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European Platform for Neurodegenerative Diseases

WP3 – SOP development

D3.3 – SOP Body Materials Collection

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[1] *Use one of the following codes:*
R: Document, report (excluding the periodic and final reports)
DEM: Demonstrator, pilot, prototype, plan designs
DEC: Websites, patents filing, press & media actions, videos, etc.
OTHER: Software, technical diagram, etc.

[2] *Use one of the following codes:*
PU = Public, fully open, e.g. web;
CO = Confidential, restricted under conditions set out in Model Grant Agreement;
CI = Classified, information as referred to in Commission Decision 2001/844/EC.

Description of Action	
V1.0	**15/10/2021**

Document History

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Abstract

The aim of the deliverable was to generate an SOP for bodymaterial collection for diverse body materials envisioned to be collected prospectively in the course of EPND. This will help reduction of variation between cohorts and biomarker results in multicentre studies.

Methods

Existing SOPs were updated (CSF, plasma) or generated (saliva, stool, urine), taking into account regulatory requirements. The following Existing SOPs and labmanuals were used as basis: Consensus protocol of the BIOMARKAPD project, Labmanual of the IMI 3TR project, that include SOPs for Stool, urine and saliva, Roche manual for Neurotoolkit evaluations, best practices from the IBBL biobank in Luxembourg. The SOP was critically reviewed by the WP3 task leader, discrepancies were discussed by email and in the WP3 meetings.

Results

SOP (see Annex below).

Discussion

There were no deviations from the Description of Action.

Conclusion

The final SOP is ready for publication. The SOP will be re-evaluated at the end of the EPND project period.

European Platform for Neurodegenerative Diseases

SOPs for body materials collection

Date: September 2022
Version 1.0

1: INTRODUCTION

Significant amounts of data and biospecimen already exist in Europe that could be used to accelerate biomarker discovery for neurodegenerative diseases including Alzheimer Disease (AD) Parkinson's Disease (PD) and Dementia with Lewy bodies (DLB). However, at this time these data and biospecimens cannot be easily found and exploited by the research community. Cohorts differ in the use of protocols for collection of clinical data and biospecimens. Variation in protocols for biospecimen collection and processing is a well-known factor that can influence biomarker results. This collection of standard operating procedures (SOPs) based on the 3TR Lab manual aims to harmonize biospecimen collection, processing, transfer and storage protocols in order to accelerate biomarker discovery for AD, PD and DLB.

Please read the SOP carefully and make sure all persons involved in biospecimen management have read it and have a copy to refer to.

Only by guaranteeing that we all collect, process, transfer and store biospecimen in the same manner we can obtain useful study results for our patients and for our project!

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2: EQUIPMENT

- A refrigerated centrifuge that can hold 5 ml, 10 ml, 15 ml and 50 ml tubes.
- A -70°C (-70°C to -86°C) freezer for temporary storage of the biospecimen to be frozen.
- Pipettes with proper tips (1000 µl and 200 µl) for transfer of biospecimen, and proper tube holder racks for laying tubes.

3: BIOSPECIMEN PROCESSING

REMEMBER: TIME ELAPSED FROM COLLECTION TO THE STORAGE OF THE ALIQUOTS SHOULD NOT EXCEED 2 HOURS FOR BOTH CSF (TEUNISSEN ET AL., 2009) AND BLOOD (VERBERK ET AL., 2022) RECORDS SHOULD BE MADE OF THESE INTERVALS.

3.1: Cerebrospinal Fluid (CSF)

ONE (1) POLYPROPYLENE TUBE of at least 12 ml (Del Campo et al., 2012; Teunissen et al., 2009) (e.g. Sarstedt, 15 ml CSF collection tubes. Cat no: Sarstedt 62.610.018) with CSF collected by lumbar puncture (LP) in the l3/l4 or l4/l5 inter-space, using 22-gauge needle and drip method or a syringe. If possible, always in the same time window at one collection site (Teunissen et al., 2009). The first 2 mls are discarded (could be used for basic CSF analysis (cell count/ blood contamination >500 RBCs/µl, to evaluate the suitability for further analysis). The next 2 ml can be collected for routine analysis, where the Sarstedt 62.554.502 is a recommended tube. The last 10 mls should then be collected using the 15 ml Sarstedt tubes mentioned above for biobanking (Teunissen et al., 2009).

CSF SHOULD **BE** frozen after processing WITHIN 2 HOURS (Teunissen et al., 2009).

