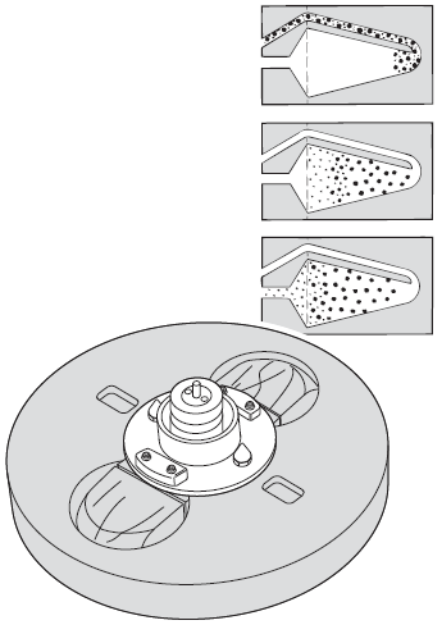
Instructions For Use

JE-5.0 Elutriation System

For Use with Avanti J-26S XP Series,
Avanti J-26 XP Series,
and J6-MI Centrifuges
Not for Use in Avanti JXN Series



PN JE5-IM-13AD
November 2020

Beckman Coulter, Inc.
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Brea, CA 92821 U.S.A.

BECKMAN COULTER

JE-5.0 Elutriation System
PN JE5-IM-13AD (November 2020)

Contact Us
If you have any questions, contact our Customer Support Center.

- Worldwide, find us via our website at www.beckman.com/support/technical.
- In the USA and Canada, call us at 1-800-369-0333.
- Outside of the USA and Canada, contact your local Beckman Coulter Representative.

Find us on the World Wide Web at: **www.beckman.com**

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May be covered by one or more pat. - see www.beckman.com/patents

Glossary of Symbols is available at beckman.com/techdocs (PN C24689).

Original Instructions

Revision History

This document applies to the latest version listed and higher versions. When a subsequent version changes the information in this document, a new issue will be released to the Beckman Coulter website. For updates, go to www.beckman.com/techdocs and download the latest version of the manual for your rotor.

Issue AC, 08/2019
Changes or additions were made to: Figure 2.4, Figure 2.5, and Figure 4.1

Issue AD, 11/2020
Changes or additions were made to: Figure 4.1, Rotor Speed and Flow Rate Nomogram.

Note: *Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.*

Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact your Beckman Coulter Representative.

Alerts for Warning, Caution, Important, and Note

⚠ WARNING

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the Important adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

The rotor materials have not been approved for *in vivo* applications.

Handle body fluids with care because they can transmit disease. No known test offers complete assurance that they are free of micro-organisms. Some of the most virulent—Hepatitis (B and C) and HIV (I–V) viruses, atypical mycobacteria, and certain systemic fungi—further emphasize the need for aerosol protection. Handle other infectious samples according to good laboratory procedures and methods to prevent spread of disease. Because spills may generate aerosols, observe proper safety precautions for aerosol containment. Do not run toxic, pathogenic, or radioactive materials in this centrifuge without taking appropriate safety precautions. Biosafe containment should be used when Risk Group II materials (as identified in the World Health Organization *Laboratory Biosafety Manual*) are handled; materials of a higher group require more than one level of protection.

Observe all cautionary information printed on the original solution containers prior to their use.

Dispose of all waste solutions according to appropriate health and safety guidelines.

The rotor and accessories are not designed for use with materials capable of developing flammable or explosive vapors. Such materials (chloroform or ethyl alcohol, for example) should not be centrifuged in or handled near the centrifuge.

Inspect the rotor body at least once a month, especially inside cavities, for rough spots or pitting, white powder deposits—frequently aluminum oxide—or heavy discoloration. If any of these signs are evident, do not run the rotor. Contact your Beckman Coulter representative for information about the Field Rotor Inspection Program and the rotor repair center.

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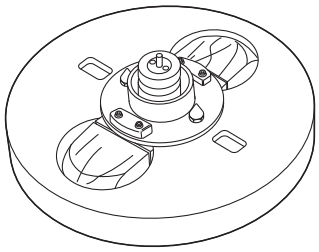
JE-5.0 System Specifications

J-E 5.0 Rotor



Maximum speed	5000 RPM
Critical speed range[a]	
J6-MI centrifuges	400 to 700 RPM
Avanti J-26S XP series and J-26XP series[b]	300 to 400 RPM
Maximum solution density	3 g/mL
Weight (without the quick-release assembly	15 kg (33 lb)
Rotor code (used with the J6-MI centrifuge)	5.2
Materials	
Rotor	black-anodized aluminum and stainless steel
Chambers	Novolac[c] clear phenolic resin
Chamber gasket	medical-grade silicone rubber
Chamber cushions	high-density polyethylene
Tubes	stainless steel
O-rings	Buna-N rubber
Seals	Tribolon[d] plastic
Harness tubing	Tygon

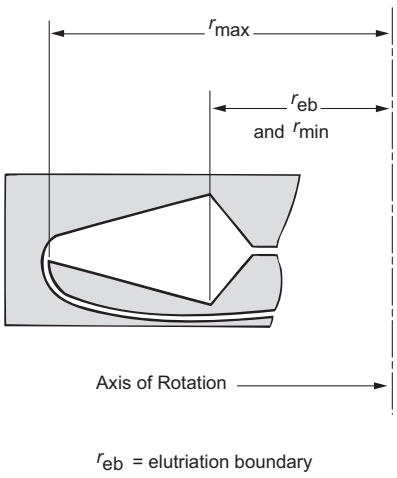
a. The critical speed range is the range of speeds over which the rotor shifts so as to rotate about its center of mass. Passing through the critical speed range is characterized by some vibration.

b. This also applies to the discontinued Avanti J-20 XP series.

c. Novolac is a name applied by Bakeland to a fusible phenolic resin.

d. Tribolon is an aromatic polymide thermoplastic whose acid/salt/base resistance is comparable to that of cellulose propriate. It is resistant to most solvents, including 6% hydrogen peroxide.

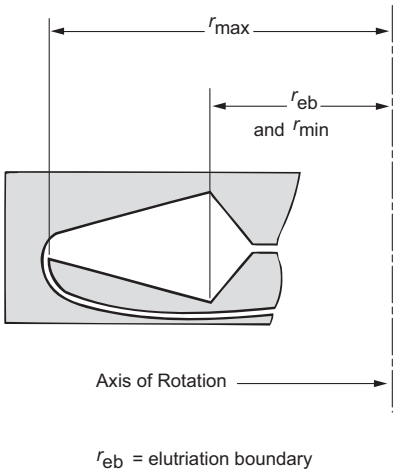
Large Chamber



Maximum speed	5000 RPM
Maximum solution density	3 g/mL
Chamber material	Novolac
Chamber capacity (one chamber)	40 mL
Relative Centrifugal Field[a] at 5000 RPM	
At r_{max} (168 mm)	$4700 \times g$
At r_{min} /r_{eb} (34.2 mm)	$2410 \times g$
volume to fill r_{min}	30 mL
Amount of medium required to elutriate one fraction of particles	500 to 1000 mL
Chamber constant	1.73×10^{-1}

a. Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed (rw^2) to the standard acceleration of gravity (g) according to the following formula: RCF = rω2/g — where r is the radius in millimeters, ω is the angular velocity in radians per second (2 π RPM /60), and g is the standard acceleration of gravity (9807 mm/s^2). After substitution: RCF = 1.12r (RPM/1000)^2

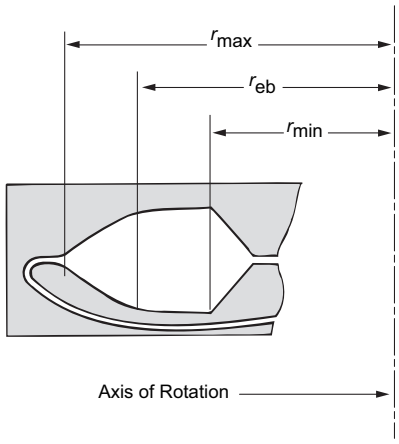
Standard Chamber



Maximum speed	5000 RPM
Maximum solution density	3 g/mL
Chamber material	Novolac
Chamber capacity (one chamber)	4 mL
Relative Centrifugal Field[a] at 5000 RPM	
At r_{max} (125 mm)	$3500 \times g$
At r_{min} /r_{eb} (86 mm)	$2410 \times g$
volume to fill r_{min}	3 mL
Amount of medium required to elutriate one fraction of particles	75 to 100 mL
Chamber constant	5.11×10^{-2}

a. Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed (rw^2) to the standard acceleration of gravity (g) according to the following formula: RCF = rω2/g — where r is the radius in millimeters, ω is the angular velocity in radians per second (2 π RPM /60), and g is the standard acceleration of gravity (9807 mm/s^2). After substitution: RCF = 1.12r (RPM/1000)^2

Sanderson Chamber



Maximum speed . . . . . 5000 RPM

Maximum solution density . . . . . .3 g/mL

Chamber material . . . . . .Novolac

Chamber capacity (one chamber) . . . . . 5.5 mL

Relative Centrifugal Field[a] at 5000 RPM

At r_{max} (125 mm) . . . . . .4230 × *g*

At r_{min} (106 mm) . . . . . .3560 × *g*

At r_{eb} (86 mm) . . . . . .2890 × *g*

volume to fill r_{min} . . . . . .3 mL

Amount of medium required to elutriate one fraction of particles . . . . . .75 to 100 mL

Chamber constant . . . . . .3.78×10^{-2}

a. Relative Centrifugal Field (RCF) is the ratio of the centrifugal acceleration at a specified radius and speed (rw^2) to the standard acceleration of gravity (*g*) according to the following formula: RCF = rω2/g — where *r* is the radius in millimeters, ω is the angular velocity in radians per second (2 π RPM /60), and *g* is the standard acceleration of gravity (9807 mm/s^2). After substitution: RCF = 1.12r (RPM/1000)^2

CHAPTER 1

The Process of Centrifugal Counterflow Elutriation

This section describes how two opposing forces, centrifugation and counterflow elutriation, combine to create a gentle means of separating cells and other particles by sedimentation rate in the JE-5.0 elutriation system.

Overview

Centrifugal elutriation in the JE-5.0 rotor combines two separation technologies: centrifugation, the process of sedimentation under the influence of a centrifugal force field, and counterflow elutriation, the process of separation by washing.

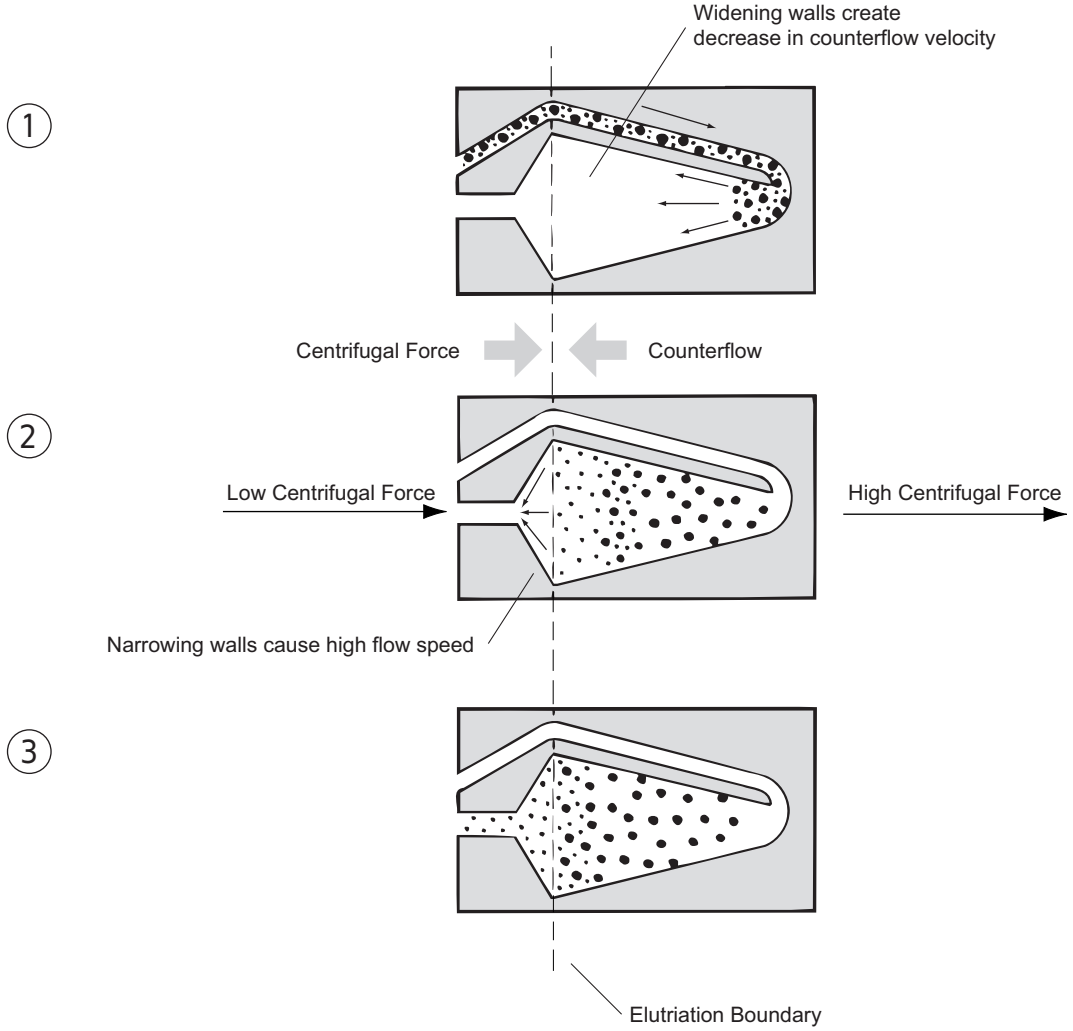
In the JE-5.0 rotor, separation takes place in a funnel-shaped elutriation chamber (see Figure 1.1). Each cell in the chamber is acted upon by two opposing forces: centrifugal force (driving it away from the axis of rotation), and fluid velocity (driving it toward the axis of rotation—"counterflow"). While the rotor is spinning in the centrifuge, a suspension of cells is pumped at a preset flow rate from outside the centrifuge into the rotor to the narrow end of the elutriation chamber. As suspended cells are introduced into the chamber, they migrate according to their sedimentation rates to positions in the gradient where the effects of the two forces upon them are balanced.

Small cells with low sedimentation rates are quickly washed toward the axis of rotation, where they are caught in an increasing flow velocity caused by the rapidly narrowing chamber walls (see Figure 1.1). These cells are washed out of the chamber, up through the rotor, and out into a collection vessel. Somewhat larger or denser cells move through the chamber more slowly and reach equilibrium at the elutriation boundary, where the centrifugal force and the velocity are relatively low and the chamber walls are at their widest point. The largest or densest cells remain near the inlet to the chamber where centrifugal force and fluid velocity are high. By increasing the flow rate in gradual steps, successive fractions of increasingly large or dense cells can be washed out of the rotor and collected. Continued incremental increase of the flow rate will finally elutriate all cells from the chamber.

During the run, you can view the processes taking place in the chamber by looking through the port in the centrifuge door. The clear resin elutriation chamber is illuminated from below by a strobe light that is synchronized with the speed of the rotor.

APPENDIX A contains detailed information regarding the principles of counterflow centrifugal elutriation, as well as the equations necessary to adjust for applications other than those discussed in this manual.

Figure 1.1 The Elutriation Process



1. Cells suspended in a medium are pumped into the chamber.
2. Centrifugal sedimentation of cells is balanced by flow velocity.
3. Flow increased — smaller, slow-sedimenting cells elutriate out of the chamber.

CHAPTER 2

System Description and Installation

This section describes the components and accessories of the JE-5.0 elutriation system. A Beckman Coulter Field Service representative will install your system after delivery; the instructions in this section will guide you in reinstalling the components.

The JE-5.0 is designed to separate and/or concentrate monodisperse suspensions of single cells or particles (approximately 2 to 50 µm in diameter) according to size. The system components include the strobe, the strobe control panel, the flow system components, the rotor body, and the quick-release assembly, which comprises the elutriation chamber, the counterbalance, and the rotating seal assembly. This system is designed to be used in Avanti J-26S XP series, J-26 XP series, discontinued J-20 XP series centrifuges, and in J6-MI centrifuges that have been modified to include a door with a viewing port.

See the Warranty at the back of this manual for warranty information.

The Strobe Assembly

The strobe assembly (Figure 2.1 and Figure 2.2) consists of a strobe flashlamp mounted to a chassis (in Avanti J-26S XP series and J-26 XP series) or installed in a molded housing (in J6-MI centrifuges). The flashlamp is covered by an opaque plastic window. During operation, a signal from the centrifuge spindle tachometer triggers the strobe once per revolution. A strobe power supply is mounted to the back of the centrifuge control head (Figure 2.3).

Contact Beckman Coulter Field Service if the strobe power cable needs to be replaced.

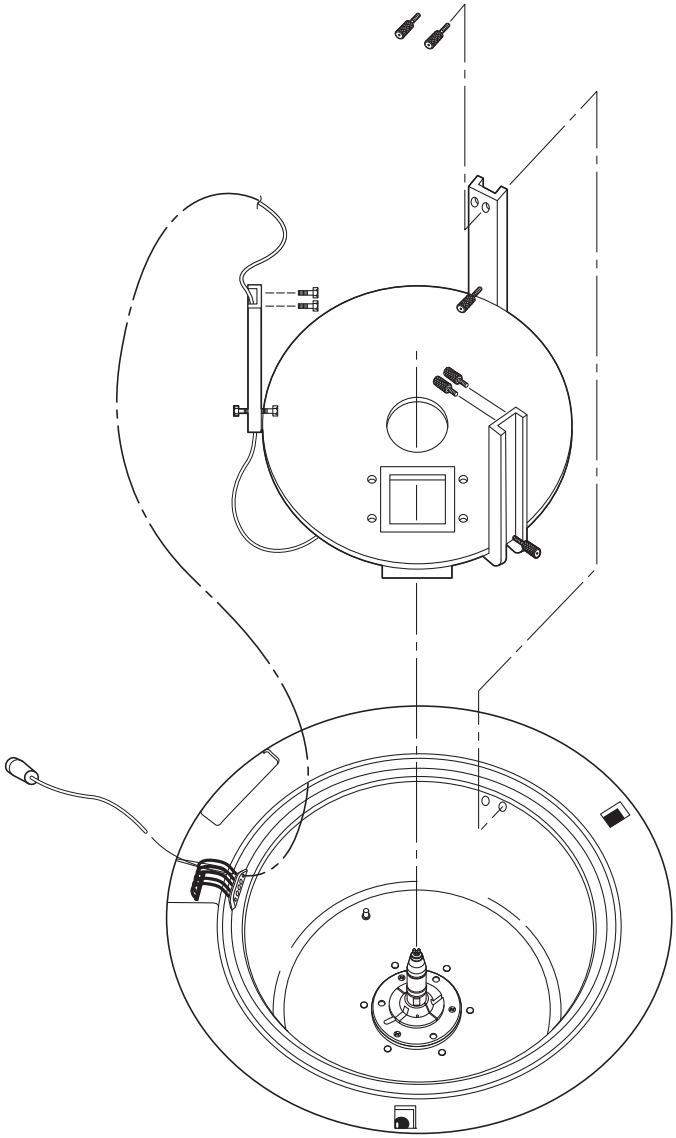
Installing and Connecting the Strobe Assembly (Avanti J-26S XP Series and J-26 XP Series Centrifuges)

⚠ WARNING

Turn the centrifuge power OFF before installing or removing the strobe power cable.

