



**I.B.AHC Biobank and Clinical Registry
for Alternating Hemiplegia of Childhood**
www.ibahc.org

**STANDARD OPERATING PROCEDURES (SOPs)
for the I.B.AHC Biobank**

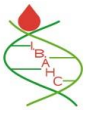
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Last revision **July 2, 2012**

Version 1



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Introduction

This document represents the set of written instructions (SOPs) that document the routine activity followed by the E. Medea authorized personnel in preparing, storing and shipping the biological material of the I.B.AHC Biobank for Alternating Hemiplegia (from now on referred to as the Biobank). This Biobank comprises the DNA blood and cell lines of AHC patients and the DNA of their parents. SOPs detail the regularly recurring work processes followed at the laboratory of Molecular Biology of the E. Medea Scientific Institute at Bosisio Parini (Lecco, Italy). The development and use of SOPs minimize variation and promote quality through consistent implementation of processes and procedures within the laboratory, even if there are temporary or permanent personnel changes.

Types of biological samples sent to the lab to be included in the Biobank.

The lab hosting the Biobank can receive blood or DNA from both AHC patients /parents to be included in the Biobank.

The blood samples from AHC patients are drawn in Na-(or K) EDTA tubes for DNA extraction and in Na-heparin for immortalization and lymphoblastoid cell line generation.

Parents' blood is drawn in Na-(or K) EDTA tubes for DNA extraction only.

- Four 4,5 ml tubes (sodium-eparine) are necessary for the patient and three 4,5 ml tubes (sodium-eparine or K-EDTA) are necessary for each of the two parents (10 tubes in total).
- In case of difficulties in shipping blood sample the Biobank can receive and store only purified DNA. Purification method and DNA concentration must be specified. In this case no cell line will be established for the patient.

The blood tubes are shipped or transported to the lab marked with the I.B.AHC identification code CID of each individual (patient or parents).

The authorized lab personnel receive the biological material and proceed with the extraction procedures or cell line generation based on the blood samples sent. The blood samples to be used for DNA preparation are frozen at -20°C to undergo the extraction procedure (see 1). The Na heparin tubes are temporarily stored at +4-8°C to undergo the immortalization procedures (see 3). These samples can be stored at this temperature for a maximum of 1 day.

1. DNA isolation from whole blood by salting out procedure

This method is rapid, safe and does not require expensive and environmentally hazardous reagents and equipment.

The cell and nuclear membranes are destroyed by the combined action of SDS and proteinase K.

The DNA, which is insoluble in the organic phase, is present in the non-miscible aqueous phase.

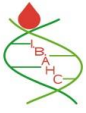
The DNA is precipitated, washed, dried and dissolved in solution in an aqueous buffer.

Equipment and Materials

Disposable gloves

Polypropylene tubes 15ml

Centrifuge refrigerant



Micropipettes
Water bath at 56°C
Freezer at + 4 or -20°C.

Reagents and solutions

Cell Shocking Solution:

8,0 g NH₄Cl (149 mM)

1,0 g EDTA (2,6 mM)

0,1 g KH₂PO₄ (0,7 mM)

Adjust to pH 7 with NaOH

Distilled water to 1 l

Autoclave to sterilize

Store ≤ 1 year at room temperature (if sterility is maintained)

Nuclear Lysis Buffer:

50 ml 2M Tris Cl, pH 7,5 (100 mM)

80 ml 5M NaCl (400mM)

4 ml 500 mM EDTA (2mM)

Adjust to pH 8,0 with NaOH

Distilled water to 1 l

Autoclave to sterilize

Store ≤ 1 year at room temperature (if sterility is maintained)

SDS 10%

Proteinase K solution (20 mg/ml):

Freeze this solution at -20°C in small aliquots.

Saturated NaCl (6M)

Absolute ethanol

70% Ethanol

TE buffer:

10 mM Tris Cl, pH 8.0

1 mM EDTA, pH 8.0

Procedure

Thaw frozen 3 ml of whole blood (with EDTA).

Resuspend the whole blood with 5 ml of Cell Shocking Solution.

Transfer in Polypropylene tubes 15ml and shake.

Centrifuge for 5 minutes at 1400 rpm.



