An important source of preanalytical error in medical laboratories: centrifugation

Abstract: Centrifugation separates particles within the specimen according to their shape, dimensions, and density and basically can be defined as a separation method. The centrifuge is an essential device in medical laboratories to prepare the serum, plasma, and urine samples for analysis. It is basically an electric device composed of the stationary (motor) and the motile (rotor) part. The centrifugation depends on two main variables: relative centrifugal force (RCF) and centrifugation time. The physical impact separating the specimen into its components in the centrifuge known as RCF is expressed as the multiples of gravitational acceleration (xg). RPM, defined as the number of rotations of the centrifuge per minute, shows the speed of the centrifuge. RCF value can be calculated by using RPM, and the centrifuge radius. Because models and sizes of centrifuges vary considerably, the use of gravity (g) forces instead of RPM is suggested. The centrifuges can be classified according to their usage, speed, technical specifications, and rotor type. An accurate and precise centrifugation process is essential to prevent errors in the preanalytical phase. The purpose of this document is to ensure the standardization of a good, precise protocol for the centrifugation process among the medical laboratories.

Keywords: centrifuge; classifications of centrifuges; preanalytical phase error; relative centrifugal force; rotation per minute.

Introduction

Centrifugation basically can be defined as a separation method. It separates particles within the specimen
according to their shape, size, and density by the centrifugal force obtained from the rotation [1]. The centrifuge is a device developed for the separation of mixtures consequently, it is commonly used in medical laboratories to prepare the serum, plasma, and urine samples for analysis. An accurate and precise centrifugation process (pre-centrifugation phase, centrifugation phase, and post-centrifugation phase) is essential to defeating errors in the preanalytical phase. The purpose of this document is to ensure the standardization of a good, precise protocol for the centrifugation process among the medical laboratories.

**Brief history of the centrifuge**

The first known centrifuge was developed to measure gunpowder force by a military engineer, Benjamin Robins (1707–1751) [2]. The analytical power of the centrifuge was firstly discovered by Friedrich Miescher. Miescher separated cellular organelles and nucleic acids by using a centrifuge and discovered deoxyribonucleic acid (DNA). Later, Theodor Svedberg developed an analytical ultracentrifugation technique and conducted protein purification studies. In the 1940s, the necessity for centrifuges gradually increased along with the widespread use of routine laboratory tests. In 1949, Spinco Corporation developed the centrifuges which could rotate 40,000 times per minute and was purchased by Beckman Coulter in 1950. In 1962, Eppendorf Corporation launched the first micro-centrifuge that centrifuges the low volume sample known as “Godet Eppendorf”. Hettich Corporation was the first to rebuild the centrifuges with computer applications. In 1976, the first microprocessor programmable centrifuges were developed and still in use in medical laboratories [3, 4].

**Applications of centrifuges in medical laboratories**

Nowadays, centrifuges are an indispensable component of medical laboratories. The preparative centrifuges use rotors with relatively large capacities to separate a specimen into various components and, commonly used in routine studies. The analytical ultracentrifugation is a technique that combines an ultracentrifuge with optical monitoring systems. The typical applications of centrifuges in medical laboratories are as follow [1].

- Precipitating the cells and other components in urine and other body fluids for microscopic and chemical investigations,
- Separation of precipitated solids from the liquid phase of a mixture that may cause the potential of interference,
- Separating antibodies and antibody bounded structures for immunochemical analysis, lipid components, cellular organelles,
- Extracting solvents, separation of liquids of varying density,
- DNA and RNA isolation.

**Principle of centrifugation**

The gravitational force of the earth can separate blood into its component. For faster and further sub-components separation the centrifugation is essential. The separation speed of the components in a specimen depends on its shape, size, density, and viscosity—denser and larger particles precipitate faster [5].

The centrifuge is an electric engine composed of two fundamental parts: stationary (stator, motor) and the rotor [6, 7]. The motor converts electrical energy into mechanical energy, and the rotor transmits the rotational motion produced by the motor. The physical impact of the spinning rotor is known as relative centrifugal force (RCF) or gravity (g). RCF depends on the speed of rotation and the distance of the particles from the center of rotation. RCF is expressed as the multiples of gravitational acceleration (×g) [1, 8]. Rotation per minute (RPM), defined as the number of rotations of the centrifuge per minute, mainly shows the centrifuge speed [1, 8]. Since the centrifuges’ radius can differ, the RCF values will be different while the RPM stays the same. Therefore, the use of RCF ensures achieving the same acceleration.

**Calculation of RCF/converting RCF and RPM**

For a correct calculation of RCF, the centrifuge’s radius should be measured properly. Radius in user manuals informed by the manufacturer can be used. If the centrifuge radius is not provided, the user can measure the radius from the center to the bottom of the tube. Measurement of the radius of fixed-angled and swinging-bucket centrifuges is shown in Figures 1 and 2 [9, 10].
Simple formulas can demonstrate the relation between RCF and RPM.

