Equipment Packet: Laboratory Centrifuge

UMDNS #: 18631

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Equipment Packet Contents:

This packet contains information about the operation, maintenance, and repair of laboratory centrifuges.

Part I: External From the Packet:

1. **An Introduction to Centrifuges:** PowerPoint

Part II: Included in this Packet:

1. Operation and Use of Centrifuges

Featured in this Section:

Malkin, Robert. Medical Instrumentation in the Developing World. Engineering World Health, 2006.

Openstax College. “Components of Blood.” From the publication: *Biology*. Rice University: 2013, pgs. 1195-1199.

Wikipedia. “Laboratory Centrifuge.” *Wikipedia*, p. 1-12. Retrieved from: https://en.wikipedia.org/wiki/Laboratory_centrifuge

WHO. “Analyzer, Hematology, Blood Grouping, Automated.” From the publication: *Core Medical Equipment*. Geneva, Switzerland, 2011.

Analyzer, Laboratory, Hematology, Blood Grouping, Automated

UMDNS		GMDN	
16817	Analyzers, Laboratory, Hematology, Blood Grouping, Automated	56712	ABO/Rh(D) blood grouping analyser IVD, automated

Other common names:
Blood type analyzer, ABO blood typing system, AB0 blood typing system;Blood Grouping System

Health problem addressed

Blood grouping systems perform basic blood processing tests that include ABO grouping and subgrouping, Rh and other red cell phenotyping, and antibody detection. These tests determine factors that can cause transfusion reactions such as red cell hemolysis, anaphylaxis, and other immunologic and nonimmunologic effects.

Product description

Floor-standing or benchtop device includes a rack or tray onto which patient blood sample tubes are loaded; the samples are mixed with reagents to determine blood type and the results are displayed on a monitor; cabinets or compartments store reagent vessels; a monitor, keyboard, mouse, and printer (or entire computer) may be connected for programming, data entry, and to view and print testing results.

Principles of operation

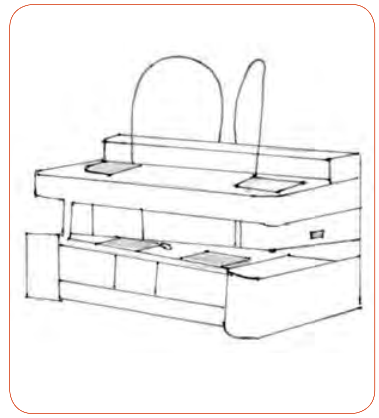
Blood tube containing ethylenediamine-tetraacetic acid (EDTA) anticoagulant is loaded onto the analyzer, and the operator usually centrifuges them to separate the RBCs from the plasma. Automated analyzers typically resuspend the RBCs in saline and load the diluted samples onto microplates to which reagents (known antisera) have been added. Blood group identity occurs when the known antiserum, containing antibodies, clumps (agglutinates) RBCs that have a corresponding antigen. Bar-code labels provides a means of sample tracking.

Operating steps

Technicians load tubes into the sample tray and keep reagents filled; tests are programmed either via a touchscreen panel on the instrument, a computer, or the required test information is on the tube's printed bar code.

Reported problems

Operators should be aware of the risk of exposure to potentially infectious bloodborne pathogens during testing procedures and should use universal precautions, including wearing gloves, face shields or masks, and gowns.



Use and maintenance

User(s): Laboratory technician

Maintenance: Biomedical or clinical engineer

Training: Initial training by manufacturer and manuals

Environment of use

Settings of use: Hospital, blood bank, clinical laboratory

Requirements: Line power, water supply, benchtop or floor space, biohazard disposal

Product specifications

Approx. dimensions (mm): 1,000 x 1,750 x 900

Approx. weight (kg): 50-500

Consumables: Reagents, blood tubes

Price range (USD): 115,000 - 225,000

Typical product life time (years): 5-7

Shelf life (consumables): EDTA: 1 year

Types and variations

Benchtop or floor-standing

WHO. "Analyzer, Hematology, Blood Grouping, Automated." From the publication: Core Medical Equipment. Geneva, Switzerland, 2011.

Introduction to Blood and Its Components

40.2 | Components of the Blood

By the end of this section, you will be able to:

- List the basic components of the blood
- Compare red and white blood cells
- Describe blood plasma and serum

Hemoglobin is responsible for distributing oxygen, and to a lesser extent, carbon dioxide, throughout the circulatory systems of humans, vertebrates, and many invertebrates. The blood is more than the proteins, though. Blood is actually a term used to describe the liquid that moves through the vessels and includes **plasma** (the liquid portion, which contains water, proteins, salts, lipids, and glucose) and the cells (red and white cells) and cell fragments called **platelets**. Blood plasma is actually the dominant component of blood and contains the water, proteins, electrolytes, lipids, and glucose. The cells are responsible for carrying the gases (red cells) and immune the response (white). The platelets are responsible for blood clotting. Interstitial fluid that surrounds cells is separate from the blood, but in hemolymph, they are combined. In humans, cellular components make up approximately 45 percent of the blood and the liquid plasma 55 percent. Blood is 20 percent of a person's extracellular fluid and eight percent of weight.

The Role of Blood in the Body

Blood, like the human blood illustrated in Figure 40.5 is important for regulation of the body's systems and homeostasis. Blood helps maintain homeostasis by stabilizing pH, temperature, osmotic pressure, and by eliminating excess heat. Blood supports growth by distributing nutrients and hormones, and by removing waste. Blood plays a protective role by transporting clotting factors and platelets to prevent blood loss and transporting the disease-fighting agents or **white blood cells** to sites of infection.

Openstax College. "Components of Blood." From the publication: Biology. Rice University: 2013, pgs. 1195-1199.

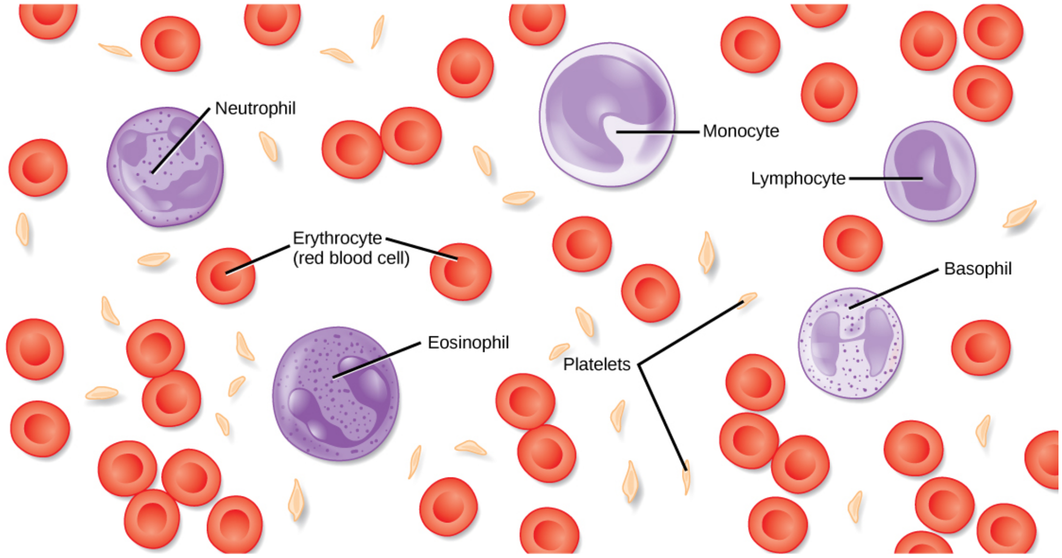


Figure 40.5 The cells and cellular components of human blood are shown. Red blood cells deliver oxygen to the cells and remove carbon dioxide. White blood cells—including neutrophils, monocytes, lymphocytes, eosinophils, and basophils—are involved in the immune response. Platelets form clots that prevent blood loss after injury.

Red Blood Cells

Red blood cells, or erythrocytes (erythro- = "red"; -cyte = "cell"), are specialized cells that circulate through the body delivering oxygen to cells; they are formed from stem cells in the bone marrow. In mammals, red blood cells are small biconcave cells that at maturity do not contain a nucleus or mitochondria and are only 7–8 µm in size. In birds and non-avian reptiles, a nucleus is still maintained in red blood cells.