Remove the supernatant, resuspend the pellet with 10 ml of Cell Shocking Solution and shake.

Centrifuge for 5 minutes at 1400 rpm.

Remove the supernatant, resuspend the cell lysates with 3 ml of Nuclear Lysis Buffer and digest with 0.2 ml of 10% SDS and 0.1 ml of proteinase K solution.

Put tube(s) in the bath at 56°C for 1 hour

Add 1ml of saturated NaCl (6M) to each tube and shake vigorously for 15 seconds.

Centrifuge for 45 minutes at 15°C and 4000 rpm.

Transfer the supernatant containing the DNA to another 15ml polypropylene tube, the precipitated protein pellet is left behind at the bottom of the tube.

Add 2 volumes of absolute ethanol and invert the tubes several times until the DNA precipitates.

Remove the precipitated DNA with a plastic spatula or pipette and transfer to a 1.5 ml microcentrifuge tube containing 100-200 microliters TE buffer (usually the final concentration is 200-500 ng/μl).

Dissolve the DNA for 1 hour at 50°C.

Store the tube at -20°C.

Check quantity/quality of DNA for the following: OD260/OD280 ratio, protein concentration and agarose gel electrophoresis to confirm the integrity.

Reference

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 1988 Feb 11;16(3):1215.

2. Quantitative and qualitative DNA analysis

Quantitative DNA analysis is performed by measuring the optical density (OD) at a wave length of 260 nm and 280 nm and then by checking DNA integrity by gel electrophoresis.

Equipment and materials

Spectrophotometer

Cuvettes

Electrophoresis apparatus

Micropipettes

Reagents and solutions

Lambda DNA:

DNA molecular weight markers at a known concentration of standard DNA fragments up to 20-30 kb such as HindIII-digested phage lambda DNA which is commercially available.



TAE electrophoresis buffer:
242 g Tris base
57,1 ml glacial acetic acid
37,2 g Na₂EDTA x 2H₂O
H₂O to 1 liter
(50X stock solution)

Agarose
Ethidium bromide

2.1 Measurement of optical density

Standard rectangular cuvettes that transmit light at every measuring wavelength may be inserted into the spectrophotometer cuvette shaft.

For a linear relationship between concentration and OD, OD values should lie between 0.1 and 0.5.

Dilute the DNA solution if necessary. An OD₂₆₀=1 corresponds to ~ 50 µg/ml. The ratio of OD₂₆₀/OD₂₈₀ should be ~1.8; smaller ratios indicate protein contamination.

2.2 Gel electrophoresis

Perform electrophoretic analysis with TAE buffer 1X in an 0.8 % agarose gel with ethidium bromide; use 1 µl of DNA isolated (see 1.) and 100 ng of Lambda DNA.

The isolated DNA should appear as a distinct band of ~20 kb (a slight smear is acceptable, yet indicative for slight degradation).

If the DNA quality control provides with positive results, the DNA is stored at -20C in a dedicated box. Temperature and general functioning of the freezer hosting the Biobank DNA are controlled by a remote alarm system.

3. Procedure for blood cells immortalization and lymphoblastoid cell line generation.

3.1 Transformation of B lymphocytes with Epstein-Barr virus (EBV)

Lymphocytes are converted to lymphoblast cells by immortalisation. This is a convenient way of obtaining unlimited quantities of patient DNA or RNA. For this purpose, it is necessary to purify lymphocytes and obtain a source of EBV to infect the cells. Virus should be produced in a facility with adequate equipment for manipulating viruses (see 10.). Once the cells are transfected, no special precautions are required to handle them.



Equipment and materials

Vertical flow sterile hood
Disposable gloves
Micropipettes
Sterile disposable 0.45 µm membrane filter
Polypropylene tubes 15ml
Centrifuge
Culture flasks 25 cm²
Incubator at 37°C and 5% CO₂
Freezer at + 4°C

Reagents, media and solutions

B95-8 monkey marmoset blood lymphocyte:
derived from a cotton-top marmoset (*Saguinus oedipus*). Releases high titres of transforming EBV. Thus it provides a source of EBV to establish continuous lymphocytic lines from human donors. The cells should be handled under laboratory containment level 2.

