The use of serum is NOT recommended by the iLS

Lipidomics Sample Collection and Tracking Form for Clinical Study < study ID >

Date, Signature	Patient-ID	Sample-ID[1]
_____	_____	_____

Collection	Date: _____ Time ____:____ *Collection window: preferably between 08:00 and 10:00 h to minimize diurnal variation* Participant fasted[2] (≥ 12 h): ☐ Yes / ☐ No If no, fasted for _____ h
Collection Tubes	1) coagulation enhancer: yes ☐ no ☐ 2)[3] ☐ <Insert specific tube name for serum <XX> mL>
Post-Draw Handling *From draw until centrifugation: max. 120 minutes; Cool at once after clotting is completed, preferably after 30 min, latest after 60 min at 4 °C (cooling rack or in ice water)![5]*	☐ Gently invert tubes 8-10x times ☐ Coagulation time at room temp.: <study specific min. time> [4] ☐ Storage at ~4°C: Time ____:____
Centrifugation	☐ Centrifuge tubes, Time ____:____ < *2,000 – 3,000[6] g* > *for 10 minutes (4 °C recommended)*
After Centrifugation *Total time from centrifugation until freezing < 1 h* *Avoid aspirating to close to the blood coagle (very high risk of contamination)*	☐ Place samples back in ice water or a cooling rack (4°C, from end of spin until transfer) ☐ Process serum into transfer tubes, homogenize, and aliquot: <Serum>: <n> * <y> mL, mark incomplete filling with "<x>" [7]
Storage[8]	☐ If possible: flash-freeze samples with liquid or gaseous N_2 ☐ Store at –80 °C, Time____:____ ☐ If unavoidable, store at –20 °C, Time____:____ *for as short as possible*
Sample Handover *Always ship samples on dry ice* *Notify receiving facility in advance* *Avoid arrival on Fridays or weekends*	☐ Sample handed over on Date_____ Time of arrival ____:____ Delivered by (Name)_____ Handed to (Name)_____ Arrival condition: ☐ frozen, Tempt [°C] _____ ☐ thawed or at room temperature
Notes *(e.g. under-fill, hemolysis, processing delay, check sample labels,…)*	

See backside for footnotes and general notes

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General notes for standard lipidomics* using serum:
Serum is NOT the recommended sample material for lipidomics! Why?
The most concerning aspect is the mandatory clotting process. This aspect is particularly concerning from the following perspective:
A) The sample tube after blood drawing must be allowed to stand at room temperature for at least 25 minutes for proper clotting
B) During the clotting process, the platelets are strongly activated and release lipids of various types (see below)
Important consequence/note: → Findings from studies investigating serum must be verified in plasma for critical lipid classes.

Lipid classes for which it is impossible to obtain valid results in serum:
Endocannabinoids and related compounds, LPA, sphingoid bases
***Most critical lipid classes** affected by mandatory **room temperature exposure** for serum generation:*
FA, LPC, LPE, LPG, LPS
***Lipid classes released by platelets during serum generation (clotting)**:*
- *Cave: platelet number is interindividual variable (e.g. thrombocytopenia, thrombocytosis)!*
- *Lipid release during platelet activation is highly stimulus-dependent. Beyond oxylipins, which play central roles in platelet signal transduction, activation induces the secretion of >2 nmol per 10^9 platelets of several additional lipid classes, including cholesteryl esters, cholesterol, PC, LPC, and PS. Although other lipid classes and species are also liberated or part of vesicles upon activation, their quantities generally remain below 2 nmol per 10^9 platelets (TG, SM, PE-P, PE, Cer).*

****Standard Lipidomics** covers the abundant lipid classes including:*
acylcarnitines, (glycosyl-) ceramides, cholesterol and cholesteryl esters, diglycerides, ether-bound phospholipids (LPC O, LPE O, PC O, PC P, PE O, PE P), free fatty acids, lysophospholipids (LPC, LPE, LPI, LPG), Phospholipids (PC, PE, PI, PG), sphingomyelins, triglycerides

The following parameters should be standardized for each study:
- tourniquet application time & venipuncture site
- brand and type of blood collection tubes and other consumables: ensure consistency across all study sites
- test consumables in advance (chemical resistance, contamination, analytical noise, etc.)
- tube collection order
- uniform sample labels using solvent-resistant cryo-labels (resistant to –80 °C or liquid nitrogen at –196 °C)
- sample mixing and centrifugation protocols (define G-force, temperature, duration, brake use, etc.)
- incident reporting and documentation (underfills, hemolysis, lipemia, events, delays), should be accessible to all study personnel
- for long-term storage (> 10 years), use internal-thread polypropylene cryovials with O-ring screw-caps
- thaw stored samples at 4 °C with standardized mixing; avoid repeated freeze-thaw cycles; mark any refrozen samples

Footnotes:
1) Sample IDs must be unique for each sample, time point and patient. It can also be called kit ID (ID for all materials prepared in package for each individual sampling). IDs should start with a letter and not leading zeros to minimize data entry errors.
2) Fasting means that only water is allowed to be consumed in the period before sample collection.
3) Specify the tube vendor and exact tube name, clotting activator is highly recommended (reduces clotting time), and fill volume in the protocol. Use tubes with ≥ 2 mL except for pediatric studies.
4) Keep clotting time consistent, also between diff. study sites! With common clotting activator coagulation time should be min. 25 min, without it should be min. 60 min. Maximum clotting time (time at room temperature) should be 60 min.
   Insufficient clotting time or premature cooling leads to microclots in the serum (even still present after centrifugation)!
5) Use an ice-water slurry (ice and water in an insulated container, more ice than water) for better and more stable temperature control than ice alone. When available, use specialized cooled storage devices.
6) Choose a fixed centrifugation force between 2000 – 3000 x g.
7) Choose a fixed aliquot volume (100–1000 µL) and set the number of aliquots on the first page, e.g. 3*0.5 mL
   Consider: accidental thawing (especially at low volumes), avoid freeze-thaw cycles, thaw time (especially for large volumes), pipetting precision. Mark incompletely filled tubes.
8) Use a consistent storage protocol. Store aliquots at –80 °C or in liquid nitrogen. Avoid storage at –20 °C due to temperature fluctuations and frequent openings of the freezer that can cause accidental thawing. Check whether the -20°C freezer has a (heating-based) self-defrosting function (regular defrost cycles!).

Example for labels:

blood sampling tube (blue):	study id Sample id 2.7 ml Serum			
aliquots (green):		study id Sample id Serum 500 µL	study id Sample id Serum 500 µL	study id Sample id Serum 500 µL

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