Three of the four elutriator portholes on the left side of the centrifuge are for liquid lines. The fourth, nearest the centrifuge control panel, is for the strobe power cable (Figure 2.1 and Figure 2.4). During installation, a Beckman Coulter Field Service Engineer will cut a slit in the rubber leading to the strobe power cable porthole, for cable insertion.

Figure 2.1 Installing the Strobe Assembly*



1 Lower the strobe assembly chassis into the centrifuge chamber as shown in Figure 2.1, routing the strobe power cable out the left side of the housing.

Place the cable behind the bracket, as shown.

2 Attach each bracket to the centrifuge chamber by first tightening the thumbscrew at the bottom of the bracket and then tightening two Phillips-head screws into the two holes at the top of the bracket.

* Part number 363738, for Avanti J-26S XP and J-26 XP Series Centrifuges.

Figure 2.2 Installing the Strobe Assembly (J6-MI Centrifuges)

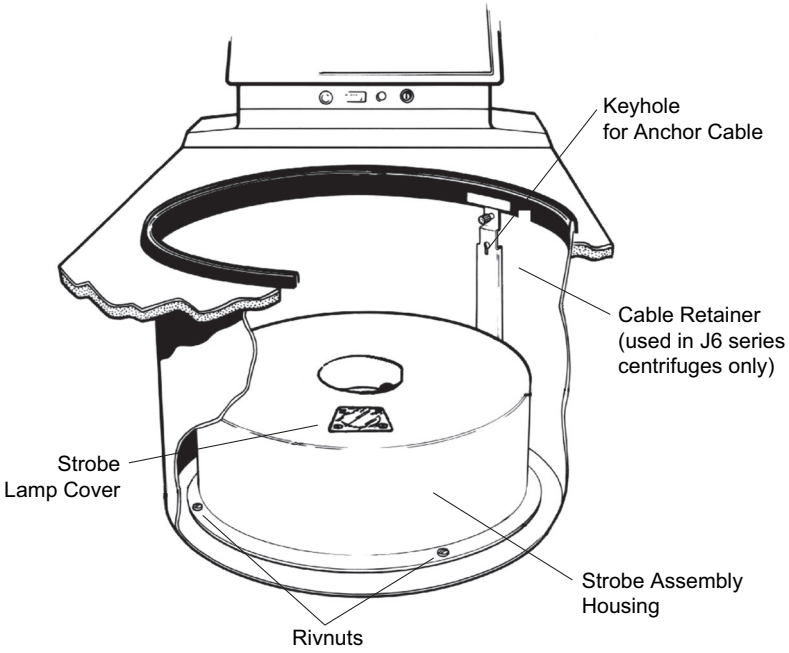


Figure 2.3 Strobe Controls (For All Centrifuge Models)

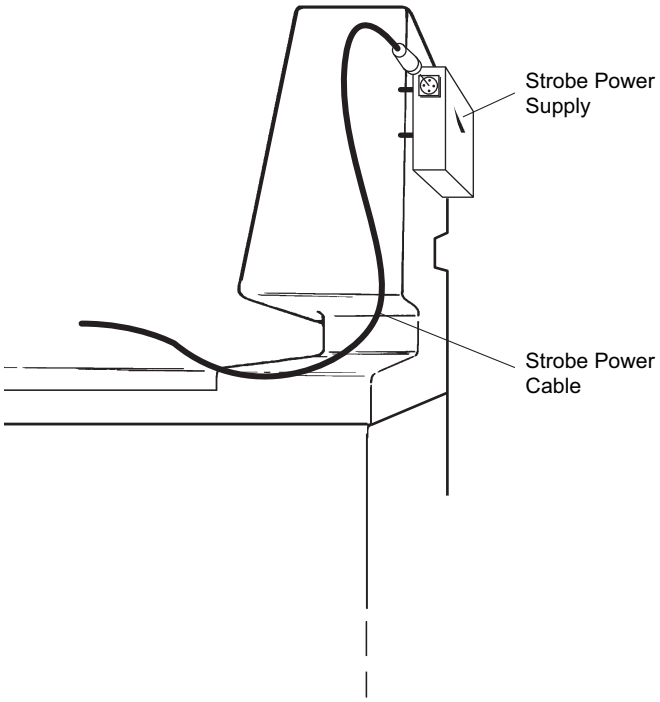
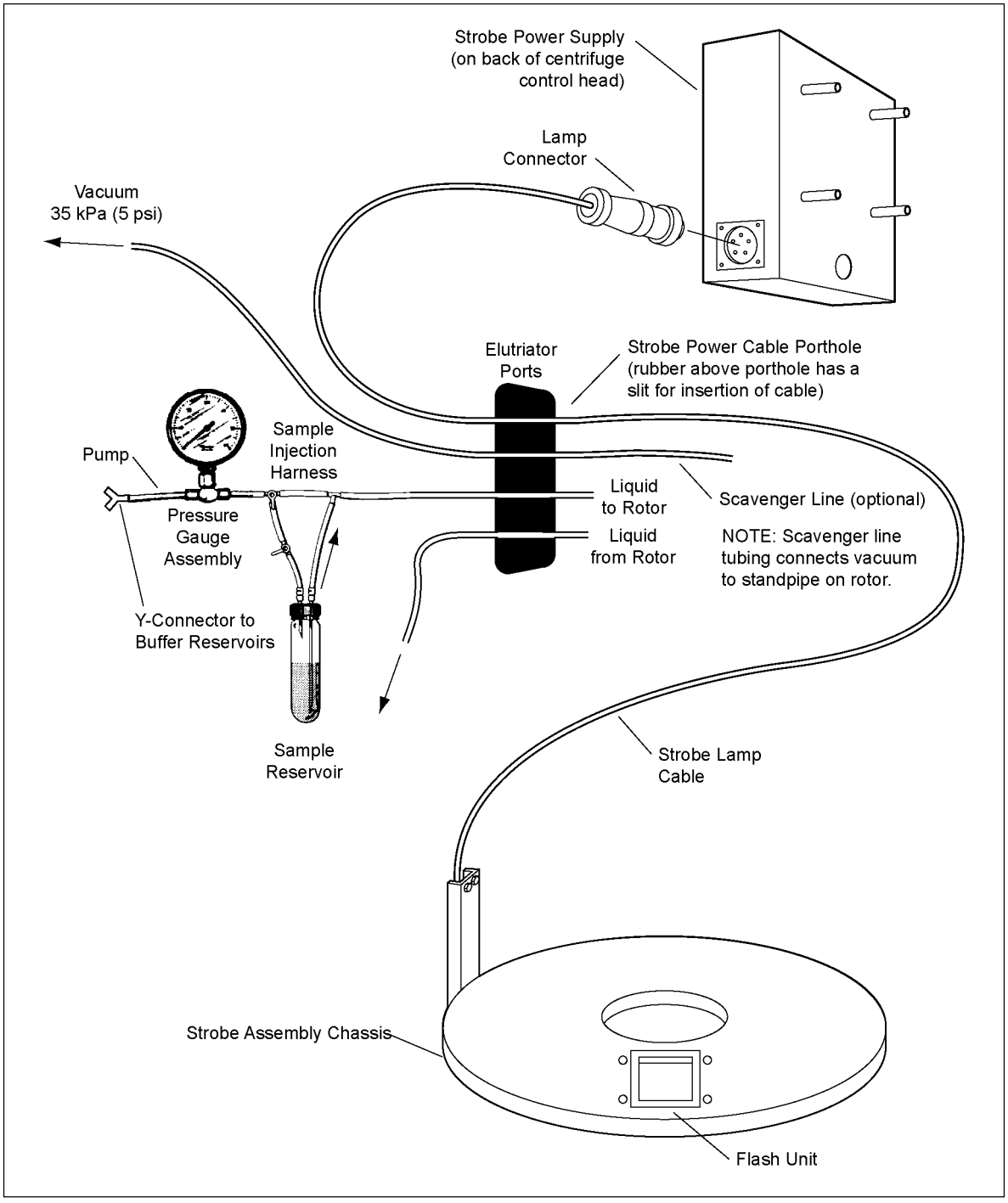


Figure 2.4 Electrical and Liquid Line Connections in Avanti J-26S XP Series and J-26 XP Series Centrifuges



3 Lightly coat the strobe cable porthole with silicone vacuum grease (306812).

(Note: the vacuum grease must be completely removed before the next non-elutriation run.)

4 Insert the strobe power cable into the porthole by pushing it through the slit in the rubber.

5 Pull the cable through the port, leaving no excess cable in the chamber.

6 Plug the strobe power cable connector into the strobe power supply on the back of the centrifuge control head (see Figure 2.2).

Installing and Connecting the Strobe Assembly (J6-MI Centrifuges)

⚠ WARNING

Turn the centrifuge power OFF before installing or removing the strobe power cable.

A tensioning bracket is mounted on the top right outer centrifuge panel (see Figure 2.5). It has four slots: two for liquid lines, one for the strobe power cable, and one for the optional scavenger line. The strobe power cable and each liquid line are each inserted into a slitted rubber stopper, and then the stopper is inserted into the tensioning bracket. The lines can be placed in the tensioning bracket in any arrangement.

1 Lower the strobe assembly housing into the centrifuge chamber as shown in Figure 2.2, routing the strobe power cable under the notch in the right lower edge of the housing.

2 To attach the housing to the bottom of the centrifuge chamber, insert four Phillips-head screws through the holes in the housing into the four mounting holes in the centrifuge chamber and tighten until snug.

3 Route the strobe power cable up the right side of the centrifuge chamber.

Place the cable retainer over the cable (see Figure 2.2).

Tighten the cable retainer captive screws into the matching rivnuts on the bottom and side of the chamber.

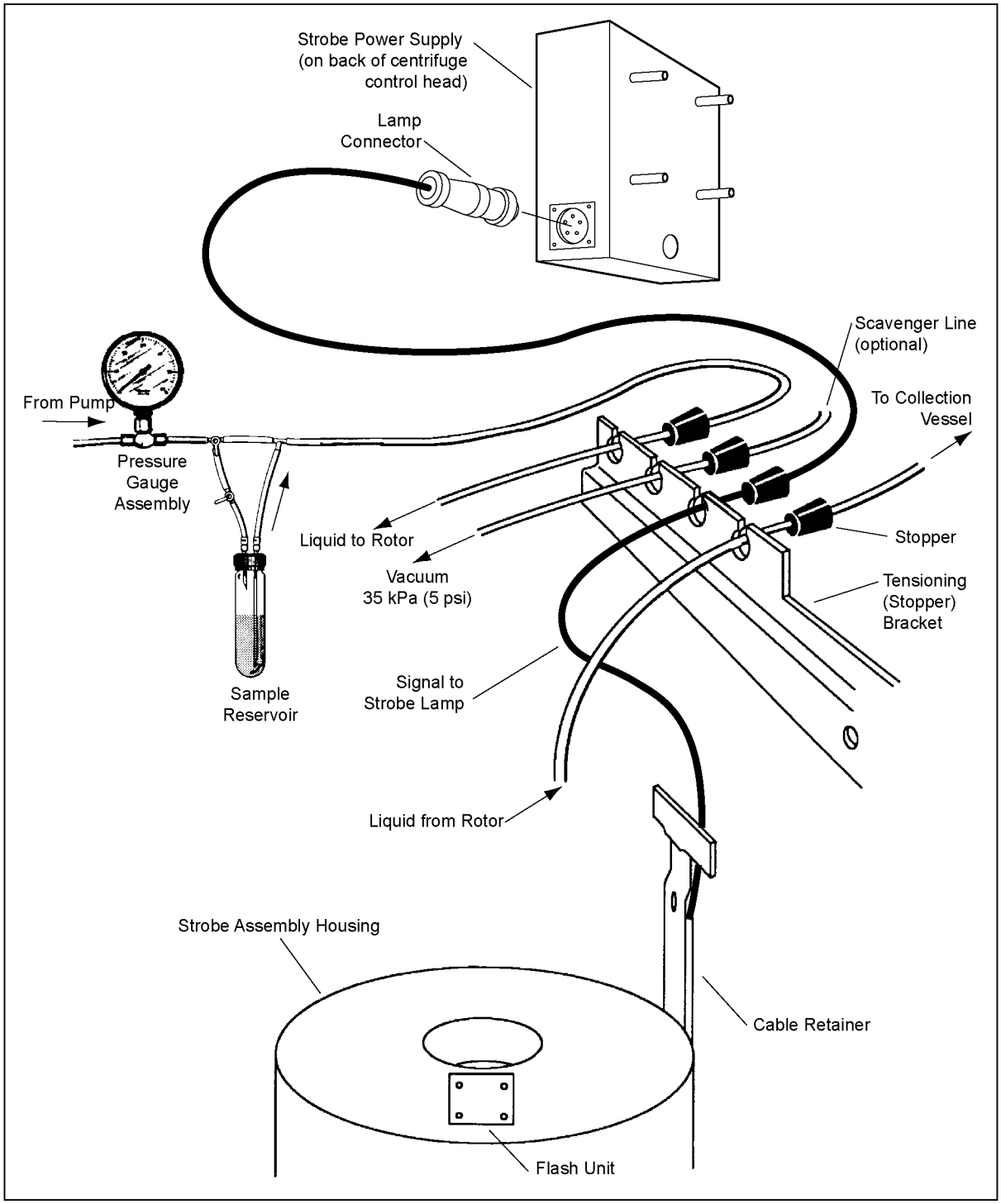
4 Insert the strobe power cable into one of the rubber stoppers, and then insert the stopper into one of the slots in the tensioning bracket.

Place the cable over the door gasket.

5 Plug the strobe power cable connector into the strobe power supply on the back of the centrifuge control head (see Figure 2.3).

6 Replace the rubber stoppers in the tensioning bracket when you have finished using the elutriation system. *The stoppers must be in place during non-elutriation centrifugation.*

Figure 2.5 Electrical and Liquid Line Connections in J6-MI Centrifuges



The Flow System

The flow system consists of a buffer reservoir, a variable-speed pump,* a gauge for monitoring back pressure in the rotor, tubing with three-way valves to route the cell suspension into and out of the rotor, a harness that serves as a combined sample injection inlet, bubble trap, and sample reservoir, and a sample collection vessel. The pump, buffer reservoir, and collection vessel are not supplied with the system.

The most commonly used tubing with this rotor is size 14 Masterflex tubing from Cole-Parmer. Other tubing can be used as well, such as Tygon. See APPENDIX B for more information on tubing.

Connecting the Liquid Lines

Avanti J-26S XP Series and J-26 XP Series Centrifuges

Cole-Parmer size 16 tubing (1/4 in.) can be inserted directly through the elutriator portholes. No adapter is required. Adapters are required for other tubing sizes as shown in Table 2.1.

Table 2.1 Tubing Adapters for Use with Avanti J-26S XP Series and J-26 XP Series Centrifuges

Tubing Description	Tubing Size (O.D.)	Beckman Coulter Adapter Part Number
Cole-Parmer size 14	4.8 mm (3/16 in.)	363830
Cole-Parmer size 15	9.6 mm (3/8 in.)	363831

To use a tubing adapter:

1. Insert the adapter completely through the elutriator porthole.
2. Cut the tubing on either side of the porthole.
3. Insert one end of tubing into one end of the adapter and the other tubing end into the other end of the adapter.

NOTE If you have difficulty inserting 6.4-mm O.D. tubing lines through a porthole, try cutting the end of the tubing at a sharp angle. After drawing the tubing through the porthole, cut the tubing end off square.

J6-MI Centrifuges

Insert tubing through the slit in one of the stoppers (see Figure 2.5) and then place the stopper in the porthole.

* See APPENDIX B for pump specifications and ordering information.

Scavenger Line (Optional)

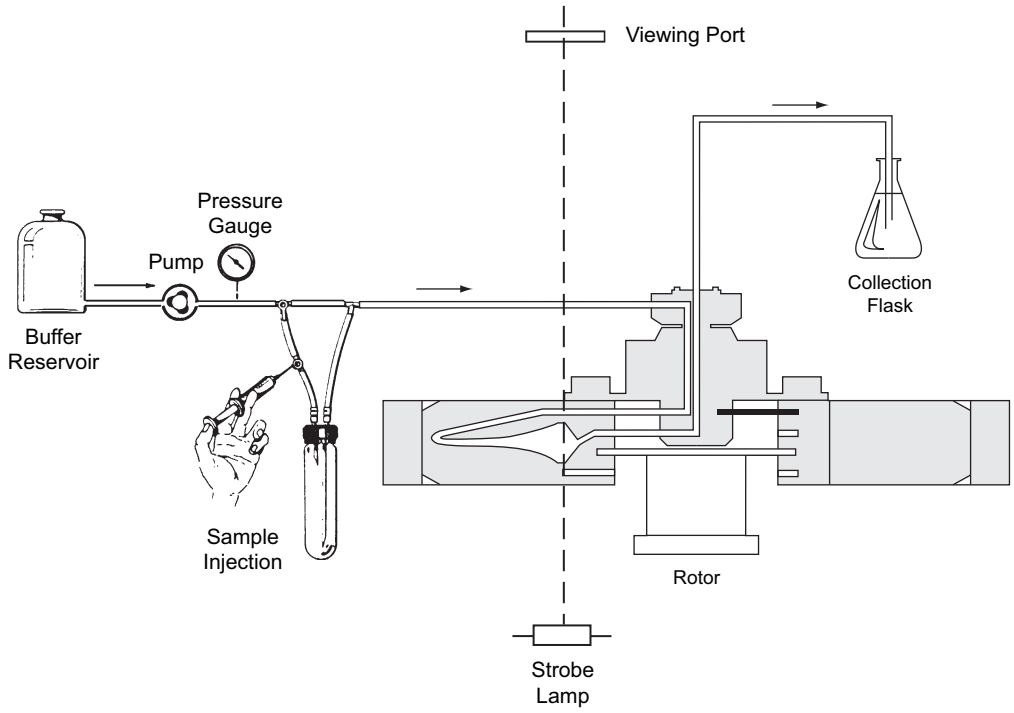
To keep the seal housing bearings dry, we recommend that you connect a third liquid line (called the scavenger line) from the standpipe on the rotor seal housing to a 35-kPa (5-psi) vacuum trap outside of the centrifuge. Pass this line through one of the elutriator ports (see Figure 2.4 and Figure 2.5).

Connecting the Reservoir and Sample Injection Harness

If you use the sample-injection harness and reservoir provided with your JE-5.0 system, connect them as shown in Figure 2.6. If you use another arrangement, note that in all cases the pressure gauge must be located between the pump and the rotor. If you use the harness, check to be sure the tubing is installed properly on the valves (Figure 2.7). Tubing between the sample-injection harness and the rotor should be no longer than one meter.

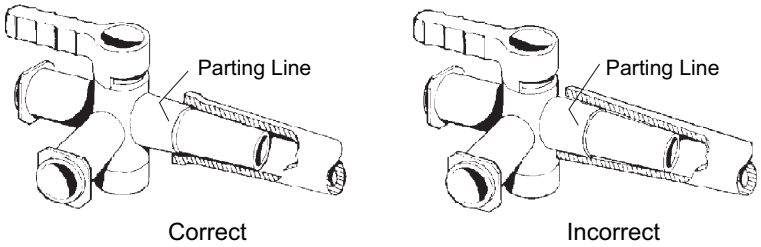
You may wish to configure your system differently in order to load sample from a separate reservoir (for example, a spinner flask). This loading method eliminates the need for the sample injection harness and may be preferable when the sample contains fragile cells. Connect tubing leading from your sample reservoir to a three-way valve inserted in the line between the buffer reservoir and the pump.

Figure 2.6 The JE-5.0 Elutriator System Components*



* Beckman Coulter supplies all necessary components except the pump, buffer reservoir, and collection flask.

