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GUIDELINE FOR CENTRIFUGE USE IN MEDICAL LABORATORIES



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1. Introduction

Centrifuges are devices developed with the purpose of analysis and centrifugation is constitutively a method of isolation. Along with that, presently it is a pre-treatment device especially to prepare the serum, plasma and urine samples to analysis, used in medical laboratories. Centrifugation is one of the most important stages of the preanalytical phase, and recognising effective variables before, during and after centrifugation is necessary to prevent preanalytical mistakes. It is one of the responsibilities of the laboratory management to create and practice instructions related to the subject.

Centrifuges should be well recognised by the laboratory workers and preanalytical impacts of centrifugation should be known well. Related information can be found dispersedly in guidelines issued by international organisations such as the World Health Organisation (WHO) and Clinical Laboratory Standards Institute (CLSI), in user manuals of the manufacturers as well as in research studies focused on special subjects. For these documents are authored in foreign languages, their national practice is limited. This document has been prepared with the purpose of creating useful, practicable guidelines for medical laboratory workers and researchers.

2. Brief History of Centrifuge

The first known centrifuge in the history is a mechanism developed to measure gunpowder force by Benjamin Robins (1707-1751) who is a military engineer. Antonin (1842-1909) and Alexander (1840-1896) Prandtl brothers designed an apparatus capable of separating the fat from the milk using rotational motion (1). Thereafter, this apparatus was improved and commercially launched by Gustaf De Laval (1845-1913).



The first one who discovered the analytical power of the centrifuge was Friedrich Miescher (1844-1895). Miescher managed to separate cellular organelles and nucleic acids through using centrifuge and to be the milestone on the way to the discovery of the DNA. Theodor Svedberg (1884-1971) conducted studies concerning protein purification by developing an analytical ultracentrifugation technique. The eponym of the svedberg unit (S/Sv) which is used in the denomination of ribosomes was Theodor Svedberg.



In the years of 1940, the need for centrifugation gradually increased along with the expansion of routine laboratory tests. In 1949, Spinco corporation developed centrifuges which could rotate 40.000 times per minute, and was purchase by Beckman Coulter in 1950. In 1962, Eppendorf corporation launched the first microcentrifuge and the capped law volume sample tubes developed by the corporation are known as “godet Eppendorf” and have still been used presently. The first corporation which applied advances in computers to the centrifuges was Hettich corporation. In 1976, they developed the first microprocessor centrifuges and thus the first programmable centrifuges were seen in medical laboratories (2,3).

3. General Information

3.1. Concepts and Terminology

Centrifugation: It is basically a separation method. Particles within the sample are separated by their shape, dimensions and density using the centrifugal force obtained from the rotational motion (4). (Figure 1)

Centrifuge: The name of the device carrying out centrifugation procedure (4).

Residue, Sediment, Pellet, Precipitate: The part of the sample precipitated in the bottom of the tube following separation. Cells, leukocytes and platelets found in the blood sample are gathered in this part in such a manner that it remains on the top of the precipitate.

Supernate, Supernatant: Part composed of serum or plasma which is supernate because of centrifugation.

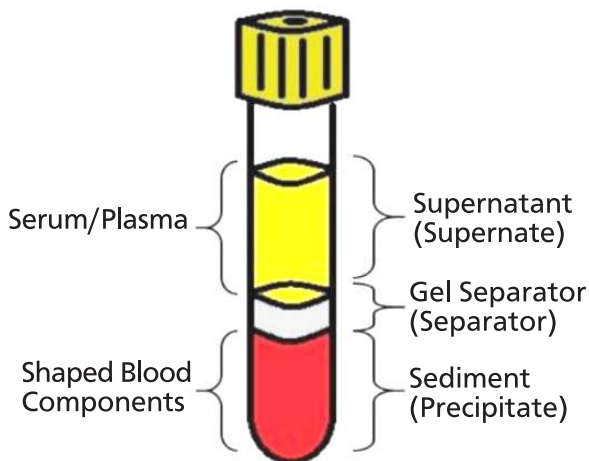


Figure 1. Serum/plasma and shaped blood constituents separated in the tube following centrifugation

RPM (Revolution / Rotation / Rounds / Rate Per Minute): Number of rotations of the centrifuge per minute and indicates the rate of centrifugation (4,5).

