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Blood Products Working Group

REVISION	DONE BY	DATE	APPROVED	DATE	ENTRY INTO FORCE
01	Blood Products Working Group	02/20/2012	Management		
Amendments:					

Collection, Processing and Storage of Cerebrospinal Fluid Samples Obtained by Lumbar Puncture

This publication is supported by the Subprogram Thematic Networks for Cooperative Research in Health of the Institute of Health Carlos III (ISCIII), within the Strategic Action on Health 2009, RD09/0076/00113

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1. ABBREVIATIONS

CSF: Cerebrospinal fluid

LP: Lumbar Puncture

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

LUMBAR PUNCTURE: is an invasive diagnostic and therapeutic procedure of paramount importance in the treatment of several neurological diseases and in neurosurgery. It is done to collect cerebrospinal fluid (CSF) from the patient. The puncture is performed aseptically into the subarachnoid space of the lumbar region. It may be required urgently or by appointment in a hospital or outpatient setting.

TURBID CSF: a CSF sample is turbid when it contains a large number of cells. The degree of turbidity depends on the number of cells it contains. It is slight when it contains 500 to 1000 cells/ml. It is evident in cases of pleocytosis above that number. It is purulent when it contains several thousands of polymorphonuclear leukocytes per ml. It occurs in diseases such as acute bacterial meningitis, abscesses, especially after rupture, and acute meningeal reactions secondary to contrast media and drugs.

HEMORRHAGIC CSF: if the puncture was difficult there may be a false positive hemorrhagic fluid (traumatic LP). To distinguish it from a real subarachnoid hemorrhage, the three tube test is done. The sample is collected in three consecutive tubes. If the discoloration due to trauma caused by the puncture disappears, the bleeding was not prior to the LP.

XANTHOCHROMIC CSF: in this case, the CSF has a yellowish color due to the increase in total protein and/or the presence of erythrocyte breakdown products. CSF with these features can be observed in many processes, most frequently in subdural hematomas, certain tumors, chronic meningitis and arachnoiditis and certain polyradiculoneuritis syndromes.

3. OBJECTIVE

The objective of this procedure is to define the course of action and to establish the basic quality guidelines with respect to collecting and handling and to the processing of cerebrospinal fluid 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 CSF samples that are obtained by lumbar puncture 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 which comply with the effective legislation: Royal Decree 664/97, following "Packing Instruction 620 (IATA - ICAO 602)"*
- *Syringes and/or material required for collecting CSF.*
- *Sterile tube for collecting CSF*
- *Gloves for protection during handling*
- *Sterile 1 milliliter Pasteur pipettes*
- *Sterile cryotubes (from 0.5 to 2 milliliters)*
- *Cryotube racks*
- *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*
- *-80º C ultra-low temperature freezer with temperature recording system and a temperature maintenance system in case of power failure (CO2 injection, internal Energy Management System (EMS), generator) and telephone alarm system*
- *Sample management software applicable to each site (Examples: BBUN application (Maxwell), Bio-e Bank application, etc.)*