Figure 2.7 Attachment of Tubing to the Three-Way Valve



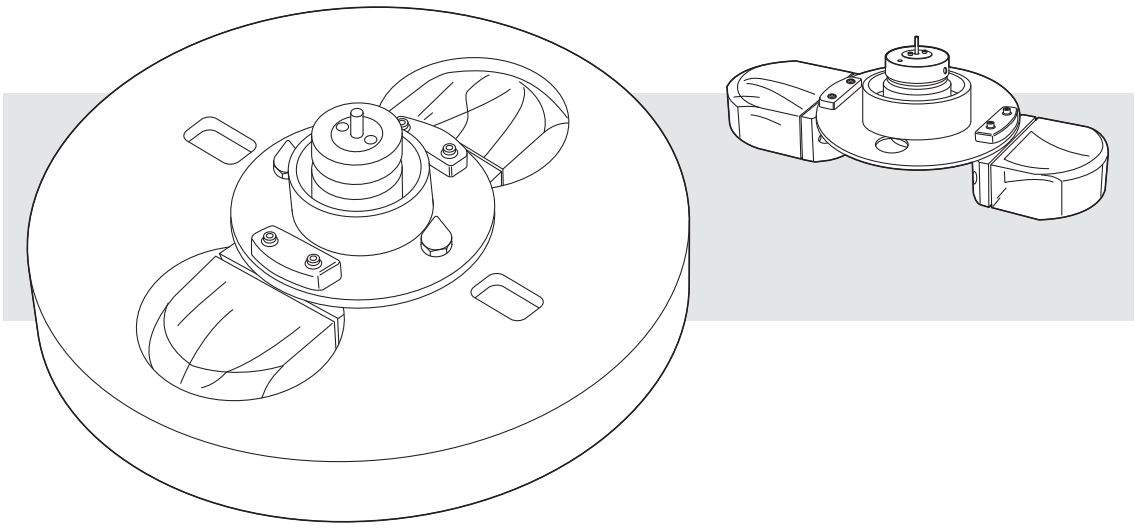
NOTE Proper position for end of tubing is just beyond the parting line on the fitting. If the tubing is pressed too far onto the valve, it will be stretched and may leak.

The Rotor Body

The rotor body is made of black-anodized aluminum and stainless steel (see Figure 2.8). Two pins in the drive hole of the rotor seat into grooves in the centrifuge drive hub and prevent the rotor from slipping during acceleration or deceleration. A reflector is located on one side of the rotor base.

The two holes in the rotor body accommodate the chamber and counterbalance (or second chamber). These holes are fitted with high-density polyethylene pads designed to cushion the quick-release assembly during centrifugation. The center opening accommodates the rotating seal assembly and the tubes linking this assembly to the elutriation chamber and counterbalance (or second chamber). The tie-down bolt that anchors the rotor to the centrifuge drive hub is located in the rotor base beneath the seal assembly through the center opening.

Figure 2.8 The JE-5.0 Rotor



Installing the Rotor Body in the Centrifuge

Install the rotor body into the centrifuge as follows.

1 Make sure the polyethylene chamber pads (356917) are installed at the outer edges of the chamber holes in the rotor body.

2 If using a J6-MI centrifuge, lubricate the centrifuge drive hub (see Figure 2.9) with Spinkote (306812).

Avanti J series centrifuge drive hubs do not require lubrication.

The rotor shaft has two pins, located parallel to the slot in the center of the body, that are designed to seat with the grooves or teeth in the centrifuge drive hub.

3 Set the rotor body (without the quick-release assembly) straight down over the centrifuge drive hub so that the slot lines up with the grooves or teeth on the hub.

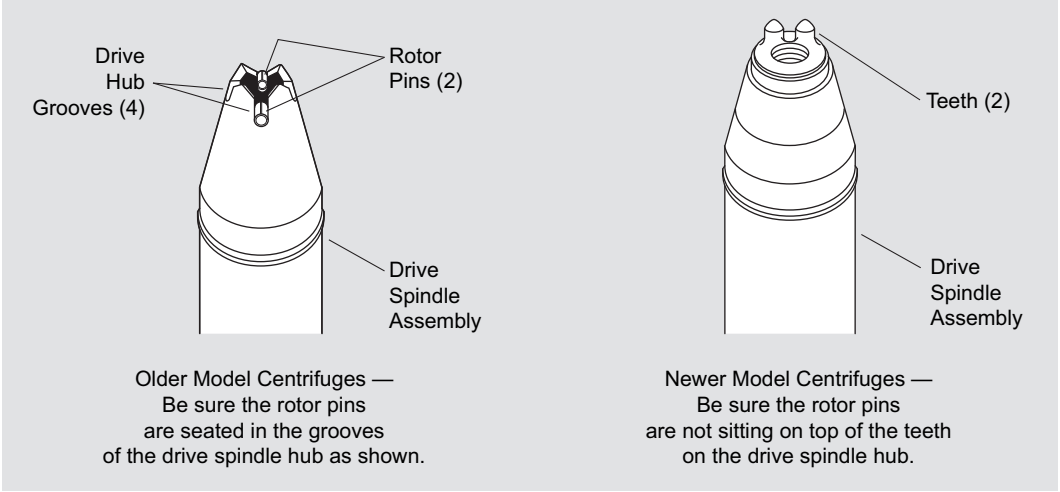
4 Use the 5/16-in. T-handle hex wrench (927766) to tighten the tie-down bolt in the center of the rotor shaft to secure the rotor to the hub.

The tie-down bolt should complete approximately four full turns.

If it will not complete at least three turns, the rotor pins are not seated in the grooves.

Loosen the bolt, turn the rotor by hand until it seats properly, and then re-tighten the bolt.

Figure 2.9 Make Sure the Rotor is Properly Seated



The Quick-Release Assembly

The components of the quick-release assembly consist of an elutriation “A” chamber, a counterbalance or a second elutriation “B” chamber, a rotating seal assembly, a round mounting plate, and four stainless steel tubes.

All of the components (except the tubes) are secured to the mounting plate (see Figure 2.8); two large holes in the plate fit over pointed spring-loaded bolts in the rotor body. The tubes connecting the chamber, counterbalance, and seal assembly are not secured: they are simply inserted into the appropriate ports in the components. Each tube has an O-ring at each end that forms a liquid-tight seal in the port, yet accommodates the radial movement of the chamber and counterbalance (or second chamber) during centrifugation.

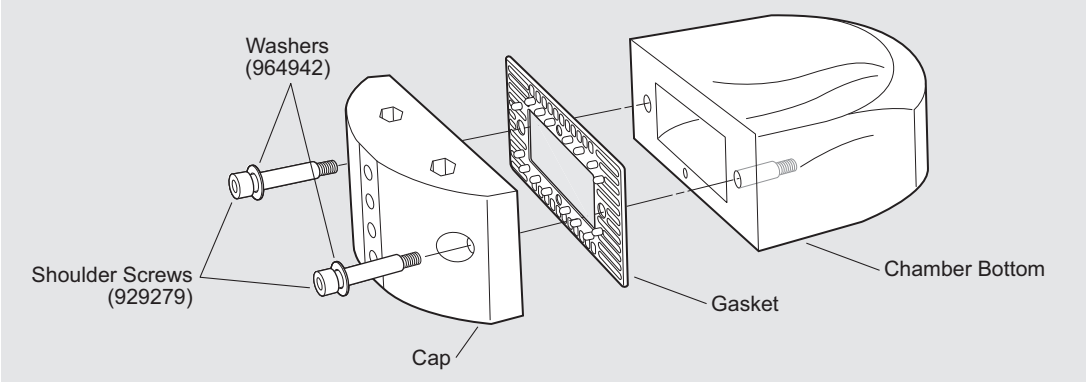
The Elutriation Chambers

The elutriation chambers are made of Novolac, a clear phenolic resin. Elutriation chambers with cavities of different sizes are available, but the basic components and outside dimensions of all chambers are the same. Specifications for the large, standard, and Sanderson elutriation chambers are contained at the front of this manual; specific information about other chambers can be found in the literature accompanying them. The elutriation chambers are warranted for 1 year (see the Warranty).

Most applications require that one elutriation chamber be used opposite a solid counterbalance; two elutriation chambers may be used in series, however. To accommodate this, two different chambers in each size are available: one (labeled "A") is designed to be used individually opposite a counterbalance, or as the first chamber when two are used in series; another (labeled "B") can be used as the second chamber in series. Chamber "B" should not be used individually.

Each chamber consists of a bottom and cap, separated by a medical-grade silicone rubber gasket. The chamber components are held together with two hexagonal socket-head shoulder screws that prevent overtightening (see Figure 2.10). The cap is marked with a letter (either "A" or "B"), and both the chamber and cap are marked with single and double dots. These markings aid in determining the orientation of the cap to the chamber depending on your application. The bottoms and gaskets of both the "A" and "B" chambers are identical. The directions of the ports and markings on the caps are different, however, as shown in Figure 2.10.

Figure 2.10 Elutriation Chamber Components



Assembling the Quick-Release Assembly

The orientation of the cap to the bottom of the chamber, as well as the configuration of the chamber(s) and seal assembly, depends on whether one or two chambers will be used during a run. Both configurations are discussed in the paragraphs that follow.

Most often one chamber is used opposite a counterbalance (see Figure 2.11a and Figure 2.11b). When the rotor is configured for two chambers to be used in series, however, fluid must always enter the "A" chamber first and the "B" chamber second, with the ports configured as shown in Figure 2.11c.

Using One Elutriation Chamber

If only one elutriation chamber is used, refer to Figure 2.12 and follow the instructions below.

---

1 To assemble the chamber, place the gasket over the screws in the cap of the chamber so that the nipples in the gasket fit into the holes in the cap.

NOTE Do not lubricate the chamber gasket. All chamber surfaces, including the gasket, must be clean, dry, and free of grease.

---

2 Place the two halves of the chamber together, matching the single dots, and tighten the chamber screws by hand using the 1/8-in. hex wrench (061223).

(The screws cannot be overtightened.)

---

3 Examine the lower portion of the seal assembly (beneath the plate): one side has two ports, the other side only one.

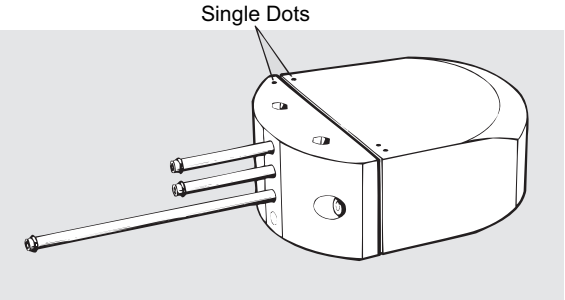
The chamber should be installed on the side with two ports.

Position the chamber so that the *single dots are facing up*.

---

4 Lubricate the O-rings of the following tubes using silicone vacuum grease or another lubricant* and place them into the chamber ports as follows:

Top port: short tube
2nd port: short tube
3rd port: long tube
Bottom port: empty



* Depending on the application, lubricants other than silicone may be preferred. Use any substance that will provide good lubrication and will help the O-rings to form a liquid-tight seal.

Figure 2.11 The "A" and "B" Chambers

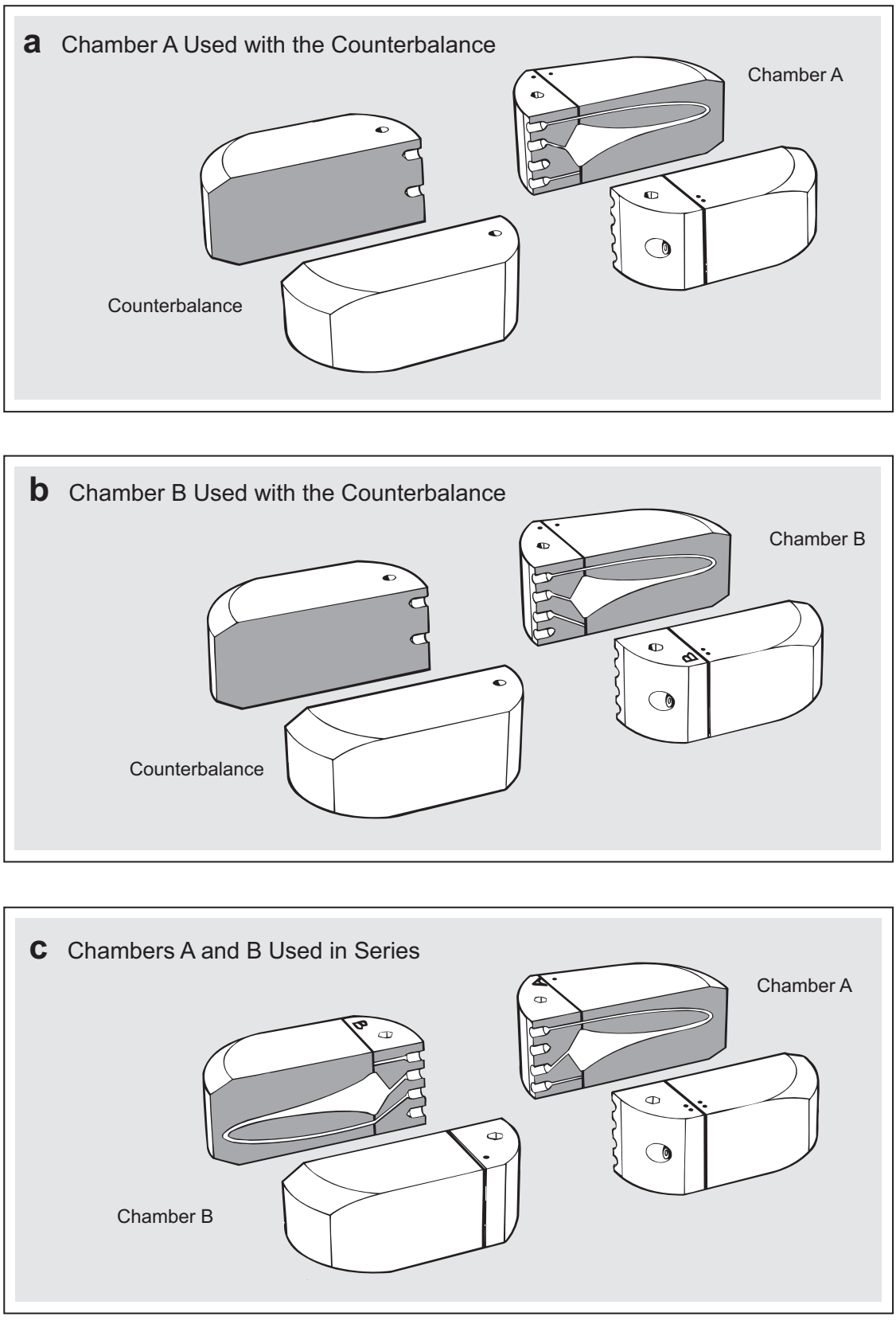
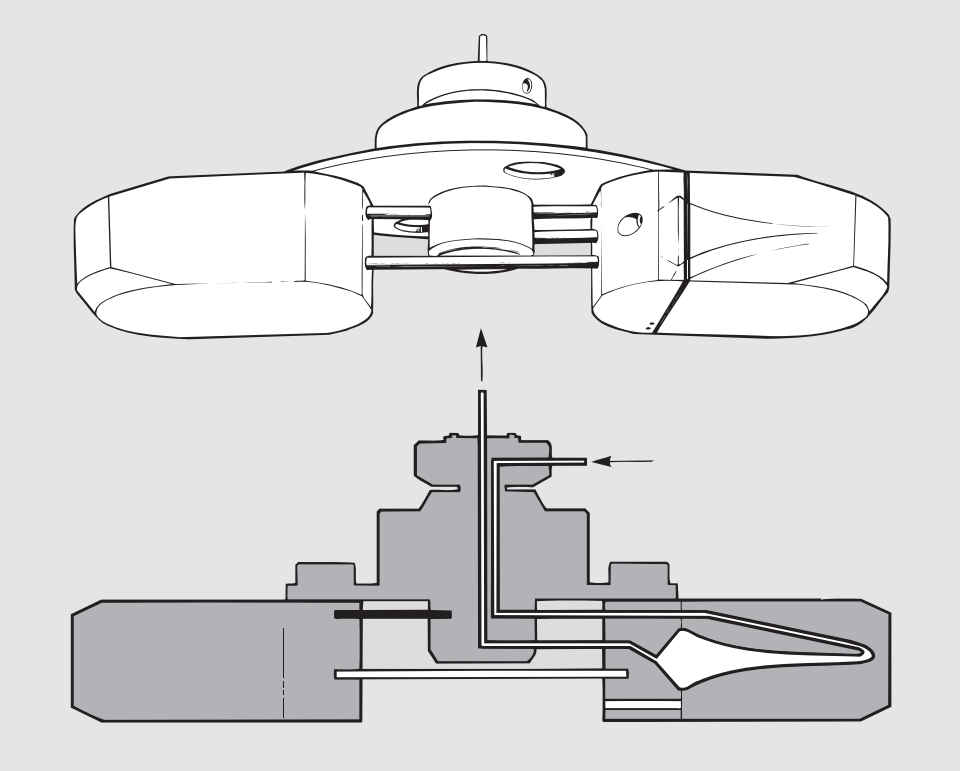


Figure 2.12 Chamber Assembly and Configuration for a Single-Chamber Run



5 Fit the two short tubes from the chamber into the two ports of the seal assembly.

The long tube should pass beneath the seal assembly.

6 Place one chamber mount (curved side outward) on the plate over the chamber mounting holes.

Place the two long screws with spring washers through the mount, the plate, and the chamber.

Carefully lift the assembly enough to allow you to insert one of the hex nuts into one of the holes on the underside of the chamber (the rounded side of the nut should face toward the outside of the hole).

7 Holding the nut in the hole, start the screw into the nut. Repeat with the other nut and screw.

Set the assembly back down.

Tighten the screws in the chamber mount using the 5/32-in. hex wrench just to the point of resistance.

Then loosen them one half turn so that the chamber can move radially.

(The chamber mount should not be loose, but should allow the chamber easy outward movement.)

8 Place the counterbalance on the bench with either side facing up.

Lubricate the O-rings of the remaining short, solid rod.

Fit this rod into the top port of the counterbalance.

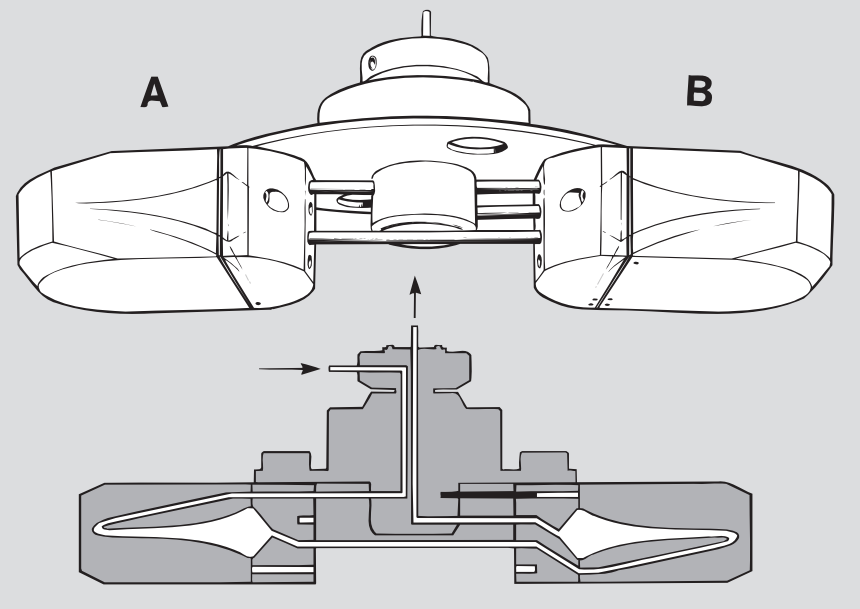
Move the counterbalance into place on the assembly opposite the chamber so that the solid rod enters the top port of the seal assembly and the long tube from the chamber fits into the third port of the counterbalance.

