. **2** Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run.
	Faulty flow sensor	Call BD.
The FACS LYSE tank liquid level is too low. Please fill the tank.	Lyse tank empty	**1** Fill the FACS LYSE tank. **2** Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run.

Software Error Messages

Message	Possible Cause	Possible Solution
The Reagent Rack does not contain all the reagents required to perform the Prep Worklist. The following reagents are missing: *<missing reagent list>*	Reagent rack missing required reagents	**1** Click **OK**. **2** Place the required reagents in the reagent rack according to the Reagent Rack Map for the panel.
The sample prep cannot run because the worklist has errors.	Incorrect data entered into the worklist	**1** Click **OK**. **2** Place your mouse on the red error icon with the exclamation point and correct the displayed error.
Sample prep has paused. Please press 'OK' button add the following reagent: *<reagent name>* and press 'Resume' button to continue	Reagent level too low	**1** Click **OK**. **2** Add specified reagent to reagent vial. **3** Click **Resume**.
Sample prep is pausing because the WASTE tank is full. 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!	Waste tank is full	**1** Empty the Waste tank. **2** Add 500 µL of Sigma Antifoam A Concentrate. **3** Click **Resume**.

Software Error Messages

Message	Possible Cause	Possible Solution
Sample prep is pausing because [DI WATER/ FACSFLOW/DIWATER 2/ LYSE] tank liquid level is too low. Please wait for the probe wash to complete. Then fill the tank and press 'Resume' button to continue!	Specified tank liquid level is too low	**1** Wait for the probe wash to complete. **2** Fill the tank. **3** Click **Resume**.
The Sample rack is disconnected. Please connect it and press 'Retry' to continue	Primary tube rack cage cable loose or disconnected	**1** Shut down the instrument. **2** Tighten or connect the cable, then engage the fastener. **3** Click **Retry**.
The Carousel rack is not loaded. Please load it and press 'Retry' to continue	Carousel rack not loaded or not fully seated on pins	• Load the carousel rack. • Verify that it is fully seated on the spindle and both pins.
The Secondary tubes are not loaded at their defined locations. <*misplaced tubes*> Please load all secondary tubes at their defined locations and press 'Retry' to continue	Missing tube	Verify that the carousel matches your worklist and your panel. Compare the worktable display to the carousel.

Software Error Messages

Message	Possible Cause	Possible Solution
The Secondary tubes are not loaded at their defined locations. The Carousel could not be scanned due to an Instrument error. Please load all secondary tubes at their defined locations and press 'Retry' to continue	Missing tube	Verify that the carousel matches your worklist and your panel. Compare the worktable display to the carousel.
	Hardware problem	Call BD Biosciences.
At least one tank needs to be filled or emptied. Please adjust liquids level and press 'Retry' to continue.	Tank full or empty	Compare tanks to display, and fill or empty tanks.
	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	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. Please wait for the probe wash to complete. Then close the Safety Cover and press 'Resume' button to continue!	Safety cover open	Close the safety cover and press **Resume**.

Software Error Messages

Message	Possible Cause	Possible Solution
Sample prep is paused because the Safety Cover could not be locked. Please wait for the probe wash to complete. Then close the cover and press 'Resume' button to continue!	Safety cover not locked	Close the safety cover and press **Resume**.
The correct help file could not be found.	File path to help file corrupted	**1** Uninstall the software. **2** 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 <*file name*> 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 Rack contains controls that are not available on the rack: <*missing control list*>	Reagent rack for selected panel is missing required controls	**1** Click **OK**. **2** Place the required controls in the reagent rack according to the reagent rack map.

Software Error Messages

Message	Possible Cause	Possible Solution
Sample cap-piercing Probe Counter exceeded 2000. The Probe needs to be replaced soon.	Probe needs replacement soon	Be aware that the probe will need to be replaced after the next 500 pierces.
Sample cap-piercing Probe Counter exceeded 2500. Immediate Probe replacement is required!	Probe needs replacement now	Perform probe replacement procedure.
Unable to lock because the Safety Cover is open Please close the cover and press 'Retry' key	Safety cover open	**1** Close the safety cover. **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 canceled because the Safety Cover was not closed.	Safety cover was not closed	**1** Click **OK**. **2** Restart the procedure.
Current procedure was canceled because the Safety Cover was not closed.	Safety cover was not locked	**1** Click **OK**. **2** Restart the procedure.

