Airfuge Ultracentrifuge
Beckman Coulter Airfuge Systems

Technical Procedure 3481

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For *In Vitro* Diagnostic Use Only

Intended Use

The Beckman Coulter Airfuge Ultracentrifuge is used to clarify lipemic serum and plasma. This clarification method clears blood samples of lipemia caused by chylomicrons, fat particles 80-500 nm in diameter, that impair accuracy of spectrophotometric test(s) and indirect ion selective electrode (ISE) analysis of electrolytes.

Clinical Significance

Chylomicron (lipemia) occurrence is usually apparent after patients consume a meal rich in fats, but also appear in 1% to 3% of sera collected from patients who have fasted or have lipid and/or lipoprotein disorders.

With samples in which these lipids are present, it is virtually impossible to obtain accurate spectrophotometric test results. The particles strongly scatter light, increasing absorbance readings and causing values to be considerably overestimated. Fat particles also displace aqueous volume and increase sample viscosity, causing concentrations to be underestimated (electrolyte determinations by indirect ISE).

Conventional centrifugation, filtration and extraction techniques are often used in attempts to diminish these errors. But these solutions take too much time, require too much sample or introduce adsorption and denaturation problems.

The Lipemic Sample Clarification System by ultracentrifugation provides a fast 10 minute spin to clarify the lipemic samples.

Description

The Beckman Coulter Airfuge® is a benchtop air-driven ultracentrifuge capable of accelerating rotors up to 110,000 rpm (revolutions per minute) in as little as 30 seconds. The instrument uses no vacuum or high-speed bearings; the rotor is supported and turned by streams of air. Rotor speed can be determined by conversion of the applied air pressure, as displayed on the pressure gauge, to rpm. This conversion is different for each type of rotor.

The Chylomicron Rotor Package is used with the airfuge for lipid clarification. The package comes with the ACR-90 Chylomicron Rotor, Rotor Stand and 3.5 mL Polyethylene Liners (pkg. of 100). Polyethylene Liners, 2.4 mL (pkg. of 100) and an adapter for 2.4 mL Liner are also available for smaller volume samples.

The rotor features an anodized base machined to fully support both chambers of the plastic liner. It has a stainless steel cover with an opening on the top, through which protrudes the dome of the inner chamber with the sample access hole. The cover is screwed down on the rotor base, compressing the liner and sealing the outer chamber from the inner chamber.

The polyethylene liner is made up of two concentric chambers—a doughnut-shaped outer chamber, and an inner chamber shaped like an inkwell, with a hole at the top for pipetting sample in and out of the liner. The 3.5 mL liner yields 2.6 mL of clarified serum, and the 2.4 mL liner yields 1.5 mL of clarified serum.

The A-100/18 fixed angle rotor can be used to clarify small volumes of lipemic serum by flotation of the chylomicrons. The rotor can hold six tubes and up to 1050 µL per run in the rotor.

The disposable 5 x 30 polypropylene tubes for the fixed angle rotor have a maximum fill volume of 175 µL and have been tested for use at temperatures between 2° and 25°C (35.6° to 77°F).

The Airfuge ultracentrifuge has been designed and tested to operate safely indoors at altitudes up to 2000 meters (6562 ft). It is an exceptionally safe ultracentrifuge because the rotor is held in place by a pressure differential created during centrifugation. An air pressure limitation of 35 psig (pounds per square inch, gauge) or 240 kPa (kilopascal) is factory set. However, do not operate the instrument at more than 30 psig (207 kPa).

Refer to the *Beckman Airfuge® Instruction Manual* for installation and setup instructions.

Methodology

During centrifugation, the inner chamber is forced downward, permitting flow between the two chambers. The low-density fat particles float to the center of the rotor, the area of lowest centrifugal force. As the rotor decelerates, the outer chamber is again sealed off by compression, isolating the fatty concentrate in the inner chamber. The fatty liquid portion is removed from the inner chamber with the rotor cover screwed down. The cover is removed so that the clarified serum/plasma can be collected from the outer chamber.

In addition to clarifying lipemic serum, this system can be used to remove fatty particles from other body fluids. It also works well for other applications where the material of concern can be isolated by flotation when centrifuged.

Controls and Indicators

Pressure Regulator Knob

The pressure regulator knob controls the driving air pressure. Pressing down on the knob and turning to the right (clockwise) closes the door. Turning the knob further brings up the air pressure and accelerates the rotor to speed.

