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Recommendations and Standard Operating Procedure for the Preparation of Human Serum from Whole Blood

1. Prerequisites

This standard operating procedure describes the preparation of serum obtained from whole blood following clotting and centrifugation. It forms part of an effort to harmonize practices in accordance with preanalytical conditions established by current best practices and manufacturers' recommendations. The table below presents blood collection tubes suitable for obtaining serum according to analytical requirements.

Cap color	Tube type	Main analytical applications	Preanalytical time interval
	Serum tube with clot activator, without gel (e.g., CAT)	▪ Clinical chemistry[1,2] ▪ Endocrinology[1,2] ▪ Serology[1,2] ▪ Therapeutic drug monitoring[1,2] ▪ Metabolomics[3–5] ▪ Proteomics[6,7] ▪ miRNomic (miRNA)[8,9]	The preanalytical interval comprises: 1. time from collection to centrifugation (TTC, time-to-centrifugation) 2. time from centrifugation to freezing (TTF, time-to-freeze) Both intervals should be as short as possible and adapted to study requirements.
	Serum separator tube with clot activator and gel (e.g., SST™)		

Additional note:

> *Regardless of the intended analytical application, tube selection must follow the guidance provided in the institution's specimen collection manual.*
> *Some manufacturers offer rapid-clotting tubes (containing silica microparticles and thrombin). When using these tubes, refer carefully to the manufacturer's preanalytical recommendations.*

The following checklist serves as a reminder of general procedures for collecting and transporting fluid specimens and, more broadly, the principles applicable to the management of biological resources. These procedures and principles should be consulted beforehand.

- ✓ International recommendations and guidelines[10–12].
- ✓ Compliance with ethical and regulatory requirements (informed consent or documented non-opposition, as applicable under French regulations).
- ✓ Trained personnel, defined responsibilities, and implementation of a quality management system (QMS) within the standards framework applicable to the institution (e.g., ISO 20387, ISO 9001).
- ✓ Controlled collection conditions (pre-collection variables, collection techniques/procedures, appropriate primary tube type and order of draw, fill volume, etc.).
- ✓ Appropriate, compliant transport (duration, temperature, and procedures).
- ✓ Unique specimen identification and a complete chain of traceability.
- ✓ Recording and retention of associated data.
- ✓ Quality controls performed at all preanalytical steps described below, with a nonconformity management system in place.

2. Reagents, Consumables, and Equipment

Consumables and small laboratory supplies

- Sterile transfer pipettes
- Micropipettes for dispensing small volumes (aliquoting)
- Sterile pipette tips
- Tube racks
- Gloves and other personal protective equipment (PPE)
- Polypropylene cryovials with 1D or 2D identification (barcodes), suited to their intended use (e.g., nuclease-free, conical-bottom, LoBind)
- Cryoboxes suited to their intended use
- Transfer tubes with properties equivalent to those of cryovials
- DASRI container

Additional note:

> *The use of cryo-resistant labels (rated for -80°C and/or liquid nitrogen) is recommended when cryovials without pre-printed identifiers are used. Follow the manufacturers' instructions and standardize the study-specific label fields (at least the specimen ID).*

Equipment

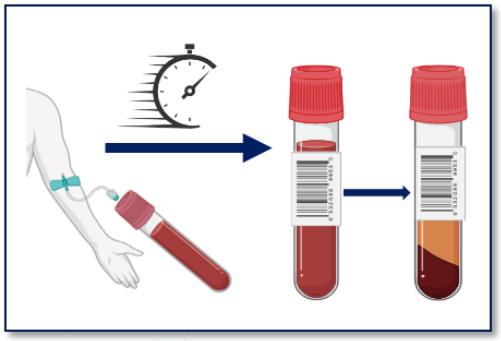
- Centrifuge, preferably fitted with a swinging-bucket rotor
- Class II biological safety cabinet if the specimen contains a known risk group 2 pathogen.

3. From Preparation to Storage

The preanalytical time interval is a critical factor affecting serum quality.
It must be kept as short as possible.
Any deviation from these recommended conditions (manufacturers, FrBioNet, published literature, etc.) must be supported by a protocol validated as fit-for-purpose for the intended use.

Step 1 – Clotting | estimated duration: minimum 30 min (2h max)

DATASET
Collection date and time



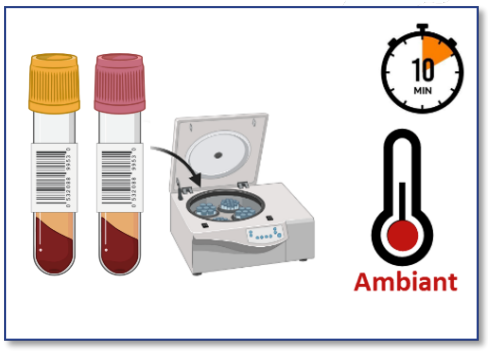
- Allow the blood to clot in the primary tube, kept upright at room temperature (preferably between 18°C and 25°C).
- After 30 minutes (the minimum interval before centrifugation[13–16]), check that clotting is complete.
- **Once clotting is complete, TTC should be as short as possible and must not exceed 2 hours from collection.**[17–20]
- Proceed immediately to Step 2.

