Appendix 2: Biospecimen standard operating procedure (SOP) guidelines:

1. Plasma and Serum

I. Acquisition of Blood Biospecimens

A. For patients with vascular access catheters (arterial line, central venous access devices) that have been placed as part of the patients' routine medical care, venous or arterial blood samples can be collected via these vascular access devices. Beware that certain biomarkers may have different levels depending on arterial vs. venous sampling (e.g., lactate). For patients without vascular access devices, trained personnel should collect blood through standard venipuncture.

B. For most purposes, whole blood will be collected using a vacutainer system. Standard vacutainer tubes for plasma collection may contain different anticoagulants (EDTA, citrate, heparin). It should be noted that if a vacutainer tube contains liquid anticoagulant (typically citrate), the vacutainer should be fully filled, otherwise the presence of liquid anticoagulant may cause differential dilution and alter the concentration of target biomarker.

C. Quantity of blood taken at each collection depends on the total number of serial collections required for your study. Table 2 provides sample guideline on maximum allowable volume of blood draw for research purposes based on the research subject's total body weight and hemoglobin.

D. It should be noted that the use of glass tubes can lead to low values for certain analytes. For the most generalizable purposes, polypropylene vacutainers and subsequent storage tubes are recommended.

II. Local Processing (Serum)

A. Blood samples should be collected in vacutainers that contain no anticoagulant for the processing of serum.

B. Samples should be sat upright at room temperature for 15-20 minutes to allow for clotting. They then should be spun at 3900 RPM at room temperature for 5-7 minutes. The cleared serum should be pipetted and stored in small aliquots.

C. Record the volume of each aliquot.

III. Local Processing (Plasma)

A. Blood samples should be collected in vacutainers that contain EDTA, citrate, or heparin when preparing plasma. Note that the anticoagulant agent may interfere with analyte detection. It is important to select a collection vacutainer that does not contain chemicals that may interfere with the planned biomarker assay. EDTA may be the most versatile anticoagulant as it is less likely to interfere with analyte detection compared with other anticoagulants.

B. Transport the original, unfrozen blood sample to the designated local laboratory as soon as possible. Freezing has significant adverse effects on plasma and its proteomic elements.

C. As soon as possible, samples should be spun at 3900 RPM at room temperature for 10 - 15 minutes. Plasma should be immediately pipetted and stored in small aliquots.

D. Record the volume of each aliquot.

IV. Local Documentation and Storage

A. Appropriate and complete documentation surrounding biospecimen collection, processing, and storage are essential and will influence the quality of research data to be obtained.

B. Bar code identification of samples with an automated date and time stamp is recommended.

C. Samples should be placed in non frost-free freezers at or below -80^{o}C. Frost-free freezers go through freeze-thaw cycles that further damage the specimen.

D. Centers at which samples are stored should institute a back-up plan for freezer failure (e.g., dry ice or liquid nitrogen). An appropriate alarm system to support freezers for longtime storage is essential.

E. An inventory system should be established for tracking provenance of samples, including the time of collection, processing, storage, and quality-control procedures carried out on each sample.

V. Shipping

A. Dry ice or cold packs must accompany the frozen specimen during air shipment. When dry ice is used, the transport time should be minimized given that dry ice sublimates at a rate of 5-10lbs per 24 hours, depending on the insulation of the shipment container.

B. Consult the local agency for proper shipping options and certified transport materials. The International Air Transportantion Association (IATA, International Air Transportation Assosciation Website) and the U.S. Department of Transportation (DOT, United States Department of Transportation Website) have legal requirements governing the packaging, labeling, and shipping of biospecimen.

   b. Category A Infectious Substances are capable of causing permanent disability, life threatening or fatal disease in humans or animals when exposure occurs.

   c. Category B Infectious Substances (also “diagnostic specimens” or “clinical specimens”) are infectious but do not meet the standard for Category A inclusion.

   d. Exempt Patient Specimens have a minimal likelihood of containing pathogens.

D. Temperature loggers can be used to monitor temperature in shipments of samples to provide confirmation and assurance that samples have been maintained at appropriate temperatures.

VI. Central Storage

A. Appropriate and complete documentation surrounding biospecimen collection, processing, storage, and shipping from the individual sites is essential and will influence the quality of the multicenter research data to be obtained.

B. Bar code identification of samples with an automated date and time stamp is recommended.

C. A formal plan for sharing the central biospecimen resource is recommended.

D. The Central Bank should maintain information of laboratories where the samples have been sent to avoid duplicative analyses and inadvertent repetitive reporting of data from the same patient.

E. The Central Bank should also maintain information regarding any stipulations on informed consent for the use of the samples. For example, in some studies participants may provide permission for their samples to be used only for studies on SAH.

2. Cerebrospinal Fluid (CSF)

I. Acquisition of CSF from an external ventricular drain (EVD) or lumbar drain (LD):

A. Document whether CSF drainage is continuous or intermittent (catheter opened only in response to intracranial hypertension). Drainage method has been shown to alter CSF protein concentration (Shore et al, 2004).

B. Recommend the use of full sterile technique for all access to the CSF collection system for CSF sampling.

C. Draw CSF directly from ventriculostomy catheter or sample from other portions of the EVD setup (e.g., below the collection cylinder) for large volume collection.

D. For larger volume collection, can let CSF collect over 30 – 60 mins in the collection cylinder, and then collect the accumulated fluid from below the cylinder, before CSF is drained into the large collection bag. Do not sample from the collection bag as it contains CSF and cell debri over time and may not represent CSF composition surrounding the brain.