Software Error Messages

Message	Possible Cause	Possible Solution
Current procedure was canceled because <*Tank Name*> tank was not filled.	The specified tank fluid level is too low	**1** Click **OK**. **2** Fill the specified tank. **3** Restart the procedure.
Current procedure was canceled because Waste tank was not emptied.	Waste tank was not emptied	**1** Click **OK**. **2** Empty the waste tank. **3** Restart the procedure.
Current procedure was canceled because an error occurred on the Instrument.	Hardware error detected	**1** Click **OK**. **2** Visually inspect the instrument worktable. Make sure all racks are seated correctly and that there are no moving parts obstructed. Make sure all cables are connected. **3** Reinitialize the instrument.
Please abort the sample prep or wait for the current Instrument operation to finish before Shutdown!	Pressed  button while sample prep running	**1** Click **OK**. **2** 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.

Software Error Messages

Message	Possible Cause	Possible Solution
Unable to load Reagent Racks file <*File Name*> <*additional Windows OS message*>	File not in expected location (moved or renamed)	Return file to original location. **1** Uninstall the software. **2** Reinstall the software.
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.	**1** Click **OK**. **2** Fill the specified tank. **3** 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.	**1** Click **OK**. **2** Fix the worklist errors. **3** Save the worklist.

Software Error Messages

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	**1** Click **OK**. **2** Select a new printer. **3** Restart the print. Alternatively, **1** Click **OK**. **2** Connect the printer. **3** Restart the print.
The worklist you are about to run contains Lot Ids and/or TruCOUNT bead information that is different from the information defined in the software. If you choose to proceed the information in the worklist will be replaced with the information from the software. The following information will be replaced: <*list of items to replace*> Would you like to proceed?	You are attempting to run a previously prepped worklist after modifying the Lot ID information.	Click **Yes** to replace specified items and proceed with run. The Lot ID information will be updated based on the latest information entered in the **Lot ID** dialog. Click **No** to cancel the run.

Software Error Messages

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	**1** Click **OK**. **2** Perform the procedure in Recovering from an X, Y, or Z-Axis Error on page 181. **3** If the instrument continues to experience step loss, or if you are unable to calibrate the probe to the reference point, call BD Biosciences.
	Instrument on unstable surface	**1** Click **OK**. **2** Place the instrument on a stable, level surface. **3** Perform the procedure in Recovering from an X, Y, or Z-Axis Error on page 181.
No liquid detected.	No reagent or not enough reagent in vial	**1** Press **Pause**. **2** Add a reagent vial with sufficient volume to the reagent rack.
	Reagents are cold	**1** Press **Pause**. **2** Allow reagents to come to room temperature.
The WASTE tank is full. Please empty the WASTE tank, and make sure to add 500 µL of Antifoam A Concentrate.	Waste tank is full	**1** Empty the waste tank and add 1 L bleach and 500 µL Antifoam A Concentrate. **2** Click **Retry** to continue. If you click **Cancel**, you will have to reinitialize the instrument prior to the next run.

Software Error Messages

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 132. While the probe is exposed, inspect it for damage. **7** Restart the worklist from the beginning.
Failure to Reinitialize the arm	The instrument has failed to reinitialize the arm.	**1** Click **OK**. **2** Perform the procedure in Recovering from a Reinitialization Error on page 182.
The instrument detected a {error type} error on the {device type} device.	Various causes	See the solution for Timeout Error above.

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9

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 218.

Method to Ensure Dispense Accuracy

Materials

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

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

Pan Balance Setup

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

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

2 Place a beaker on the balance.

3 Tare the balance.

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

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

6 Repeat steps 3 to 5 until all 20 tubes have been weighed.

Use the following worksheet to record your weights.

Secondary Tube No.	Weight (g)		
	Empty	Full	Fluid = (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.

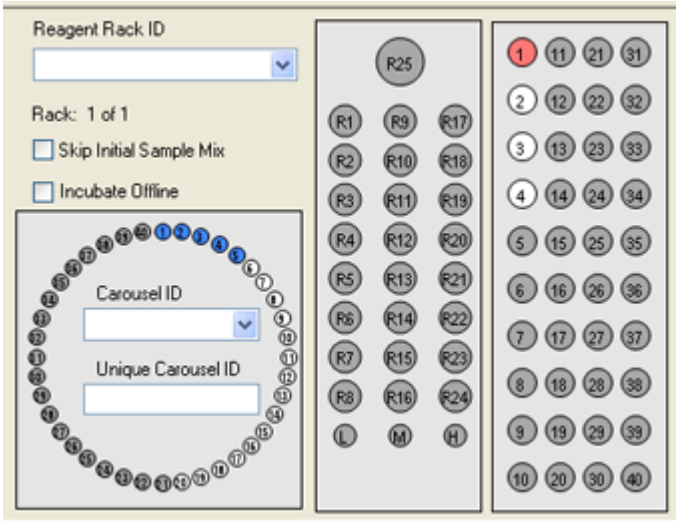
Specify the **Sample ID** and **Primary Tube Type**. Use the default Primary Rack Position 1, 2, 3, and 4.