Growth Medium:

RPMI 1640 500 ml
2 mM L-Glutamine
Antibiotics (100 µl/ml streptomycin; 100 IE/ml penicillin)
20% heat-inactivated Fetal Bovine Serum (FBS)
(Store at +4°C and heat at +37°C before use)
Complete growth medium is prepared under the hood using disposable sterile plastic ware and filtered using 0.22 micrometer filters. The cap and neck of the bottle containing the sterilized medium are protected with Parafilm.

Cyclosporin A (2 mg/ml), store at + 4°C and heat at +37°C before use.

Ficoll-hypaque gradient:

is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.077± 0.001 g/ml (store in freezer at + 4°C and protect from light).

Procedure

3.2 Culture of the lymphoid starter cell line B95-8 and purification of EBV

Cultivate B95-8 monkey cells (EBV producers) to a density about 1 x 10⁶ cells/ml as suspension cultures in 25 cm² flasks in 20 ml of RPMI 1640 supplemented with 20% FBS, 2 mM Glutamine and antibiotics (100 µl/ml streptomycin; 100 IE/ml penicillin).

Leave in 5% CO₂ incubator at + 37°C.

Subculture the cells to obtain sufficient medium containing EBV for the transformation.

When the culture has reached the desired density, harvest EBV-containing supernatant medium



and centrifuge at low speed (1300 rpm) to remove cells.

Filter the supernatant twice through 0.45 µm membrane filter to remove all viable cells of the marmoset cell line.

Store filtered virus pools at + 4°C for at most 4-6 month.

Dilute the filtrate 1:1 with fresh RPMI 1640, supplemented with 20% FBS, 2 mM Glutamine and antibiotics (100 µl/ml streptomycin; 100 IE/ml penicillin), immediately before use.

Care should be taken at two important steps during this transformation: first ensure removal of all cells of the starter line B95-8 from the filtrate used transformation; second, do not freeze the virus-containing supernatant because this will cause loss of transformation efficiency.

3.3 Ficool separation of unfractionated mononuclear leukocytes obtained from whole blood

Mix 3,5 ml fresh Na²⁺-heparinized blood sample 1:1 with RPMI 1640.

Centrifuge the blood-RPMI 1640 mixture over Ficool-hypaque gradient (1:1) for 45 min at 1300 rpm without brake.

Isolate the leukocytes and wash three times with RPMI 1640.

Determine the number of cells obtained (see **9.**).

3.4 Establishment of cultures

Resuspend the pellet of leukocytes in the 1 ml B95-8 diluted 1:1 with RPMI 1640 supplemented with 20% FBS, 2 mM Glutamine and antibiotics (100 µl/ml streptomycin; 100 IE/ml penicillin) at a cell concentration of 2×10^6 cells/ml in 25 cm² flask.

Add 2 µg/ml of Cyclosporin A and incubate at + 37°C with 5% CO₂.

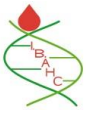
Thereafter change medium once a week by removing half of the supernatant and replacing it by fresh medium containing 1 µg/ml of Cyclosporin A.

The first subcultivation after starting the cultures can usually be carried out after 2-3 weeks.

Criteria indicating successful transformation: there are two distinct morphologic features that can be observed usually as early as approximately 24h post-infection with EBV indicating successful transformation of B cells: 1. blastogenesis becomes evident resulting enlargement of the lymphocytes; 2. there is increasing development of cell aggregates of proliferative lymphoblast cells.

Reference

Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet.* 1986 Aug;73(4):320-6.



3.5 Freezing, cryopreservation, storage and reactivation of cell lines

Equipment and materials

Disposable gloves
Polypropylene tubes 15ml
Centrifuge
2 ml cryogenic vials
Freezer at – 20°C and – 136°C
Water bath at 37°C
Incubator at 37°C and 5% CO₂

Reagents, media and solutions

RPMI 1640

Growth Medium:

RPMI 1640 500 ml
2 mM Glutamine
Antibiotics (100 µl/ml streptomycin; 100 IE/ml penicillin)
20% heat-inactivated Fetal Bovine Serum (FBS)

Dimethyl sulfoxide (DMSO)

3.6 Freezing, cryopreservation and storage of cell lines

Cultures for cryopreservation should be healthy, free of contamination and in log phase growth for several days before freezing.