9 Repeat steps 6 and 7 to mount the counterbalance.

Using Two Elutriation Chambers

If two elutriation chambers are used in series, refer to Figure 2.13 and follow the instructions below.

Figure 2.13 Chamber Assembly and Configuration for a Two-Chamber Run



1 To assemble the chambers, place a gasket over the screws in the cap of each chamber so that the nipples in the gasket fit into the holes in the cap.

NOTE Do not lubricate the chamber gasket. All chamber surfaces, including the gasket, must be clean, dry, and free of grease.

2 Place the two halves of each chamber together, *matching the double dots*, and tighten the screws by hand using the 1/8-in. hex wrench.

(The screws cannot be overtightened.)

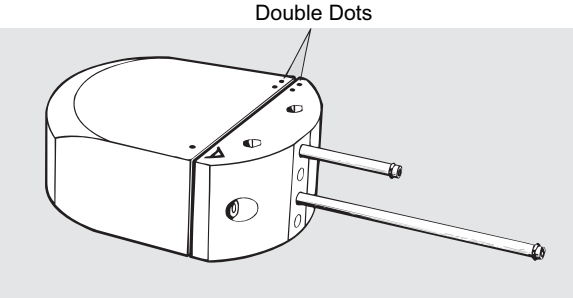
3 Examine the lower portion of the seal assembly (beneath the plate): one side has two ports, the other side only one.

The first chamber (labeled on the cap with the letter "A") should be installed on the side with one port; the second chamber (labeled "B") should be installed on the side with two ports.

4 Place the first chamber on the bench with the letter "A" facing up.

Lubricate the O-rings of the following tubes and place them into the chamber ports as follows:

Top port: short tube
2nd port: empty
3rd port: long tube
Bottom port: empty



5 Fit the top tube from the first chamber into the single port on the seal assembly.

The long tube should run beneath the seal assembly.

6 Place a chamber mount (curved side outward) on the plate over the chamber mounting holes.

Place the two long screws with spring-washers through the mount, the plate, and the chamber.

Carefully lift the assembly enough to allow you to insert one of the hex nuts into one of the holes on the underside of the chamber.

(The rounded side of the nut should face down, toward the outside of the hole.)

7 Holding the nut in the hole, start the screw into the nut.

Repeat with the other nut and screw.

Set the assembly back down.

Tighten the screws in the chamber mount, using the 5/32-in. hex wrench, just to the point of resistance.

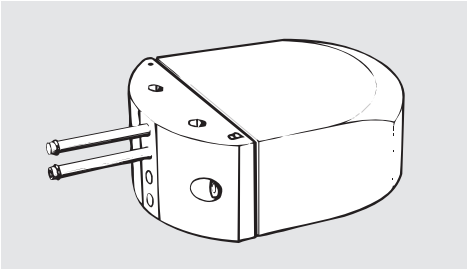
Then loosen them from one half to three quarters of a turn so that the chamber can move radially.

(The chamber mount should not be loose, but should allow the chamber easy outward movement.)

8 Place the second chamber on the bench with the letter "B" facing up.

Lubricate the O-rings of the following tubes and place them into the chamber ports as follows:

Top port: short, solid rod
2nd port: short tube
3rd port: empty
Bottom port: empty



9 Move the second chamber into place on the assembly opposite the first chamber.

The short, solid tube fits into the top port of the seal assembly and the long tube from the first chamber fits into the third port of the second chamber.

10 Repeat steps 6 and 7 to mount the second chamber.

Installing the Quick-Release Assembly in the Rotor Body

1 Lift the assembly by the plate and position it so that the holes are over the bolts.

2 Push the plate down until the bolts spring back, locking it in place.

Connecting the Flow System to the Quick-Release Assembly

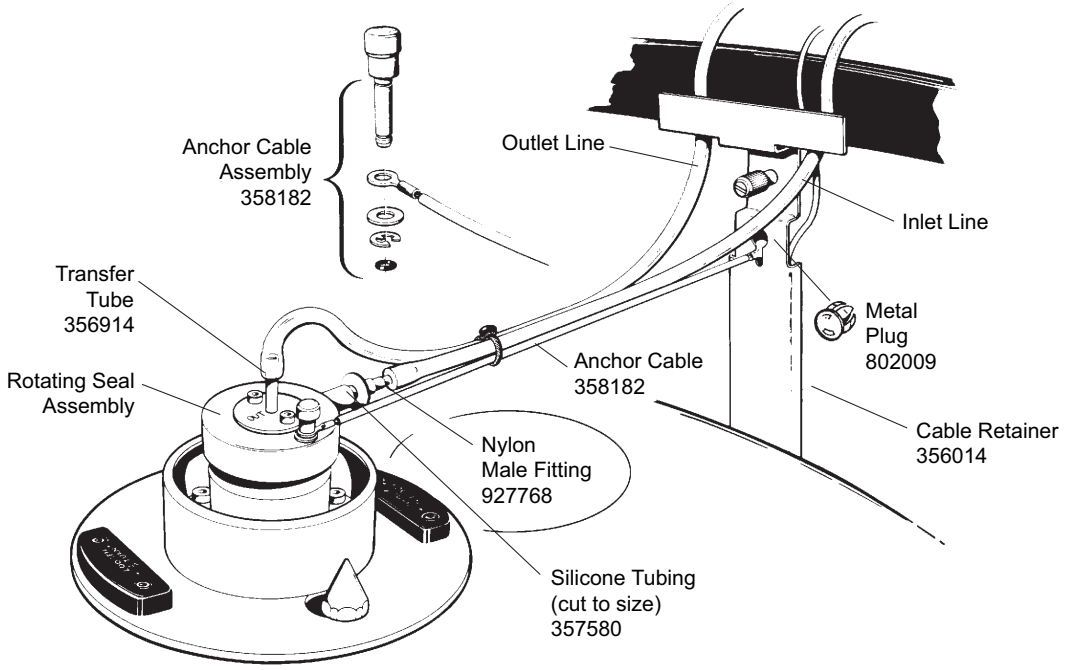
The tubing lines should be attached to the seal assembly as shown in Figure 2.14. To attach the inlet line, perform these steps.

1 Locate the 1-ft (30.5-cm) piece of larger-diameter(3/16-in. I.D. × 5/16-in. O.D.) silicone tubing provided (357580).

2 Insert this tubing into the port on the side of the seal housing as far as it will go.

(Note that this is the only place where the larger-diameter tubing is used.)

Figure 2.14 Attaching Tubing to the Seal Assembly (J6-MI Centrifuge Shown)



3 Cut the tubing so that about 1/4 in. (6.4 mm) protrudes from the seal assembly.

4 Insert one end of the nylon male fitting (927768) as far as it will go into this short tubing.

5 Attach the inlet line coming from the pump to the other end of the male fitting.

6 Attach the outlet line to the transfer tube (356914) at the top of the seal assembly.

7 *Avanti J-26S XP series and J-26 XP series centrifuges:* Route both lines behind the top of the bracket, pulling them back from the rotor so that most of the slack is eliminated.

J6-MI centrifuges: Route both lines behind the tabs at the top of the cable retainer (see Figure 2.13), pulling them back from the rotor so that most of the slack is eliminated.

The lines exit over the centrifuge gasket.

8 Attach the anchoring cable as follows.

a. *Avanti J-26S XP series and J-26 XP series centrifuges*: Slide the ball end of the anchor cable into one of the screw holes. Insert the screw over the cable and tighten it.

J6-MI centrifuges: Slide the ball end of the anchor cable into the keyhole in the cable retainer. Place the metal plug (802009) into the keyhole to hold the cable in place.

b. *All centrifuges:* Remove the pin from the top of the rotating seal assembly. Place the pin through the metal eyelet on the other end of the cable.

c. *All centrifuges:* Reinsert the pin into the hole in the top of the seal assembly.

NOTE In J6-MI centrifuges, the tubing lines may flatten slightly when the centrifuge door is closed, impeding the flow of fluid to and from the rotor. To prevent this, route the tubing through two 1-mL disposable auto-pipette tips. The tips provide extra support for the tubing. *Carefully* cut the pointed ends off of two tips. Route the incoming line through one of the tips, and the outgoing line through the other. Place the protected section of each tubing line over the centrifuge gasket.

CHAPTER 3

Preparing for Operation

This section describes the procedures necessary to prepare for a run. Refer to the centrifuge instruction manual for centrifuge operation.

Preparing for a Sterile Run

All rotor and system components can be steam autoclaved, and/or flow-through sterilized.

Autoclaving

The rotor body and the quick-release assembly can be autoclaved at 121°C for up to 20 minutes. *Do not autoclave above 121°C, as some components may deform.*

Before autoclaving, loosen the chamber screws (841747) with the angled hex wrench (007350) as described below to avoid warping the chamber cap.

1. Insert the short end of the wrench and turn the wrench one turn to loosen the screws.

   Do not remove the screws.

2. Introduce a small amount of water (0.5 to 1.0 mL) into the chamber.

3. Retighten the chamber screws when autoclaving is complete.

Using a Sterilizing Solution

1. Clamp off the sample mixing chamber.

2. Start the centrifuge and spin the rotor to 2000 RPM.

3. Introduce into the system one of the following solutions:
   - Hydrogen peroxide, 6% solution, 100 mL
   - Bleach (5% sodium hypochlorite), 100 mL

To prove the efficacy of chemical disinfection, sterile culture media can be pumped through the rotor and incubated to establish that this method of sterilization is valid. This procedure need be done only the first time chemical sterilization is accomplished.

Preparing the Centrifuge

Temperature Control

Centrifuge temperature control settings for this rotor vary, depending on which centrifuge is used and at what speed it is run.

- *Avanti J-26S XP series and J-26 XP series*: Set the required run temperature. Temperature compensation settings are adjusted automatically.
- *J6-MI*: Set the required rotor temperature and enter rotor code **5.2**. Then adjust the temperature compensation setting to ensure that the rotor reaches the set temperature. Table 3.1 lists temperature compensation settings for various speeds.

Table 3.1 Temperature Compensation Settings for the J6-MI Centrifuge

Speed (RPM)	Compensation Setting
up to 3000	3
3000 to 4000	4
4000 to 5000	5

Preparing the Sample

Sample

Cells that occur in single-cell suspension, such as peripheral blood cells, may be introduced directly into the rotor through the sample reservoir. Cells obtained from tissue must be dispersed to a single-cell suspension before processing by elutriation. A variety of enzymes may be used for the dissociation of tissues into single-cell suspensions.

Enzymatic methods of cell dispersion are subject to variation between lots of enzymes, and performance may vary from one enzyme manufacturer to another. In addition, any enzymatic dispersion must not allow cell surfaces to show a proclivity for cell-to-cell adhesion, otherwise cell clumping will occur during the elutriation procedure and cause a loss of efficiency in the separation of fractions.

⚠ WARNING

Handle body fluids with care because they can transmit disease. No known test offers complete assurance that they are free of micro-organisms. Some of the most virulent—Hepatitis (B and C) viruses and HIV (I–V), atypical mycobacteria, and certain systemic fungi—further emphasize the need for aerosol protection. Handle other infectious samples according to good laboratory procedures and methods to prevent spread of disease. Because spills may generate aerosols, observe proper safety precautions for aerosol containment. Do not run toxic, pathogenic, or radioactive materials in this centrifuge without taking appropriate safety precautions. Biosafe containment should be used when Risk Group II materials (as identified in the World Health Organization *Laboratory Biosafety Manual*) are handled; materials of a higher group require more than one level of protection.

Buffer

Any appropriate buffer can be used for elutriation as long as it is less dense than, and physiologically compatible with, the cells to be separated. Typical buffers include physiological saline or phosphate buffered saline solutions.

Avoiding Cell Clumping and Adherence

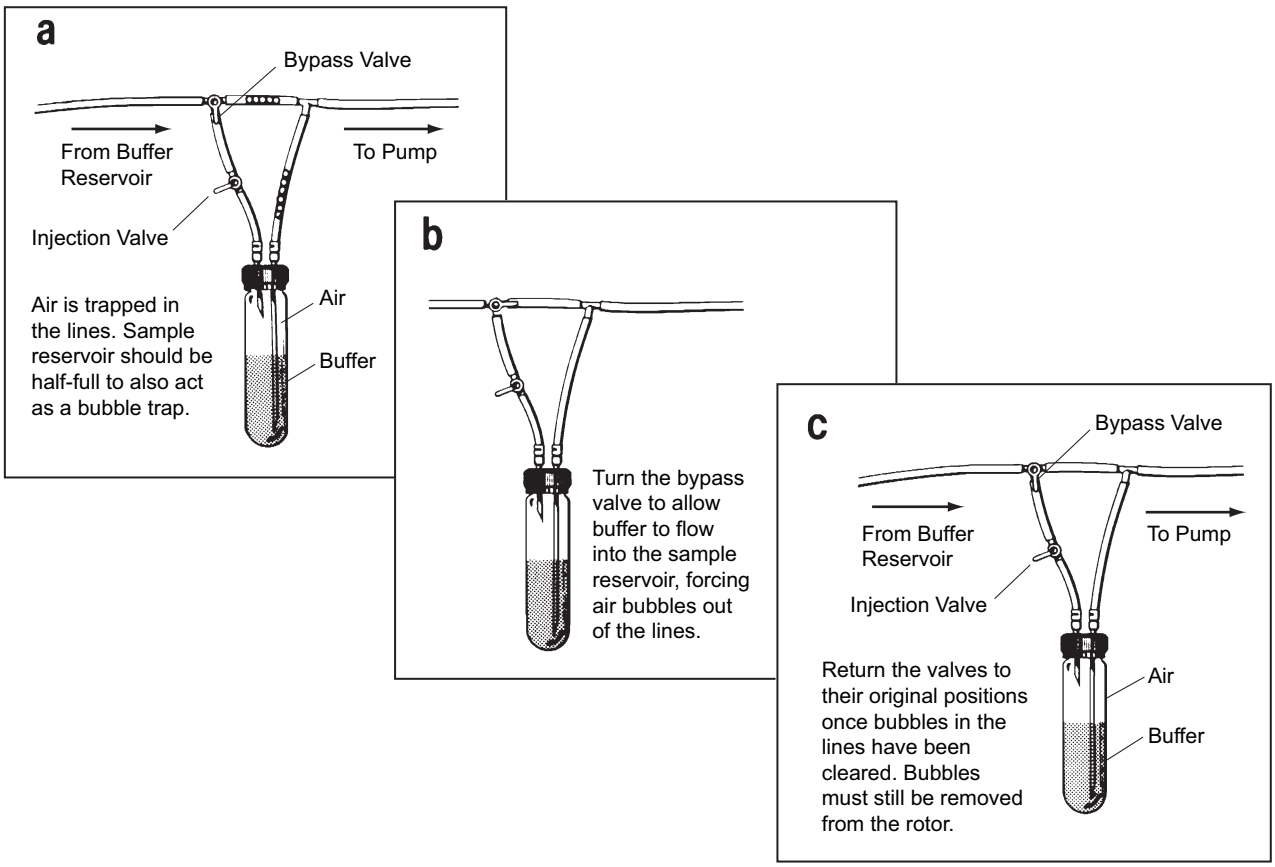
Cell clumping occurs for a variety of reasons and can cause problems with the separation. Adding a chelating agent such as EDTA (1 to 10 mM) to the elutriation buffer will remove the cations Ca^{++} and Mg^{++} that are involved in cell-to-cell attachment. Adding DNase (1 to 20 µg/mL) to the medium reduces clumping that occurs when DNA released from damaged cells forms a matrix, trapping separated cells. Consideration must often be given to the order in which cells are exposed to various methods of disaggregation. For example, DNase requires Mg^{++} and is maximally active in the presence of both Mg^{++} and Ca^{++}; thus, inclusion of both EDTA and DNase in the same medium would reduce or eliminate enzyme activity, depending on the relative concentration of each.

Cell-to-cell attachment may be caused by a variety of factors, some chemical and some physical in nature, which may or may not respond to correction by EDTA or DNAse. Published methodology will sometimes give insight into how to achieve success in cell disaggregation.

Purging Air from the System

During system installation, air bubbles may become trapped in the lines as shown in Figure 3.1. If air bubbles are not removed from the system, the flow of liquid in the rotor will be restricted and high back pressure (greater than 10 psi) may result. Visually check the tubing for the presence of air bubbles and perform the steps below to remove them before introducing sample into the system.

Figure 3.1 Purging Air from the System



1 Set the valves as shown in Figure 3.1a. With the rotor stationary, start the pump flowing at one of the following flow rates:

- large chamber: about 200 mL/min.
- standard and Sanderson chambers: about 20 mL/min.

2 Turn the bypass valve* as shown in Figure 3.1b so buffer flows into the sample reservoir and lines.

The sample reservoir should be approximately half full to allow it to function as a bubble trap.

3 When the air has been removed from the lines of the harness, turn the bypass valve back to its original position (Figure 3.1c).

4 To remove air from the rest of the system, first spin the rotor to the right (clockwise) by hand.

You will see bubbles exiting the rotor into the collection vessel.

* The handle of a three-way valve points to the line that is closed; the lines in both other directions are open.

Then, to ensure that all air is removed, set the centrifuge for 1000 RPM and start it running while the pump continues to operate.

When the rotor reaches 1000 RPM, turn off the centrifuge.

NOTE If air is inadvertently introduced into the system when sample is in the chamber, stop the system and then follow the above procedure, remembering to collect the sample as it is washed out of the rotor. After the air bubbles have been removed, reintroduce the sample and proceed with the separation.

Purging Air from the Pressure Gauge

Before beginning the elutriation procedure, it is important to purge any air that may become trapped inside the pressure gauge, as follows. When the rotor is filled but before cells are introduced into the line, invert the gauge and tap it a few times in the inverted position. This will force air out of the gauge.

Calibrating the Pump

To facilitate reproducible elutriation protocols, it is necessary to calibrate the pump periodically to ensure that it can be set to proper flow rates. Skip this procedure if you are using a self-calibrating pump.

1 Accelerate the rotor to operating speed and fill the rotor by pumping buffer through the system.

2 Set the pump to a flow rate that is approximately at the lower end of the flow rate range to be used for the elutriation procedure.

If the range being used is 6 to 35 mL/min, set the flow rate approximately at 6 mL/min, using the vernier gauge on the pump flow control knob.

3 When the system is fully primed, that is, when the buffer fills the rotor and exits the tubing, place the end of the tubing into a graduated cylinder and immediately start timing the buffer entering the graduated cylinder.

At the end of 1 minute of flow, stop the pump and immediately remove the tubing from the graduated cylinder.

4 Read the volume of fluid in the graduated cylinder.

This is the volume of fluid pumped in 1 minute, so the flow rate is this volume per minute.

5 Empty the graduated cylinder and repeat steps 3 and 4 for incremental pump settings that increase in steps approximately 25% of the range of flow rates to be used for the elutriation.

Repeat a total of three times for a total number of four measurements.

6 Plot all four measured flow rates on a graph, using the X-axis as the pump vernier setting, and the Y-axis as the flow rate.

7 Draw a straight line through the four data points.

The pump should be linear if it is working properly.

If not linear, check the tubing, and repeat the calibration procedure in steps 3 through 6.

8 Use this graph as the calibration curve for the pump.

It will allow you to match vernier settings to flow rates for your pump.

CHAPTER 4

System Operation

This section describes how to determine run parameters (rotor speed and initial flow rate) and sample loading and collection methods. Once the rotor components have been sterilized and the air has been removed from the rotor and tubing lines, you are ready to begin operation.