RCF (Relative Centrifugal Force/Field) or Gravity (g): Physical impact separating the sample placed into the centrifuge into its components. RCF is composed of the change in the direction of centrifugal force affecting the rotating particle and expressed as the multiples of gravitational acceleration (xg) (4,5).

3.2. Parts of the Centrifuge

Centrifuge is basically an electric engine. It is composed of two fundamental parts: stationary (stator) and the motile (rotor) (6,7) (Figure 2).

Engine: The device converting electrical energy to mechanical energy and producing rotational motion.

Rotor: The part of the centrifuge which is pivoting. It transfers the rotational motion produced by the engine.

Bucket (Basket): The part where the tubes and tube holders are placed.

Tube Holder: The part that ensures the tubes to stand steady and motionlessly and is placed in the basket.

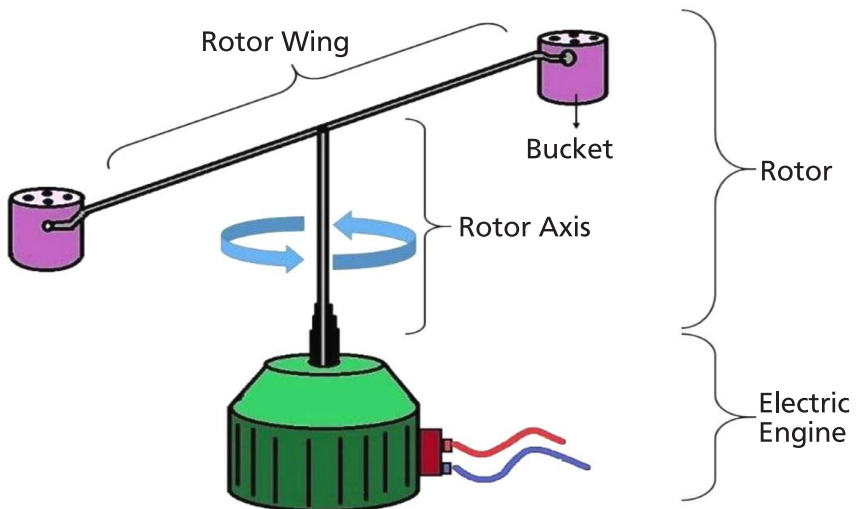


Figure 2. Basic parts of the centrifuge

Control Panel: Centrifuges bear a control panel to be programmed or controlled while operating. The buttons on the control panel are usually the buttons for programming, accelerating, braking, starting and emergency stop. By following centrifuge instructions, variables such as RCF/RPM, time, temperature, acceleration and brake can be adjusted and these values can be monitored on the screen which is found on the panel while the device is operating. (Figure 3)

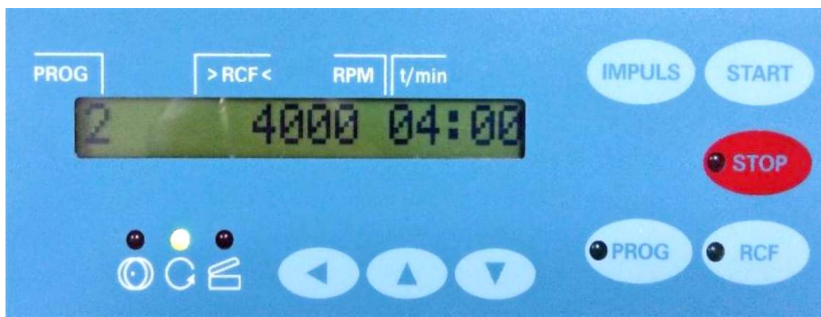


Figure 3. Centrifuge Control Panel (illustration)

3.3. Classification of Centrifuges

Centrifuges can be classified differently according to their usage, speed and technical specifications. Usage areas of centrifuges can be categorised under subtitles as in the following (4):

- Separating shaped components of blood to obtain serum/plasma,
- Precipitating cells and other components in urine and other body fluids for microscopic and chemical investigations,
- Removing the precipitates that have the potential of interference,
- Separating antibodies and antibody bounded structures for immunochemical analysis,
- Extracting solvents,
- Separating lipid component (chylomicrons, lipoproteins) from serum.