1. CSF obtained is normally centrifuged at 1800-2000 g for 10 min at RT (Del Campo et al., 2012; Teunissen et al., 2009).
2. When cells are to be obtained, centrifuge at 400g, 10 min at RT.
3. The supernatant should be gently mixed to remove gradient effects before aliquoting (Del Campo et al., 2012).
4. CSF is aliquoted in polypropylene vials (500µl/vial)
    - Recommended use of polypropylene tubes. Preferably, make aliquots of 0.5 ml (eg. Sarstedt (Cat. No.: 72.730.005).
    - A minimum of two aliquots is recommended and Tubes are preferably 75% filled.((Deisenhammer et al., 2015))

3.2: Blood

3.2.1: Serum

ONE (1) BLOOD TUBE WITHOUT ANTICOAGULANT (red cap, BD Vacutainer tubes, REF. 367896) of 8.5 ml to obtain serum. Gently invert the tubes 5 times after blood collection. SHOULD BE PROCESSED AND FROZEN <2 HOURS.

1. Maintain the tube vertically at room temperature, avoiding direct exposure to light, in a rack for its coagulation for 30 minutes until processing (in the case that 30 min hasn´t elapsed from time of collection).
2. Centrifuge the tube at 1800-2000 g for 10 min at room temperature. (18-25 degrees C) Serum tubes can only be centrifuged once..
3. Carefully aspirate the upper fraction of light-yellow appearance (serum) without touching the interface gel and generate aliquots of 500uL (~4 serum aliquots) in freezing vials (Sarstedt Cat. No.: 72.730.005).
   Properly seal tubes to get a tight seal.
4. Discard primary tube with blood pellet.
5. Store serum aliquots immediately at -70°C.

3.2.2: Peripheral Blood Mononuclear Cells (PBMCs) (if collected, optional)

ONE (1) K2EDTA BLOOD TUBE (lavender cap, BD Vacutainer, REF 367525) **of 10 ml** to obtain Peripheral Blood Mononuclear Cells (PBMCs). Gently invert the tubes 8 to 10 times after blood collection to ensure the anticoagulant works. SHOULD **NOT** BE FROZEN, BUT PROCESSED IMMEDIATELY.

1. Dilute the biospecimen 1:1 with PBS (1 volume of PBS per 1 volume of blood) into 50 ml tube.
2. Perform Ficoll protocol for mononuclear cell separation:

    a. Pipette 1 volume Ficoll per 2 volumes of diluted biospecimen into 50 ml tube (divide the material in multiple falcons, if necessary).
    b. Carefully layer diluted blood biospecimen onto Ficoll solution (avoiding mixing the phases).
    c. Centrifuge 2000 rpm, RT, 15 min, with deceleration set to 0 (i.e. breaks turned off).
    d. Collect the white interphase of mononuclear cells and add to a clean 15 ml tube.

3. Wash twice with 10 ml PBS, with centrifugation 1800-2000g, RT, 5 min.
4. Resuspend in 10 ml PBS and count the cells.
5. Divide the single cell suspension in different 1.5 ml tubes.
6. (Prepare tubes with 1 million cells or 10 million cells)
7. Centrifuge the tubes 5 min at 500 x g.
8. Carefully remove the supernatant.
9. Resuspend the cell pellet slowly in 500 ul FBS (100%).
10. Add 500 ul freezing medium (80% FBS + 20% DMSO (cold)) and mix by pipetting (2x).
11. Transfer to cryotubes.
12. Put the cryotubes into a Mr. Frosty.
13. Store at -70ºC (between 4h to 74h) until to be transferred to liquid nitrogen.

3.2.3: Plasma and DNA / RNA

ONE (1) K_2 EDTA BLOOD TUBE (lavender cap, BD Vacutainer, REF 367525) **of 10 ml** to obtain plasma and DNA. Gently invert the tubes 8 to 10 times after blood collection. SHOULD **NOT** BE FROZEN, BUT PROCESSED at room temperature IMMEDIATELY.

1. Centrifuge the tubes at 1800-2000 g for 10 min at room temperature. Carefully remove from centrifuge without disturbing any platelets and/or cell pellet that might be on the bottom or sides of the tubes.
2. Carefully transfer plasma from tube avoiding pellet collection to 2 identically labeled 15 ml transparent tubes (62.554.502 • Sarstedt® Screw Cap Tube). Cap the tubes.
3. Re-spin plasma biospecimen at 2500 g for 10 min at room temperature.
4. Avoiding pellet collection, generate aliquots of 0.5 ml (8 plasma aliquots) in freezing vials(0.5mL Sarstedt tubes, cat.no 72.730.005) .
5. Properly seal tubes to get a tight seal.
6. Store primary tubes with blood pellets plus plasma aliquots immediately at -70°C.

ONE (1) TEMPUS™ RNA TUBE (blue cap, Applied Biosystems™ REF 4342792) to obtain RNA. Shake the tube vigorously or vortex the tube for 10 seconds, to get a perfect mix between blood and the stabilizing agent.