For information on creating a worklist, see Preparing Primary Tubes on page 99, Panel Selection on page 107, and Carousel Rack Setup on page 107.

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

Sample ID	Case Number	Primary Tube Type	Primary Rack Position	Panel Name
AP 1		BD Vacutainer	1	A & P Dispense
AP 2		BD Vacutainer	2	A & P Dispense
AP 3		BD Vacutainer	3	A & P Dispense
AP 4		BD Vacutainer	4	A & P Dispense

7 Place the primary tubes containing 3 mL of DI water in primary tube rack positions 1–4.



8 Close the safety cover and click **Run**.

9 Save the worklist when prompted.

The probe dispenses the water to all 20 tubes.

10 Immediately weigh each of the 20 tubes, individually.

IMPORTANT: Use forceps to handle the secondary tubes.

a Place a beaker on the balance.

b Tare the balance before each tube.

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

11 Click **Close** to end the process.

Analysis

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

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

Calculate the mean:

$$\bar{X} = \frac{\Sigma X}{N}$$

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:

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

Example:

X = 0.0495 g; density of water = 1 g/mL

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

$$\text{fluid volume} = 49.5 \text{ µL}$$

Calculate the standard deviation:

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

Use the following formula to calculate the %CV:

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

You can use a spreadsheet to do these calculations.

Configure the spreadsheet like the following.

	A	B	C	D	E
1					
2					
3				Weight (g)	
4					
5		Secondary	Empty	Full	Fluid
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		12			=D18-C18
19		13			=D19-C19
20		14			=D20-C20
21		15			=D21-C21
22		16			=D22-C22
23		17			=D23-C23
24		18			=D24-C24
25		19			=D25-C25
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 189.

10

Technical Specifications

This chapter covers the following topics:

Instrument

Dimensions	Height: 76.2 cm (30 in.) Width: 63.5 cm (25 in.) Width (with fluidics tower): 88 cm (35 in.) Depth: 66 cm (26 in.)
Required workspace dimensions	Height (with safety cover open): 94 cm (37 in.) Unit designed to fit lab bench: 76 cm (30 in.) depth
Weight	64 kg (≤140 lb)—instrument only, excluding computer 100 kg (≤220 lb)—including packaging
Power requirements	100–240 VAC (50–60 Hz)
Power consumption	150 W
Fuses (2)	Type T 5.0 Amp–250 V

Environment

Storage temperature	–20°C to 50°C
Operating temperature	18C° to 28°C (64F° to 82°F)
Operating relative humidity	20–80% (non-condensing)
Noise level	• ≤60 dBA (idle mode) • ≤75 dBA (run mode)
Facilities	No special room requirements

Sample Loading

Primary tube rack compatibility	• 13-mm rack • 16-mm rack
Reagent rack	Standard
Carousel rack compatibility	Sample-prep ready BD FACS™ Loader carousel racks, numbers 1–16

Tube Compatibility

Carousel rack	Accommodates up to 40 uncapped 12 x 75-mm tubes • Corning Falcon® polystyrene test tubes • BD Trucount™ Tubes
Primary tube rack	Accommodates up to 40 tubes in the following sizes: BD Vacutainer® • 13 x 75 mm • 13 x 100 mm • 16 x 75 mm • 16 x 100 mm Use BD Hemogard™ closures or standard rubber stoppers. Sarstedt S-Monovette • 2.7 mL, 11 x 66 mm • 2.6 mL, 13 x 65 mm • 3.4 mL, 13 x 65 mm • 4.9 mL, 13 x 90 mm • 4.0 mL, 15 x 75 mm • 5.5 mL, 15 x 75 mm

Reagent rack	Accommodates up to • 24 standard BD Biosciences reagent vials (22.9 mm in diameter), uncapped • Three BD Trucount™ Control vials (15.9 mm in diameter), uncapped • One 60-mL BD FACSClean™ vial (38.9 mm in diameter), uncapped
Tube adapters	Allows use of 11-mm and 13-mm tube in a primary tube rack
Labels	≤5 mils (127 microns) thick

Pre-Programmed Dispense Volumes

Sample	50 µL
Reagent	20 µL
Lyse	450 µL
BD Trucount™ Control	50 µL

Pre-Programmed Incubation Times

Lyse	15 minutes
Reagent	15 minutes

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 of 20 µL:

Secondary Tubes	Excess Volume	Conditioning Volume
1 tube	4 µL	7 µL
2–6 tubes	7 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 (multi-dispense) of a BD-defined panel equals:

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

Conclusion

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

Minimum Sample Volumes

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

Sample Volume Definitions

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 10-1 For BD Vacutainer® Tubes

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

Table 10-2 For Sarstedt Tubes

Sample Volume (µL)	Number of Secondary Tubes	Minimum Sample Volume (µL)					
		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	5–6	1,000	700	1,000	1,000	1,000	1,000
50	7–8	1,100	800	1,100	1,200	1,100	1,200

Performance

BD Multitest/BD Tritest/Absolute Count Panels

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

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

- Recapped tubes
- Clogged probe (from rubber particles from cap piercing or clotted blood)

BD Multitest 6-Color TBNK Method Comparison

Table 10-3 Lymphocyte Subset Absolute Counts

Site (n)	Site 1		Site 2		Site 3		Site 4		Criteria
Lymphocyte Subset	Mean Bias	95% CI	Mean Bias	95% CI	Mean Bias	95% CI	Mean Bias	95% CI	
$CD3^+CD4^+$	0.5	(–1.3, 2.3)	0.9	(–0.1, 1.9)	1.2	(0.3, 2.2)	0.29	(–0.9, 1.5)	±10%
$CD3^+CD8^+$	–0.1	(–1.8, 1.7)	0.8	(–0.04, 1.7)	–0.01	(–0.6, 0.6)	0.47	(–0.9, 1.9)	±10%
Total $CD3^+$	0.2	(–1.5, 1.9)	0.7	(0.04, 1.4)	–0.02	(–0.6, 0.5)	0.67	(–0.4, 1.8)	±10%
$CD3^-CD19^+$	0.7	(–1.4, 2.8)	3.0	(1.3, 4.7)	1.7	(0.5, 3.0)	2.45	(0.3, 4.6)	±20%
$CD3^-(CD16 + CD56)^+$	4.1	(1.9, 6.3)	3.8	(2, 5.6)	2.5	(0.7, 4.2)	3.76	(1.3, 6.2)	±20%

Table 10-4 Lymphocyte Subset Percent Positives

Site (n)	Site 1			Site 2			Site 3			Site 4		
Lymphocyte Subset	Mean Bias	95% CI	Criteria *	Mean Bias	95% CI	Criteria *	Mean Bias	95% CI	Criteria *	Mean Bias	95% CI	Criteria *
$CD3^+$ $CD4^+$	0.02	(–0.1, 0.2)	±3	–0.5	(–1.1, 0.1)	±10 %	0.3	(0.1, 0.4)	±3	– 0.18	(–0.5, 0.1)	±10 %
$CD3^+$ $CD8^+$	–0.5	(–0.9, –0.1)	±10 %	–0.5	(–1.1, 0.2)	±10 %	0.4	(–0.1, 0.9)	±10 %	– 0.67	(–1.6, 0.3)	±10 %
Total $CD3^+$	–0.2	(–0.5, – 0.0002.)	±10 %	–0.6	(–0.8, – 0.3)	±10 %	0.4	(0.04, 0.7)	±10 %	– 0.47	(–0.9, 0.0)	±10 %
$CD3^-$ $CD19^+$	– 0.04	(–0.2, 0.1)	±3	0.1	(0.1, 0.2)	±3	0.1	(0.1, 0.2)	±3	0.12	(–0.1, 0.3)	±3
$CD3^-$ $(CD16 + CD56)^+$	0.4	(0.2, 0.5)	±3	0.1	(0, 0.3)	±3	0.2	(0.01, 0.3)	±3	0.36	(0.1, 0.6)	±3

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

NOTE Data were acquired using BD FACSCanto™ II.

BD Multitest™ IMK Method Comparison

Table 10-5 Lymphocyte Subset Absolute Counts

Tube Type (n)	Tube Type 1		Tube Type 2		Criteria
Lymphocyte Subset	Mean Bias	95% CI	Mean Bias	95% CI	
$CD3^+CD4^+$	-3.3	(-4.3, -2.3)	0.9	(-0.6, 2.4)	±10%
$CD3^+CD8^+$	-3.2	(-4.1, -2.2)	0.0	(-1.4, 1.4)	±10%
Total $CD3^+$	-3.5	(-4.2, -2.7)	-1.0	(-1.9, -0.2)	±10%
$CD3^-CD19^+$	-3.7	(-5.6, -1.8)	-1.5	(-3.3, 0.3)	±20%
$CD3^-(CD16^+{+}56^+)$	-1.5	(-2.9, -0.1)	1.8	(-0.6, 4.1)	±20%