The red coloring of blood comes from the iron-containing protein hemoglobin, illustrated in **Figure 40.6a**. The principal job of this protein is to carry oxygen, but it also transports carbon dioxide as well. Hemoglobin is packed into red blood cells at a rate of about 250 million molecules of hemoglobin per cell. Each hemoglobin molecule binds four oxygen molecules so that each red blood cell carries one billion molecules of oxygen. There are approximately 25 trillion red blood cells in the five liters of blood in the human body, which could carry up to 25 sextillion (25×10^{21}) molecules of oxygen in the body at any time. In mammals, the lack of organelles in erythrocytes leaves more room for the hemoglobin molecules, and the lack of mitochondria also prevents use of the oxygen for metabolic respiration. Only mammals have anucleated red blood cells, and some mammals (camels, for instance) even have nucleated red blood cells. The advantage of nucleated red blood cells is that these cells can undergo mitosis. Anucleated red blood cells metabolize anaerobically (without oxygen), making use of a primitive metabolic pathway to produce ATP and increase the efficiency of oxygen transport.

Not all organisms use hemoglobin as the method of oxygen transport. Invertebrates that utilize hemolymph rather than blood use different pigments to bind to the oxygen. These pigments use copper or iron to the oxygen. Invertebrates have a variety of other respiratory pigments. Hemocyanin, a blue-green, copper-containing protein, illustrated in **Figure 40.6b** is found in mollusks, crustaceans, and some of the arthropods. Chlorocruorin, a green-colored, iron-containing pigment is found in four families of polychaete tubeworms. Hemerythrin, a red, iron-containing protein is found in some polychaete worms and annelids and is illustrated in **Figure 40.6c**. Despite the name, hemerythrin does not contain a heme group and its oxygen-carrying capacity is poor compared to hemoglobin.

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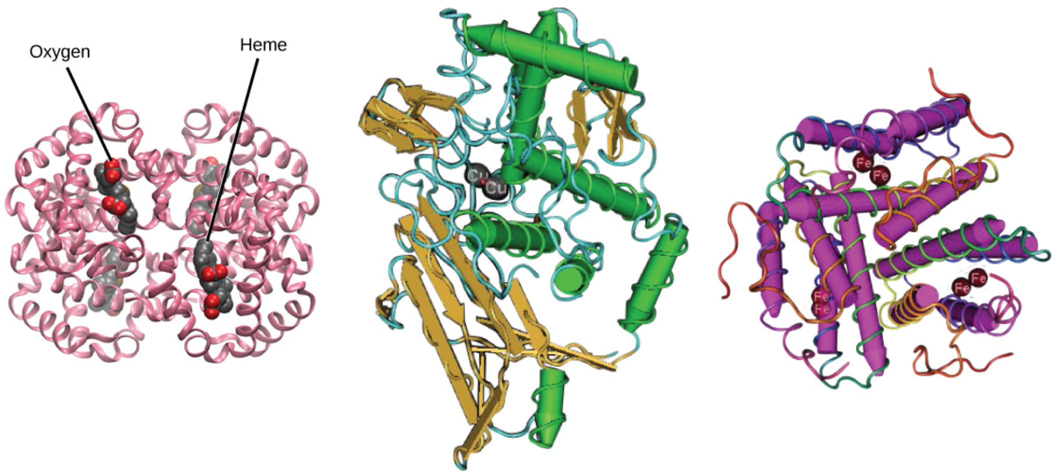

Figure 40.6 In most vertebrates, (a) hemoglobin delivers oxygen to the body and removes some carbon dioxide. Hemoglobin is composed of four protein subunits, two alpha chains and two beta chains, and a heme group that has iron associated with it. The iron reversibly associates with oxygen, and in so doing is oxidized from Fe^{2+} to Fe^{3+}. In most mollusks and some arthropods, (b) hemocyanin delivers oxygen. Unlike hemoglobin, hemolymph is not carried in blood cells, but floats free in the hemolymph. Copper instead of iron binds the oxygen, giving the hemolymph a blue-green color. In annelids, such as the earthworm, and some other invertebrates, (c) hemerythrin carries oxygen. Like hemoglobin, hemerythrin is carried in blood cells and has iron associated with it, but despite its name, hemerythrin does not contain heme.

The small size and large surface area of red blood cells allows for rapid diffusion of oxygen and carbon dioxide across the plasma membrane. In the lungs, carbon dioxide is released and oxygen is taken in by the blood. In the tissues, oxygen is released from the blood and carbon dioxide is bound for transport back to the lungs. Studies have found that hemoglobin also binds nitrous oxide (NO). NO is a vasodilator that relaxes the blood vessels and capillaries and may help with gas exchange and the passage of red blood cells through narrow vessels. Nitroglycerin, a heart medication for angina and heart attacks, is converted to NO to help relax the blood vessels and increase oxygen flow through the body.

A characteristic of red blood cells is their glycolipid and glycoprotein coating; these are lipids and proteins that have carbohydrate molecules attached. In humans, the surface glycoproteins and glycolipids on red blood cells vary between individuals, producing the different blood types, such as A, B, and O. Red blood cells have an average life span of 120 days, at which time they are broken down and recycled in the liver and spleen by phagocytic macrophages, a type of white blood cell.

White Blood Cells

White blood cells, also called leukocytes (leuko = white), make up approximately one percent by volume of the cells in blood. The role of white blood cells is very different than that of red blood cells: they are primarily involved in the immune response to identify and target pathogens, such as invading bacteria, viruses, and other foreign organisms. White blood cells are formed continually; some only live for hours or days, but some live for years.

The morphology of white blood cells differs significantly from red blood cells. They have nuclei and do not contain hemoglobin. The different types of white blood cells are identified by their microscopic appearance after histologic staining, and each has a different specialized function. The two main groups, both illustrated in **Figure 40.7** are the granulocytes, which include the neutrophils, eosinophils, and basophils, and the agranulocytes, which include the monocytes and lymphocytes.

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Figure 40.7 (a) Granulocytes—including neutrophils, eosinophils and basophils—are characterized by a lobed nucleus and granular inclusions in the cytoplasm. Granulocytes are typically first-responders during injury or infection. (b) Agranulocytes include lymphocytes and monocytes. Lymphocytes, including B and T cells, are responsible for adaptive immune response. Monocytes differentiate into macrophages and dendritic cells, which in turn respond to infection or injury.

Granulocytes contain granules in their cytoplasm; the agranulocytes are so named because of the lack of granules in their cytoplasm. Some leukocytes become macrophages that either stay at the same site or move through the blood stream and gather at sites of infection or inflammation where they are attracted by chemical signals from foreign particles and damaged cells. Lymphocytes are the primary cells of the immune system and include B cells, T cells, and natural killer cells. B cells destroy bacteria and inactivate their toxins. They also produce antibodies. T cells attack viruses, fungi, some bacteria, transplanted cells, and cancer cells. T cells attack viruses by releasing toxins that kill the viruses. Natural killer cells attack a variety of infectious microbes and certain tumor cells.

One reason that HIV poses significant management challenges is because the virus directly targets T cells by gaining entry through a receptor. Once inside the cell, HIV then multiplies using the T cell's own genetic machinery. After the HIV virus replicates, it is transmitted directly from the infected T cell to macrophages. The presence of HIV can remain unrecognized for an extensive period of time before full disease symptoms develop.

Platelets and Coagulation Factors

Blood must clot to heal wounds and prevent excess blood loss. Small cell fragments called platelets (thrombocytes) are attracted to the wound site where they adhere by extending many projections and releasing their contents. These contents activate other platelets and also interact with other coagulation factors, which convert fibrinogen, a water-soluble protein present in blood serum into fibrin (a non-water soluble protein), causing the blood to clot. Many of the clotting factors require vitamin K to work, and vitamin K deficiency can lead to problems with blood clotting. Many platelets converge and stick together at the wound site forming a platelet plug (also called a fibrin clot), as illustrated in **Figure 40.8b**. The plug or clot lasts for a number of days and stops the loss of blood. Platelets are formed from the disintegration of larger cells called megakaryocytes, like that shown in **Figure 40.8a**. For each megakaryocyte, 2000–3000 platelets are formed with 150,000 to 400,000 platelets present in each cubic millimeter of blood. Each platelet is disc shaped and 2–4 μm in diameter. They contain many small vesicles but do not contain a nucleus.

Openstax College. "Components of Blood." From the publication: Biology. Rice University: 2013, pgs. 1195-1199.