SHOULD BE FROZEN AFTER BLOOD COLLECTION at -20ºC.

3.2.4: Plasma collection for Alzheimer-Studies using NTK, Roche

For the collection of EDTA-Plasma the 9 ml S-Monovette® K_3 EDTA (REF 02.1066.001) from Sarstedt should be used. SHOULD **NOT** BE FROZEN, BUT PROCESSED at room temperature IMMEDIATELY.

1. Centrifuge the tubes at 1800-2000 g for 10 min at room temperature. Carefully remove from centrifuge without disturbing any platelets and/or cell pellet that might be on the bottom or sides of the tubes.
    a. The collected blood in the EDTA plasma collection devices has to be left in the collection device at room temperature for 30 minutes at minimum and no longer than 60 minutes after venipuncture.

   b. Centrifugation no later than 60 minutes after blood collection for the measurement of β-amyloid (1-40) or β-amyloid (1-42) individually, and no later than 120 minutes for the measurement of the β-amyloid (1-42) / β-amyloid (1-40) ratio.

2. Carefully transfer plasma from tube avoiding pellet collection to a 15 ml polypropylene tube (e.g. from Sarstedt, VWR, TPP) for biospecimen pooling and mix by turning the capped tube upside down several times.
3. Aliquots of 0.5 ml EDTA are aliquoted into cryotubes.

Recommendations:

   a. 0.5ml polypropylene cryotube (72.730.003, Sarstedt)
   b. 2 ml polypropylene cryotube (72.609.001, Sarstedt)

4. The biospecimen aliquots must be frozen immediately at -70°C, but not later than 2 hours after centrifugation.
   a. The EDTA-plasma can be frozen and thawed up to 3 times without any influence of the Aß42 levels
   b. Ship biospecimen on dry ice where necessary.
   c. Limit the number of transfers to a new tube to a maximum of 2.

3.3: Urine

This section details procedures for the collection and preparation of urine biospecimen for the analysis of microbiome, metabolomics, lipidomics, proteomics/proteins, biomarkers, parent drugs and/or their metabolites using methodologies such as immunoassays and mass spectrometry platforms.

Before the visit, the participants should be advised not to drink extra fluids and not to take food and drinks that are diuretic, e.g. alcoholic beverages, coffee, tea, energy drinks and soft drinks. Normal diuresis is usually sufficient for collecting enough material. If larger volumes of urine are produced due to excessive drinking, the analytes of interest may be diluted below detection limits. It is only if the subject is dehydrated or it is very warm and humid that extra fluid intake may be required.

The division of the biospecimen into twenty aliquots is important as it allows future distribution of aliquots for biomarker measurements using multiple platforms and multiple laboratories.

ONE (1) STERILE URINE COLLECTION CONTAINER OF 100 ML (SARSTEDT CAT. NO. 75.562.105) FOR URINE (ABOUT 80 ML). SHOULD NOT BE FROZEN, BUT PROCESSED.

TIME ELAPSED BETWEEN URINE COLLECTION TO THE STORAGE OF URINE ALIQUOTS SHOULD NOT EXCEED 2 HOURS.

urine procedure:

Ask the subject to pass midstream urine directly into a collection container.

- Urine recovery:
  - Transfer urine to 6 ml tubes (Greiner-bio one, cat no 456065) and spin at 1800-2000g, 10 min, room temperature.
  - Fill six (6) Sarstedt 5 ml-tubes (cat no 72.383.004) with 3 ml volume.
  - Close the tubes carefully with the provided caps and place it, if possible, immediately in vertical position in a freezer (-70°C). Biospecimen may be stored temporarily at 4°C for a short period of time (≤ 6 h) pending transfer to the freezer. If so, make sure to transfer the frozen biospecimen within 72 hours to a -70˚C freezer.

- Discard the remaining urine volume.

- Pellet recovery:
  1. Transfer 40 ml of urine biospecimen to 1 labeled 50 ml Falcon tube. Centrifuge the tube at 2500 g for 10 min at 4 ºC to remove any debris.
  2. Discard excess supernatant avoiding pellet collection and make 2 urine pellet aliquots in pre-coded freezing vials (Sarstedt Cat. No.: 72.730.005): Properly seal tubes to get a tight seal.
  3. Store urine pellet aliquots at -70°C.

3.4: Stool

TWO (2) STERILE UNIVERSAL CONTAINERS OF 30 ml (Sarstedt cat no 80.623.022) for Stool.