Run Procedure

Determining Run Parameters

1 Before beginning operation, consult the nomogram (Figure 4.1) or a published method using the same cell/buffer system to determine the initial flow rate for elutriation.*

Find the appropriate rotor speed on the nomogram.

Then find the approximate size (in micrometers) of the smallest particle in suspension.

Lay a straightedge across the nomogram from the rotor speed (RPM) through the particle size.

The point where the line crosses the appropriate column on the right (for the chamber you are using) shows the pump flow rate required to retain the smallest particle in the chamber.

2 Determine an initial flow rate that will ensure that all size ranges of particles to be fractionated are retained.

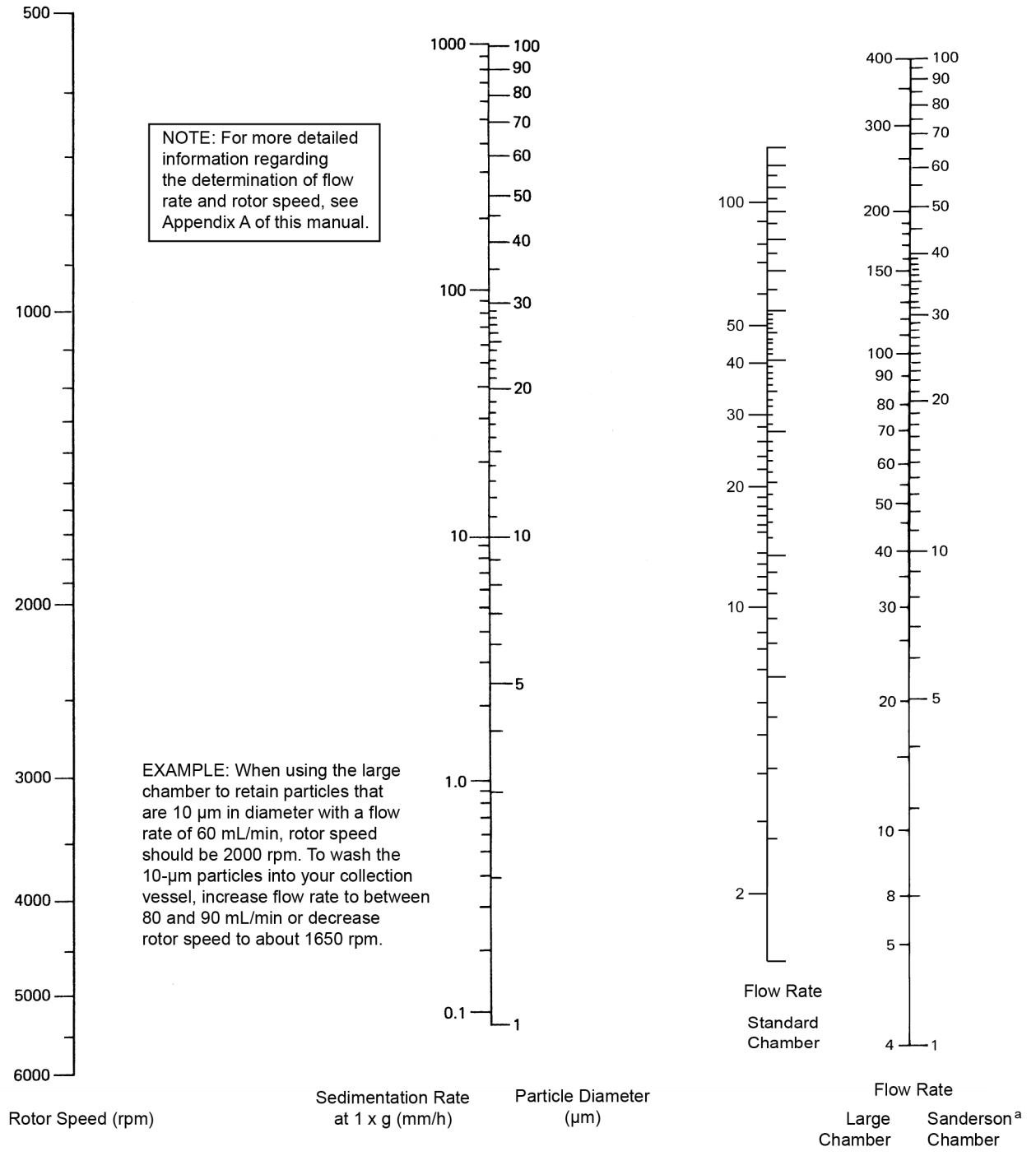
All smaller particles, such as small cells and cell debris, will wash through the chamber as the sample is introduced.

3 Set the controls of the centrifuge and close the centrifuge door.

Then turn on the pump and set the flow rate to the appropriate setting.

* The nomogram assumes spherical particles and a particular density difference between the particles and the buffer. More exact determination of flow rate may be necessary, depending on your application. APPENDIX A lists several equations that may be useful in more precisely determining flow rate based on particle diameter and sedimentation rate.

Figure 4.1 Rotor Speed and Flow Rate Nomogram*



[a] To get a Sanderson chamber flow rate from a known standard chamber flow rate, multiply the standard flow rate by 0.74. Conversely, to get a standard chamber flow rate from a known Sanderson chamber flow rate, multiply the Sanderson flow rate by 1.35.

* Use a straightedge to connect a flow rate and rotor speed so that this line intersects the particle axis at a point corresponding to the smallest, lightest particles to be retained for the chamber you are using.

4 Press the power switch of the strobe control to **ON**. When the rotor speed has stabilized, look through the port in the centrifuge door.

Turn the **DELAY ADJUST** knob on the strobe control completely to the left (counterclockwise); then slowly turn it to the right (clockwise) until the cell is in view.

(The chamber will appear to be motionless.)

5 Determine the number of cells to introduce into the rotor at one time.

The numbers listed in Table 4.1 are approximations based on average-sized mammalian cells; actual numbers may vary if your sample cells are smaller or larger.

If two chambers are used in series, the amounts may be doubled.

With the standard and Sanderson chambers, the volume of buffer normally required for separation is approximately 100 mL × the number of fractions to be collected. With the large chamber, the volume of buffer normally required is 1000 mL × the number of fractions to be collected. Be sure to include enough extra buffer to flush the system at the beginning of a run.

Table 4.1 Sample and Buffer Loading Volumes[a]

	Large Chamber	Standard Chamber	Sanderson Chamber
Sample volume: minimum maximum	 2×10^8 cells 10^{10} cells	 2×10^7 cells 10^9 cells	 2×10^6 cells 10^9 cells
Amount of buffer required to elutriate one fraction	~500 to 1000 mL	~75 to 250 mL	~75 to 250 mL
Air purge parameters: Pump rate (then spin rotor to 1000 RPM)	200 mL/min	20 mL/min	20 mL/min
Pump calibration	up to 400 mL/min (in the range you will be using)	up to 250 mL/min (in the range you will be using)	up to 250 mL/min (in the range you will be using)

a. Air urge parameters, and pump calibration rates for the large, standard, and Sanderson chambers

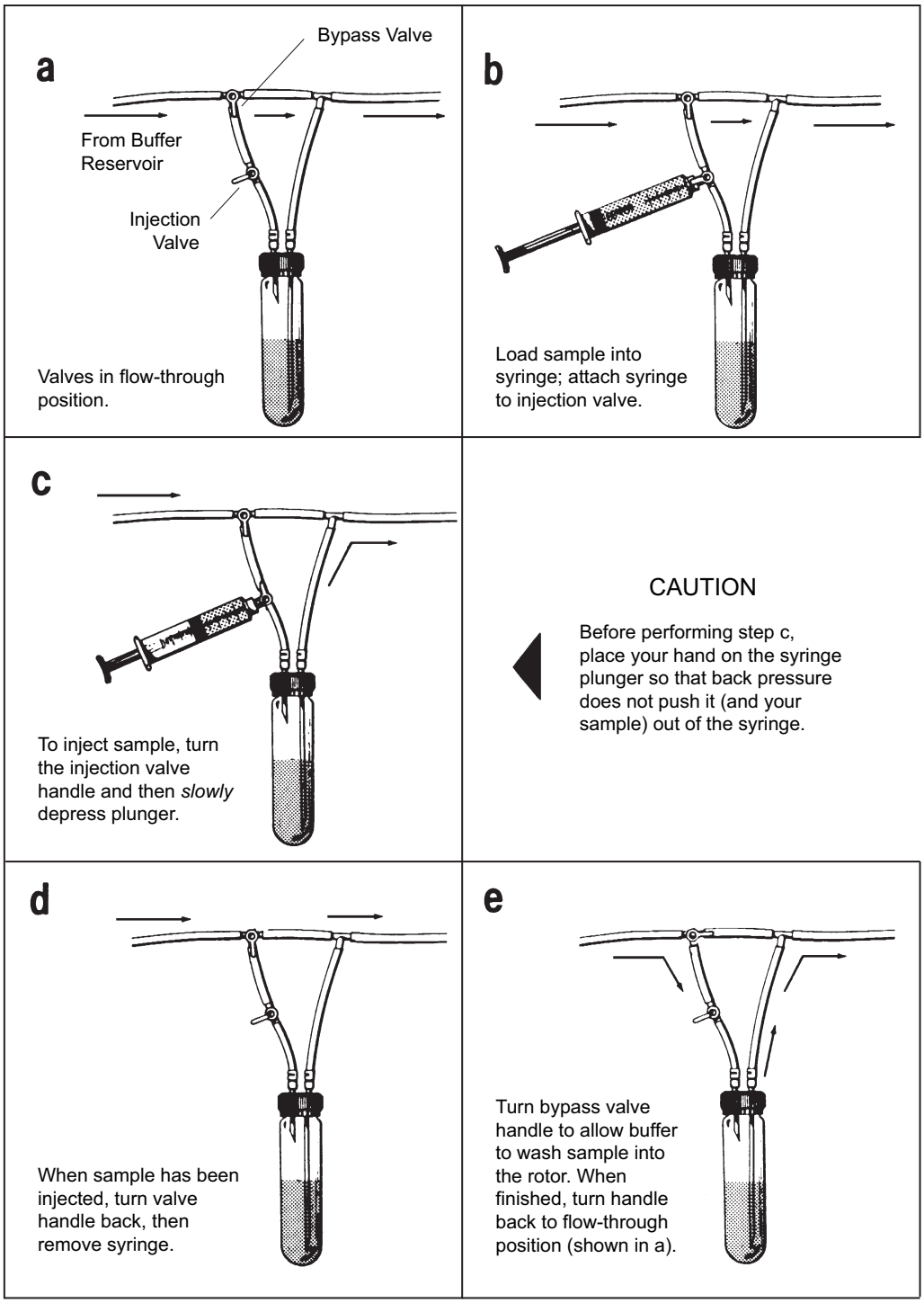
Loading the Rotor

Sample may be loaded into the rotor either by injection, using a syringe with a luer fitting attached to an injection port on the system, or by placing the sample in a separate reservoir and pumping it into the rotor. Introducing the sample from a separate reservoir may be preferable when the sample contains fragile cells.

To inject the sample from a syringe:

1 Make sure the bypass and injection valves are set as shown in Figure 4.2a.

Figure 4.2 Sample Injection Procedure



2 Load the sample into a syringe with a luer fitting.

3 Attach the fitting to the port on the sample reservoir as shown in Figure 4.2b.

4 Holding the plunger of the syringe steady, turn the injection valve as shown in Figure 4.2c.

5 Slowly press the plunger of the syringe to inject the sample.

Take care not to force air out of the sample reservoir into the line leading to the rotor.

6 After all of the cells are in the reservoir, turn the injection valve back again (Figure 4.2d).

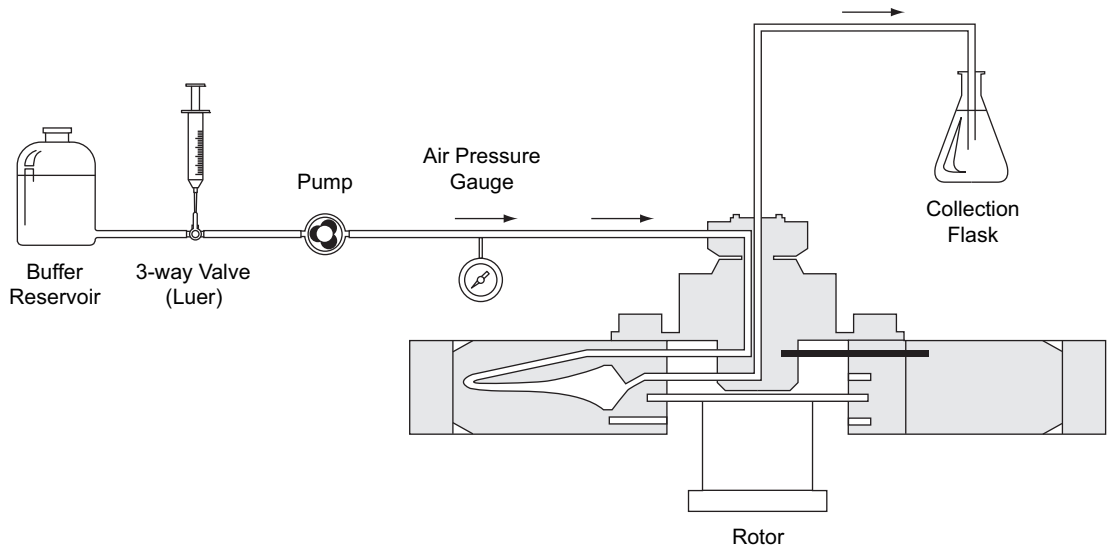
Then turn the bypass valve to allow the buffer to wash the cells into the rotor (Figure 4.2e).

Alternate method of injecting the sample from a syringe:

1 Use a 10- to 30-mL syringe.

Set up the equipment as shown in Figure 4.3.

Figure 4.3 Alternate Sample Injection Procedure



2 Load sample into the syringe; attach the syringe to the injection valve.

Make sure that the stopcock valve handle is pointing toward the syringe to block the flow of sample into the line.

3 Turn the bypass valve toward the buffer reservoir, shutting off the flow from the buffer reservoir.

The pump will then automatically draw the syringe plunger down, injecting the sample into the rotor.

You do not have to manually depress the syringe.

4 Just before the barrel reaches the bottom of the syringe, turn the bypass valve back toward the syringe to stop the sample flow.

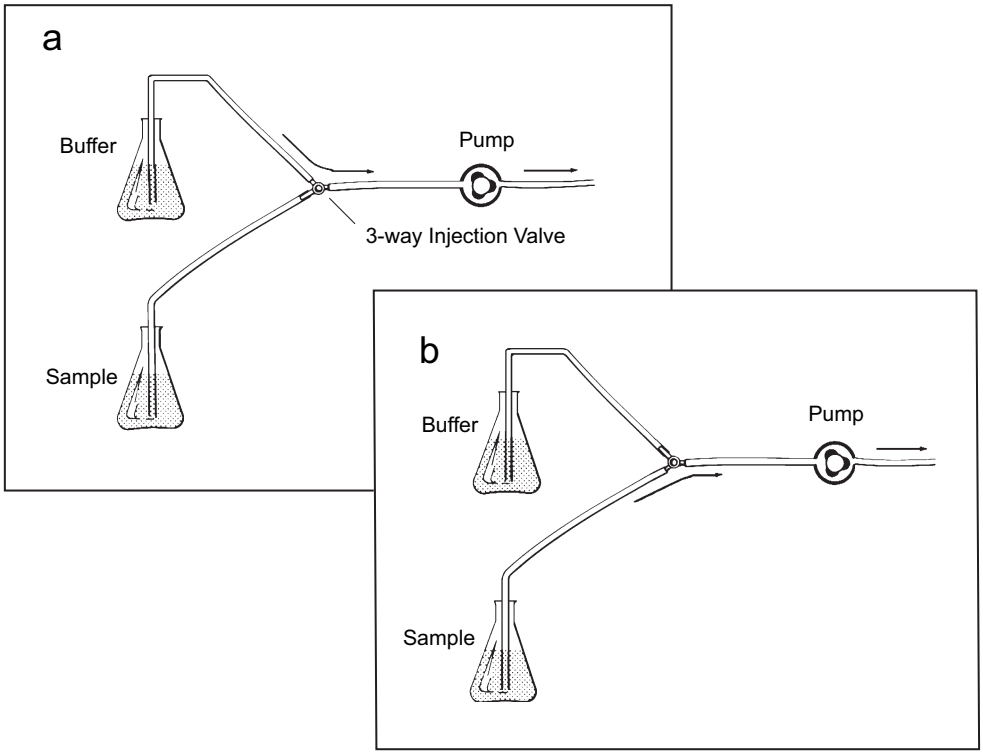
This will prevent any air trapped in the syringe from entering the line.

To load a sample from a separate reservoir:

1 Turn the handle of the three-way injection valve to the position shown in Figure 4.4a.

The pump will draw sample from the reservoir into the rotor.

Figure 4.4 Introducing Sample from a Separate Reservoir



2 When most of the sample has been drawn past the three-way valve, turn the valve back to the position shown in Figure 4.4b.

This avoids introducing air into the system; a small amount of sample will remain in the line behind the valve.

3 Look through the viewing port in the door to see the cells as they enter the elutriation chamber and move toward the elutriation boundary.

If you cannot see the leading edge of the cells, the cells may be pelleted rather than in suspension.

If this is the case, increase the flow rate slightly or decrease the speed slightly to resuspend the cells in the chamber.

If the chamber is not observed at first glance, adjust the centrifuge strobe delay slightly to bring the chamber into view.

Collecting Discrete Fractions

Remove discrete fractions of cells from the chamber by adjusting the flow rate in slight increments. See Table 4.1 for operating parameters.

Concentrating the Largest Fraction in the Chamber

If the particles required are the largest in the sample,

1 Use the nomogram (Figure 4.1), or a published method using the same cell/buffer system, to determine an approximate flow rate and rotor speed that will elutriate all but the largest particles.

2 Start the centrifuge and pump to the settings determined in step 1 and run the system until all but the largest particles have exited the rotor.

3 Collect the particles remaining in the chamber by turning off the centrifuge and washing out the remaining fraction into a collection reservoir.

CHAPTER 5

Care and Maintenance

This section describes routine and occasional care and maintenance procedures. Exploded views of the rotor components are provided to aid you with assembly and disassembly.

NOTE It is your responsibility to decontaminate the rotor before requesting service by Beckman Coulter Field Service.*

⚠ WARNING

Do not use alcohol or other flammable substances in or near operating centrifuges.

Maintenance

General Information

- The rotor body can be left in the centrifuge between runs. Before each run, make sure that the rotor body is tied securely to the centrifuge drive hub.
- Do not use sharp tools on the rotor, since corrosion begins in scratches and may open fissures in the metal with continued use. If the rotor body has been scratched, ask your local Beckman Coulter office about having it reanodized.
- Periodically inspect the rotor components for rough spots, pitting, white powder deposits, or heavy discoloration. If any of these signs are evident, *do not run the rotor*. Show it to your Beckman Coulter Field Service representative. Your Beckman Coulter representative provides contact with both the Field Rotor Inspection Program and with the rotor repair center.

After a Run

1 Flush the lines and rotor with sterile, distilled water to remove any remaining buffer or cells.

If the elutriation chambers require cleaning, follow the instructions in *Cleaning The Elutriation Chamber*, below.

Dirty or contaminated tubing cannot be cleaned; discard it and use new tubing for the next run.

* In the United States, call 1-800-742-2345. Outside the U.S., contact your local Beckman Coulter office or visit us at www.beckman.com.