Procedure

Change medium 24 h before freezing by removing half of it and replacing it with fresh medium.

Count the number of viable cells which should be in log phase (as described in **10.**).

Centrifuge cell suspension for 5 min at 1300 rpm to pellet cells. Use a pipette to remove the supernatant without disturbing the pellet.

Resuspend cell pellet at a concentration high as $5-10 \times 10^6$ cells/ml in RPMI 1640 supplemented with 20% FBS, 2 mM Glutamine, antibiotics and 10% DMSO (a cell concentration of less than 5×10^6 cells/ml per tube results in a delay of growth after thawing).

Prepare aliquots of 1,8 ml per sterile cryotubes.

Write date, cell line code and passage number on cryotube.

Place tubes briefly on ice: start the freezing procedure within 5 minutes.

The cells should be frozen slowly (at -1°C/min). Transfer the tubes from ice to an a freezer at -20°C; then place in -80°C freezer for one or two days. Finally transfer the cryotubes to the in -136°C freezer tank for long-term storage. Both types of freezer, (-80 and -135C) are subject to



temperature and functioning control by a remote alarm system.

3.7 Reactivation of cell lines

Cryopreserved cells are fragile and require gentle handling.

Procedure

Remove the cryotubes from -136°C freezer and thaw in a water bath at 37°C (requires a few minutes).

Add 5 ml cold RPMI 1640 medium immediately after complete thawing to decrease the DMSO concentration.

Centrifuge for 5 min at 1300 rpm and remove DMSO containing medium.

Resuspend cell pellet in approximately 2 ml fresh medium and incubate for 24 h at 37°C and 5% CO₂ to allow recovery of cells.

Add further 2-6 ml of medium or subcultivate.

Reference

Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet.* 1986 Aug;73(4):320-6.

3.8 Security instructions for the transformation of B lymphocytes with EBV

- To avoid EBV infection of personnel handling EBV-transformed cell lines the following security instructions are practised:
- All persons handling lymphoblastoid cell lines are tested for anti-VCA-titers. Only seropositive persons are allowed to handle these cells.
- A vertical flow sterile hood is used for all manipulations.
- All materials and solutions which come into contact with lymphoblastoid cells or their medium are sterilized before it is discarded.

Reference

Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet.* 1986 Aug;73(4):320-6.



4. Manual DNA isolation from lymphoblasts

Starting material for DNA isolation is a frozen cell pellet. Then the Extraction procedure follows the same protocol as the whole blood (see1.).

Equipment and materials

Disposable gloves
Polypropylene tubes 15ml
Centrifuge
Freezer at -80°C
Micropipettes
Water bath at $+56^{\circ}\text{C}$

Reagents, media and solutions

RPMI 1640

Nuclear Lysis Buffer:

50 ml 2M Tris Cl, pH 7.5 (100 mM)

80 ml 5M NaCl (400mM)

4 ml 500 mM EDTA (2mM)

Adjust to pH 8.0 with NaOH

Distilled water to 1 l

Autoclave to sterilize

Store ≤ 1 year at room temperature (if sterility is maintained)

SDS 10%

Proteinase K solution (20 mg/ml):

Freeze this solution at -20°C in small aliquots.

Procedure

4.1 Preparing cell lines for DNA isolation

Grow lymphoblast cells up to 10^8 - 10^9 cells in total.

Centrifuge cell suspension into tube for 5 min at 1300 rpm.

Aspirate media and suspend the cell pellet with 5 ml RPMI 1640.

Centrifuge for 5 min at 1300 rpm.

Put dry cell pellet in at -80°C freezer for 1-2 h.



4.2 DNA isolation

Thaw frozen cell pellet.

Suspend cell pellet in 3 ml of Nuclear Lysis Buffer and digest with 0.2 ml of 10% SDS and 0.1 ml of proteinase K solution.

Put tube(s) in the bath at 56°C for 1 hour and treated under the same conditions as for whole blood (see1.).

Reference

Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet.* 1986 Aug;73(4):320-6.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* 1988 Feb 11;16(3):1215.