3.3.1. Centrifuges by usage

In general, they are classified in two groups (8):

1. **Preparative Centrifuges:** Preparative centrifuges are divided into two groups as differential centrifuges and density gradient centrifuges. While differential centrifuges are rather used for separating particles, density gradient centrifuges are used to separate two different fluids.
2. **Analytical Centrifuges:** They are rather from the group of ultracentrifuges. They can qualify or quantify separated components in the sample by combining other measurements methods such as refractometry, fluorometry.

Centrifuges may also be named due to their technical specifications. **Fixed angle centrifuges** are rather preferred for preparing urine samples in routine works. **Swinging-bucket centrifuges** are preferred for preparing blood and urine samples in routine works. **Temperature controlled centrifuges** are the devices where internal temperature of the centrifuge can be controlled during processing. **Microcentrifuges** are designed to centrifuge low volume samples (1-2 mL) and run relatively faster (10000 - 15000 x g).

3.3.2. Centrifuges by Speed

- a. **Very low speed centrifuges** (RCF <4000 x g): Counter top centrifuges used in sample preparation in medical laboratories are from this group.
- b. **Low speed centrifuges** (RCF <5000 - 10000 x g): Centrifuges used in the preparation of erythrocyte suspension, fresh frozen plasma at blood banking is from this group.
- c. **High speed centrifuges** (RCF 10000 - 50000 x g): Centrifuges used in DNA, RNA studies are from this group.
- d. **Ultracentrifuges** (RCF 100 thousand – 1 million x g): Ultracentrifuges are the centrifuges with very high speed spinning numbers and used for analytical purposes. For example, lipoprotein analysis can be conducted using ultracentrifuge.

3.3.3. Centrifuges by rotor type

Rotor type of the centrifuge specifies the location of the rotating/spinning tubes. According to this, centrifuges can be classified as swinging-bucket (horizontal angled), fixed-angled and right-angled. In routine laboratory practice, mostly swinging-bucket and fixed-angled ones are used.

In swinging-bucket centrifuges, tubes become horizontal under the influence of centrifugal force while the rotor is running (becomes perpendicular to the rotational axis), and as the centrifuge stops it returns to the vertical position. Sediment surface is smooth, parallel to the rotational axis and well packed.

In fixed-angled rotors, tubes rotate with a 25-40 degrees fixed angle to the perpendicular rotational axis. Therefore, sediment (pellet) precipitates towards the side and bottom of the tube under the influence of centrifugal force. The sediment formed is not well packed as in the swinging-bucket centrifuges. Overall, fixed-angled rotors can reach to higher centrifugal forces than swinging-bucket centrifuges. For the centrifugal force has effects on tubes from different directions, location of layers separated varies inside the tube (9). (Figure 4)

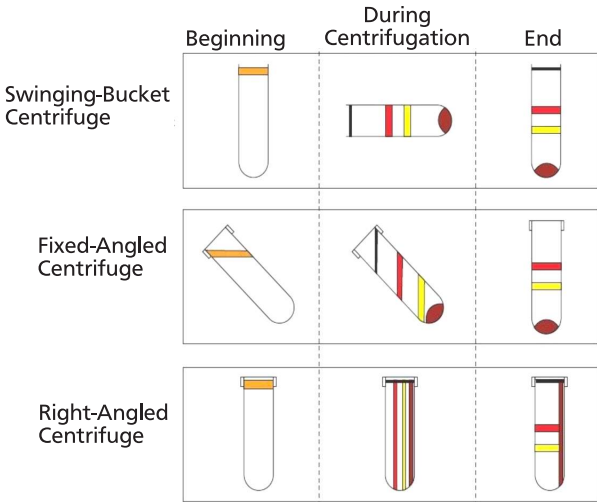


Figure 4. Shapes of delamination of the samples centrifuged by rotor type (9).