RCF calculation formula:

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

$R=\text{centrifuge radius (centimeter), } 1.118 \times 10^{-5}$ is a constant value calculated from angular velocity, frequency, acceleration, and angular acceleration formulas.

RPM calculation formula:

$$\text{RPM} = \sqrt{\frac{RCF \times 10^5}{1.118 \times r^2}}$$

The conversion of RPM and RCF could easily be done by nomograms (Figure 3) or the calculator applications on the web [11, 12].

### Classifications of centrifuges

Centrifuges can be classified according to their usage, speed, technical specifications, and rotor type [1, 9]. Classifications of centrifuges are shown in Table 1.

### Important issues in the centrifugation

The centrifugation can be evaluated in three phases, similar to the total laboratory process; the pre-centrifugation phase, the centrifugation phase and, the post-centrifugation phase. Especially an appropriate blood drawing procedure is essential, and attention should be paid to the instructions for proper, precise centrifugation [13].

### Pre-centrifugation phase

The time period between collection and centrifugation is the first issue to be noticed. Waiting too long between collection and centrifugation may cause a lowering of glucose concentration at an estimated rate of 5–7% per hour [14]. Clotting time, fibrin, and tube balance in the centrifugation are also other important issues before centrifugation.

To obtain plasma from specimens with EDTA, heparin, fluoride, or citrate, blood can be centrifuged without delay. The specimen should be allowed to complete clot formation in an adequate time at room temperature before centrifugation for serum. The period of time to allow the blood to clot recommended by the manufacturer based on the tube type should be considered. If there is no specific time recommended by the manufacturer, the specimen should be allowed to clot for a minimum of 30 min and should not exceed 60 min [10, 15, 16]. Specimen clotting time has been substantially reduced by the new tube types containing “clot activator” or “clot accelerator” [17]. Keeping the specimen in the refrigerator, shorter clotting time than the recommended, and anti-coagulation treatment of the patient might cause delayed fibrin [10].

Tubes should be placed in balance on the opposite side of the centrifuge, and, if necessary, balance tubes should be used. The filling volume of the tubes, especially with the additives, should be checked. Tubes should always be kept capped until analysis [10] (see Table 2 Recommendations-I for pre-centrifugation phase).
Centrifugation phase

The centrifugation depends on two main variables: RCF and centrifugation time. World Health Organisation (WHO) and Clinical Laboratory Standard Institution (CLSI) (H21-A5) recommends the duration for the centrifugation of all blood samples [18, 19, 20]. There are partial differences between centrifugation force and time recommended by different manufacturers and institutions (see Table 3 for recommended centrifugation time). There are also studies reporting the centrifugation time of citrated samples can be decreased to 5 min [21]. In high RCF values, the platelet activation has been observed and may result in erroneous coagulation test results [22]. However, in RCF values lower than recommended, it was reported that centrifugation has a procoagulant effect [23].

Since the temperature inside the centrifuge could reach 50 °C, it is recommended to use temperature-controlled centrifuges [24]. Unless a specific temperature is indicated, the recommended centrifuge temperature is 20–22 °C [10, 25].

The centrifuge should be placed on a stable, non-shaking bench or a floor in a balanced manner. The centrifugation process should not be performed unattended. Unplugging the centrifuge or cutting the power is not recommended. The centrifuge should not be opened or forced to open without displaying a lid opening sign (See Table-2 Recommendations-II for centrifuge phase).
Table 1: Classification of the centrifuges.

A. Centrifuges by usage
(1) Preparative centrifugation
   a. Differential centrifugation (separation of particles)
(2) Analytical centrifugation
   a. Density gradient centrifugation
      i. zonal (or rate zonal) centrifugation
      ii. isopycnic or sedimental equilibrium centrifugation
B. According to rotor type and technical properties
(1) Fixed angle centrifuge
(2) Swinging-bucket centrifuge
(3) Right-angled centrifuge
(4) Temperature controlled centrifuge
(5) Vacuum centrifuges
C. Centrifuges by speed
(1) Very low speed centrifuges (RCF < 4,000 × g) (in routine usage)
(2) Low speed centrifuges (RCF = 5,000–10,000 × g) (blood bank)
(3) High speed centrifuge (RCF = 10,000–50,000 × g) (DNA–RNA isolation)
(4) Ultracentrifuge (RCF = 100,000–1,000,000 × g) (lipoprotein analysis)

Table 2: Recommendations for pre-centrifuge, centrifuge, post-centrifuge and, maintenance.

Pre-centrifugation Phase

Recommendations-I
Plasma, samples with EDTA, heparin, fluoride, or citrate can be centrifuged without any delay for blood clotting.
For the serum samples, the tube type and the clotting time that is recommended by the manufacturer should be considered.
The sample should be allowed to complete the clot formation in an upright position at room temperature.
Tubes should always be kept capped during centrifugation.
Tubes should be placed in the centrifuge in balance.