2 Autoclave or chemically disinfect the quick-release assembly and other components, if required.

3 Disassemble, clean, and dry the components of the quick-release assembly.

Check the condition of the O-rings and gaskets and replace any that are damaged.

4 When reassembling the components, lightly coat all O-rings with silicone vacuum grease.

Do not lubricate the chamber gasket.

5 Wipe away any condensation on the rotor body, especially on or near the reflector located on the base.

Cleaning

The Rotor Body

Wash the rotor body and quick-release assembly after each use to prevent buildup of residues. Clean the rotor immediately after use if you have run salt solutions or other corrosive materials, or if spillage has occurred. Do not allow corrosive materials to dry on the rotor components. Refer to *Chemical Resistances* (publication IN-175) for the chemical compatibilities of rotor and rotor component materials.

⚠ CAUTION

Strongly alkaline solutions will damage the rotor.

Solution 555 (339555) is a mild detergent for use with rotors and accessories. Dilute the detergent with water (10 parts water to 1 part detergent) before use. A Rotor Cleaning Kit (339558) is available, which contains two quarts of Solution 555 and two brushes.

⚠ CAUTION

Do not use Solution 555 on the elutriation chambers.

The Elutriation Chamber

To achieve optimal results, it is necessary to thoroughly clean all inside surfaces of the elutriation chambers. This helps to maintain an unobstructed pathway for cells entering the chamber, and to prevent problems of cell contamination and clumping caused by the presence of endotoxins and other contaminants on the chamber surface. However, the geometry of the elutriation chamber makes it difficult to mechanically clean its internal surfaces. The following method, which uses a

detergent solution acting in an ultrasonic cleaner, achieves rapid, thorough cleaning of all interior surfaces of the chamber (including the narrow inlet tube).

1. Place a volume of 2% Micro brand liquid laboratory cleaner sufficient to cover the entire elutriation chamber with liquid into the stainless steel tub of a Branson Model 3200 (or equivalent) ultrasonic cleaner.*

2. Disassemble the elutriation chamber and place the parts into the apparatus so that the chamber internal surfaces and inlet tube are completely covered by detergent (chamber is on its side, inlet tube down).

3. Turn on power to the ultrasonic cleaner and allow the cleaning process to proceed for one-half to one hour, depending on the amount and nature of the material to be removed from the chamber surfaces.

4. Remove the chamber parts and rinse them under rapidly flowing clean tap water to clear out residual detergent and debris.

   Direct the water pressure into the lower chamber base so that water flushes outwards through the inlet tube.

5. Rinse all cleaned parts thoroughly in deionized water.

6. Dry the chamber parts by blowing laboratory air into the inlet tube and over the interior surfaces of the chamber.

   (Laboratory air should be clean and oil-free, or it should be filtered.)

   The cleaned chamber may also be dried in ambient air in a clean, dust-free environment.

The chamber may then be autoclaved, or flow-through sterilization may be performed when the chamber is reassembled onto the rotor.

Disassembly and Cleaning

After each run, disassemble, clean, and dry the components of the quick-release assembly (at least the chamber(s), tubes, and top part of the rotating seal assembly) following the procedure below. While it is disassembled, check the condition of all O-rings, gaskets, and seals. Figure 5.1 shows an exploded view of the assembly.

To remove the quick-release assembly from the rotor:

* Branson Ultrasonic Cleaning Equipment Company, Danbury, CT.

1 Place the fingers of each hand under the sides of the plate and press the pointed bolts toward the center of the rotor with your thumbs.

2 Lift the assembly up over the bolts and place it on a flat surface.

Removing and Disassembling the Chamber and Counterbalance

1 Use the 5/32-in. hex wrench (029840) to loosen the screws holding the chamber mounts, chamber, and counterbalance to the plate; remove them by pulling the chamber mounts up, bringing both screws and washers up with each mount.

Beneath the chamber and counterbalance are two nuts: lift the assembly to expose the nuts and set them aside.

Pull the chamber and counterbalance outward to remove them from the tubes; pull any remaining tubes loose from the seal assembly, chamber, and counterbalance and set the tubes aside.

2 To disassemble the chamber, use the 1/8-in. hex wrench (016223) to loosen the chamber mounting screws and remove them.

Pull the two halves apart.

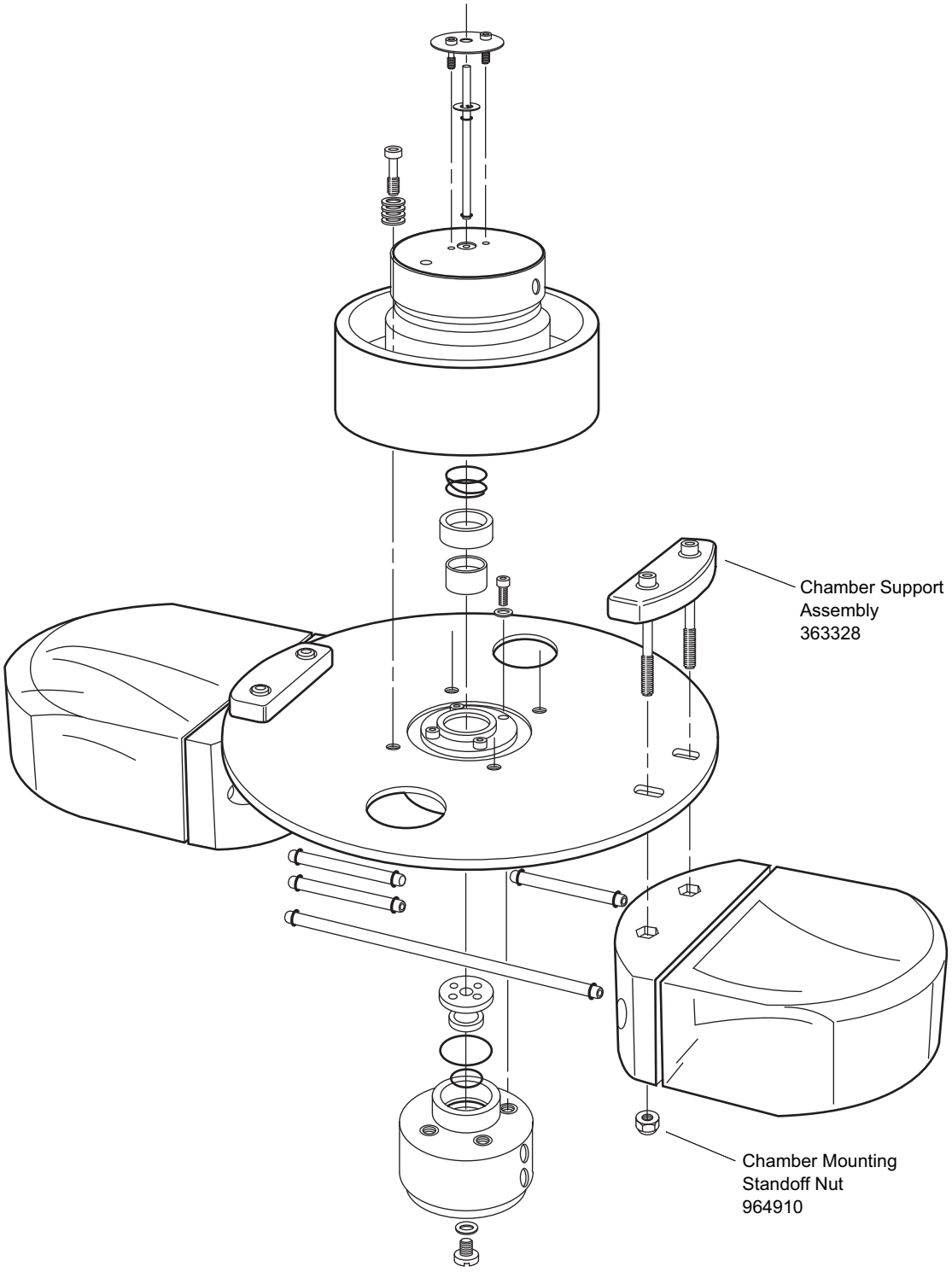
Remove the gasket.

Disassembling the Rotating Seal Assembly

1 Remove the small retaining plate around the transfer tube by using a slotted screwdriver to loosen the captive screws and washers.

2 Pull up on the transfer tube to remove it (the retaining clip near the top will be removed with the tube).

Figure 5.1 Exploded View of the JE-5.0 Quick-Release Assembly



3 Use the 1/8-in. hex wrench (016223) to loosen the captive screws around the seal housing until you can lift the entire housing free of the plate beneath.

4 Remove the spring, seal guide, and black seal from the seal assembly.

The lower part of the seal assembly beneath the plate usually does not require disassembly.

The procedure is given here, however, should you wish to remove the white seal for inspection or cleaning (refer to Figure 5.1).

5 Remove the four screws securing the bottom part of the seal assembly (containing the white seal) to the plate using the 9/64-in. hex wrench (817305).

Remove the slotted screw and nylon washer beneath the bottom part of the seal assembly and insert one of the long chamber mounting screws into this screw hole.

Continue to turn the longer screw into the hole until the white seal rises free of the assembly.

Remove the seal and set it aside; remove the long screw.

Removing the Bearings

The bearings do not need to be removed and cleaned unless moisture is found during inspection. If moisture is found, refer to Figure 5.2 and follow the procedure below.

Pliers (A11129) are used to disassemble and reassemble the bearings. These pliers are adjustable from internal to external, enabling them to expand or compress the retaining rings in the bearings as required. Turn the locking screw one-quarter turn to release halves, then switch positions of the halves and turn the locking screw one-quarter turn to re-lock.

1 Two bearings are contained within the seal housing.

Turn the housing upside down.

Around the center shaft is a retaining clip that must be removed.

Adjust the pliers to external position.

Insert the tips of the pliers into the two holes of the retaining clip.

While pressing down on the housing to release the internal spring tension, spread the jaws of the pliers apart, opening the clip (as shown in Figure 5.2a).

Keep even tension on the open clip and lift it out of the assembly.

NOTE Do not spread the clip apart more than is necessary to remove it. Excess spreading can cause the clip to deform or break.

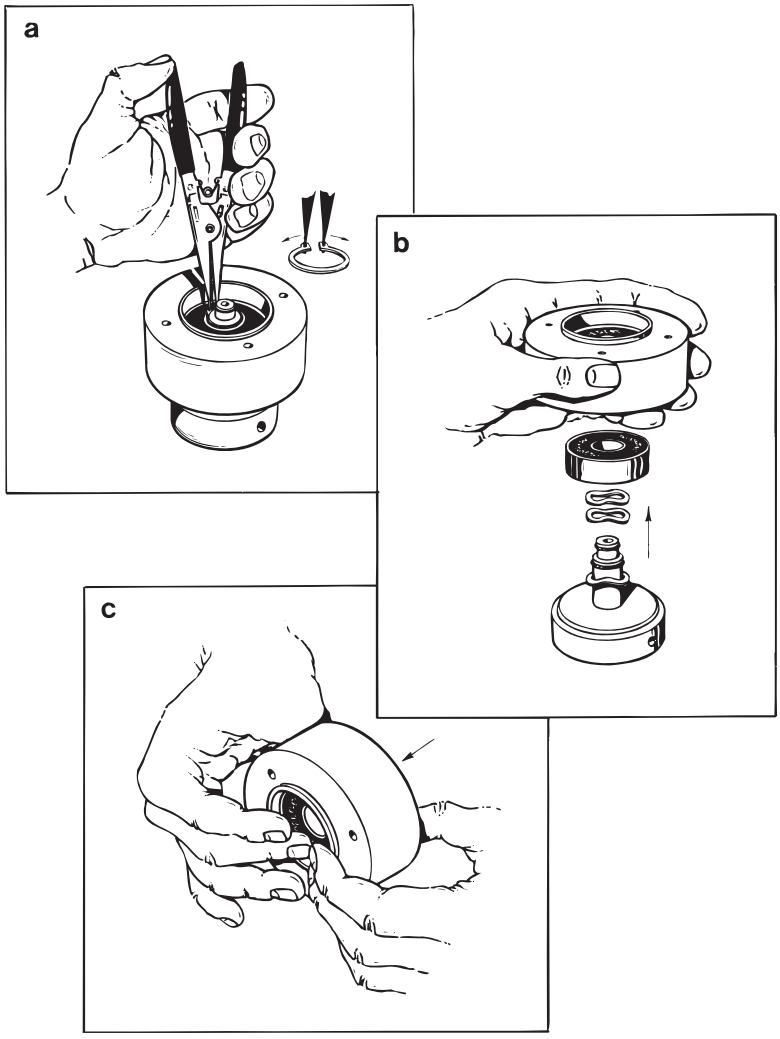
2 Pull the housing up until it is free from the top part of the assembly (see Figure 5.2b).

The top bearing may be loose enough to fall out of the housing; if it does not, turn the housing right side up, insert your finger in the hole, and lift the bearing out of the housing.

Remove the three spring washers from around the center shaft of the top part of the assembly and set them aside.

3 Push the bottom bearing out of the housing as shown in Figure 5.2c.

Figure 5.2 Removing the Bearings



Sterilization and Disinfection

Autoclaving

The rotor body and the quick-release assembly can be autoclaved at 121°C for up to 20 minutes. *Do not autoclave above 121°C, as some components may deform. Do not autoclave the bearings.*

Before autoclaving, loosen the chamber screws (841747) with the angled hex wrench (007350) as described below to avoid warping the chamber cap. *If pathogenic substances were used, however,*

do not loosen the chamber screws. Place the quick-release assembly in the autoclave for 20 minutes. To loosen the chamber screws,

1 Insert the short end of the wrench and turn the wrench one turn to loosen the screws.

Do not remove the screws.

2 Introduce a small amount of water (0.5 to 1.0 mL) into the chamber.

3 Retighten the chamber screws when autoclaving is complete.

NOTE Autoclaving may cause the sample reservoirs to become opaque or to develop white striations (crazing), but these conditions will not affect performance.

Using a Sterilizing Solution

Hydrogen peroxide (6%) or bleach (5% sodium hypochlorite) can be used on the rotor as follows.

1 Clamp off the sample mixing chamber.

2 Start the centrifuge and spin the rotor to 2000 RPM.

3 Introduce into the system one of the following solutions:

- Hydrogen peroxide, 6% solution, 100 mL
- Bleach (5% sodium hypochlorite), 100 mL

4 Introduce into the system at least one liter of sterile, pyrogen-free distilled water or buffer.

To prove the efficacy of chemical disinfection, sterile culture media can be pumped through the rotor and incubated to establish that no contamination of the rotor is present. This procedure need be done only one time.

While Beckman Coulter has tested these methods and found that they do not damage the rotor or components, no guarantee of sterility or disinfection is expressed or implied. When sterilization or disinfection is a concern, consult your laboratory safety office regarding proper methods to use.

Decontamination

If the rotor (and/or accessories) becomes contaminated with radioactive material, it should be decontaminated using a solution that will not damage the anodized surfaces. Beckman Coulter has tested a number of solutions and found two that do not harm anodized aluminum: RadCon Surface Spray or IsoClean Solution (for soaking),* and Radiacwash.†

While Beckman Coulter has tested these methods and found that they do not damage components, no guarantee of sterility or disinfection is expressed or implied. Consult your laboratory safety officer regarding the proper decontamination method to use.

Reassembling the Rotor

Assembling the Bearings

Be sure all parts are clean and dry. Lightly coat all O-rings with silicone vacuum grease. *Do not lubricate the chamber gasket*. All chamber surfaces, including the gasket, must be clean, dry, and free of any grease or lubricant.

Do not store the bearings in any fluid, such as oil. The must be stored in a clean, dry environment before and after use.

Reinstall the bearings as follows (refer to Figure 5.3).

1 Insert the white seal in the cavity so that the four small holes are toward the top.

Push it down into the cavity with your finger until it will not go further.

Insert and hand-tighten the slotted screw and nylon washer under the bottom half of the seal assembly.

2 Place the plate over the lower seal assembly so that the holes in the seal assembly are oriented in the same direction as the long, narrow chamber mounting holes in the plate.

Insert and hand-tighten the four short hex screws with washers using the 9/64-in. hex wrench.

3 Place the black seal over the white seal so that its cavity faces up.

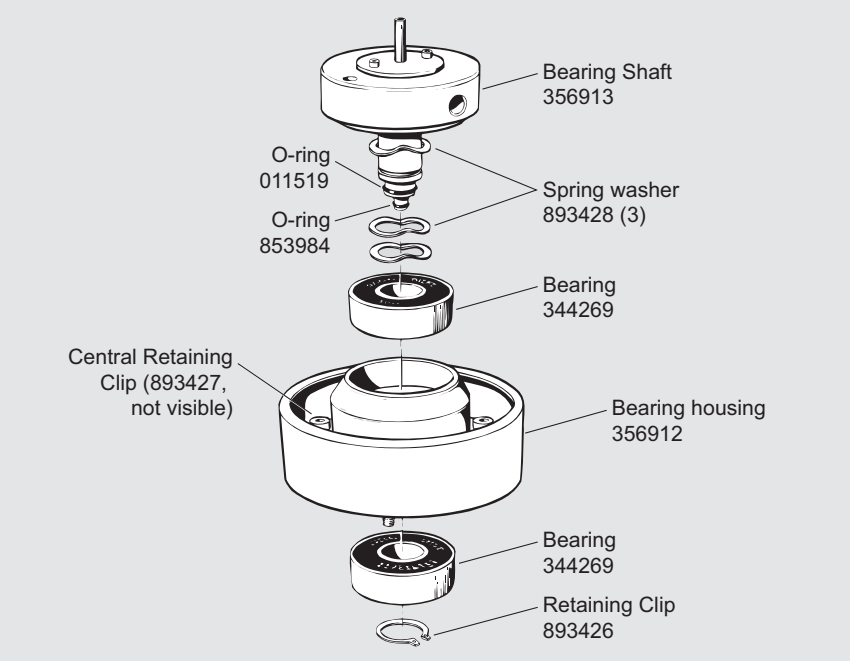
Place the seal guide over the black seal so that its largest cavity faces up.

Place the spring in the large cavity of the guide.

* n U.S.A., contact Nuclear Associates (New York); in Eastern Europe and Commonwealth States, contact Victoreen GmbH (Munich); in South Pacific, contact Gammasonics Pty, Ltd. (Australia); in Japan, contact Toyo Medic Co. Ltd. (Tokyo).

† n U.S.A., contact Biodex Medical Systems (Shirley, NY); internationally, contact the U.S. office to find the dealer nearest you.

Figure 5.3 Exploded View of the Bearing Assembly



4 Looking at the underside of the seal housing, gently place the housing over the seal assembly so that the cavity in the middle of the upper housing rests over the seal and spring.

Turn the housing by hand until the captive screws align with the holes in the plate.

Use the 1/8-in. hex wrench to tighten the screws.

5 Lubricate the O-rings of the transfer tube and insert it (with the retaining clip toward the top of the tube) into the housing.

Push down on the transfer tube until the retaining clip is flush with the top of the housing.

6 Place the small retaining plate over the transfer tube; align the screws with the holes on the top of the seal assembly and use a slotted screwdriver to tighten them.

Attaching the Chamber and Counterbalance

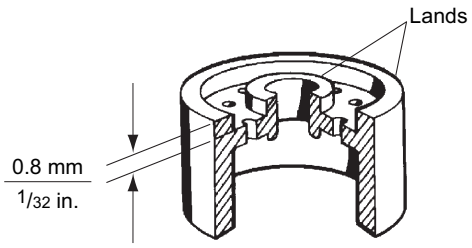
CHAPTER 2, *System Description and Installation*, describes the procedure for configuring and assembling the quick-release assembly.