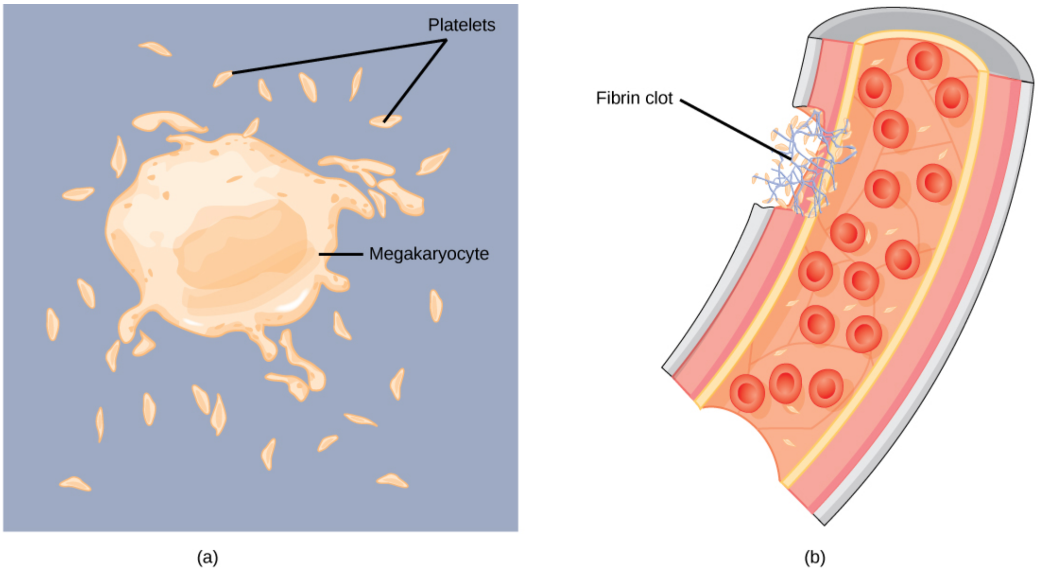


Figure 40.8 (a) Platelets are formed from large cells called megakaryocytes. The megakaryocyte breaks up into thousands of fragments that become platelets. (b) Platelets are required for clotting of the blood. The platelets collect at a wound site in conjunction with other clotting factors, such as fibrinogen, to form a fibrin clot that prevents blood loss and allows the wound to heal.

Plasma and Serum

The liquid component of blood is called plasma, and it is separated by spinning or centrifuging the blood at high rotations (3000 rpm or higher). The blood cells and platelets are separated by centrifugal forces to the bottom of a specimen tube. The upper liquid layer, the plasma, consists of 90 percent water along with various substances required for maintaining the body's pH, osmotic load, and for protecting the body. The plasma also contains the coagulation factors and antibodies.

The plasma component of blood without the coagulation factors is called the **serum**. Serum is similar to interstitial fluid in which the correct composition of key ions acting as electrolytes is essential for normal functioning of muscles and nerves. Other components in the serum include proteins that assist with maintaining pH and osmotic balance while giving viscosity to the blood. The serum also contains antibodies, specialized proteins that are important for defense against viruses and bacteria. Lipids, including cholesterol, are also transported in the serum, along with various other substances including nutrients, hormones, metabolic waste, plus external substances, such as, drugs, viruses, and bacteria.

Human serum albumin is the most abundant protein in human blood plasma and is synthesized in the liver. Albumin, which constitutes about half of the blood serum protein, transports hormones and fatty acids, buffers pH, and maintains osmotic pressures. Immunoglobin is a protein antibody produced in the mucosal lining and plays an important role in antibody mediated immunity.

Openstax College. "Components of Blood." From the publication: Biology. Rice University: 2013, pgs. 1195-1199.

Laboratory centrifuge

A tabletop laboratory centrifuge	
Uses	Separation
Related items	Gas centrifuge Ultracentrifuge

A **laboratory centrifuge** is a piece of laboratory equipment, driven by a motor, which spins liquid samples at high speed. There are various types of centrifuges, depending on the size and the sample capacity.

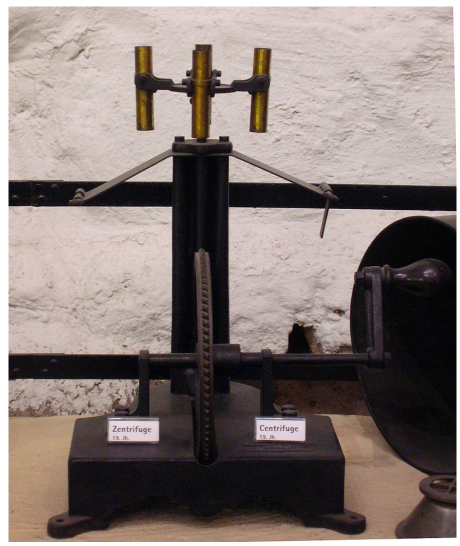
Like all other centrifuges, laboratory centrifuges work by the sedimentation principle, where the centripetal acceleration is used to separate substances of greater and lesser density.

Operation

Increasing the effective gravitational force will more rapidly and completely cause the precipitate ("pellet") to gather on the bottom of the tube. The remaining solution is called the "supernate" or "supernatant".

The supernatant liquid is then either quickly decanted from the tube without disturbing the precipitate, or withdrawn with a Pasteur pipette. The rate of centrifugation is specified by the acceleration applied to the sample, typically measured in revolutions per minute (RPM) or *g*. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity.

The use of a centrifuge is known as centrifugation.



A 19th century hand cranked laboratory centrifuge.

Types

Laboratory centrifuge

There are various types of centrifugation:

- Differential centrifugation, often used to separate certain organelles from whole cells for further analysis of specific parts of cells
- Isopycnic centrifugation, often used to isolate nucleic acids such as DNA
- Sucrose gradient centrifugation, often used to purify enveloped viruses and ribosomes, and also to separate cell organelles from crude cellular extracts

There are different types of laboratory centrifuges:

- **Microcentrifuges**

(devices for small tubes from 0.2 ml to 2.0 ml (micro tubes), up to 96 well-plates, compact design, small footprint; up to 30.000 g)

- **Clinical centrifuges**

(devices used for clinical applications like blood collection tubes, low-speed devices)

- **Multipurpose benchtop centrifuges**

(devices for a broad range of tube sizes, high variability, big footprint)

- **Stand alone centrifuges**

(heavy devices like ultra-centrifuges)

Many centrifuges are available with (regrigerated device)or without cooling function. There are different providers of laboratory centrifuges like Eppendorf, Thermo-Heraeus, Thermo-Sorvall, Hettich, Beckmann-Coulter or Sigma.

History

English military engineer Benjamin Robins (1707-1751) invented a whirling arm apparatus to determine drag. In 1864, Antonin Prandtl invented the first dairy centrifuge in order to separate cream from milk. In 1879, Gustaf de Laval demonstrated the first continuous centrifugal separator, making its commercial application feasible.

Different sizes of centrifuges were developed. The range of applications varied from Liter-scale to Milli-Liter-scale.

Regarding the laboratory microcentrifuge, in 1962 the Hamburg-based company “Netheler & Hinz Medizintechnik” (nowadays known as “eppendorf”) developed the “Microliter System” for laboratory usage. Besides the first piston stroke pipette, based on the work of Dr. Schnittger (Marburg/ Germany), the plastic-made micro test tube and the first microcentrifuge (model 3200) were introduced for applications in routine analysis labs in microliter scale. This first real microcentrifuge had one control knob for the time and space for up to 12 micro test tubes in a fixed-angle rotor. Common up-to-date features like cooling, programming, automatic imbalance detection, noise reduction, or changeable rotor systems were completely missing.

The “Microliter System” was the starting point for a broad range of tools for the molecular lab, developed by all different kinds of biotech and labware companies.

Design


A large laboratory centrifuge.

Laboratory centrifuges are used in chemistry, biology, and biochemistry for isolating and separating solids from liquids in a suspension. The solids can be insoluble compounds, biomolecules, cell organelles, or whole cells. They vary widely in speed and capacity. They usually comprise a rotor containing two, four, six, or many more numbered wells within which centrifuge tubes may be placed.

When a suspension in a centrifuge tube is centrifuged, the solids settle at the bottom of the centrifuge tube; having a tapered wall helps to concentrate the solids, making it easier to decant the supernatant solution, leaving the solids.

Generally spoken, there are two main types of rotors:

Fixed-angle rotor

The rotor (mainly made of aluminium) is very compact. There are boreholes with a specific angle (like 45°) within the rotor. These boreholes are used for the sample tubes.

Swing-out rotor (= horizontal rotor)

The rotor looks like a cross with gondolas, called buckets. Within these buckets, different tubes can be centrifuged. For a safe centrifugation, a specific adadpter for every tube shape is mandatory.