Table 10-6 Lymphocyte Subset Percent Positives

Tube Type (n)	Tube Type 1		Tube Type 2		Criteria[a]
Lymphocyte Subset	Mean Bias	95% CI	Mean Bias	95% CI	
$CD3^+CD4^+$	-0.03	(-0.2, 0.1)	0.1	(-0.1, 0.3)	±3
$CD3^+CD8^+$	0.3	(0, 0.7)	-0.5	(-1.1, 0.1)	±10%
Total $CD3^+$	0.2	(0, 0.4)	-0.3	(-0.5, -0.1)	±10%
$CD3^-CD19^+$	-0.1	(-0.2, 0)	0	(-0.1, 0.1)	±3
$CD3^-(CD16^+{+}56^+)$	0.1	(0, 0.2)	0.2	(0, 0.4)	±3

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

NOTE Data were acquired using BD FACSCalibur™.

BD Multitest Assay Precision

Table 10-7 Precision of Lymphocyte Subset Absolute Counts

Lymphocyte Subset	CDL[a] Upper CV		CDN[b] Upper CV		Criteria
	Within Run	Within Device	Within Run	Within Device	
$CD3^+CD4^+$	8.2	8.1	5.0	4.9	≤10%
$CD3^+CD8^+$	5.1	5.2	5.3	5.9	≤10%
Total $CD3^+$	5.0	5.1	4.1	4.5	≤10%
$CD3^-CD19^+$	6.4	6.3	6.3	6.4	≤20%
$CD3^-(CD16 + CD56)^+$	8.1	8.1	6.9	7.7	≤20%

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

Table 10-8 Precision of Lymphocyte Subset Percent Positives

Lymphocyte Subset	CDL[a] Upper SD		CDN[b] Upper SD		Criteria
	Within Run	Within Device	Within Run	Within Device	
$CD3^+CD4^+$	0.78	0.74	1.08	1.06	≤2.5
$CD3^+CD8^+$	1.38	1.51	1.06	1.08	≤2.5
Total $CD3^+$	1.24	1.38	1.03	1.00	≤2.5
$CD3^-CD19^+$	0.95	1.01	0.64	0.65	≤2.5
$CD3^-(CD16 + CD56)^+$	1.11	1.11	0.66	0.69	≤2.5

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

NOTE Data were acquired over 21 days, using three instruments.

Pipetting Accuracy and Precision

Table 10-9 Pipetting Accuracy

	n	Acceptance criteria	Volume dispensed (µL)			Pass/Fail
			SPA III A	SPA III B	SPA III C	
Blood (single dispense) 50 µL	20	50 µL ±3%	50.42	50.55	49.76	Pass
Blood (multi-dispense) 50 µL	20	50 µL ±3%	49.98	49.87	50.07	Pass
Reagent 20 µL	20	20 µL ±7%	19.98	20.39	19.55	Pass

Table 10-10 Pipetting Precision

	n	Acceptance criteria	90% CI of CV (upper limit)			Pass/Fail
			SPA III A	SPA III B	SPA III C	
Blood (single dispense) 50 µL	20	≤3% CV	1.58	0.4	0.63	Pass
Blood (multi-dispense) 50 µL	20	≤3% CV	0.74	0.75	0.75	Pass
Reagent 20 µL	20	≤5% CV	1.27	1.58	2.54	Pass

Sample and Reagent Carryover

Sample and reagent carryover were each tested with two equations:

- **Equation 1:** Carryover = [(First Low – Third Low)/(Third High – Third Low)] x 100

    - From Section 5.3 and Appendix 4 of Guidelines for the Evaluation of Blood Cell Analysers including those used for Differential Leucocyte and Reticulocyte Counting and Cell Marker Applications, *Clinical Laboratory Haematology* 16:157–174 (1994).

- **Equation 2:** Carryover = [(First Low – Third Low)/(Third High – First Low)] x 100

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

Sample Carryover

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

Equation	Manual Preparation		SPA III		Acceptance criteria
	minimum	maximum	minimum	maximum	
1	–0.47	0.08	–0.12	0.12	≤0.2%
2	–0.47	0.08	–0.12	0.12	≤0.2%

Reagent Carryover

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

Equation	Manual Preparation		SPA III		Acceptance criteria
	minimum	maximum	minimum	maximum	
1	–0.011	0.001	–0.021	0.006	<0.01
2	–0.011	0.001	–0.021	0.006	<0.01

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

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