3.4. Running Principle of Centrifuge

Gravitational force of the earth can separate blood into its components. Erythrocyte sedimentation rate is an analytical application of this fact. However, as the samples are desired to be separated faster and into further sub-components, centrifugation is needed. Separation speed of the components in the sample depends on the shape, size, density and viscosity of the milieu. Denser and bigger particles precipitate faster (9). (Figure 5)

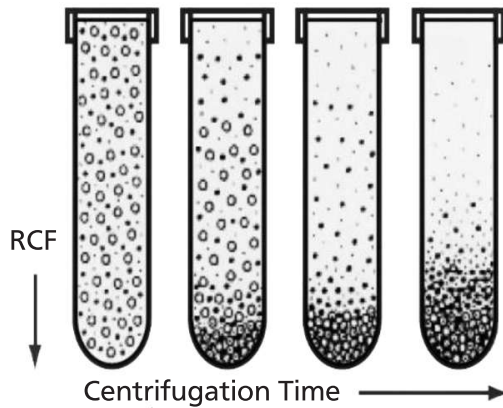


Figure 5. Separation and precipitation of particles in the tube during centrifugation (9).

Thrust created by rotational motion acts on the particles radially from the centre to the particles in the sample and it is known as RCF (Figure 6). RCF is indicated as the multiples of gravitational acceleration (xg).

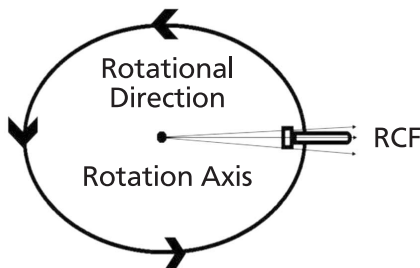


Figure 6. Rotational motion and RCF formation

RCF and RPM are the concepts that are often used instead of each other by mistake. It is important to communicate between laboratories using the same criterion and standards and the same expressions. For radii of different centrifuges vary, RCF values they operate even if they have the same RPM values; comparing with their RPM values causes repeatability problems regarding preanalytical procedures. RCF provides the same acceleration to be obtained in centrifuges with different radii. In Figure 7, changes in RCF value in centrifuges with different radii in the same RPM values can be seen.

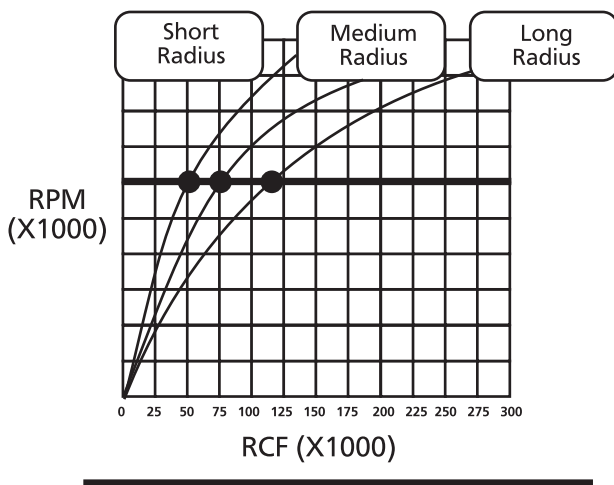


Figure 7. Change in RPM and RCF value in different radii

3.4.1. Where the centrifuge radius should be measured from?

For a correct calculation of RCF, radius value of the centrifuge should be measured properly. Radius values informed by the manufacturer in user manuals can be used while calculating the RCF value. If this value is not known, also the user can measure the radius. The distance between the centre and the bottom of the tube is considered as the radius of the centrifuge. How to measure the radius of fixed-angled and swinging-bucket centrifuges is shown in Figure 8 and Figure 9 (10).

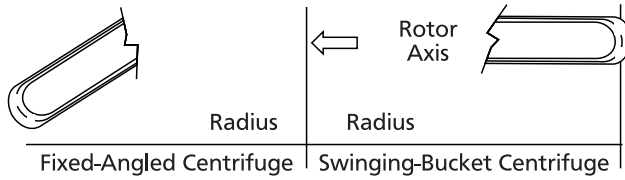


Figure 8. Measuring the radius of the centrifuge (8).