The rotor is closed by a rotor lid. The rotor is located in a rotor chamber which is covered by a metall centrifuge lid. The open lid prevents the motor from turning the rotor when the rotor chamber is open. During the run, the lid is locked. The lid protects the user from being injured by touching a rapidly spinning rotor. The rotor chamber and the lid of high quality centrifuges are robust enough to survive a rotor failure at full speed. This robustness protects the user and the laboratory from crashing fragments in case the rotor fails catastrophically. After a rotor crash, a centrifuge should not be reused as the enormous forces during a crash may have damaged essential parts of the device.

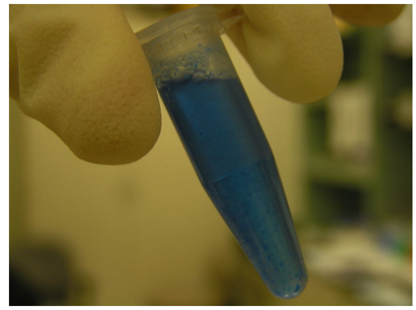
The rotor must be balanced by placing samples or blanks of equal mass opposite each other. Since most of the mass is derived from the solvent, it is usually sufficient to place blanks or other samples of equal volume. As a safety feature, some centrifuges may stop turning when wobbling is detected (automatic imbalance detection, see Safety).

Centrifuge tubes

Centrifuge tubes or **centrifuge tips** are tapered tubes of various sizes made of glass or plastic. They may vary in capacity from tens of millilitres, to much smaller capacities used in microcentrifuges used extensively in molecular biology laboratories. The most commonly encountered tubes are of about the size and shape of a normal test tube (~ 10 cm long). Microcentrifuges typically accommodate microcentrifuge tubes with capacities from 250 µl to 2.0 ml. These are exclusively made of plastic.

Glass centrifuge tubes can be used with most solvents, but tend to be more expensive. They can be cleaned like other laboratory glassware, and can be sterilized by autoclaving. Plastic centrifuge tubes, especially microcentrifuge tubes tend to be less expensive. Water is preferred when plastic centrifuge tubes are used. They are more difficult to clean thoroughly, and are usually inexpensive enough to be considered disposable.

Microcentrifuge tubes



Microcentrifuge tube with Coomassie Blue solution

Microcentrifuge tubes or **microfuge tubes** are small, cylindrical plastic containers with conical bottoms, typically with an integral snap cap. They are used in molecular biology and biochemistry to store and centrifuge small amounts of liquid. As they are inexpensive and considered disposable, they are used by many chemists and biologists as convenient sample vials in lieu of glass vials; this is particularly useful when there is only a small amount of liquid in the tube or when small amounts of other liquids are being added, because microcentrifugation can be used to collect the drops together at the bottom of the tube after pipetting or mixing.

Made of polypropylene,[1] they can be used in very low temperature (-80 °C to liquid nitrogen temperatures) or with organic solvents such as chloroform. They come in many different sizes, generally ranging from 250 μL to 2.0 mL. The most common size is 1.5 mL. Disinfection is possible (1 atm, 120 °C, 20 minutes) and is commoly performed in works related to DNA or microbes, where purity of the sample is of utmost importance. Due to their low cost and the difficulty in cleaning the plastic surface, they are usually discarded after each use.

Eppendorf tube has become a genericized trademark for *microfuge tubes* or *microcentrifuge tubes*. Eppendorf is a major manufacturer of this item, but is not the only one.

Three microcentrifuge tubes: 2 mL, 1.5 mL and 200 μL (for PCR).

Four screw-top microcentrifuge tubes.

Safety

The load in a laboratory centrifuge must be carefully balanced. Small differences in mass of the load can result in a large force imbalance when the rotor is at high speed. This force imbalance strains the spindle and may result in damage to centrifuge or personal injury. Some centrifuges have an automatic rotor imbalance detection. The control software immediately discontinue the run when imbalance appears.

Before starting a centrifuge, an accurate check of the rotor lockage as well as the lid lockage is mandatory. Centrifuge rotors should never be touched while moving, because a spinning rotor can cause serious injury. Modern centrifuges generally have features that prevent accidental contact with a moving rotor as the main lid is locked during the run.

Because of the kinetic energy stored in the rotor head during high speed rotation, those who have experienced the loss of a rotor inside of an ultracentrifuge compare the experience to having a bomb explode nearby.

When handling dangerous samples(like biohazard), the lid of the rotor needs to have a special gasket (aerosol-tight) to prevent contamination of the laboratory. The rotor can be loaded with the samples within a hood and the rotor lid is fixed on the rotor. Afterwards, the aerosol-tight system of rotor and lid is transferred to the centrifuge. Fixation of the rotor within the centrifuge is done without opening the rotor lid again. After the run, the rotor (incl. lid) is removed from the centrifuge to the hood (closed system) for further steps.

Theory

Protocols for centrifugation typically specify the amount of acceleration to be applied to the sample, rather than specifying a rotational speed such as revolutions per minute. The acceleration is often quoted in multiples of g, the acceleration due to gravity at the Earth's surface. This distinction is important because two rotors with different diameters running at the same rotational speed will subject samples to different accelerations.

The acceleration can be calculated as the product of the radius and the square of the angular velocity.

Relative centrifugal force is the measurement of the force applied to a sample within a centrifuge. This can be calculated from the speed (RPM) and the rotational radius (cm) using the following calculation.

$$g = \mathrm{RCF} = 0.00001118 \times r \times N^2$$

where:

g = Relative centrifuge force

r = rotational radius (centimetre, cm)

N = rotating speed (revolutions per minute, r/min)

To avoid having to perform a mathematical calculation every time, one can find nomograms for converting RCF to rpm for a rotor of a given radius. A ruler or other straight edge lined up with the radius on one scale, and the desired RCF on another scale, will point at the correct rpm on the third scale. Example [2] Based on automatic rotor recognition, up to date centrifuges have a button for automatic conversion from RCF to rpm and vice versa.

See also

- Centrifuge
- Centrifugation
- Gas centrifuge
- Separation
- Ultracentrifuge

External links

- RCF Calculator and Nomograph [3]
- Centrifugation Rotor Calculator [4]
- Selection of historical centrifuges [5] in the Virtual Laboratory of the Max Planck Institute for the History of Science

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[1] "Chemical Stability of Disposables" (http://www.eppendorfna.com/utilities/enewsletter.asp?ENLUID=e200606&REFUID=AP04) (pdf). *Applications Note 05*. Eppendorf. June 2005. .
[2] http://aquaticpath.umd.edu/nomogram.html
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[4] http://www.changbioscience.com/cell/rcf.html
[5] http://vlp.mpiwg-berlin.mpg.de/technology/search?-max=10&-title=1&-op_varioid=numerical&varioid=3

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3.3 Centrifuges and Electrical Motors

3.3.1 Use and Principles of Operation

If a liquid contains particles, the particles will eventually sink to the bottom under the force of gravity. A centrifuge more rapidly separates particles from liquid by rotating a liquid to simulate a higher force of gravity. Either a liquid/liquid or a liquid/solid mixture can be separated with the substance of higher density migrating towards the outer part of the centrifuge. Centrifuges vary in size, in speed of the rotation, how long they will run, temperature and angles of rotation of the samples.

A small, table-top, electric centrifuge is common in the developing world. However, smaller clinics may have only a hand-cranked centrifuge.

A centrifuge consists of a base and an inner spinning cylinder in which the substance to be separated is placed. Some centrifuges have timers that automatically turn off after a set period of time and some also have high precision speed regulators to control the speed with which the centrifuge spins. Centrifuges can be used to prepare a substance for analysis or to analyze the particle content. There are two types of preparative centrifuges: mechanical and electrical. Of the analytical centrifuges, the only one used in medicine is the microhematocrit, used for separating plasma from the blood suspension.

With the lid of this centrifuge tipped back, you can see the four tubes where the specimen or dummy tubes would be placed. When the rotor turns, the tubes will tip out at an angle. The small round dot just beyond the white interior (center bottom) is an interlock that prevents the unit from spinning if the lid is open

The simplest centrifuges have a single speed motor, a mechanical timer and a rotor that holds the samples at a preset angle of 20 to 40 degrees. For user safety, the lid of the centrifuge should have an interlock on it so that the unit will not spin with the lid in the up position.

A simple rotor is made from metal with 4, 6 or 8 holes drilled into it at an angle where the samples are placed. Balancing the rotor is very important. If the user has only a few samples to be spun down they may have to use "dummy tubes" to properly balance the load. Since the motor shaft is attached to the rotor, uneven loads can cause motor damage and uneven speeds. Another type of rotor has sample carriers that are vertical at rest but when spun move out to 20 to 40 degrees.

