



## Serum Isolation from Human Peripheral Blood

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## Serum Isolation from Human Peripheral Blood

### 1. Subject

This Standard Operation Procedure (SOP) describes a method to isolate serum from blood using centrifugation.

### 2. Application

Isolation of serum from peripheral blood using centrifugation.

### 3. Definitions and Abbreviations

PBMC = peripheral blood mononuclear cells  
RT = room temperature  
v/v = volume/volume  
rpm = rotations per minute  
ml = milliliter  
EDTA = ethylenediaminetetraacetic acid

### 4. Principle

Blood plasma is the liquid component of blood, in which the blood cells are suspended. It makes up about 60% of total blood volume. It is composed of mostly water (90% by volume), and contains dissolved proteins, glucose, clotting factors, mineral ions, hormones and carbon dioxide (plasma being the main medium for excretory product transportation). Plasma is the supernatant fluid obtained when anti-coagulated blood has been centrifuged. The blood is mixed with an appropriate amount of anticoagulant like heparin, oxalate or ethylenediaminetetraacetic acid (EDTA). This preparation should be mixed immediately and thoroughly to avoid clotting. Process samples as soon as possible. Blood serum is blood plasma without fibrinogen or the other clotting factors. Serum is clearer than plasma because of fewer proteins. Proteins are sometimes considered as interfering substances in some tests as they react with the reagent and thereby yield inaccurate results. Serum is the preferred specimen in clinical testing as the interference that may be caused by a plasma specimen because of the presence of an anticoagulant, is eliminated. If storage is necessary prior to processing, store the blood at room temperature, shielded from light. DO NOT refrigerate the cells.

### 5. Safety precautions

Treat all blood and synovial fluid samples as infectious material. Wear disposable gloves.

### 6. Equipment and Accessories tools

#### 6.1 Equipment

- Centrifuge Hettich Rotanta 46 (UMC# 99-000-2142)
- Easypet pipet (Eppendorf, Germany, 4006173)
- Pipets 10-1000 µl (Gilson, The Hague, The Netherlands)



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### 6.2 Accessories

- 1.8 ml sterile polypropylene Nunc cryotubes (Nalge Nunc, Roskilde, Denmark, 375418)
- 15 ml sterile polypropylene conical tubes (Falcon/ Becton Dickinson, Erembodegem, Belgium, 352096)
- 10 ml sodium-heparin vacutainer blood collection tubes (Becton Dickinson, Erembodegem, Belgium, 366480)
- 5 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357543)
- 10 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357551)
- Disposable gloves (Kimberly Clark, Zaventem, Belgium)
- Easyload 200 µl (741035) and 1000 µl (741000) sterile pipettips (Greiner Bio-one, Germany)

### 7. Samples

#### 7.1 Sample Collection

Whole blood should be drawn in sodium–heparin tubes with a venoject vacuum system.

#### 7.2 Sample Processing

**All handlings of the sample should be done in a biohazard safety cabinet will wearing gloves**

Store the serum at –80°C (room KC01.081.1).

### 8. Procedure

1. In a centrifuge capable of safely spinning blood tubes, spin the blood at roughly 2500 rpm for 10 minutes at room temperature.
2. Remove the tubes from the centrifuge, and in a clean and safe environment, open the tubes to access the serum located at the top of the specimen.
3. Using a sterile transfer pipet or a 1000 µl pipettor, carefully transfer 2x 1.5 ml of the serum into appropriately labeled screw-cap cryovial tube. If 2x 1.5 ml is not possible, transfer as much as reasonably possible without disturbing or collecting any of the pelleted cells.
4. As soon as possible, place and store the collected serum at -80° C.

### 10. Processing of the Results

#### 10.1 Documentation of cells

- Log the sera in the excel file plasma storage



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### 11. Documentation

Fill in the patient form as accurate as possible. Document each sample in the Isolation Notebook as follows

Patient → Protocol code (consists of protocol code in combination with a serial number)  
Isolate by → Person who isolated the samples  
Plasma → amount of serum per cryovial.

### 14. Accompanied Forms/ Attachments/ Document

Instruction manual centrifuge  
Instruction manual safety cabinet  
Instruction manual freezer  
Instruction manual refrigerator

### 15. Remarks

Do not close the Nunc cryovials to tight, as this will break the inner seal. Screw top firmly but stop when resistance is experienced.

\*\*\*END\*\*\*