Smoothing (Lapping) the Black Seal

With normal use, the black seal (shown in Figure 5.4) will give several thousand hours of service. However, the seal is made of graphite-filled polyimide plastic (Tribolon) which can become nicked or scratched; such damage allows leakage between the inlet and the outlet of the rotor. The seal can be re-polished using one of the methods below.

NOTE The raised rings (lands) on the seal must be at least 0.8 mm (1/32 in.) high for surfaces to seal properly (see Figure 5.4). If they are worn below this measurement, replace the seal.

Figure 5.4 The Black Seal



Recommended Method

The recommended method is to polish the seal by running the rotor as described below.

1. Remove all liquid from the rotor system (pump all liquid out).

2. Remove the black seal and wipe the sealing surface (with the rings).

   Clean and dry it, if necessary.

   Do not lubricate the seal.

3. Reassemble the seal assembly and install the quick-release assembly in the rotor (with or without chambers).

4. Attach the anchor cable to the top of the seal assembly to hold it stationary.

   Attach the other end of the anchor cable through the keyhole in the cable retainer and insert plug 802009 (see Figure 2.13).

5. Set the centrifuge temperature to 20°C or less and set the speed to 2000 RPM.

6. Run the rotor dry for three to four hours.

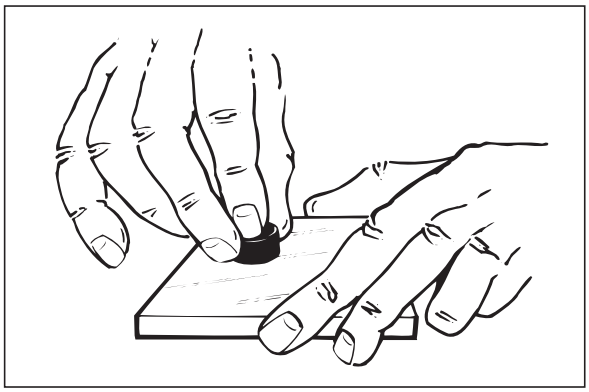
   This should polish the black seal.

(For older seals, this procedure may need to be repeated.)

Alternate Method

An alternate method is to polish the ringed surface by turning it against the etched glass plate (870671) provided with the rotor (see Figure 5.5).

Figure 5.5 Lapping the Black Seal



1. Pour a small amount of water on the rough surface of the glass plate.
2. With two fingers resting firmly on the seal, trace a figure eight on the plate with the sealing surface ten or twelve times.
3. Rotate the seal 90 degrees and repeat the procedure.
4. Do this two more times, each time rotating the seal 90 degrees in the same direction.

The combination of the figure-eight pattern and the 90-degree rotation will ensure that the seal is polished evenly. Properly lapped rings should reflect light smoothly and evenly. Note that the glass plate will eventually need to be replaced.

Troubleshooting

Possible malfunctions that may occur are described in Table 5.1 along with probable causes and recommended actions. Perform the recommended corrective action in sequence, as listed. If you are unable to correct the problem, call Beckman Coulter Field Service.

Table 5.1 Troubleshooting Chart

Problem	Probable Causes	Recommended Action
Separations are of poor quality; first collected fractions are mixed populations of cells, not pure cell types	Most likely: a leak in the rotating seal. To confirm leakage, inject dye into the input buffer stream and observe its path through the rotor. If dye bypasses the elutriation chamber, then the rotating seal is probably leaking.	Disassemble the rotor and make sure that the seal is seated properly and freely clears the housing assembly. Reassemble the rotor and retest using the dye.
Total number of cells recovered from the rotor is less than the number injected (i.e., 1×10^8 injected vs. 5×10^7 recovered in all fractions combined	Most likely: leaks from the chamber, chamber connections, or rotating seal. Cells may be aggregating. To diagnose the problem, pump buffer through the rotor when it is in static condition, then pump with the rotor running at 2000 RPM. If leakage occurs in static condition, then the problem is probably with a chamber gasket or chamber connector O-rings. If leakage occurs in running condition, the problem is most likely with the rotating seal.	Lubricate the chamber connector O-rings with silicone vacuum grease and make sure that the chamber gasket is properly seated and correctly placed in the chamber. *Do not lubricate the chamber gasket*. Make sure that the rotating seal is properly seated and free of the housing assembly. Tighten the chamber, reassemble the rotor, and retest as before.
Operating pressure too high; as the rotor reaches operating speed, the pressure displayed on the gauge rises above 10 psi	Air is probably not purged from the rotor, or the chamber is blocked. Stop the rotor while the pump is running. If the pressure goes down when the rotor decelerates and air exits the rotor, then trapped air was most likely causing the problem. If the pressure remains high or rises, then most likely the chamber is blocked.	Decelerating the rotor will usually clear trapped air from the rotor and the pressure will remain low when the rotor accelerates to operating speed. A blocked chamber can be cleaned with a sonicator, using strong detergent. Reassemble the rotor after cleaning and retest.
Monocytes clump; a large pellet is observed at the distal end of the rotating chamber	Monocytes are probably activated. Cells exiting the chamber are not as pure as expected; fewer cells are recovered from rotor than were injected.	Thoroughly clean the chamber. Use a sonicator and avoid ethanol. Also, use a 1% fetal calf serum, draw blood in ACD, and use citrate buffer throughout wash and elutriation.
Leakage	**1.** Chamber screws loose **2.** Chamber gasket missing or damaged **3.** O-rings need more lubrication **4.** O-rings damaged **5.** Poorly connected tubing **6.** Seal stuck to the O-ring in the seal assembly	**1.** Tighten screws by hand. **2.** Replace gasket. *Do not lubricate*. **3.** Coat the O-rings lightly but evenly with silicone vacuum grease. **4.** Replace the O-rings. **5.** Check tubing connections. **6.** The black seal may be worn improperly: spring tension may be too strong or too weak. Replace the spring. If the seal is badly worn, lap or replace it.

Table 5.1 Troubleshooting Chart

Problem	Probable Causes	Recommended Action
Liquid accumulates in the well around the seal assembly	**1.** Seal dirty	**1.** Stop the rotor and pump; blot up the liquid. Start the pump again; check to see if liquid accumulates. *If no liquid is seen*, start the rotor spinning at 2000 to 3000 RPM for 5 minutes. Stop the rotor and again check for liquid in the well. *If liquid is seen*, disassemble and check the seal assembly. Clean or lap the seal as required.
	2. Seal worn unevenly	**2.** Same as step 1.
	3. inched or blocked lines	**3.** Check lines for blockages; disassemble and clean rotor.
	4. Air bubbles in system	**4.** Follow the instructions in *Purging Air from the System* (CHAPTER 3).
Mixing of inlet and outlet liquid	**1.** Cross-leakage over the face of the seal due to: **a.** excessive back pressure (greater than 10 psi) **b.** damaged or unevenly worn seal face **c.** defective spring in the shaft assembly	**1.** See below. **a.** Purge air from the lines and rotor. Inlet and outlet lines should be 1/8-in. diameter. Check installation of chambers. **b.** Lap or replace the seal. **c.** Replace the spring.
	2. O-rings missing/damaged	**2.** O-rings should be lubricated and in good condition.
Poor separation	**1.** Sample not prepared properly	**1.** Disperse sample to a single-cell suspension.
	2. Tubing too long	**2.** Reduce length of tubing between sample injection point and the rotor.
	3. Pump pulsating	**3.** Use pump that pulsates less than 5%.
	4. improper run parameters	**4.** Recheck nomogram in CHAPTER 4 for flow rate and rotor speed or use formula in Table 4.1.
	5. Changing flow rate too fast	**5.** Incremental changes in flow rate should not exceed 1 to 2 mL/min.
It is difficult to synchronize the elutriation chamber in the viewing port	You are turning the flash delay knob too quickly	Start at zero delay, with the knob fully counterclockwise. Slowly increase the delay adjust (clockwise) until the appropriate chamber is in view.
No light output from the strobe assembly	Lamp is burned out	Call Beckman Coulter Field Service for lamp replacement.
Strobe operating problems	Damaged strobe cable	Do not use strobe. Call Beckman Coulter Field Service for replacement. Inspect the cable regularly.

Table 5.1 Troubleshooting Chart

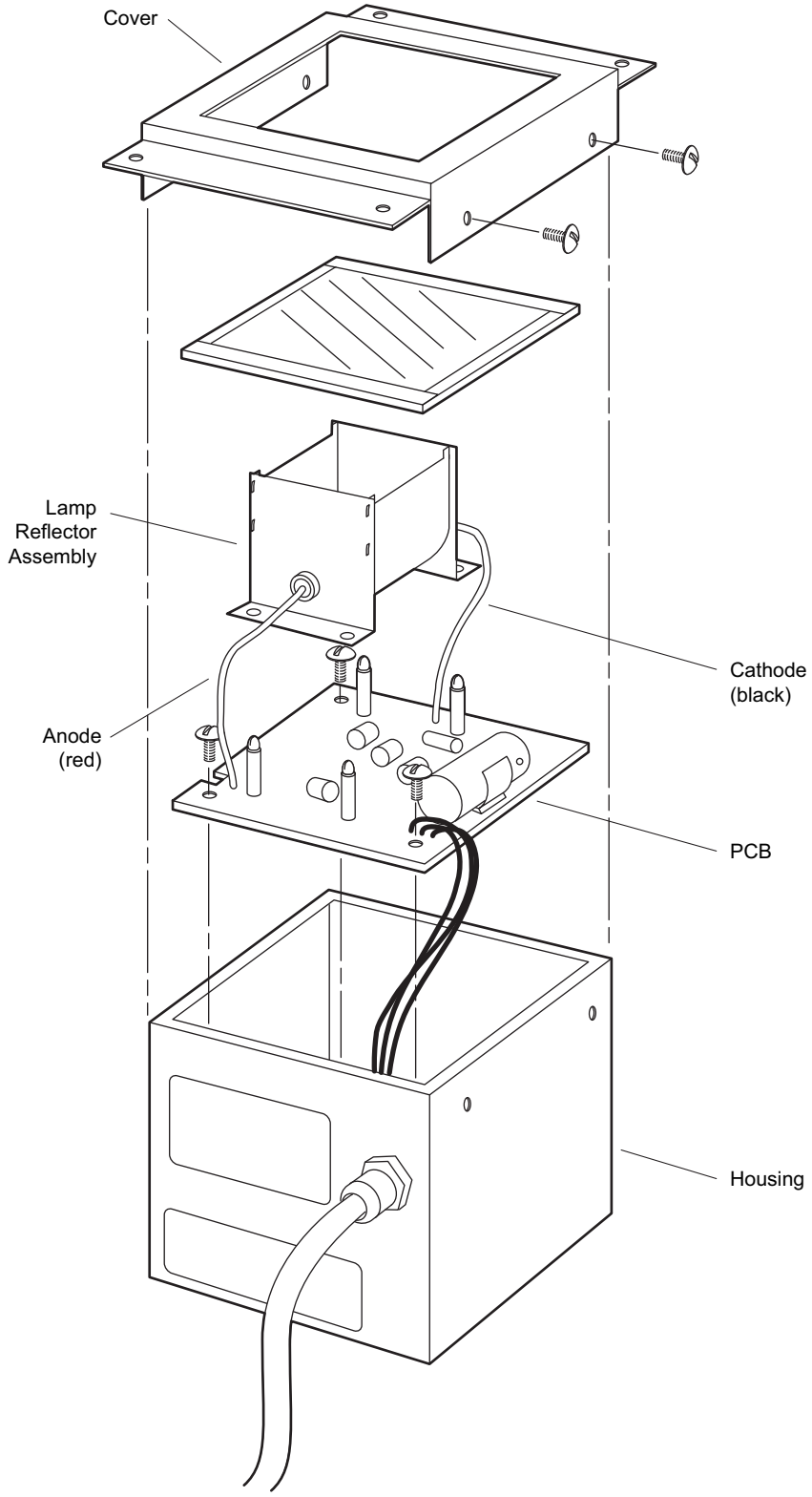
Problem	Probable Causes	Recommended Action
Rotor vibration	Air in the rotor	Follow the instructions in *Purging Air from the System* (CHAPTER 3).
	Empty or partially empty chamber	Check rotor/tubing assembly.
	Improperly assembled rotor	Recheck assembly procedures.
	Bent drive shaft	Call Beckman Coulter Field Service.

Stroboscopic Problems

If it is difficult to synchronize the rotor in the viewing port, first make sure that the elutriation chamber is installed in the **ELUTE** position on the rotor body. To adjust the flash delay control, start with the flash delay fully counterclockwise (left, zero delay) and then slowly rotate clockwise (to the right, increasing the delay) until the rotor chamber is in view.

The lamp is sealed inside the lamp reflector assembly (see Figure 5.6). When the lamp burns out, this entire assembly must be replaced by Beckman Coulter Field Service. Attempts by the user to remove the lamp reflector assembly may damage the assembly and surrounding components. The lamp has a long expected life (approximately 5 years). *Do not touch the lamp, as this can shorten lamp life.*

Figure 5.6 Exploded View of Strobe Assembly



Vibration

Vibration can be caused by air in the rotor (see *Purging Air from the System* in CHAPTER 3), an empty or partially empty chamber, a bent rotor shaft (contact Beckman Coulter Field Service to replace), or an improperly assembled rotor (see Figure 5.1).

Returning a Rotor

Before returning a rotor or accessory for any reason, prior permission must be obtained from Beckman Coulter, Inc. A return form may be obtained from your local Beckman Coulter sales office. The form should contain the following information:

- rotor type and serial number,
- history of use (approximate frequency of use),
- reason for the return,
- original purchase order number, billing number, and shipping number, if possible,
- name and email address of the person to be notified upon receipt of the rotor or accessory at the factory,
- name and email address of the person to be notified about repair costs, etc.

To protect our personnel, it is the customer's responsibility to ensure that all parts are free from pathogens and/or radioactivity. Sterilization and decontamination must be done before returning the parts. Smaller items (such as tubes, bottles, etc.) should be enclosed in a sealed plastic bag.

All parts must be accompanied by a note, plainly visible on the outside of the box or bag, stating that they are safe to handle and that they are not contaminated with pathogens or radioactivity. ***Failure to attach this notification will result in return or disposal of the items without review of the reported problem.***

Use the address label printed on the return form when mailing the rotor and/or accessories.

Customers located outside the United States should contact their local Beckman Coulter office.

Parts and Supplies

Contact Beckman Coulter Sales* or visit www.beckman.com for information about ordering parts, supplies, or publications. Refer to the *High Performance, High Speed, High Capacity Rotors, Tubes & Accessories catalog* (BR-8102, available at www.beckman.com). For your convenience, a partial list of rotor supplies is given below. Many replacement components and their part numbers are shown in Figure 2.14, Figure 5.3, and Figure 5.7.

Description	Part Number
Anchor cable assembly for Avanti J-26S XP series and J-26 XP series centrifuges	366922
Anchor cable assembly for the J6-MI centrifuge	358182
Bearing, stainless steel	366190
Bearing	344269

Description	Part Number
O-ring, transfer tube	010426
Plate, chamber/seal support	356910
Pressure gauge assembly	340148
Retaining plate, transfer tube	356915

* In the United States, call 1-800-742-2345. Outside the U.S., contact your local Beckman Coulter office or visit us at www.beckman.com.

Description	Part Number
Cable retainer for the J6-MI centrifuge	356014
Plug for cable retainer	802009
Chamber, large (40-mL), “A”	356940
Chamber, large (40-mL), “B”	356941
Chamber, standard (4-mL), “A”	356943
Chamber, standard (4-mL), “B”	356944
Chamber, Sanderson (5.5-mL), “A”	356945
Chamber, Sanderson (5.5-mL), “B”	356946
Chamber mounting standoff nut (qty/2)	964910
Chamber support spacer (qty/2)	363328
Chamber pad, polyethylene	356917
Chamber gasket, 40-mL	356907
Chamber gasket, 4-mL	356935
Chamber gasket, Sanderson, 4-mL	359399
Chamber shoulder screws (2 per chamber)	929279
Chamber shoulder screw washers	964942
Chamber tube (solid)	356921
Counterbalance	356942
E-ring, retaining, center tube	853696
Fitting, inlet line (to seal assembly), 3/16 in. I.D.	927768
Fuse, strobe, 1.0 A-TD 250 Vac	000148
Glass plate for lapping seal (4 × 5 in.)	870671
Housing, bearing	356912
Housing, seal	356911
O-ring, small, seal housing	870655
O-ring, small, bearing shaft	011519
O-ring, large, seal housing	868638
O-ring, center tube	853984

Description	Part Number
Retaining clip, external	893426
Retaining clip, internal	893427
Sample reservoir, 30-mL	335213
Sample reservoir, 75-mL	335197
Screw, cap (support plate to seal housing)	803993
Screw, slotted, stainless steel (retaining plate)	964972
Screw, housing	824905
Screw, shoulder (chamber assembly)	929279
Seal, rotating, black	347526
Seal, stationary, white	335145
Syringe assembly	347543
Shaft (bearing)	356913
Spacer (spring/seal)	357459
Spring (seal assembly)	340145
Strobe lamp assembly, Avanti J-26S XP series and J-26 XP series	363738
Strobe chassis assembly, Avanti J-26S XP series and J-26 XP series	366386
Tube, long	356920
Tube, short	356919
Tube, transfer	356914
Tubing, silicone	357580
Washer, flat (bearing housing to support plate)	887438
Washer, flat (seal housing)	889303
Washer, spring (retaining plate)	927730
Washer, spring (support plate to seal housing)	927731
Washer, spring (bearing assembly, 3 required)	893428
Windage Reduction Plug Assembly kit for Avanti J centrifuges	367855
Stoppers, modified with slit for the J6-MI centrifuge	336403

Supplies

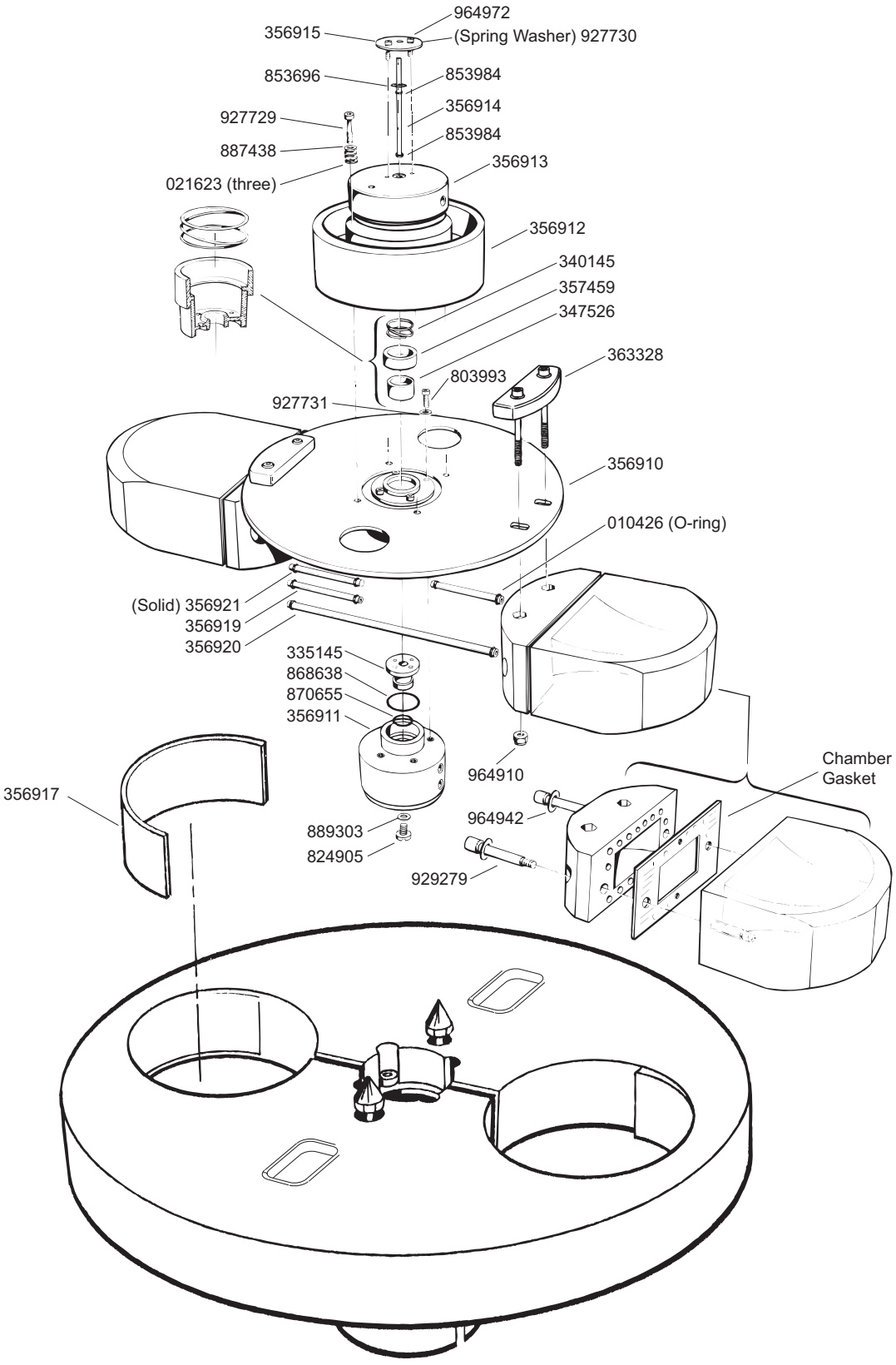
NOTE For MSDS information, go to the Beckman Coulter website at www.beckman.com.