The simplest centrifuges have a single speed ranging from 2,500 to 10,000 RPM. Low speed centrifuges have RPM rates up to 12,000 RPM, high speed units go up to 35,000 RPM and the ultrahigh speed can reach 125,000 RPM. The simplest variable speed centrifuges will have a rheostat speed control, which may be non-linear. Most of the newer variable speed centrifuges have built in tachometers that provide the users with a speed indication. More sophisticated speed control systems can involve SCR's, stepper motors and servo systems.

Most high speed and all ultrahigh speed units are refrigerated because the friction caused by the air on the samples will dry them out and change the results.

Centrifuges have a timer that is either electronic or mechanical built into the controls. Depending upon the centrifuge, the time can be set from seconds to days. If no time is selected the centrifuge will probably not run. Also, the centrifuge may have a time delay on the start where it will not start to spin for several seconds after the RPM rate and timer are set and the start button pushed.

3.3.2 Common Problems

Any part of the centrifuge may cause a problem. However, not every part of the centrifuge can or should be repaired. After eliminating the timer, rotor and most of the rest of the machine, the only repairable part of the centrifuge which needs much further explanation is the motor.

If the timer is at fault, often the only practical solution in the field is to bypass the timer (so the centrifuge always turns when switched on), and instruct the staff to use manual timing. As personnel are generally plentiful in the developing world hospital, this solution is typically well accepted by the staff, especially if they have been attempting to operate without any centrifuge at all.

If the rotor is cracked or bent, it should not be repaired. There are tremendous forces developed in a centrifuge. If the rotor is weakened or off balance by being bent, the machine could be destroyed and the staff injured in the process.

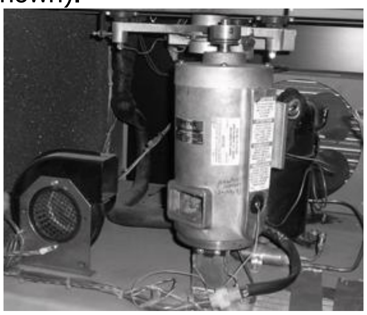
Mechanical centrifuges typically only need lubricating and cleaning to return them to use. If a piece is broken, it often cannot be repaired.

All centrifuges produced after 1990 are required to have an interlock system that does not allow the rotor to spin unless the cover is closed. Some of these interlock systems are very simple; a solenoid that pushes a rod through a hole in the cover latch is common. Others are more complicated and may involve several solenoids, flexible cables and a clock. The clock can be tied to the RPM indicator and will not release the solenoids until a set time has elapsed after the speed drops to zero. These timed units may give the appearance of failure because the operator cannot immediately open the lid. Check the manual, if available, to confirm both the delay and if that delay is adjustable.

There is always a temptation to defeat a broken interlock system. This should only be done for clinical laboratory departments that have no alternative centrifuge and then only after careful consultation with the technician who will be using the machine. Affix a picture on top of the machine showing a damaged finger and an open lid so that all future users will know about this danger.

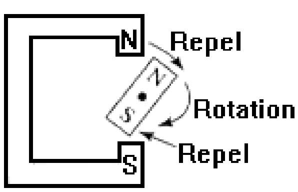
3.3.3 Motors

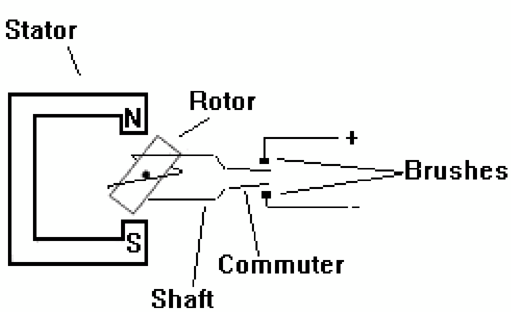
The inside of a simple centrifuge is nothing more than a motor and a few switches. This more sophisticated centrifuge includes two fans (additional motors) and a small bit of electronics (not shown).



The heart of the centrifuge is the motor. Almost all variable speed electrical motors in the developing world work on the same principle, whether they are in a centrifuge or any other piece of equipment (fixed speed motors, such as pumps and compressors are often of the induction type, not discussed here). The motor works by passing electrical current through electro-magnets attached to a rotating shaft. Stationary, permanent magnets attract or repel the electro-magnets depending on the orientation of the magnetic fields. The fields' orientations are switched such that the electro-magnets are progressively attracted to the permanent magnets around the circle, bringing the shaft of the motor around with them.

If a magnet is placed within another magnet, it can be made to rotate around a shaft by aligning the magnets to repel each other. If the polarity of the rotating magnet is then switched, the shaft and magnet will continue to rotate, and the device will be a motor. In order to switch the polarity, brushes contact commuters, the brushes and commuter forming switches. As the shaft rotates, the brushes contact different commuter parts, alternating the polarity of the rotating magnet.





In general, the engineer in the field will not be called upon to rebuild an electrical motor. Almost every major city in the developing world has a shop that can accomplish this task. However, most motors use carbon brushes to make electrical contact with the electro-magnets on the rotating part of the motor. These brushes wear down over time and need to be replaced. The brushes can be replaced by the field engineer.

Brushes should only be replaced with brushes of the same size. Do not use undersize brushes as they may wear unevenly and score the shaft of the motor. Brushes are held against the shaft via spring pressure, if the spring weakens, breaks or is missing the motor may not spin. If the caps holding the brushes in place become loose or cracked, that can also cause the brushes to lose contact and the motor will not run at all or will not run consistently.

Brushes that are installed properly and with the correct tension make the brushes wear evenly and have a bright almost shinny look on the contact end. If the brushes are defective or not making good contact the contact face of the brush will be dull and not smooth. Both brushes should be removed from the unit and compared when troubleshooting.

The shaft is held in the center of the permanent magnets by bearings. These are not often the cause of the problem, but in certain cases they can cause noise as the shaft rattles instead of being held in place. Bearings can be removed and replaced. Most developing world cities have motor repair shops which can replace or repair the bearings.

Besides the brushes and the motor bearings, many motor systems, including centrifuges, will have braking systems. If the rotor of a centrifuge, for example, was left to stop on its own, it could take a long period of time for the rotor to drop from 100,000 RPM to zero. To cut the time most units have a brake. The brake is not a mechanical device, as on your car. In some systems, the brake is a resistor that is temporarily placed across the motor. The motor is essentially operating as generator, with the mechanical energy coming from the spinning rotor and the electrical energy dumping into the resistance.

Motors are sometimes used in a 50 Hz country, despite being designed for 60 Hz use. In general, this causes few problems in centrifuges. In other applications, it can cause overheating. If possible lower the voltage about 10% on these motors to reduce heating.

In more sophisticated systems, the brake reverses the electrical field in the electro-magnets to make them attempt to spin the rotor in the opposite direction. The operator has to energize the switch and should only hold the switch in the reverse or stop position for a few seconds at a time.