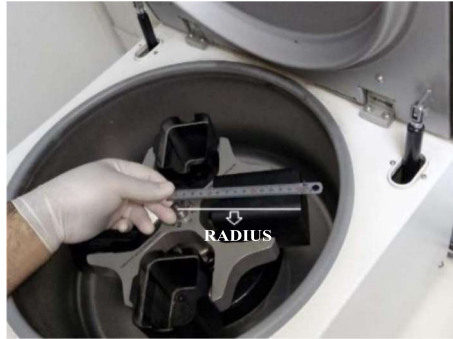


Figure 9. Measuring the radius of the centrifuge

3.4.2. Physical Basics of Centrifuge

The motion of the particle within the rotating tube in the centrifuge keeps up with regular circular movement and can be explained by Newton physics. Angular velocity (ω) is the rate of change of angular displacement of a spinning particle in time. In other words, the angle tracked in unit time by the radius vector which connects the particle to the origin is called angular velocity. As the radius vector connecting a particle which is rotating to the origin completes a full spin, it tracks 2π radian angle and meanwhile about a period of time (T) passes.

$$\text{Velocity} = \frac{\text{Distance}}{\text{zaman}}$$

$$\omega = \frac{2\pi}{T}$$

Frequency (f) is the winding number per one second of the particle which has regular circular motion and expressed in Hertz. There is a $f = 1/T$ relation between period and frequency. RPM is the expression of frequency in minutes.

It can be shown as $\text{RPM} = 60/f$. Angular velocity can be formulated according to the RPM as in the following.

$$\omega = \frac{(2\pi \times \text{RPM})}{60}$$

Acceleration (a) is the derivative of velocity by time. Acceleration is manifested as any change occurs in the speed or direction of a particle moving.

$$\text{Acceleration (a)} = \frac{\text{Velocity}(\Delta V)}{\text{Time}(\Delta t)}$$

Linear velocity of the particle that has a regular circular motion is constant, but any change in its direction causes gaining acceleration. Angular acceleration formula is as in the following:

$$\text{Angular acceleration (a)} = \omega^2 \times r$$

RCF is the rate of centripetal force acting on circulating particle to gravitational force and is shown with multiples of gravitational acceleration (**x g** (980.665 cm/s²)). RCF formula is obtained as in the following:

$$\text{RCF} = \frac{F \text{ centrifuge}}{F \text{ gravity}}$$

$$\text{RCF} = \frac{m \times a}{m \times g}$$

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

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

If angular velocity is shown clearly and the formula is rearranged, RCF formula is as in the following:

$$\text{RCF} = \frac{\left(\left(\frac{2\pi \times \text{RPM}}{60}\right)^2 \times r\right)}{g}$$

$$\text{RCF} = \frac{\left(\frac{4\pi^2 \times (\text{RPM})^2}{3600}\right) \times r}{g}$$

When constant values included in the formula:

$$RCF = \left(\frac{4 \times (3.14)^2 \times (RPM)^2}{3600 \times 980} \right) \times r$$

The formula can be shown simpler by multiplying constant values by each other.

$$RCF = 1.118 \times 10^{-5} \times r \times (RPM)^2$$

Therefore the relation between RCF and RPM is demonstrated by the formula. The value 1.118×10^{-5} is not an empirical constant value. It is a value obtained from the formula.

To give an example of converting from RPM to RCF for the following conditions;

Centrifuge rotor radius = 14 cm

Recommended RPM = 3100 for the used tube.

$$RCF = \left(\frac{4 \times (3.14)^2 \times (3100)^2}{3600 \times 980} \right) \times 14$$

It becomes $RCF = 1504 = 1500 \times g$.

And the formula while converting to RPM from RCF is:

$$RPM = \sqrt{\frac{RCF \times 10^5}{1.12 \times r^2}}$$

An example of conversion from RCF to RPM is as in the following,

Rotor diameter is: 14 cm

Recommended RPM = 1500 x g for the used tube.

Thus RPM is;

$$\text{RPM} = \sqrt{\frac{1500 \times 10^5}{1.12 \times 14^2}}$$

= 3093 rpm, 3000 or 3100 rpm can be used.

