Institute of Health Carlos III

Red Nacional de Biobancos
Spanish National Biobank Network

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SOP
Serum

Blood Products Working Group

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Collection, Processing and Storage of Serum Samples

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1. ABBREVIATIONS

IATA: International Air Transport Association

ICAO: International Civil Aviation Organization

EMS: Energy Management System

g: gravitational acceleration (unit of measurement of the RCF: Relative Centrifugal Force)

2. DEFINITIONS

BLOOD SERUM: Blood fraction resulting after coagulation and removal of the fibrin clot and other components. Equivalent to blood plasma but without the proteins involved in coagulation.

HEMOLYZED SAMPLE: the rupture of red blood cells that release hemoglobin and other substances into the plasma/ serum due to which it acquires a pink to red color.

CLOTTED SAMPLE: occurs due to a difficult and long extraction, not properly mixing the blood in the tubes or patient characteristics.

JAUNDICED SAMPLE: serum or plasma containing elevated bilirubin levels, resulting in a more intense yellow color.

LIPEMIC SAMPLE: serum or plasma with a high fat content and a milky and/or cloudy appearance; it may occur in samples from patients who have not fasted as recommended and who had an abundant food intake.

3. OBJECTIVE

The objective of this procedure is to define the procedure and to establish the basic quality guidelines with respect to collecting and handling and to the processing of serum samples that will be deposited in biobanks belonging to any center or hospital affiliated to the National Biobank Network.

4. SCOPE

This procedure applies to all serum samples that are obtained in order to be stored in a biobank. This protocol does not detail the occupational health and safety processes regarding biohazardous materials and/or chemical products, and it is recommended that the personnel follow the Health and Safety rules established in each center.

5. MATERIALS AND SERVICES

- Courier Service holding a permit for the transport of biological materials*:

Material	UN Classification		Packing instructions				Comments
	Class	No.	ADR	RID	ICAO	IMDG	
Infectious samples affecting humans	6.2	2814	620	620	692	620	Materials groups 2, 3, 4
Diagnostic specimens	6.2	3373	650	650	650	----	Materials groups 1, 2, 3

**For further information refer to the section on Related Documentation (1)*

- For non-infectious samples: Bag or container for internal transport in the hospital.
- For infectious or hazardous samples: Transport container for dangerous substances that complies with the effective legislation: Royal Decree 664/97, following "Packing Instruction 620 (IATA - ICAO 602)"
- Syringes and/or material required for collecting blood
- 4ºC cool box
- Vacuum blood extraction tube with safety cap and separating gel
- Gloves for protection during handling
- Sterile 1 milliliter Pasteur pipettes
- Sterile cryotubes (from 0.5 to 2 milliliters)
- Blood collection tube racks
- Racks for cryotubes
- Cryo storage boxes
- Labels appropriate for the type of cryotubes
- Sterile tips with or without filter that fit the type of pipettes used
- Pipettes (to collect volumes between 0.2 and 1 milliliter)
- Filter paper
- Centrifuge with adapters suitable for the type of collection tubes used
- Printer for labeling samples
- -80ºC ultra-low temperature freezer with temperature recording system and a temperature maintenance system in case of power failure (CO_2 injection, internal Energy Management System (EMS), generator) and telephone alarm system
- Sample management software applicable to each center (Examples: BBUN application (Maxwell), Bio-e Bank application, etc.)

6. DEVELOPMENT

6.1 PRIOR CONSIDERATIONS

- The type of centrifuge used may affect the stability of the gel barrier. The use of a centrifuge with a swinging bucket rotor instead of a fixed angle rotor allows obtaining a more stable gel barrier. Centrifugation must be done in a refrigerated centrifuge (15- 24ºC).
- Re-centrifuging tubes with gel separators is strictly prohibited because it may lead to changes in analytical results (e.g. potassium)
- For research involving Biomarkers
    - The time of extraction of the sample is especially important in studies of biomarkers that are influenced by the circadian rhythm.
    - Degradation processes in serum are time and temperature-dependent. For research of biomarkers that are highly sensitive to these two factors it is essential to process the sample in the shortest possible time. This is the case for studies focused on finding biomarkers by metabolomics and/or proteomics.
- Freeze-thaw cycles may alter the concentration of certain biomarkers. This is true to the extent that samples that have been repeatedly frozen and thawed (two or more cycles) should not be used in certain studies and should be kept separate for testing.

6.2 BLOOD EXTRACTION

6.2.1 This must be done after the patient signed the informed consent (for a specific study and/or for the biobank)

6.2.2 Collection is done in blood collection tubes with gel and clot activator particles as an additive. There are different tube formats, from 5 ml to 8.5 ml, that may be used (Vacuette; Vacutainer, etc.).

6.2.3 After obtaining the blood the tube must be inverted several times gently to promote clotting by making all the blood come into contact with the clot activator particles (maximum 4 times). Place the tube in an upright position thereafter.

6.2.4 The perfectly labeled sample and the request are transported to the laboratory together with the informed consent, while following the safety guidelines established by each center for the transport of biological material. It is recommended that the time between blood collection and freezing at -80ºC be defined based on the type of studies for which the sample is intended; thus, based on preliminary tests, it has been determined that:

a) optimum time for cell studies: maximum 1.5 hours after extraction; and

b) optimum time for virological studies: maximum 24 hours after extraction.

6.3 RECEIPT OF THE SAMPLE IN THE LABORATORY

6.3.1 Check the information and identification of the tubes and ensure the correct relationship between tubes and patient information, in accordance with the confidentiality commitment required by the Data Protection Act.

6.3.2 Label and record the sample according to the sample management process used by the center.

6.3.3 Fill in the minimum data sheet with the data necessary for proper storage of the sample(*). It is advised to collect the maximum possible information concerning the sample at the time of extraction:

- Date and time of withdrawal.
- Type of anticoagulant.
- Incidents not related to the protocol.

* *There are various Data Collection Sheet models, depending on the biobank receiving the samples: they are adapted to the specific characteristics of its functioning and its internal management.*

6.4 PROCESSING: SERUM COLLECTION AND STORAGE OF THE SAMPLES

6.4.1 Processing of peripheral blood to obtain serum should be done in the shortest time possible; the optimum processing time is 1.5 h ± 30 min.

6.4.2 To prevent platelet activation, after extraction samples must be stored at room temperature until centrifugation and direct exposure to light must be avoided.

6.4.3 Upon receipt of the sample in the laboratory place the blood tube upright in a rack and let coagulate for 30 min, counted from the time of extraction. If the sample is not centrifuged after this time, store it upright at 4ºC until further processing and storage (maximum 2 h); enter this incident on the laboratory sheet.

6.4.4 Centrifuge the sample between 1500 and 2000xg at room temperature for 10 min.

6.4.5 Carefully aspirate the clear and transparent yellowish upper fraction (serum), using a sterile Pasteur pipette or a micropipette of adequate volume without touching the gel interface.

6.4.6 Divide all the serum into aliquots of at least 0.3-0.5 ml over suitable cryogenic vials that are properly labeled and identified. Close the tubes properly to obtain an airtight seal. Record the number of aliquots obtained for each sample.

6.4.7 Record the location of the sample in the sample management software used by the biobank.

7. REFERENCE DOCUMENTATION

- *Standard ISO 9001:2008. Quality management systems. Requirements.*
- Organic Law 15/1999 of 13 December on the Protection of Personal Data (LOPD).
- Law 14/2007, of 3 July, on Biomedical Research (LIB).

8. RELATED DOCUMENTATION

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