Description	Part Number
Silicone vacuum grease (1 oz)	335148
Solution 555 cleaning concentrate (1 qt)	339555
Spinkote lubricant (for rotor/shaft interface) (2 oz)	306812
Tubing, tygon, 1/8 in. (I.D.) × 1/4 in. (O.D.)	357520
Tubing, silicone, 3/16 in. (I.D.) × 5/16 in. (O.D.) (for rotating seal assembly connection)	357580
Tubing harness kit	356938

Tools

Description	Part Number
Pliers for internal and external retaining clips (bearing removal)	A11129
Screwdriver	807371
Wrench, 1/8-in. hex driver	016223
Wrench, 1/8-in. hex angle	007350
Wrench, 5/16-in. hex T-handle	927766
Wrench, 5/32-in. hex driver	029840
Wrench, 5/64-in. hex driver	001884
Wrench, 9/64-in. hex angle	817305

Figure 5.7 Exploded View of JE-5.0 Parts with Part Numbers



APPENDIX A

Elutriation Principles, Equations, and Example Methods

Introduction

To fully understand separation by elutriation, several variables of the elutriation process must be considered:

1. The strength of the centrifugal force field in the elutriation chamber (*g* force).
2. The effect of the counterflow buffer stream in the elutriation chamber (counterflow velocity).
3. The size distribution (in microns) of the cells in the mixture.
4. The geometry of the elutriation chamber (especially the cross-sectional area of the chamber at its widest point).
5. The density of the elutriation buffer.

The relationship of these variables is expressed in the formula (Stokes' Law):

$$SV = \left(\frac{d^2(\rho_p - \rho_m)}{18\eta}\right)\omega^2 r$$ **EQ 1**

where

SV	=	the sedimentation velocity
d	=	the diameter of the particle
ρ_p	=	the density of the particle
ρ_m	=	the density of the buffer
η	=	the viscosity of the buffer
r	=	the radial position of the particle
ω	=	the angular velocity in radians/second

Although Stokes' law accurately describes the behavior of rigid spherical particles, it is somewhat less accurate in describing the sedimentation velocity of cells/particles that are not rigid and occasionally not spherical. Nevertheless, it is useful because it deals with those aspects of the system that influence the behavior of a sedimenting particle. Note that two properties of a cell affect its sedimentation velocity: its size and its density. Size plays a more important role, however, since the diameter value is raised to the second power. Because cell populations often do not differ much with respect to density, cell separation by sedimentation velocity is mostly based on size differences.

In counterflow centrifugal elutriation, the forces that result in cell sedimentation in a radial direction are balanced by the velocity of fluid flowing in the opposite direction. The flow velocity (V) at any point is equal to the flow rate (F), divided by the cross-sectional area at that point (A).

$$V = \frac{F}{A} = \left(\frac{d^2(\rho_p - \rho_m)}{18\eta}\right)\omega^2 r \qquad \textbf{EQ 2}$$

The flow rate F, in the chamber is the same at every point, i.e., $V_1A_1 = V_2A_2$. Therefore changes in the cross-sectional area produce changes in the flow velocity. Where the cross-sectional area is small (for example, near the r_{max}), the flow velocity is highest. At the elutriation boundary, where the cross-sectional area is greatest, the fluid velocity is lowest. Thus there is a velocity gradient in the elutriation chamber.

Similarly, there is a gradient of centrifugal force, increasing from the elutriation boundary r_{eb}, to r_{max}. Where the centrifugal force field is greatest, the fluid velocity is also greatest; as *r* decreases, the cross sectional area of the chamber increases and the fluid velocity decreases. Under the influence of the equal but opposing forces of the gravitational field and the fluid flow, small (lower sedimentation velocity) cells are in equilibrium nearest r_{eb} where the centrifugal force field and fluid velocity are low. Thus separations are the result of cells of different sedimentation velocities being in equilibrium at different radial positions in the chamber. When the flow rate is increased (or the speed is decreased), cells that were in equilibrium near the elutriation boundary are washed out of the chamber and the distribution of cells at equilibrium shifts toward the center of rotation. Subsequent increases in flow rate and/or decreases in speed wash populations of cells out of the chamber in increasing order of size.

The nomogram (Figure 4.1) allows you to determine flow rate and speed combinations with which cells of a given size will either be retained or swept out of the chamber. It is based on EQ 2 where F/A is substituted for V and the relationship solved for F:

$$F = Ad^2\left(\frac{\rho_p - \rho_m}{18\eta}\right)\omega^2 r \qquad \textbf{EQ 3}$$

Assuming that $\rho_p - \rho_m = 0.05$ g/mL, $\eta = 1.002$ mPa/s, and combining these with A (the cross sectional area of the chamber at the elutriation boundary), *r* = the radius at the elutriation boundary, and constants that convert ω to RPM, yield a chamber constant, C. **EQ 3** then becomes:

$$F = Xd^2\left(\frac{RPM}{1000}\right)^2 \qquad \textbf{EQ 4}$$

an expression relating flow rate, cell diameter and RPM.

Chamber Constants for Various Chambers	
40 mL large chamber	1.73×10^{-1}
4 mL standard chamber	5.11×10^{-2}
5.5 mL Sanderson chamber	3.78×10^{-2}

The sedimentation velocity (SV) scale of the nomogram assumes that the sedimentation velocity of the cell was measured in a fluid having the same density and viscosity as the fluid being used for elutriation. If this is not so, the sedimentation velocity should be adjusted for elutriation conditions using the following formula:

$$SV_{(adj)} = SV_{(determined)} \left(\frac{\Delta\rho(\text{elutriation fluid})}{\Delta\rho(\text{SV determined})}\right)\left(\frac{\eta(\text{SV determined})}{\eta(\text{elutriation fluid})}\right) \quad \textbf{EQ 5}$$

When the particle diameter scale is used instead of the sedimentation velocity scale, flow rates from the graph may require adjustment if the viscosity of the elutriation medium and the difference in density between the particle and the medium differ significantly from the assumptions made to construct the nomogram (1.002 mPa.s and 0.05 g/mL, respectively). The following formula should be used for that adjustment:

$$SV_{(adj)} = SV_{(determined)} \left(\frac{\Delta\rho(g/mL)}{0.05(g/mL)}\right)\left(\frac{1.002(mPa \bullet s)}{\text{viscosity}(mPa \bullet s)}\right) \quad \textbf{EQ 6}$$

Elutriation Chambers

Elutriation chambers are available in different sizes and shapes, depending on the desired application. This manual discusses using the large, standard, and Sanderson elutriation chambers.

Example 1

We wish to derive a set of elutriation parameters for the separation of two particular cell species in a heterogeneous mixture (e.g., lymphocytes and monocytes) derived from a Ficoll density gradient separation of peripheral blood. The gradient separation yields a mixture having the following components: 32% monocytes, mean cell diameter 9.0 µ; and, 68% lymphocytes, mean cell diameter 4.5 µ.

We want to use PBS as the elutriation buffer and run the elutriation at a constant 2500 RPM in a 5mL Sanderson chamber. We calculate the buffer flow rates using EQ 4:

$$F = 0.0378 * (4.5)^2 * \left(\frac{2500}{1000}\right)^2 = 4.78 \text{ mL/min. for the 4.5µ lymphocytes} \quad \textbf{EQ 7}$$

$$F = 0.0378 * (9.0)^2 * \left(\frac{2500}{1000}\right)^2 = 19 \text{ mL/min. for the 9.0µ monocytes} \quad \textbf{EQ 8}$$

We can now set the initial buffer flow to 5 mL/min., which will establish elutriation boundary conditions for the lymphocytes when they are loaded into the elutriation chamber. Increasing the flow rate by 1 or 2 mL/minute after the elutriation boundary is established will then wash the lymphocytes out of the chamber but retain cells whose diameter exceeds 4.5 µ. After we collect a 150 mL fraction of the 4.5µ cells, we then increase the buffer flow rate to 20 to 21 mL/min. and collect a 150 mL fraction of the 9.0 µ monocytes.

Example 2

We wish to separate 6.0 µ to 7.5 µ cells from a mixture with a range of cell sizes from 2.5 µ to 10.3 µ. There are two ways to accomplish this:

1. We can use a one-step elutriation method that removes the cell range directly by varying the flow rate,

   or:

2. We can use a two-step protocol that requires us to collect and reprocess cells.

Both of these methodologies find proponents in the current literature.

One-Step Method

By using the formulas for flow rate as in Example 1, we calculate that at 2500 RPM the flow rate for eluting 5.9 µ cells is 8.0 mL/min., and for 7.5 m cells it is 13 mL/min. Therefore, we load at 8.0 mL/min. and collect a load fraction of 150 mL. This fraction is discarded. We then increase the buffer flow to 13 to 14 mL/min. and collect a second fraction of 150 mL. This fraction contains the 6.0 m to 7.5 m cells. What remains in the chamber is washed out by stopping the rotor and allowing the buffer flow to continue. This fraction is discarded.

The effect of increasing rotor speed is to increase the range of flow rates required to elute cells of differing size. For example, to elute the same 6.0 m to 7.5 m cell population at 3400 RPM would require flow rates of 16 mL/min. to 25 mL/min. (vs. 8 mL/min. to 13 mL/min. for 2500 RPM). This fact can be used to advantage if separating cells whose size differences are small; that is, the higher the RPM, the easier to resolve the two cell lines due to the greater difference in flow rate to wash the cells free of the chamber.

Two-Step Method

We know that the flow rate at 2500 RPM for eluting cells up to 7.5 µ is 13 mL/min. The cells are therefore loaded at 13 mL/min. and 150 mL of the load fraction is collected and saved. What remains in the chamber is washed out by stopping the rotor and allowing the buffer flow to continue. These are cells above the 7.5 µ range and this fraction is thus discarded. The first fraction (load fraction) is spun to concentrate the cells to 10 mL and reinjected into the rotor at 13 mL/min., but at an increased rotor speed of 3200 RPM. This higher speed determines that the flow rate of 13 mL/min. elutes cells up to 5.9 µ, but not larger. A load fraction of 150 mL is collected and discarded. The chamber contents are then washed out by stopping the rotor and allowing the buffer flow to continue. These washed out cells are in the range of 6.0 µ to 7.5 µ.

There are no compelling reasons to choose either of these elutriation methods over the other. For any individual protocol evolution, consideration must be given to ease of using the protocol, survival rates of cells subjected to constant handling, effects of time and elutriation buffer on cell functions, and so on. In general, the less the cells are handled and the less time they spend out of culture conditions, the better the cell survival rates and the less the normal cell function is disrupted.

It is important to control all variables as closely as possible when confirming flow rate and speed parameters. For example, buffer temperature must be constant from reservoir to elutriation chamber if an accurate chamber temperature is to be derived for elution parameters. The

centrifuge must maintain accurate temperature and speed for the rotor, and this should be calibrated periodically. All air should be purged from the rotor before loading cells into the chamber (verified by a "0" reading on the in-line pressure gauge). Elutriation chambers and rotors must be kept clean and free of endotoxins and contamination. Pumps need calibration routinely to ensure accurate flow rates, and electronic particle counters and sizers need routine calibration to insure proper readout.

First-Time Operation

The following steps should be followed when first attempting a new protocol:

1. Connect the elutriation apparatus as described in CHAPTER 2, and as shown in Figure 2.14, *Attaching Tubing to the Seal Assembly*.

2. Fill the rotor with elutriation buffer.

   Bring the rotor to operating speed and continue the flow of elutriation buffer through the rotor while stopping and starting the rotor at least twice to purge air from the system.

   When all air is purged, the pressure gauge will read "0," and no air will visibly exit the rotor.

3. Determine the speed and flow rate to be used for the protocol.

   Bring the rotor to operating speed.

4. Set the buffer flow to the speed necessary to establish elutriation boundary conditions for the smallest-diameter cells to be elutriated from the cell mixture.

   Introduce the mixture into the elutriation chamber in an injection volume of 10 mL.

   You can view the separation taking place in the chamber by looking through the port in the centrifuge door.

   The clear resin elutriation chamber is illuminated from below by a strobe light that is synchronized with the speed of the rotor.

   Observe the cells entering the elutriation chamber and check to see that the elutriation boundary has been formed and is stable.

5. Increase the buffer flow 1 to 2 mL/min and collect a 50-mL sample of the effluent at this buffer flow.

   Perform a cell differentiation and count on a Coulter cell sizer (or equivalent) and record the results.

6 Increase the buffer flow in 1- to 2-mL/min increments, collecting 50-mL samples for each incremental increase in buffer flow. Determine the mean cell size and number for each sample when it is collected. When the proportion of cells of desired size in the mixture reaches 50%, then this becomes the penultimate fraction and a 0.5- to 1.0-mL increase in buffer flow should be used to elute this size population of cells from the chamber. A collection volume of 150 mL should be sufficient to wash the entire cell population from the chamber.

7 Proceed to increase the buffer flow to the rate that establishes elutriation boundary conditions for the next size cell population to be eluted from the mixture, and perform steps 5 and 6, above, to clear this population of cells from the chamber.

8 Repeat steps 5 through 7 above until all the desired cells are cleared from the chamber.

9 Stop the centrifuge and wash the remaining cells from the chamber by continuing the buffer flow and collecting at least 100 mL of effluent wash volume.

10 Note the flow rates for each population of cells. This is the elutriation protocol.

APPENDIX B

Pump and Tubing Selection

The Pump and Pump Head

Required Pump Specifications

- Flow rate from 5 to 400 mL/min (for the large chamber) or from 2 to 100 mL/min (for the standard and Sanderson chambers)
- Minimal pulsation (<5%)
- Electrical specifications to match available power source

Any type and brand of pump may be used with the JE-5.0 rotor, as long as it meets the above specifications. We recommend the Cole-Parmer Masterflex L/S series pumps because they have been tested at Beckman Coulter. Listed below is one Masterflex standard pump; other Masterflex pump models will work with the JE-5.0. See the Cole-Parmer catalog for a complete list of compatible pumps with a variety of features and prices

NOTE Cole-Parmer part numbers are subject to change. Verify the pump type and specifications when you place your order.

Cole-Parmer Part Number	Power (50/60 Hz)	
	VAC	Amperes
EW-07520-40	90 to 130	1.5
EW-07520-47	200 to 260	0.8
EW-07523-60 dual voltage	90 to 130 and 190 to 260	1.5 at 115 VAC; 0.8 at 230 VAC

To order, contact:

Cole-Parmer Instrument Company
625 East Bunker Court
Vernon Hills, IL 60061 U.S.A.
1-800-323-4340 (United States only)
Fax: 847-247-2929 (United States only)
Website: www.coleparmer.com
E-mail: info@coleparmer.com

Recommended Pump Heads

In addition to the pump, one of the following stainless steel pump heads must also be ordered.

Type of Pump Head	Cole-Parmer Part Number	Used with Tubing Size
Standard	G-07016-21	16
Easy-Load	G-07518-10	accepts all sizes and types of tubing
Quick Load	G-07021-24	accepts all sizes and types of tubing
Easy-Load II	EW-77201-60	accepts all sizes and types of tubing

Tubing

Tubing shipped with the JE-5.0 system is 1/8 × 1/4-in. food-grade Tygon; an additional piece of 3/16 × 5/16-in. silicone tubing is used in the rotating seal assembly connection. Both sizes of tubing are available by the foot from Beckman Coulter and in larger quantities from Cole-Parmer.

Beckman Coulter, Inc.
JE-5.0 Elutriator Rotor Warranty, Seal Assembly Warranty, and Chamber Warranty

Subject to the conditions specified below and the warranty clause of the Beckman Coulter terms and conditions of sale in effect at the time of sale, Beckman Coulter agrees to correct either by repair, or, at its election, by replacement any defects of material or workmanship which develop within seven (7) years after delivery of a JE-5.0 elutriator rotor to the original buyer by Beckman Coulter or by an authorized representative, provided that investigation and factory inspection by Beckman Coulter discloses that such defect developed under normal and proper use. Should a Beckman Coulter centrifuge be damaged due to a failure of a rotor covered by this warranty, Beckman Coulter will supply free of charge all centrifuge parts required for repair.

Elutriation chambers, counterbalances, and except for moving parts (rotating seal and bearings), the seal assembly employed with the JE-5.0 rotor are warranted for one (1) year against defects in materials and workmanship. Moving parts should be expected to give reasonable service for a reasonable period of time. What constitutes either reasonable service or a reasonable period of time shall be determined solely by Beckman Coulter.

Conditions

1. Except as otherwise specifically provided herein, this warranty covers the rotor only and Beckman Coulter shall not be liable for damage to parts external to the rotor or ancillary supplies including but not limited to (a) bearings, (b) rotating and stationary seals, (c) O-rings, (d) tubing, or (e) rotor contents.
2. This warranty is void if the rotor is operated with a rotor drive unit or in a centrifuge unmatched to the rotor characteristics or operated in a Beckman Coulter centrifuge that has been improperly disassembled, repaired or modified.
3. This warranty is void if the rotor has been subjected to customer misuse such as operation or maintenance contrary to the instructions in the Beckman Coulter rotor or centrifuge manual or the chamber bulletin.

Disclaimer

IT IS EXPRESSLY AGREED THAT THE ABOVE WARRANTY SHALL BE IN LIEU OF ALL WARRANTIES OF FITNESS AND OF THE WARRANTY OF MERCHANTABILITY AND THAT BECKMAN COULTER, INC. SHALL HAVE NO LIABILITY FOR SPECIAL OR CONSEQUENTIAL DAMAGES OF ANY KIND WHATSOEVER ARISING OUT OF THE MANUFACTURE, USE, SALE, HANDLING, REPAIR, MAINTENANCE, OR REPLACEMENT OF THE PRODUCT.

Related Documents

Avanti J-26S XP
PN B10087

Avanti J-26S XPI
PN B10093

Avanti J-26 XP
PN J326XP-IM-5

Avanti J-26 XPI
PN J326XPI-IM-4

Model J6-MI
PN J6MI-IM-10

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