3.3.4 Suggested Testing

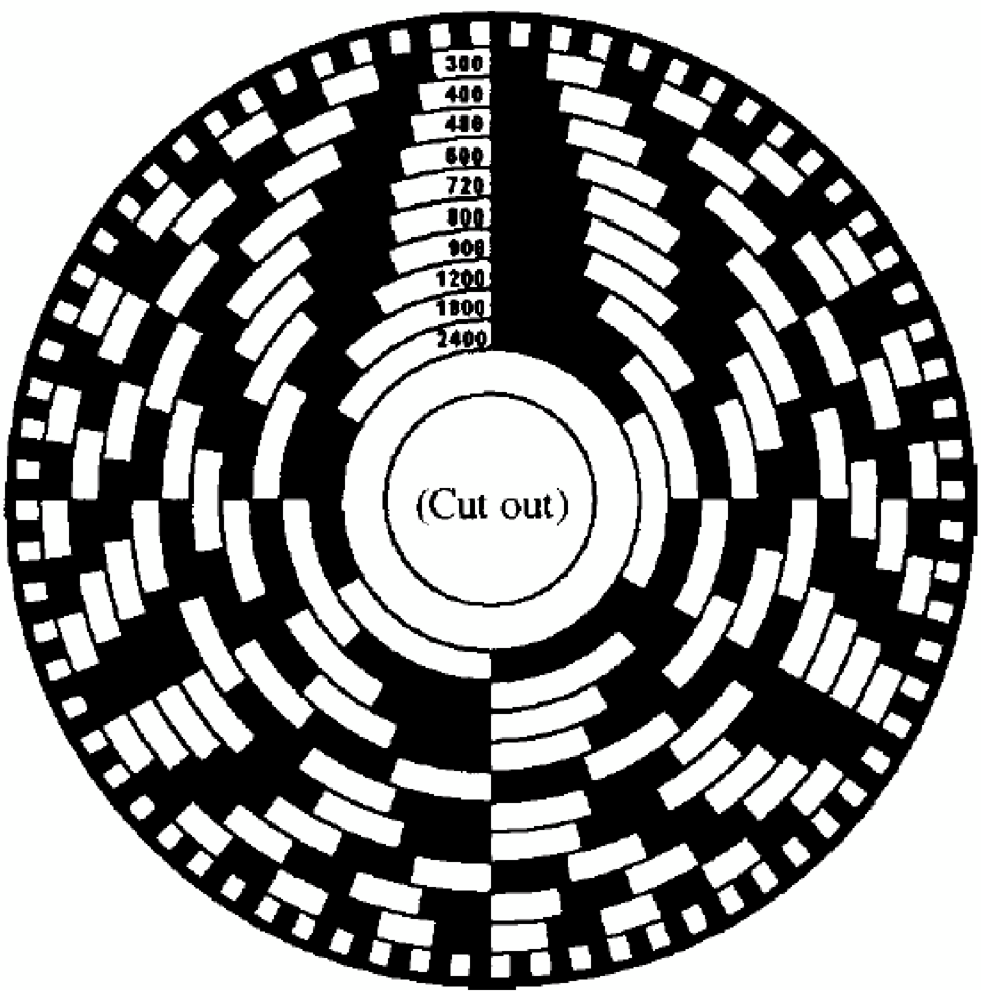
The centrifuge creates tremendous forces inside the vessel when in use. If the rotor were to break or become dislodged, it could damage the machine or injure the user. Therefore, you should perform some safety testing before releasing the device for use.

First, check that the lid cannot be opened when the rotor is turning. Never release a centrifuge which can be opened while the rotor is turning without a thorough discussion of the dangers with the staff. If this is the only centrifuge that the hospital has at its disposal, you may have to release the device for use without a safety interlock.

Second, you should insure that the device can spin up to speed and brake without excessive noise. Be sure to balance the rotor (with equal amounts of water-filled vials on each side) before turning it on. Check the rotor for cracks or bends before starting the centrifuge. Particular attention has to be paid to centrifuges that have rotors that can be changed out. The users have been known to not fully tighten down the knob securing the rotor to the motor shaft causing severe damage to the device and lab when the rotor broke loose while spinning.

The ideal test for a centrifuge is a tachometer used to verify the rpm. However, you can make an approximate measurement of the centrifuges speed without one. Under light from a fluorescent bulb that runs on 60 Hz. current, the gage shown below will give you an accurate reading when you are running at one of the speeds on the gage. The "flashing" of the fluorescent bulb at 120 Hz will cause one of the bands to appear to stop moving at the RPM indicated by that band. The gage will not work with an incandescent light bulb.

To use the gage, photocopy it, cut it out, and place it on the spindle. You may need to cover it in clear packing tape to make it stiffer. Spin up the centrifuge until one of the bands has stopped, mark that spot on the speed control knob. Count the bands from the inside to note which band has stopped. You can increase the speed and find the next time that this same band stops. This speed corresponds to twice the marked RPM. Likewise, you can find speeds which are three, four or more times what is marked by counting the number of times the bands stop as you increase the RPM's. To determine the RPM, stop the centrifuge, read the band, and multiply by the number of times it stopped as you were increasing the rotation. You'll need to try this a few times before getting consistent results.



2. Diagrams and Schematics of Centrifuges

Featured in this Section:

Malkin, Robert. *Medical Instrumentation in the Developing World*. Engineering World Health, 2006.

OpenStax College. "1901 Composition of Blood.jpg." *Wikimedia Commons*. Retrieved from: https://commons.wikimedia.org/wiki/File:1901_Composition_of_Blood.jpg

Figure 1: Centrifuge Components

given radius [r] and the force of gravity [g] is known as the *relative centrifugal field or [RCF]*[2].

$$RCF = \frac{r\omega^2}{g}$$

The RCF is the tool which allows rotors of different specifications to be compared when equivalent centrifugal effects are required.

COMPONENTS OF THE CENTRIFUGE

The most important components of a centrifuge are the following[3]:

The electric/electronic control which generally has the following elements:

1. On and off control, operation time control (timer), rotation speed control (in some centrifuges), temperature control (in refrigerated centrifuges), vibration control (safety mechanism) and brake system.

Sectional diagram of a centrifuge (numbers correspond to descriptions in the text above)

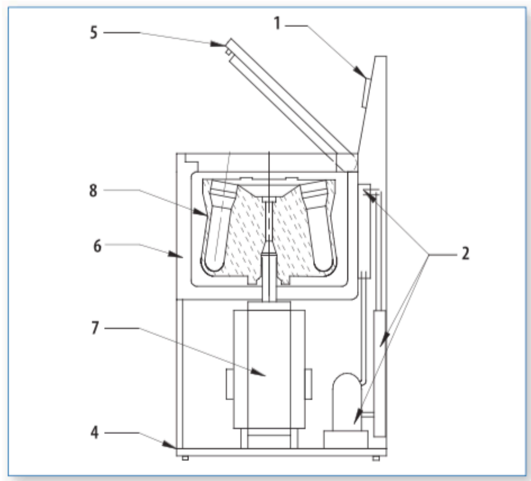
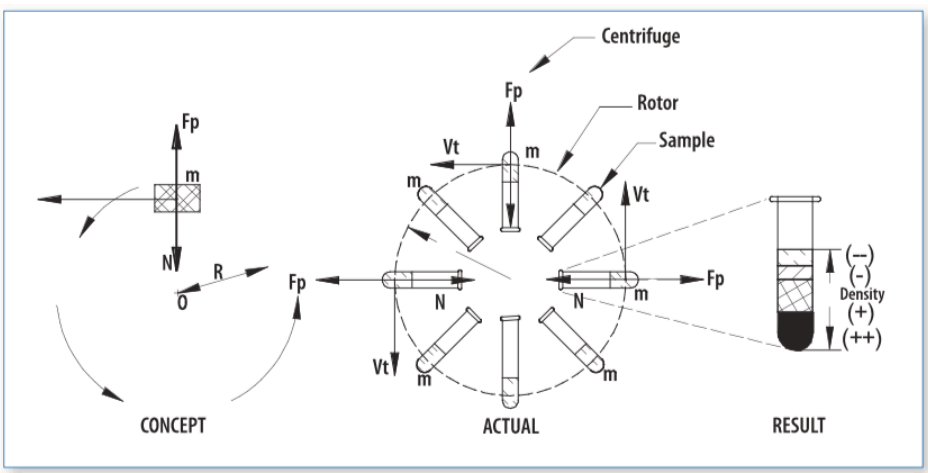


Figure 20. Centrifugal force concept



[1] Newton's law of movement, together with the explanation of the inertia marks of reference can be consulted in books on physics, chapters on uniform circular movement.
[2] RCF. Relative Centrifugal Field.
[3] The numbers identifying each component correspond to those in the the sectional diagram of the centrifuge.

WHO. "Chapter 7: Centrifuge." From the publication: Maintenance Manual for Laboratory Equipment, WHO: 2008, pgs. 45-49.