Centrifugation Phase

Recommendations-II
Settings should be done over RCF.
Recommended centrifugation time by different manufacturers or international institutions should be applied.
For the heat-sensitive analyses the temperature-controlled centrifuges should be used.
20–22 °C is the recommended temperature for the centrifugation unless a specific temperature is not indicated.
The centrifuge should be placed on a stable, non-shaking bench or on a floor in a balanced manner.
The centrifugation process should not be performed unattended.

Post-centrifugation Phase

Recommendations-III
For the tests that will be analyzed within 48 h, samples can be kept in the refrigerator (2–8 °C). The re-centrifugation of whole blood samples is not recommended in general (except for special cases).

Maintenance

Recommendations-IV
Instructions of the centrifuge manufacturers should be read and applied carefully.
Daily cleaning of the centrifuges should be performed.
The outer surfaces of the centrifuge, the boiler, and the accessible parts of the rotor must be cleaned by wiping with sodium hydrochloride (bleach) prepared with 1:10 normal dilution.
The balance of the centrifuges should be checked periodically at recommended intervals.
The accuracy of the timer of the centrifuge should be checked by using a chronometer by the operator.
Periodical maintenance of the device should be carried out in intervals as recommended by the manufacturer.
The calibration of the centrifuge should be obtained and documented.
Table 3: Recommendations centrifugation time by international institutions and manufacturers.

<table>
<thead>
<tr>
<th></th>
<th>BD</th>
<th>Greiner</th>
<th>WHO</th>
<th>CLSI</th>
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</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Gold:</td>
<td>10 min</td>
<td>10 min</td>
<td>15 min</td>
</tr>
<tr>
<td></td>
<td>1,300–2,000×g</td>
<td>1,800–2,000×g</td>
<td>2,200×g</td>
<td>1,500×g</td>
</tr>
<tr>
<td></td>
<td>Red:</td>
<td>10 min ≤1,300×g</td>
<td>1,500×g</td>
<td>2,200×g</td>
</tr>
<tr>
<td></td>
<td>Orange: 3–10 min</td>
<td>1,500–4,000×g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubes</td>
<td>PET:</td>
<td>10 min–15 min</td>
<td>10 min</td>
<td>15 min</td>
</tr>
<tr>
<td></td>
<td>2,000–2,500×g</td>
<td>1,500×g</td>
<td>1,500×g</td>
<td></td>
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<tr>
<td>with citrate</td>
<td>Glass: 15 min</td>
<td>2,000×g</td>
<td>1,500×g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,500×g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubes</td>
<td>Non-gel: 10 min</td>
<td>15 min</td>
<td>15 min</td>
<td>1,500×g</td>
</tr>
<tr>
<td></td>
<td>≤1,300×g</td>
<td>2,200×g</td>
<td>1,500×g</td>
<td>considered appropriate by the manufacturer</td>
</tr>
<tr>
<td>with heparin</td>
<td>Geb: 10 min</td>
<td>1,300–2,000×g</td>
<td>1,800–4,000×g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mechanical separator: 3–10 min</td>
<td>1,800–4,000×g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aThe gel tubes and the barricore tubes of the BD company had different centrifugation duration.

Post-centrifugation phase

Procedures after the centrifugation affect the durability of the parameters that will be tested. In general, if the measurement will be done within 48 h, samples can be kept in the refrigerator (2–8°C). Each analyte has its own stability. It is recommended that the samples (serum/plasma) that will not be tested within 48 h should be frozen and be kept at –20°C.

If re-centrifugation is applied, intracellular fluid leaks into the serum/plasma and changes the analytes’ concentration. Therefore, the re-centrifugation of samples is not recommended [10]. Specimens collected for the tests performed with whole blood containing K₂EDTA or K₃EDTA should not be centrifuged. If centrifugation is performed by mistake, in obligatory conditions, the specimen can be used by remixing gently. It was reported that platelet values decrease in whole blood samples that have been centrifuged [26] (see Table 2 Recommendations-III for post-centrifugation phase).

Maintenance of centrifuges

Many manufacturers recommend daily cleaning of the centrifuges. The outer surfaces of the centrifuge, the boiler, and the accessible parts of the rotor must be cleaned by wiping with sodium hydrochloride (bleach) prepared with 1:10 standard dilution (1/10 bleach preparation:1 measure of bleach + 9 parts of water [5,000–6,000 ppm releases chlorine]) [27].

The balance of the centrifuges should be checked periodically at recommended intervals. The accuracy of the centrifuge timer can be checked by using a chronometer by the operator while the speed of the centrifuge could be checked with special apparatus such as a tachometer. The calibration of the device should be obtained and documented (see Table 2 Recommendations-IV for maintenance).

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References