Additional note:

Check the fill volume. Never uncap the primary tube or transfer blood from one primary tube to another.

Step 2 – Centrifugation | estimated duration: 10 min

DATASET
Centrifugation start date and time
Centrifugation speed
Centrifugation temperature
Centrifugation duration



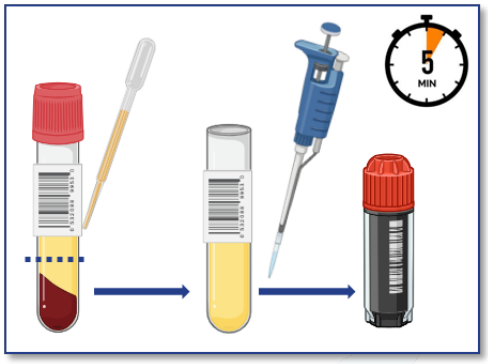
- The proposed harmonized centrifugation conditions are **2,000g for 10 minutes, with brake on**, at room temperature.[7,13–16,21]
- Visually check that the resulting supernatant is acceptable (two distinct phases and HIL index).
- Proceed immediately to Step 3.

Additional note:

A second centrifugation may be needed for analyses of serum miRNAs.
Major manufacturers, recommended centrifugation forces range from 1,300 g to 2,000 g for 10 minutes.

Step 3 – Aliquoting | estimated duration: 5 min

DATASET
Aliquot volume
Storage container
Unique tube identifier



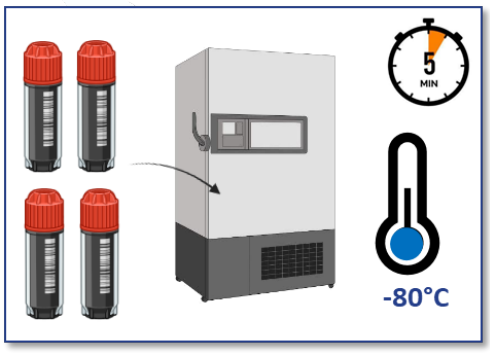
- Adjust aliquot volumes to the intended downstream use (where known), to avoid repeated thawing. If the intended use is unknown, we recommend preparing aliquots of 200-500 µL.
- To ensure that the aliquots prepared are homogeneous, it is recommended to transfer the upper serum phase to a secondary tube without disturbing the interface (leave a safety margin above the blood cell pellet).
- **Homogenize** and dispense into barcoded cryovials.
- Proceed immediately to Step 4.

Additional note:

Some manufacturers recommend that the primary tube not be centrifuged a second time[22].
When using a gel-containing primary tube (SST type), leave an adequate margin above the gel barrier to avoid contaminating the serum (which may affect, for example, proteomic applications).

Step 4 – Freezing | estimated duration: 5 min

DATASET
Storage start date and time
Storage temperature
Storage location
Freeze-thaw cycle



- **Long-term storage**: freeze immediately at -80°C. Storage in liquid nitrogen may also be considered.
- **Short-term storage**:

→ Storage at +4°C may be considered depending on the intended use.

→ Freezing at −20°C is permissible depending on the intended application and project requirements (*e.g., it is not recommended for metabolomics*).

- Avoid all freeze–thaw cycles.

⚠ *Freeze–thaw cycles and prolonged storage can lead to significant alterations in some biomarkers, warranting strict limitation of these cycles and monitoring of storage conditions for each project. Thawing events must be accurately recorded, including their number and duration.*

Additional note:

For long-term freezing, -80°C is recommended.
Regardless of its setpoint temperature, the storage unit must be routinely monitored and its performance verified.

4. Nonconformities and Recommendations in the Event of Anomalies

We recommend referring to the nonconformity terminology list available in the laboratory manual and additionally recording serum-specific specimen characteristics in the following situations:

- Clot not formed/incomplete clotting;
- Contamination caused by aspiration of the cell pellet.

5. Quality control

We recommend consulting the online biological specimen qualification tool developed by IBBL: https://www.findmyassay.com.

Most of the essential checks are visual (e.g., gel integrity, clot formation, and serum color and appearance).

The most important of these is the **HIL index** (hemolysis/icterus/lipemia),[23] used as a quality control tool to detect analytical interferences:

- **Hemolysis:** release of intracellular components, producing a pink-to-red color. Classified as mild, moderate, or severe according to hemoglobin levels[24].
- **Icterus**: bilirubin-related interference, producing a dark yellow-to-brown color.
- **Lipemia**: lipid-related interference, producing a turbid, milky appearance.

6. Traceability and SPREC

The following tools must be implemented to ensure complete specimen traceability and management of associated data:

- ✓ Specimen transfer requisition / collection form (laboratory manual and accompanying template).
- ✓ Laboratory Information Management System (LIMS).
- ✓ At a minimum, the data fields listed for each preparation step above.

We recommend using SPREC (Standard PREanalytical Code)[25] to standardize and document specimen preanalytical conditions, in addition to the LIMS traceability system. The SPRECalc release notes providing guidance on implementing the tool; version 4.0[26] will soon be available.

A recommended SPREC code for serum preparation will be proposed in an appendix in due course.

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