Pressure Gauge

Operating air pressure is displayed on this gauge. Recommended operating air pressure is 30 psig (207 kPa).

Run Light

The run light comes on when the timer is set and remains lit until the braking system is engaged.

Time-Delay Knob

This knob, located on the ultracentrifuge bottom panel is used to set a time-delay period that allows the rotor to coast after the timer reaches zero at the end of a run. The run light goes out and a brake pin is released at the end of the set delay period. A setting of 120 seconds on the knob (or about 3.5 on older instruments) represents the 2-minute delay period.

Brake Tension Screw

The brake spring wire forces the brake pin up into the rotor bottom during braking. Occasional adjustment of the spring tension, using the BRAKE TENSION adjusting screw located on the back panel, may be necessary.

Levitation Air Screw

When the timer reaches zero at the end of a run, the driving air is turned off and levitation air supports the rotor during its deceleration. If the flow of levitation air is too high and the rotor fails to stop completely, or too low and the rotor stops too fast, adjustment of the LEVITATION AIR screw located on the rear panel may be necessary.

Equipment

Ultracentrifuge

Beckman Coulter Airfuge® Air-Driven Ultracentrifuge. Beckman-Coulter, Brea, California. For technical assistance, call Beckman-Coulter Sales and Service: 1-800-742-2345.

Refer to the Beckman Coulter *Airfuge® Air-Driven Ultracentrifuge Instruction Manual* for detailed instructions for installation, set up, detailed parts description, operation, general maintenance and troubleshooting.

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Rotors

ACR-90 Rotor

ACR-90 rotor uses 3.5-mL and 2.4-mL disposable liners, and is used to clarify lipemic serum/plasma. Lipemic samples can be clarified by a 10-minute spin at 90 000 rpm at 30 psig (207 kPa) air pressure.

- Chylomicron Rotor Package (part# 341260): includes ACR-90 Chylomicron Rotor (part# 341259), Rotor Stand (part# 341252), and 3.5 mL Polyethylene Liners, pkg. of 100 (part#341251).
- Polyethylene Liners, 3.5 mL (pkg. of 100). Part number: 341251
- Polyethylene Liners, 2.4 mL (pkg. of 100). Part number: 342634
- Adapter for 2.4 Liner. Part number: 342635

A-100/18 Fixed Angle Rotor

The fixed angle rotor uses 5 x 20 mm polypropylene tubes to clarify small volumes of lipemic serum/plasma by flotation of the chylomicrons

- A-100/18 Fixed Angle Rotor Assembly. Part number: 347593
- 5 x 20 mm Polypropylene tubes (pkg/100). Part number: 342630
- Rotor caps (pkg/20). Part number: 339643

Supplies

1. SAMCO Scientific Transfer pipets, extended Fine Tip, Std bulb: Catalog no.: 13-711-31, Thermo Scientific no: 235
2. Disposable gloves

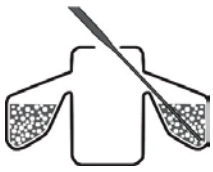
Procedure

Verify centrifuge is plugged in and air supply is on.

ACR-90 rotor

1. Determine amount of sample available for ultracentrifugation then use the appropriate polyethylene liner size for that volume. Maximum fill for 3.5 mL liner is 2.6 mL, and maximum fill for 2.4 mL liner is 1.5 mL.
2. Place open rotor on rotor stand. Insert adapter ring if using the smaller 2.4 mL liner in rotor.

3. Using the disposable extended fine tip transfer pipette, fill outer chamber with serum/plasma.



4. Screw stainless steel rotor lid onto rotor.

5. Lightly touch the brake pin to be sure it operates freely and check the stator pad in ultracentrifuge. Stator pad must be clean and in good condition. A worn stator pad is smooth and shiny on the inside surface. Replace a worn stator pad.

Worn stator pad.

New stator pad

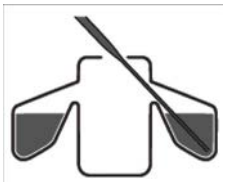
6. Place the rotor on the stator pad and close the instrument door.