4.3 Cell culture check for bacterial and/or fungal contamination

All cell lines that need to be put in culture for shipment to other labs are checked for bacterial and fungal contaminations. These are generally visible to the naked eye and detected by sudden increase in turbidity and colour change of the culture medium as the result of a change in pH. Both bacterial and fungal contaminations are readily confirmed microscopically. The cell culture may survive for a short time but the cells will eventually die.

Continuous use of antibiotics and antifungal agents may sometimes mask the presence of these organisms and can lead to the development of resistant strains that are difficult to eliminate. It is important therefore to check periodically for the presence of contamination.

Once contamination has been ascertained the medium and all other reagents used for this cell line must be discarded. The contaminated culture is destroyed with sodium hypochlorite.

4.4 Cell Counting and assessment of cell viability with haemocytometer

For the manipulations of cell cultures it is necessary to quantify the number of living cells prior to use.

Cell numbers are determined with a haemocytometer, an instrument that allows to calculating the number of cells per unit volume of a cell suspension.

The haemocytometer consists of two chambers, each of which is divided into nine 1.0 mm squares. A cover glass is supported 0.1 mm over these squares so that the total volume over each square is 1.0 mm x 0.1 mm or 0.1 mm³, or 10⁻⁴ cm³. Since 1 cm³ is approximately equivalent to 1 ml, the cell concentration per ml will be the average count per square x dilution factor x 10⁴. A common convention is to count cells that touch the middle lines (of the triple lines) to the left and top of the square, but do not count cells similarly located to the right and bottom.

To differentiate viable cells from non-viable using trypan blue. Only non-viable cells absorb the dye which appear blue and asymmetrical under the microscope, while healthy viable cells are refractory to the dye and rounded.



The accuracy of the count with a haemocytometer mainly depends on the accurate mixing of the sample, number of chambers counted and number of cells counted (the concentration of cell suspension should be adjusted so that count is 200-500 per 0.1 mm³).

Equipment and materials

Disposable gloves
Haemocytometer
Polypropylene tubes
Micropipettes
Inverted Microscope

Reagents and solutions

Phosphate buffered saline 1X (PBS 1X)
Trypan blue

Procedure

Prepare a uniform cell suspension of the cell culture to be counted
Dilute trypan blue with PBS (dilution 1:5)
Transfer 0.5 ml of agitated cell suspension to a tube and add 0.5 ml of diluted trypan blue
Place cover glass over the two haemocytometer chambers
With a micropipet, fill both chambers of the haemocytometer by capillary action.
Place the haemocytometer on the stage of an inverted microscope at X100 magnification.
Count the cells in the central square and in the four squares at the corners. Count separately viable (refractory) and non-viable (blue) cells.
Calculate the number of cells per ml, and the total number of cells, in the original culture as follows:
Cells/ml = average count per square x dilution factor x 10⁴.
Total cells = cells per ml x total volume of cell preparation from which the sample was taken.
Repeat counting to assure reproducibility.

References

Sanders C., Skerry DW. The distribution of blood cells on haemocytometer counting chambers with special reference to the amended British Standards Specification 748 (1958). *J Clin Pathol.* 1961 May;14:298-304.

Sanders C. The total leucocyte count with special reference to the haemocytometer. *J Med Lab Technol.* 1955 Jul;13(3):144-50.



5. Good cell culture practice

Good cell culture practice is achieved when all procedures are carried out to a standard that precludes contamination by bacteria, fungi and mycoplasma and cross- contamination with other cell lines.

Equipment and materials

Disposable gloves

Vertical flow sterile hood

Flow sterile hood are checked every six months to ensure that they are safe to use in terms of product and user protection. These tests confirm that the airflow is correct and that the filters are functioning properly.

Incubator (37°C, 5% CO₂)

Cell cultures require a strictly controlled environment in which to grow.

Incubator is used routinely to provide the correct growth conditions, such as temperature, degree of humidity and CO₂ levels in a controlled and stable manner.

Humidified incubators are a particular area for concern due to the potential for fungal and bacterial growth in the water trays. This will create a contamination risk that can only be avoided by regular cleaning of the incubator.

The temperature of an incubator are regularly checked and the temperature adjusted as necessary.

Incubator CO₂ levels should also be regularly checked to ensure the levels are being correctly maintained.

