SPAN STANDARD EXPERIMENT

#1	LAB INFORMATION

Report Title	SPAN: MRI Pipeline Validation
Report Author	Patrick Lyden Cenk Ayata
Dates of Experiment	March 2021-June 2021
Responsible Person(s)	Patrick Lyden Cenk Ayata Ryan Cabeen
Location of Experiment	At Sites

#2	Purpose

The Stage 1 MRI pipeline yields several morphometric measures, including total cerebral volume, lesion volume, ventricular volume, and midline shift. The values obtain from each time point scan will be compared to:

Chronic Lesion comparison: Day 29 scan will be compared to morphometric values obtained from Cresyl Violet stained sections from Stage 1 banked brain tissue.

Acute Lesion comparison: TTC imaged sections obtained immediately after Day 2 MRI scan from 4 young mice (60 min MCAo) at each site. Each site performs 4 animals =total sample size is 24.

#3	REFERENCES TO OTHER SOPS

SPAN SOP 6 Mouse Middle Cerebral Occlusion
SPAN SOP 17 Reperfusion
SPAN SOP 35 MRI Acquisition Stage 1
SPAN SOP 45 Tissue Banking
SPAN SOP 59 TTC Staining

#4	METHODS

Chronic Lesion (Stage 1 Day 30 Scans vs Cresyl violet)	Notes/Observations
1. CC will select brains from tissue bank to cover a range of lesion sizes. Selection will be made via LONI image analyst (Ryan Cabeen) 2. Sites will perform Histology for total n=20 mouse brains, approximately n=3-4 per site 3. 25 micron thick, 2 sections taken every 500 microns, exhaustive sectioning from frontal pole to midbrain 4. Cresyl violet staining 5. Mount/cover slip/image 6. Using Image J or other software, delineate area of hemispheres (ipsi and contra), ventricles and visible lesion.	Retrospective analysis of Stage 1 subjects already obtained. **SPAN SOP 45** Tissue Banking **Note:** For scanned images **Do Not** have the lesion/hemispheric outlines permanently embedded on the images
Acute Lesion (Day 2 scans vs TTC)	**Notes/Observations**
1. Each site to perform 60 min. right side MCAo on **n=4** young mice. 2. Perform 48-hour MRI followed **immediately** (or within 12 hrs. of scan) with sacrifice and TTC staining. 3. Follow SOP 59 for TTC Staining procedure. 4. Upload the D2 MRI Scan in IDA with the visit code: "**MRI Validation D2**". Email the CC spancc@usc.edu when files have been uploaded. Include the following details in your email: ☐ Animal ID ☐ Date of Scan	**SPAN SOP 6** Mouse Middle Cerebral Occlusion **SPAN SOP 17** Reperfusion **SPAN SOP 35** MRI Acquisition Stage 1 **SPAN SOP 59** TTC Staining Note: if sites have an existing TTC protocol in current use, they may use their own protocol

Data Transfer	
1. Sites will transcribe data onto a CSV 2. Send CSV upon completion to the Coordinating Center (spancc@usc.edu) 3. Include images of slides with the CSV	**Note:** For scanned images **Do Not** have the lesion/hemispheric outlines permanently embedded on the images
Power Analysis	
1. Brain and lesion volumes will be compared MRI vs histology using Pearson's or Spearman's correlation coefficient. 2. Power analysis with power 80%, alpha 0.05, with n = 20 gives sufficient sample size to detect whether the correlation coefficient is greater than 0.97	

#4	**RESULTS**
#5	**LESSONS LEARNED/ NEXT STEPS**