Calculated RCF value means maximal RCF. However, the same RCF value is not used for the whole content of the tube. Minimal RCF is the calculated RCF value as the radius is taken as the distance between the rotor axis and the surface of the fluid inside the tube. There may be significant differences between maximal and minimal RCF values. If so, it should be kept in mind that calculations can be done over the radius in average. Radius in average is measured as the distance between the mid-height of the fluid inside the tube from the bottom and the axis of the rotor. On the other hand, even if the RCF value is the same, for the swinging-bucket rotors, higher centrifuge force is applied to the tube compared to the force applied to the fixed-angled centrifuges.

3.5. Converting RCF and RPM to Each Other

RCF and RPM values can be converted to each other through the formula as well as by using nomograms (Figure 10) (11). In the web, there are calculators that can perform this conversion easily (12).

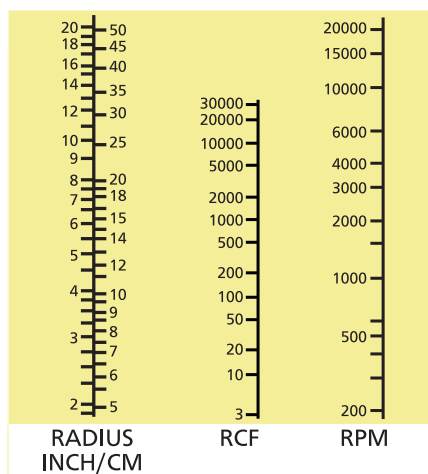


Figure 10. Nomogram providing to reach the RCF value by using centrifuge radius and RPM values (11).

4. Points to Consider Before Centrifugation

Centrifugation is one of the substantial stages of pre-analytical period and the rules to be obeyed should be taken into consideration to perform centrifugation in compliance with the method (13). For a proper and healthy centrifugation, blood should be taken, transferred to the laboratory and placed into the centrifuge properly.

4.1. Sample Staying Time Prior to Centrifugation

For obtaining plasma, samples with EDTA, heparin, fluoride or citrate can be centrifuged without waiting.

To obtain serum, considering the type of the tube and the time recommended by the manufacturer, the sample should wait;

- at room temperature and
- until the clotting is completed



If there is no time recommended by the manufacturer, serum samples must wait for at least 30 minutes before centrifugation (10, 14). Waiting time should not exceed 1 hour.

4.2. Delayed (Residual) Clot Formation

Delayed clot formation causes residual clot within the serum/plasma and is one of the significant preanalytical mistake resources. Residual clots included in the sample may cause to obstructions in the probes and tubing of the analyser and interferences during measurements; for example, it has been reported that it may cause to false positive results in troponin I/T measurements (15).

Circumstances leading to delayed intratubular coagulation are:

- Holding the sample in refrigerator,
- Receiving anticoagulant treatment by the patient from whom the sample has been collected,
- Holding the sample shorter than the recommended time.

Removing residual clot from the tube with a tool such as wooden stick etc. is a malpractice observed in laboratories. Even if residual clot is removed from serum/plasma, small particles may remain included in the tube. In addition, such a practice is reported to cause adverse events such as haemolysis. It is a choice to re-centrifuge the samples which have residual clots. And the best practice will be to refuse the sample and get a new blood collection (10).

4.3. Tubes Shortening Intratubular Clotting Time and Their Use

Intratubular clotting time is a significant factor that prolongs the test cycle in the laboratory. With the purpose to shorten this time, tubes including “clot activator” or “clot accelerator” has been brought into use (Figure 11) (16). Pre-centrifugation sample waiting time varies according to the type of the clot accelerator tube. While using these tubes, recommendations of the manufacturer should be considered or the tubes should be held for durations mentioned as in the following:

- Serum tubes without gel: 60 minutes
- Glass or silica coated tubes: 15-30 minutes
- Tubes with thrombin additive: 5 minutes
- Tubes with venom additive: 2 minutes

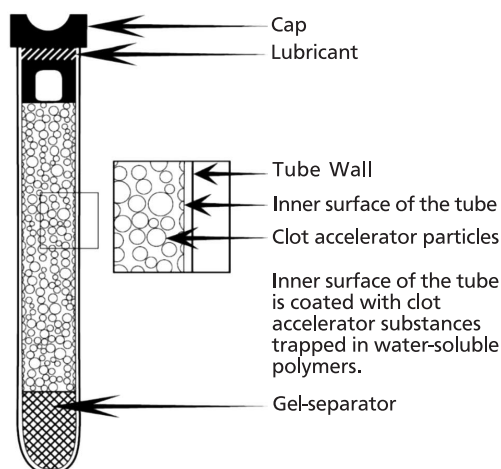


Figure 11. Structure of the clot accelerator tubes (16)

4.4. Position of the Tubes at Waiting Time

The tubes must be held in vertical position while being transported or waiting for the completion of clotting (Figure 12).