PATIENTS' – PROTOCOL (PLEASE TRANSLATE THIS TO YOUR LANGUAGE):

1. **General procedure for stool sampling – without stabilizer (Sarstedt cat no 80.623.022)**

a) Stool sample collection

You received a small stool collection tube with a brown lid to collect your stool samples. A plastic spoon is attached to the brown lid of the tube. Using this spoon, please collect little portions from two different areas of your stool and transfer this into the tube. Please fill the tube with at least 4 spoons of stool. Please then close the lid.

b) Stool sample collection

Please clean the outside of the stool collection tube if necessary. You can store these tubes overnight in a refrigerator (4°C). **Please do not freeze (<0°C) the stool samples!**

c) Shipment of samples

Please return the filled stool tube back to your clinic within 24h after sample collection.

SITE – PROTOCOL:

Upon arrival of the sample at the site, please directly store it at -70°C

3.5: Saliva

This document details procedures for the collection and preparation of saliva biospecimen for the analysis of microbiome, metabolomics and RNA & protein analyses.

Before the visit, the participants should be advised one (1) hour prior to the biospecimen collection NOT to:

- Brush their teeth or use oral hygiene products
- Smoke
- Eat (including gum)
- Drink (only water is allowed)

For saliva collection, bubble formation should be avoided. The collection tube's opening must never be touched with bare hands. All tubes and collection devices must always be placed on a clean and dry surface

3.5.1: Microbiome

ONE (1) DNA GENOTEK OMNIGENE SALIVA COLLECTION KIT (OME-505).

1. Hand over the Omnigene tube to the donor
2. The donor will:
    a. rinse 2X their mouth with tap water
    b. wait 5' before starting biospecimen collection
    c. remove the cap from the Omnigene saliva collection tube
    d. spit gently their saliva in the tube until a final volume of 2ml has been reached
3. The donor will re-cap the Omnigene tube
4. Place the tube in a box for RT transport (never freezing conditions or T<25°C)
5. Delivery no longer than 7 days after biospecimen collection
6. At processing site, biospecimen can be stored at RT until aliquoting
7. After aliquoting, transfer biospecimen to -70°C.
8. Ship biospecimen on dry ice to the biobank.

3.5.2: metabolomics

ONE (1) SALIMETRICS SALIVA COLLECTION TUBE (CAT NO 5016.04) AND ONE (1) 4ML GRADUATED CRYOVIAL (E.G. SARSTEDT 72.383.992)

1. Hand over the 4ml cryovial and the saliva collection device tube to the donor.

2. The donor will:
    a. Remove the cryovial cap and insert the Salimetrics tube into the cryovial
    b. Rinse 2X their mouth with tap water and immediately start the collection
    c. Let the saliva passively flow into the cryovial, making use of the Salimetrics device as a straw
    d. Stop the collection when a final volume of 2ml has been reached
3. The donor will re-cap the 4ml cryovial tube
4. Place the tube in a transport box containing ice packs (2-8°C)
5. Delivery no longer than 2 hours after biospecimen collection
6. After aliquoting, transfer biospecimen to -70°C.
7. Ship biospecimens on dry ice to the biobank.

3.5.3: RNA & protein

ONE (1) RNAPRO·SAL™ SPLIT BIOSPECIMEN KIT FOR LIQUID BIOPSY (cat no #RPSAL-701).

1. Hand over the RNAPro·Sal Split Biospecimen Kit tube to the donor
2. The donor will:
    a. rinse their mouth with tap water
    b. wait 5' before starting biospecimen collection
    c. attach the two 'Collection Tubes' to the base of the 'Splitting Unit'
    d. attach the 'Compression Tube' into the top end of the 'Splitting Unit' firmly
    e. collect saliva in their mouth
    f. place the tip of the 'Collector' pad where the saliva has been pooled
    g. collect the saliva until the appearance of the 'Biospecimen Volume Adequacy Indicator' (SVAI) will change from white to fully red (the SVAI can be read by removing the collector from the mouth. Collection should resume immediately afterwards)
    h. place the white absorbent pad into the 'Compression Tube', holding the 'Collector' in an upright position
    i. push firmly the plunger downwards to transfer the saliva from the pad into the 'Collection Tubes'
    j. push and hold for 30''
    k. remove gently the 'Collection Tubes' from the 'Splitting Unit'
    l. The donor will re-cap the 'Collection Tubes'
3. Add immediately:
        A) a protein stabilising agent, or
        B) an RNA stabilising agent according to the planned analysis
4. Close tightly the lids of both 'Collection Tubes'
5. Invert each tube 2-3X
6. Allocate the tubes in a box for transport containing ice packs (2-8°C)
7. Delivery no longer than 2 hours after biospecimen collection
8. After aliquoting, transfer biospecimen to -70°C.

9. Ship biospecimen on dry ice to the biobank.

References

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