E. Target CSF collection within the first 24 hours of admission, recording time of collection from SAH onset and time of day. Ideally, the first collection should be as close to the SAH onset as feasible.

F. If CSF is available through external collection system, recommend repeated sampling for at a minimum frequency of every 24 h for biokinetic studies.

A. Clearly document source of CSF collection (EVD v.s. LD). Note that there may be a 2.5-fold difference in protein concentration in ventricular CSF compared to lumbar CSF (Hühmer et al, 2006). Comparison of CSF from different sources is generally

discouraged. If a comparison must be made, the difference in CSF composition by region should be taken into account in the interpretation of results.

Acquisition of CSF from a Lumbar Puncture (LP)

B. In patients who are unlikely to receive ventriculostomy. CSF can be collected via lumbar puncture (LP) with informed consent. However, comparison with EVD CSF is discouraged given a 2.5-fold lower protein concentration than in LP CSF (Hühmer et al, 2006).
C. Atraumatic spinal needle LP kits should be used to minimize risk of post-LP headache. Draw with a sterile polypropylene syringe, or allow flow under gravity.
D. Target CSF collection within the first 24 hours of admission, recording time of collection from SAH onset and time of day. Ideally, the first collection should be as close to the SAH onset as feasible.
E. Collect 1-mL fractions and place in ice bath, with a maximum of 25 mL per time point. Send the first 2 mL for clinical laboratory analysis. It is important to match fractions when comparing across patients, as protein concentration varies depending on the draw volume (Blennow et al, 1993).
F. Patient should rest in a recumbent position for 1 h post-LP receive liberal fluid intake, and avoid exertion for 24-48 h to minimize risk of headache.

II. Processing and Storage of CSF

A. Transport and process CSF immediately after collection as significant cell lysis contamination will occur within 1 hour.
B. Collect CSF samples into low protein binding polypropylene tubes (e.g., Eppendorf brand LoBind® tubes). Avoid polystyrene and glass tubes, which will result in significant protein loss (Hesse et al, 2000).
C. Centrifuge CSF at 3900 RPM at room temperature for 15 mins. Immediately remove supinant in aliquots in new, low binding tubes. Lable each tube using low-temperature resistant laboratory lables (e.g., Cryo-Babies by USA Scientific). Document number of

aliquots and volume of each aliquot. Immediately place aliquots on dry ice or into -80°C freezer for storage.

D. Additives or preservatives (e.g., protease and phosphatase inhibitors) may be combined with CSF depending on experimental objectives.

E. Store all CSF samples at or below -80°C to minimize proteolytic breakdown (Wagner et al, 2007). Avoid repeated freeze and thawing cycles and storage at -20˚C (Carrette et al, 2005).

Tabel 1 MAXIMUM ALLOWABLE TOTAL BLOOD DRAW VOLUMES

Body Wt (Kg)	Body Wt (lbs)	Total blood volume (mL)	Maximum allowable volume (mL) in one blood draw (= 2.5% of total blood volume)	Maximum volume (clinical + research) (mL) in a 30-day period	Minimum Hgb required at time of blood draw	Minimum Hgb required at time of blood draw if subject has respiratory/CV compromise
1	2.2	100	2.5	5	7.0	9.0-10.0
2	4.4	200	5	10	7.0	9.0-10.0
3	6.3	240	6	12	7.0	9.0-10.0
4	8.8	320	8	16	7.0	9.0-10.0
5	11	400	10	20	7.0	9.0-10.0
6	13.2	480	12	24	7.0	9.0-10.0
7	15.4	560	14	28	7.0	9.0-10.0
8	17.6	640	16	32	7.0	9.0-10.0
9	19.8	720	18	36	7.0	9.0-10.0
10	22	800	20	40	7.0	9.0-10.0
11-15	24-33	880-1200	22-30	44-60	7.0	9.0-10.0
16-20	35-44	1280-1600	32-40	64-80	7.0	9.0-10.0
21-25	46-55	1680-2000	42-50	64-100	7.0	9.0-10.0
26-30	57-66	2080-2400	52-60	104-120	7.0	9.0-10.0
31-35	68-77	2480-2800	62-70	124-140	7.0	9.0-10.0
36-40	79-88	2880-3200	72-80	144-160	7.0	9.0-10.0
41-45	90-99	3280-	82-90	164-180	7.0	9.0-10.0

Body Wt (Kg)	Body Wt (lbs)	Total blood volume (mL)	Maximum allowable volume (mL) in one blood draw (= 2.5% of total blood volume)	Maximum volume (clinical + research) (mL) in a 30-day period	Minimum Hgb required at time of blood draw	Minimum Hgb required at time of blood draw if subject has respiratory/CV compromise
		3600				
46-50	101-110	3680-4000	92-100	184-200	7.0	9.0-10.0
51-55	112-121	4080-4400	102-110	204-220	7.0	9.0-10.0
56-60	123-132	4480-4800	112-120	224-240	7.0	9.0-10.0
61-65	134-143	4880-5200	122-130	244-260	7.0	9.0-10.0
68-70	145-154	5280-5600	132-140	264-280	7.0	9.0-10.0
71-75	156-185	5680-6000	142-150	284-300	7.0	9.0-10.0
76-80	167-176	6080-6400	152-160	304-360	7.0	9.0-10.0
81-85	178-187	6480-6800	162-170	324-340	7.0	9.0-10.0
86-90	189-198	6880-7200	172-180	344-360	7.0	9.0-10.0
91-95	200-209	7280-7600	182-190	364-380	7.0	9.0-10.0
96-100	211-220	7680-8000	192-200	384-400	7.0	9.0-10.0

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