Fibrin formed in the tubes held in horizontal position may stick to the cap or side walls of the tube and may not leave serum despite centrifugation.

Mispositioning of the tubes during holding time causes fibrin formation and increases haemolysis likelihood (10).



Figure 12. Tubes must be held in perpendicular position during transportation and in the laboratory after collecting blood.

4.5. Points to Consider While Placing the Tubes into the Centrifuge

Tubes should be placed in balance in the reciprocal scales of the centrifuge and, if necessary, balance tubes should be used (Figure 13). Tubes should be labelled; sample level should be checked in tubes with additives.

Tubes should always be kept capped. Keeping the tubes capped prevents droplet formation inside the centrifuge by evaporation of the fluid contained in the tube and contamination of potential infectious agents.

In tubes which are uncapped, pH increases due to carbon dioxide loss. In some tests, pH increase causes false results (pH [increases], ionised calcium [decreases], acid phosphatase activity [decreases]). Keeping the tubes capped during centrifugation also prevents changes in analyte concentrations due to evaporation (10).



Figure 13. Tubes should be placed in balance in the reciprocal scales of the centrifuge and should be capped.

5. Things to Consider During Centrifugation

5.1. Selecting Correct Centrifugation Time and RCF Value

Separation by centrifugation depends on two main variables:

- RCF,
- Centrifugation time

Higher value of RCF is limited with tube resistance and cannot be increased in very high values. Centrifugation time is the easiest factor to change.

There are partial differences between centrifugation times recommended by different manufacturers and institutions (Table 1).

WHO recommends centrifuging all blood samples for 15 minutes (17, 18).

CLSI (H21-A5) recommends centrifuging samples with citrate for 10-15 minutes (19).

However, there are also studies reporting that centrifugation time can be decreased to 7 minutes (20).

Table 1. Centrifugation time recommended by different manufacturers or international institutions

	BD	Greiner	WHO	CLSI
Serum	Gold: 10 min 1300-2000 x g Red: 10 min ≤1300 x g Orange: 3-10 min 1500-4000 x g	10 min 1800-2200 x g	15 min 1500 xg	Conditions considered appropriate by the manufacturer
Tube with citrate	PET: 10-15 min 2000-2500 x g Glass: 15 min 1500 x g	10 min 1500-2000 x g	15 min 1500 xg	15 min 1500 x g
Tube with heparin	Non-gel: 10 min ≤1300 x g Gel: 10 min 1300-2000 x g Mechanical Separator: 3-10 min 1800-4000 x g	15 min 2200 x g	15 min 1500 xg	Conditions considered appropriate by the manufacturer

In samples centrifuged in high RCF values, it was reported that platelet activation has been observed (21). However, in RCF values lower than recommended RCF values, it was reported that centrifugation has had a procoagulant effect (22).

5.2. Centrifuge Interior Temperature

Rotor rotating as the centrifuge runs causes heat release by rubbing against air friction.

Due to the friction, it was reported that the temperature inside the centrifuge could reach to the levels of 50°C (23). Therefore, it is recommended to use temperature controlled centrifuges. It is important to use temperature controlled centrifuges to measure especially heat-sensitive parameters such as ACTH, ammonia, cAMP. Unless it is indicated a specific temperature for a specific analyte, 20-22°C is recommended for the centrifuge temperature (10, 24). Becton Dickinson (BD) recommends centrifugation at temperatures 20-25°C for SST II Advance serum tubes (25).

5.3. Points to Consider During the Centrifuge is Running

Centrifuge should be placed on a durable, steady, firm counter or floor in a balanced manner.