7. Set the TIME dial for the required run time. The dial indicates up to 5 hours of run time in 5-minute increments. For runs shorter than 30 minutes, turn the dial past the 30-minute point, then back to the recommended run time of **10 minutes** to prevent significant evaporative loss.
8. Secure the instrument door by turning the pressure regulator knob to the right (clockwise), pushing down, until the air pressure indicated on the PRESSURE gauge brings the rotor to the required speed. Optimal run speed is achieved at 30 psig (207 kPa). (This is the only recommended operating air pressure for the ACR-90 rotor.) Note that some low-speed rotor vibration is normal at the beginning of the run.

CAUTION

If the rotor makes an unusual noise, turn the timer to zero. When the rotor stops, open the chamber door and remove the rotor. Check to make sure the rotor is loaded correctly. Correct the problem before restarting the run.

9. When the timer reaches or is turned to zero, the rotor will coast for about 2 minutes. After the 2-minute delay period, the run light goes out and the brake pin engages the rotor. The rotor will stop about 1 to 3 minutes later, depending on the rotor.
10. After the rotor has stopped, turn the pressure regulator knob to the left (counterclockwise) until the PRESSURE gauge reading is zero.
11. Open airfuge door, remove rotor and place in rotor stand. Gently push down against and grasp rotor steel cover and turn left (counterclockwise) to unscrew cover form rotor. Rotor stand will prevent rotor body from turning.
12. Using another disposable extended fine tip transfer pipette, aspirate clarified serum/plasma from outer chamber liner.

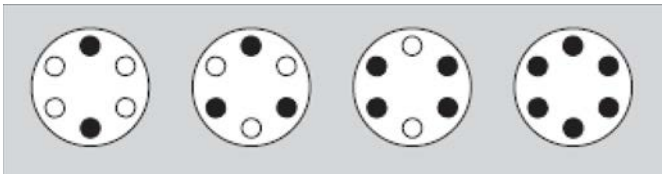


13. When done, dispose of chamber liner and pipet and decontaminate airfuge if any sample has been spilled.
14. Close airfuge door lid but do not tighten the pressure regulator knob between runs.

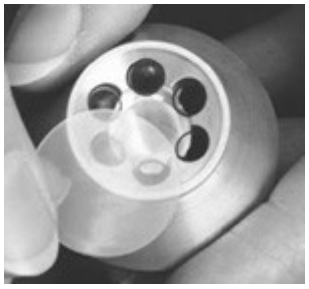
A-100/18 Fixed Angle Rotor

The fixed angle rotor can be used to clarify small volumes of lipemic serum/plasma by flotation of the chylomicrons. The rotor can hold six tubes; up to 1050 µL of gradient and sample volume can be centrifuged per run.

1. Before using the rotor, inspect the rotor bushing. Replace a worn bushing (refer to *Maintenance* section in *Instruction Manual for A-100/18 Rotors*).
2. Using a disposable extended fine tip transfer pipette, fill at least one polypropylene tube with sample. Do not exceed the maximum fill volume (175 µL) of the tube. If only using one tube for sample, fill another tube of equal volume with water to balance. Use forceps to load the filled tubes into the rotor. If fewer than six tubes are being run, they must be arranged symmetrically in the rotor. Opposing tubes must be filled to the same level with liquid of the same density.



3. Install a rotor cap by rubbing it into place in the rotor groove.



CAUTION
Do not run the rotor without a cap. Use a new cap for each rotor use.

4. Follow steps 5 through 10 for ACR-90 rotor.

> **CAUTION**
> **If disassembly reveals evidence of leakage, you should assume that some fluid escaped the rotor. Apply appropriate decontamination procedures to the centrifuge and accessories.**

5. Remove the rotor from the instrument.
6. Remove the rotor cap by pressing in the center of the cap to free an edge, then use forceps or your finger to grasp the cap.
7. Remove the tubes from rotor using forceps.
8. Using another disposable extended fine tip transfer pipette, minimize disturbance of lipemic layer and carefully move pipette tip through lipemic layer to aspirate clarified serum/plasma.
9. When done, dispose of chamber liner and pipet and decontaminate airfuge if any sample has been spilled.
10. Close airfuge door lid but do not tighten the pressure regulator knob between runs.

References

1. Airfuge® Air-Driven Ultracentrifuge Instruction Manual, AF-IM-13, Beckman Coulter, Inc., Brea, California 92821, 2009.
2. The Lipemic Serum Clarification System, DS-18212B, B2013-14280, Beckman Coulter, 2013
3. Instructions for Using the A-100/18 and A-100/30 Rotors, AF-TB-009AK (02/2014), Beckman Coulter, Brea, California 92821