6. DEVELOPMENT

6.1. PRIOR CONSIDERATIONS

- An extraction volume of 10 to 12 ml sample is recommended. The concentration of biomarkers may vary with the extraction volume; it is less when the volume is equal to or less than 2 ml.
- Storing several CSF samples obtained during the course of the LP can lead to errors in the results. It is advised to use the first 2 ml for basic CSF analysis as part of the diagnosis. The rest of the sample collected in various tubes must be pooled before dividing it into aliquots.
- When a CSF sample is hemorrhagic, one should be aware that the presence of erythrocytes in the CSF can give false positives when biomarkers are studied. Furthermore, the presence of blood can alter CSF protein patterns when it is studied using proteomic techniques. Blood cells are usually recovered by the centrifugation step that is done before storing the samples (section 6.4). A red blood cell count before freezing the sample is therefore essential. It is not advisable to use a CSF sample with an amount of erythrocytes equal to or more than 550/µl for biomarker studies.
- There is no evidence that the type of needle used in the LP may alter biomarker concentrations. However, the use of atraumatic needles is recommended because they are better tolerated by patients, who have a lower risk of post-puncture headache.
- The use of polypropylene tubes with leak-proof screw caps is recommended because their walls have a low protein binding capacity, and for safety reasons to prevent spilling of the sample.
- The concentration of biomarkers in blood often influences their composition in the CSF. It is therefore advisable to store serum, plasma and DNA samples together with the patient's CSF samples. They must be taken at the same time. In many studies the patient's serum is used as a comparative sample for the composition of the CSF.
- Quantification by nephelometry of each stored sample is recommended, as a quality control measure over time. Quantification can be done at certain periods as a quality measure, by verifying the state of the sample at least at the level of albumin, its most abundant protein.
- 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. CEREBROSPINAL FLUID EXTRACTION

6.2.1. This is done by lumbar puncture, after the patient has signed the informed consent (for a specific study and/or for a biobank)

6.2.2. CSF is collected in tubes that are specific for this purpose. Sterile 12 ml screw cap tubes without any additive.

6.2.3. After collecting the sample, it can be kept at 4ºC in a refrigerator or at temperatures up to 22ºC (room temperature) until processing and storage. There is currently no evidence for a preferred temperature that must be maintained prior to sample processing. Keep the tube upright during transport, as the closure of the tube is not leak proof.

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 extraction of the CSF and freezing at -80ºC is between 1 and 2 hours after extraction. If the cells are intended to be stored or if cytometry studies are to be done, it is recommended that sample processing takes place in the shortest time possible, as the number of cells decreases rapidly (1 hour).

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, while keeping 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 gather the maximum amount of information possible concerning the sample at the time of extraction:

- Date and time of extraction (to be taken into account in studies of biomarkers that are influenced by the circadian rhythm).
- Suspected diagnosis
- Age of the patient (the barrier dysfunction of patients is closely related to their age)
- Total volume extracted (required for cell count)
- Visual anomaly of the CSF (normal CSF is crystal clear)
- Incidents not related to the protocol.

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

6.4. CSF PROCESSING AND SAMPLE STORAGE

6.4.1. Processing of CSF consists of separating the cells it may contain in order to: count them, analyze them by flow cytometry, store them under biobank conditions and to prevent their interference in the diagnostic tests that are carried out.

6.4.2. Upon receipt of the sample in the laboratory, it is passed to a 15 ml Falcon tube for centrifugation. If the cells are to be stored for later RNA isolation, it is recommended to centrifuge at 400xg for 10 min at room temperature to prevent cell rupture. If CSF volume less than 4 ml it can be centrifuged in two Eppendorf tubes, followed by removal of the supernatant and storage of the cells. Freezer space is managed optimally with this process. If it is not foreseen that RNA will be extracted, we recommend centrifugation at 2000xg for the same time and at the same temperature.

6.4.3. After centrifugation, the supernatant is carefully removed and transferred to a clean and sterile collection tube (either another type of Falcon tube or a tube with the same characteristics as the one used for the collection of CSF when it starts to flow after the LP).

It is important to leave the smallest possible volume of liquid at the bottom. Cells are frozen directly at -80ºC, or the appropriate buffer is first added for the desired processing afterwards and then they are frozen at -80ºC. The number of cells is calculated based on the volume of sample stored in the biobank, based on the cell counting performed in the laboratory and the volume of CSF allocated for diagnosis.

6.4.4. Divide all the CSF into 0.3-0.5 ml aliquots in 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. Store within 2 hours at -80ºC.

6.4.5. 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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11. *ISBER Best Practices for repositories: Collection, Storage, Retrieval and Distribution of Biological Materials for Research. Cell Preservation Technology 6(1), 3-58, 2008 http://www.isber.org/Pubs/BestPractices2008.pdf*

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