It is necessary to stand over the centrifuge as it is running.

Centrifuge must be stopped by pressing on the stop button when there is a unexpected sound, vibration, smoke odour or smoke itself. For unplugging the centrifuge or cutting the power will deactivate the brake system, it is not recommended.

Centrifuges used in laboratories usually warns the user with a beep as the centrifugation time has been programmed comes to its end. Centrifuge gate should not be opened or forced to open unless the centrifuge stops and an opening notice is released.

6. Points to Consider After Centrifugation

Practices after the centrifugation have influence on the durability of the parameters that will be tested. As a rule, serum or plasma obtained after centrifugation must be separated from cellular elements as soon as possible. Tubes with gel reagent that are used presently provide convenience by making a barrier between serum/plasma and packaged cells. CLSI recommends testing samples of serum/plasma separated by centrifugation and kept in room temperature within 8 hours of centrifugation. If the measurement will be done within 48 hours, samples can be kept in refrigerator (2-8°C). Samples (serum/plasma) that will be tested longer than 48 hours are recommended to be kept by freezing at -20°C. Since blast freezers that freezes very rapidly may destroy the sample, they are not recommended to be used. Samples should be frozen slowly and while analysing should be thawed slowly at room temperature. If there is any turbidity in defrosted sample, the sample is centrifuged and precipitated the sediment, and the test is performed with the clear sample. Manufacturer's instructions should be applied to the procedures of freezing-thawing in the primary tube. In fact, durability of each analyte is different. Different stabilities may be specified for the same analytes by different measurement methods. Every laboratory should check analyte stability from all accessible sources and perform its own validation studies (10).

6.1. Re-centrifugation of Samples

Due to high RCF during centrifugation, membrane structures of the cells may be damaged. If re-centrifugation is applied, intracellular fluid leaks into the serum/plasma and changes the concentration of analytes. Therefore, as a general rule, re-centrifugation of samples is not recommended (10). If the samples will be re-centrifuged, it will be a better practice to re-centrifuge samples following transferring serum/plasma separated into a new empty tube.

6.2. Erroneous Centrifugation of the Samples

Samples collected for the tests performed with complete blood containing K2 or K3 EDTA such as complete blood count, HBA1c, cyclosporine

should not be centrifuged. If centrifugation is performed by mistake, the sample should not be thrown in obligatory conditions and remixed gently. If another negative event such as haemolysis and precipitation is not observed, sample can be processed (10).

It was reported that platelet values decrease in complete blood samples that has been centrifuged by mistake (26).

It was indicated that changes have not been observed in HbA1c samples that were centrifuged by mistake (10).

6.3. Centrifuge Maintenance

Centrifuges collect dusts on and inside while running. These cumulants may cause both the performance of the centrifuge to reduce and serious malfunctions and accidents. Besides, the centrifuge may get dirty due to leakage or drip from the fluids placed into the centrifuge in daily usage. Therefore, many manufacturers recommend daily cleaning of centrifuges. Outer surfaces, vessel, places of the rotor within reach of the centrifuge should be cleaned by wiping (Figure 14) (27).



Figure 14. Daily centrifuge cleaning

The balance of the centrifuges should be checked by levelling in certain intervals.

User manuals provided by the manufacturer should be read carefully and recommendations included in the instructions should be applied.

Periodical maintenance and controls of the devices should be carried out in intervals recommended by the manufacturer. Accuracy of the timer of the centrifuge can be checked by using chronometer by the

user. However, for checking the speed of the centrifuge, special apparatus such as a tachometer is used. Centrifuge speeds should be tested in certain intervals or by technical staff trained about the matter when needed; and if required, calibrations should be done and documented.

7. Summary

1. Warnings of tube and centrifuge manufacturers should be read and applied carefully;
2. Settings should be done over RCF;
3. Tubes should wait for the recommended time at room temperature;
4. Tubes should be held in perpendicular position;
5. Tubes should be always capped;
6. Tubes should be placed in the centrifuge in balance;
7. Residual clots in the tube should not be removed with sticks;
8. Tubes should not be recentrifuged (except for special cases);
9. Daily cleaning and periodical maintenance of centrifuges should be performed.

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