Inverted microscope with phase contrast

Centrifuge

The centrifuges are situated where it can be easily accessed for cleaning and maintenance. Centrifuges are checked frequently for signs of corrosion.

Water bath 37°C

Haemocytometer

Micropipette, with tips (sterile, nucleic acid and nuclease free)

Polypropylene tubes 15ml

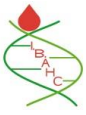
Pasteur pipettes

Pipettes 5-10 ml

Culture flasks 25 cm²

Cryotubes 1.5 ml

Freezer -20°C, -80°C and -136°C



Procedure

Disinfect the flow hood with 70% ethanol.

Clean the surfaces of all the materials in use before putting them into the hood.

Bottles with growth media and reagents are opened only inside the hood. Before closing the bottles, their necks and caps are flamed on a Bunsen.

Cultures and media should be examined daily for evidence of gross bacterial or fungal contamination.

After completing work, equipment and materials are disinfected before they are removed from the hood. Work surfaces inside the hood are sprayed with 70% ethanol; the gloves are discarded.

Incubators, cabinet, centrifuges and microscopes are cleaned regularly.

Reference:

Coecke S et al. Guidance on good cell culture practice. a report of the second ECVAM task force on good cell culture practice. *Altern Lab Anim.* 2005 Jun;33(3):261-87.

6. Shipment of AHC DNA samples and lymphoblast cell lines

Transport at room temperature.

6.1 Preparation of the cell lines for transport

The day before shipment, the medium is diluted with half volume of fresh complete medium (a culture flasks 25 cm²).

Equipment and materials

Vertical flow sterile hood
Disposable gloves
Culture flasks 25 cm²
Polypropylene tubes 15ml
Centrifuge
Parafilm

Reagents, media and solutions

Culture flasks 25 cm² to confluence

Growth Medium:

RPMI 1640 500 ml

2 mM L-Glutamine

Antibiotics (100 µl/ml streptomycin; 100 IE/ml penicillin)

20% heat-inactivated Fetal Bovine Serum (FBS)

(heat at +37°C before use)



Procedure

Transfer the cell suspension in 15ml polypropylene tubes.

Centrifuge for 8 min at 1300 rpm.

Discard the supernatant.

Resuspend in at least 14 ml of fresh complete medium.

Protect the cap and neck of the tube with Parafilm.

6.2 Preparation of the DNA for transport

Equipment and materials

Disposable gloves

Spectrophotometer

tubes 1.5 ml

Parafilm

Reagents and solutions

DNA resuspended in TE 1X (see 1.)

Procedure

Dissolve the DNA for 1 hour at 50°C in agitation.

Quantify the DNA (see Quantitative and qualitative DNA analysis)

Transfer the suitable volume in a tube 1.5 ml

Protect the cap and neck of the tube with Parafilm.

6.3 Preparation of the package

The packaging material is approved by the Regulation on Transport of Biological Material (ADR) in force as of January 1st 2005.

The documents included in the package are as follows:

The list of samples IDs, with their amount and concentration,

- the custom declaration (if travelling to foreign countries)
- the material transfer agreement to be faxed back to the sender by the recipient lab.

Equipment and materials

The tubes containing the suspension of biological cells are packed in a triple packaging system. The three components of a triple packaging system are:

1. The primary receptacle holds the biological material and must be leak-proof: the polypropylene tubes 15ml with the cell suspension (for cell lines) and tubes 1.5 ml (for DNA).



2. The secondary container is leak-proof container that encloses and protects the primary receptacle. The secondary container must contain enough absorbent material to absorb all of the fluid from the primary receptacle in case of breakage.
3. The outer container is a rigid container that houses the secondary container. The outer package should be properly marked and labelled. It should be able to withstand outside influences such as physical damage while in transit.

An itemized list of package contents must be included between the outer and secondary container.

Procedure

Place the primary container in the secondary and tightly close the screw -top lid.

Between the outer and secondary include the following documents: an itemized list of package contents, the Transport Document and the Safety Instructions Document.

Indicate on outer container the sender and the recipient's address clearly.

6.4 Transport of Samples

The package containing the samples prepared in this way is picked up by the express courier.

The carrier must be accredited for the transport of dangerous goods.

For transport to foreign countries, both the sender and the recipient must have an import-export authorization for biological material.