Figure 2: Centrifuge Rotors

Types of rotors

Centrifuges use many different types of rotors. Among the most commonly used are the following:

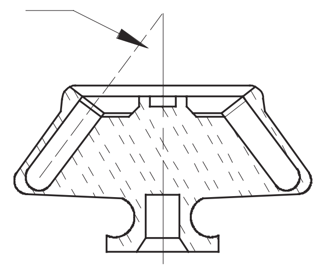
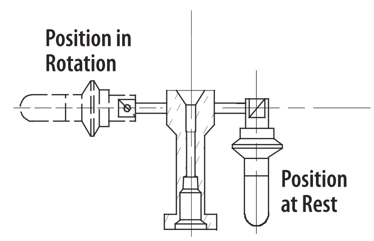
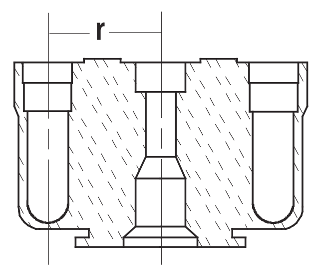
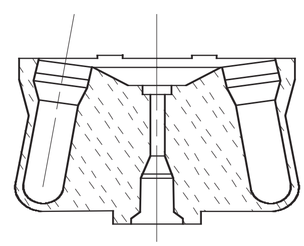
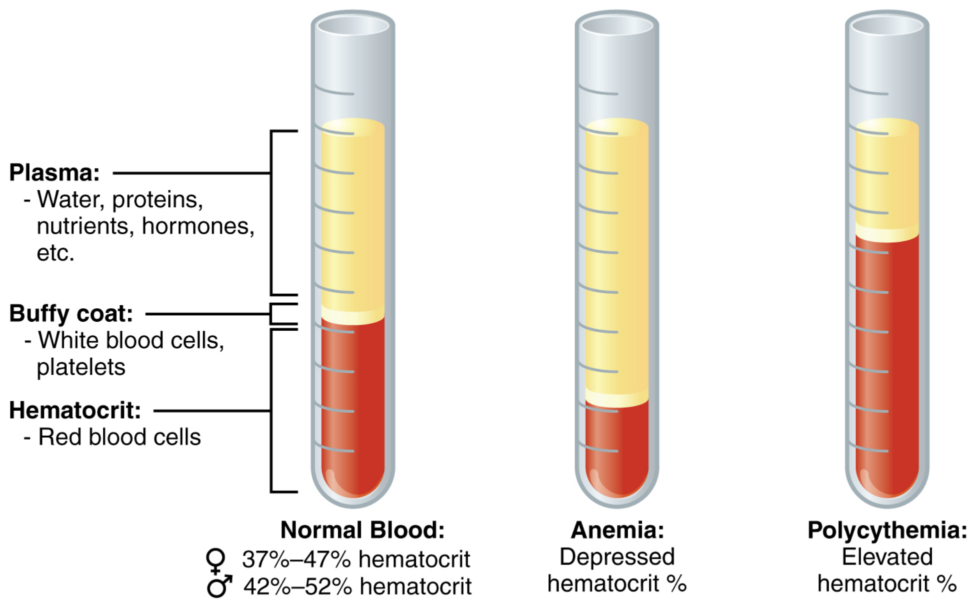
Type of rotor	Characteristics	Transversal cross-section
Fixed angle rotors.	These are general purpose rotors. They keep tubes at a fixed angle [α] which by design, is specified between 20 and 45 degrees. They are used for sediment sub-cellular particles. The angle shortens the trajectory of the particles and the centrifugation time compared to the swinging buckets rotors.	
Swinging buckets rotors.	These are used for carrying out isopycnic studies (separation by density) and rate-zonal studies (separation by sedimentation coefficient), where maximum resolution of the zones is required for the sample.	
Vertical tube rotors.	This type of rotor keeps tubes parallel to the rotational axis. Thus, separate bands are formed across the tube's diameter, not its length. These rotors are used for carrying out isopycnic studies and in some cases, zonal limit separations where a short centrifugation time is important. These rotors use specially designed tubes.	
Almost vertical tube rotors.	This type of rotor is designed for gradient centrifugation when some sample components do not participate in the gradient. The small angle of these rotors reduces the centrifugation time in comparison to fixed angle rotors.	

Figure 3: Composition of the Blood



OpenStax College. “1901 Composition of Blood.jpg.” Wikimedia Commons. Retrieved from: https://commons.wikimedia.org/wiki/File:1901_Composition_of_Blood.jpg

3. Preventative Maintenance and Safety of Centrifuges

Featured in this Section:

Cooper, Justin and Alex Dahinten for EWH. “Centrifuge Preventative Maintenance.” From the publication: *Medical Equipment Troubleshooting Flowchart Handbook*. Durham, NC: Engineering World Health, 2013.

Occupational Safety and Health Administration. “Laboratory Safety: Centrifuges.” *OSHA*. Last update: August, 2011. Retrieved from: https://www.osha.gov/Publications/laboratory/OSHAquickfacts-lab-safety-centrifuges.pdf

EQUIPMENT

Centrifuge Preventative Maintenance

Preventive Maintenance Checklist

1. Lubricate and clean motor.
2. Clean case.
3. Inspect power cords and plugs.
4. Inspect controls and switches.
5. Insure appropriate menu settings for proper use.
6. Insure tightness of rotor.
7. Check lights and indicators.
8. Verify that alarms are operating properly.
9. Insure interlock is functioning.
10. If refrigerated, insure temperature reading is working.
11. Replace/repair gaskets, seals, and vacuum pump (if applicable).

Cooper, Justin and Alex Dahinten for EWH. "Centrifuge Preventative Maintenance." From the publication: Medical Equipment Troubleshooting Flowchart Handbook. Durham, NC: Engineering World Health, 2013.

OSHA® QuickFacts

Laboratory Safety
Centrifuges

Centrifuges, which operate at high speed, have great potential for injuring users if not operated properly. Unbalanced centrifuge rotors can result in injury or death. Sample container breakage can release aerosols that are harmful if inhaled.

The majority of all centrifuge accidents result from user error. To avoid injury, workers should follow the manufacturer's operating instructions for each make and model of centrifuge that they use.

Follow these steps for the safe operation of centrifuges:

- Ensure that centrifuge bowls and tubes are dry.
- Ensure that the spindle is clean.
- Use matched sets of tubes, buckets and other equipment.
- Always use safety centrifuge cups to contain potential spills and prevent aerosols.
- Inspect tubes or containers for cracks or flaws before using them.
- Avoid overfilling tubes or other containers (e.g., in fixed angle rotors, centrifugal force may drive the solution up the side of the tube or container wall).
- Ensure that the rotor is properly seated on the drive shaft.
- Make sure that tubes or containers are properly balanced in the rotor.
- Only check O-rings on the rotor if you are properly trained.
- Apply vacuum grease in accord with the manufacturer's guidelines.
- Do not exceed the rotor's maximum run speed.
- Close the centrifuge lid during operation.

continued on page 2

The majority of all centrifuge accidents result from user error.

For assistance, contact us. We can help. It's confidential.

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OSHA 3406 8/2011
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Occupational Safety and Health Administration. "Laboratory Safety: Centrifuges." OSHA. Last update: August, 2011. Retrieved from: https://www.osha.gov/Publications/laboratory/OSHAquickfacts-lab-safety-centrifuges.pdf

OSHA® QuickFacts

Laboratory Safety
Centrifuges

continued from page 1

- Make sure that the centrifuge is operating normally before leaving the area.
- Make sure that the rotor has come to a complete stop before opening the lid.

When centrifuging infectious materials, wait 10 minutes after the rotor comes to a complete stop before opening the lid. If a spill occurs, use appropriate decontamination and cleanup procedures for the spilled materials. Report all accidents to your supervisor immediately.

When centrifuging infectious materials, wait 10 minutes after the rotor comes to a complete stop before opening the lid.

For assistance, contact us. We can help. It's confidential.

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Occupational Safety and Health Administration. "Laboratory Safety: Centrifuges." OSHA. Last update: August, 2011. Retrieved from: https://www.osha.gov/Publications/laboratory/OSHAquickfacts-lab-safety-centrifuges.pdf

4. Troubleshooting and Repair of Centrifuges

Featured in this Section:

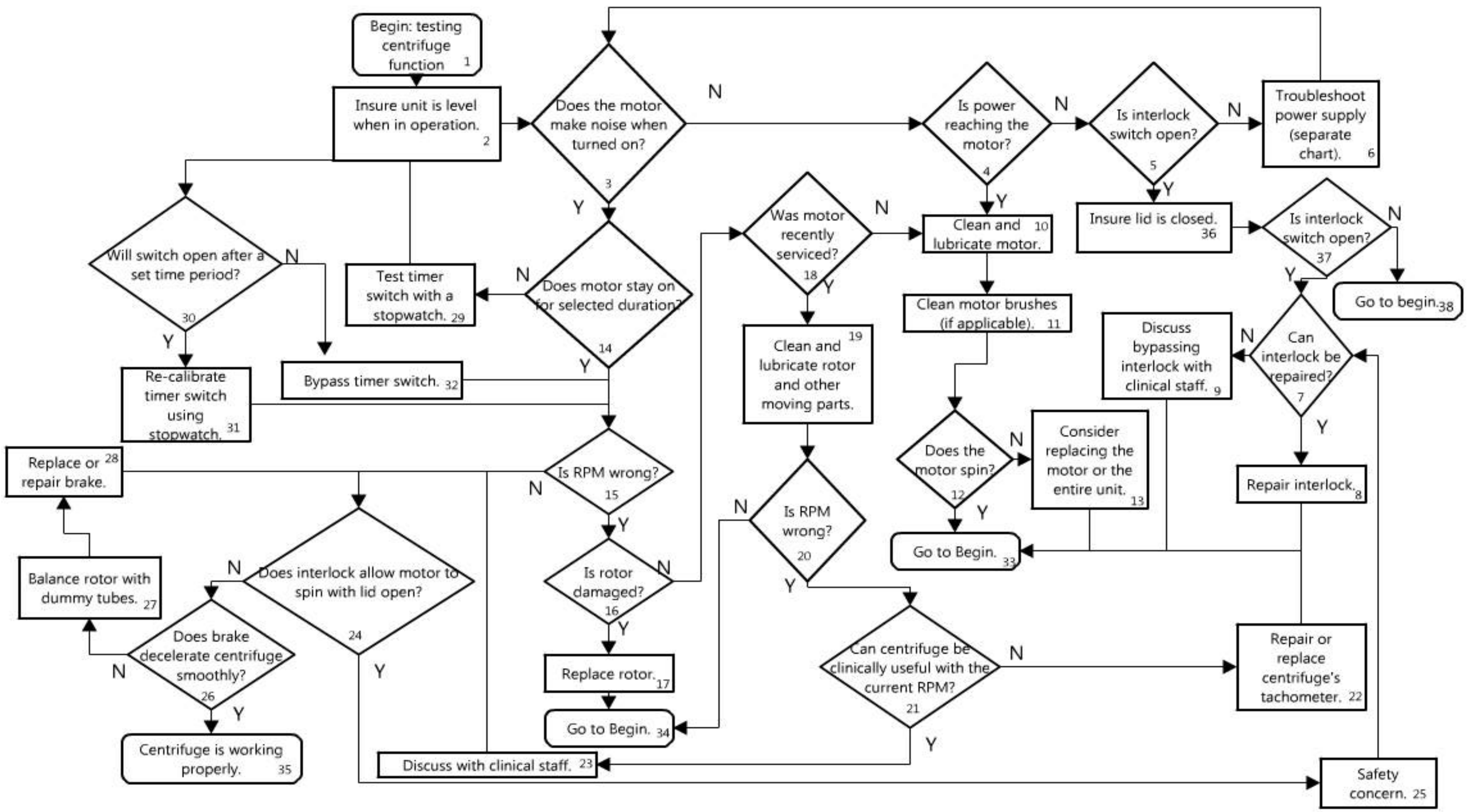
Cooper, Justin and Alex Dahinten for EWH. “Autoclave Troubleshooting Flowchart.” From the publication: *Medical Equipment Troubleshooting Flowchart Handbook*. Durham, NC: Engineering World Health, 2013.

Malkin, Robert. *Medical Instrumentation in the Developing World*. Engineering World Health, 2006.

Centrifuge Troubleshooting Flowchart

Centrifuge Troubleshooting

Diagnostic flowchart



Cooper, Justin and Alex Dahinten for EWH. "Centrifuge Troubleshooting Flowchart." From the publication: Medical Equipment Troubleshooting Flowchart Handbook. Durham, NC: Engineering World Health, 2013.

Centrifuge Troubleshooting Table

TROUBLESHOOTING TABLE		
Rotors[1]		
PROBLEM	**PROBABLE CAUSE**	**SOLUTION**
Severe vibration.	The rotor is unbalanced.	Balance the rotor's load. Fill all the opposite tubes with the same level of liquid of same density.
		Distribute the weight of the opposite tubes symmetrically.
		Load fixed angle or vertical tube rotors symmetrically.
	The speed selected is near the rotor's critical speed range.	Select a rotation outside of the critical speed range.
	The rotor is incorrectly mounted.	Verify the rotor's assembly. Test that it is well adjusted.
	There is a lack of lubrication in the rotor's supports.	Lubricate the pivoting axis according to the manufacturer's recommendation. For e.g. each 250 centrifugation procedures.
Rotor covers, canister or cubes difficult to loosen after centrifugation.	A vacuum is being produced during centrifugation.	Open the ventilation line in the upper part of the rotor or bucket to eliminate the vacuum.
	The rings are contaminated with filth, dried lubricants or metallic particles.	Perform routine cleaning of the rings and lubricate. Use recommended products recommended by the manufacturers.

[1] *Rotors and Tubes for Beckman Coulter J2, J6 and Avanti® J series centrifuges, User's Manual,* Palo Alto, California, The Spinco Business Center of Beckman Coulter, 2001.

WHO. "Chapter 7: Centrifuge." From the publication: Maintenance Manual for Laboratory Equipment, WHO: 2008, pgs. 45-49.

Centrifuge Troubleshooting Table

Tubes

PROBLEM	PROBABLE CAUSE	SOLUTION
The tubes leak.	The covers are badly secured.	Adjust the covers.
	The tubes are too full.	The meniscus must be lower in order to prevent leaks.
	The maximum recommended level has been exceeded in the open tubes.	Verify the volume and speed recommendations for the centrifugation.
	A deficient seal is presumed in the rapid seal tubes.	Press lightly, after heat sealing (only if the contents are not affected). If leaks are visible, seal again.
The tubes are cracked or broken.	The tubes can be broken or become fragile if they are used below the recommended temperature.	If the sample is frozen, warm to 2 °C before centrifuging. Evaluate how the tubes behave at low temperatures before centrifuging.
	The tubes become fragile with age and use.	Discard expired tubes, use new ones.

Various systems

PROBLEM	PROBABLE CAUSE	SOLUTION
The main switch is in the on position but the centrifuge is not functioning.	There is no power to the instrument.	Verify the power supply.
The centrifugue cover cannot be opened.	The centrifuge is off.	Turn the centrifuge ON. Press the handle and open the cover.
The balance indicator is activated.	The load to be centrifuged is unbalanced.	Balance the load to centrifuge.
	The centrifuge is not levelled.	Level the centrifuge.
There is a vibration at low speed.	The rotor adjustment mechanism is slack.	Correctly adjust the fastening system.
	The load is unbalanced.	Verify the balance of the load to be centrifuged.
	The selected speed is close to the rotor's resonance point.	Select a more elevated rotation speed or use a different type of rotor.
There are fluctuations in the rotation speed.	The transmission belts are in a bad condition (*).	Turn off the centrifuge. Verify the condition and state of the belts. The belts must be tempered.
The rotation speed does not reach the selected speed.	The brushes are defective.	Turn off the centrifuge. Verify the condition of the brushes. If this is the problem, put new brushes with the same specifications as the originals.
	The speed control calibration is maladjusted.	Adjust the speed control calibration.
The chamber is cold but the rotor is warm.	The temperature is incorrectly selected.	Verify the temperature selection.
The display which signals the state of the brushes is on.	The brushes are in a bad condition.	Turn off the centrifuge. Verify the condition of the brushes. Substitute the brushes by others with the same specification.

(*) Valid procedure in centrifuges with potential belt transmission system.

WHO. "Chapter 7: Centrifuge." From the publication: Maintenance Manual for Laboratory Equipment, WHO: 2008, pgs. 45-49.

5. Resources for More Information about Centrifuges

Featured in this Section:

Lab Tricks, "How to Use a Centrifuge Safety." Posted January 18, 2010. Retrieved from: https://www.youtube.com/watch?v=IhJNFGfsUus

WHO. "Centrifuges: Basic Principles." *Maintenance and Repair of Laboratory, Diagnostic Imaging, and Hospital Equipment* (WHO: 1996).

Resources for More Information:

Internal Resources at library.ewh.org: For more information about maintenance and repair of laboratory centrifuges please see these resources in the BMET Library!

1. WHO. "Centrifuges: Basic Principles." *Maintenance and Repair of Laboratory, Diagnostic Imaging, and Hospital Equipment* (WHO: 1996).

External Resources:

2. **Centrifuge Operation and Common Mistakes:** This video demonstrates the proper usage of a centrifuge, while also highlighting common mistakes. The four major points of this video are: 1. Use the right tubes 2. Balance the tubes 3. Secure the rotor lid 4. Check centrifuge settings. **Lab Tricks, "How to Use a Centrifuge Safety." Posted January 18, 2010. Retrieved from: https://www.youtube.com/watch?v=IhJNFGfsUus**

Centrifuge Bibliography:

Cooper, Justin and Alex Dahinten for EWH. "Centrifuge Preventative Maintenance." From the publication: *Medical Equipment Troubleshooting Flowchart Handbook*. Durham, NC: Engineering World Health, 2013.

Cooper, Justin and Alex Dahinten for EWH. "Centrifuge Troubleshooting Flowchart." From the publication: *Medical Equipment Troubleshooting Flowchart Handbook*. Durham, NC: Engineering World Health, 2013.

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