AAPS Interlaboratory Study-Instructions

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1. Overall Objective

The goal of this study is to characterize protein degradants of two stressed monoclonal antibodies, an IgG1 and an IgG2, in multiple labs using particle counting/characterization, liquid chromatography, mass spectroscopy, electrophoresis, and spectroscopy tools and examine assay variability. The originator labs will generate and distribute the samples and provide a harmonized set of analytical protocols to the participants.

2. Participants' Roles

Participants will receive samples of unstressed and stressed mAbs supplied by Amgen (Shipment #1) and NIST (Shipment #2), aliquot them based on the methods their organization will perform for Shipment #1 samples or analyze them directly for Shipment #2 samples. They will provide the results back to NIST using the data reporting templates provided. NIST will anonymize the data and assess variability of the data among the labs.

Participant responsibilities:

1. Participants should read and understand the goals of the study. Please reach out to Srivalli Telikepalli (aapsinterlab@nist.gov) for any questions or clarifications.

2. Upon receival of the two packages, notify Srivalli Telikepalli (aapsinterlab@nist.gov) and Kimya Nourbakhsh (at knourbak@amgen.com) the condition of the packages and samples (thawed, broken, leaking vials, etc.) when received. Shipment #1 will arrive from Amgen and Shipment #2 will arrive from NIST.

3. If the samples are received in good condition, participants can thaw and aliquot the samples (as shown in Figure 1) or directly analyze the samples as appropriate for the sample type. There are two sets of samples, and the working lifetime of the samples when thawed is limited—please read the instructions carefully before aliquoting and planning your measurements.

4. Participants will evaluate the samples by multiple methods, (see Table 1 for the methods for each lab), as previously determined by their organization/PI. They will be requested to follow the method protocols supplied to them (if available) and record method parameters and results as fully as possible in the accompanying data collection templates.

5. Once all the data has been collected, finalized, and reviewed, 1 participant from each lab can collate the data collection sheets for all the methods and email them to aapsinterlab@nist.gov. It is strongly recommend that the data entry is cross-checked by a

second person before submitting the data. The data will be anonymized after it has been received by NIST. Data should be returned **3 months** after receipt of the samples.

6. Participants are requested to **retain all raw data** for the duration of this study.

7. Some participants have volunteered to be the subject matter experts (SME) for specific methods (see the bottom of each protocol). Those participants may be contacted if a question arises regarding a specific method. Anonymized questions and SME answers will be distributed to all participants.

3. Important Considerations When Planning Experiments

1. **Samples are time sensitive**. Time the data acquisition accordingly so that once samples are thawed, they can be immediately aliquoted or analyzed. It is important to set up the instrument and perform any required control measurements before the samples are thawed. In general, to save time, run all buffers and controls before the protein samples are fully thawed. Record all method parameters and data in the accompanying data collection templates.
2. **Perform Tier 1 methods first.** Tier 1 methods are the top priority of the study. Complete the Tier 2 methods once all the Tier 1 data has been acquired successfully.
3. **Balance guidelines provided in protocols with general, routine practices in your organization.** If any step in the protocols is inconsistent with your routine practices, reach out to aapsinterlab@nist.gov. Remember, the protocols are not meant to be Standard Operating Procedures but aim at aligning key measurement parameters and data output while allowing the researcher to follow their routine workflow and operate with compatible instrumentation available in their laboratory.

Table 1: Participating labs and the methods they will complete. The first row shows the number of replicates suggested for each method. The approximate amount of material allotted (in µL) is shown for each method and per lab (for a 1 mg/mL mAb). All methods will be run on unstressed and stressed material; CG-MALS, DSC, and DSF will only be performed on unstressed material.

	Tier 1. Highest Priority Methods											Tier 2: Sizing/Characterization									
	VI	DLS	LO	MFI	FlowCam	(SEC)/SEC-MALS	NTA	UV-Vis	Near UV CD	FTIR	rCE-SDS and/or nrCE-SDS	cIEF or IEC	batch SLS	AF4/H F5	Intrinsic fluor.	Extrinsic fluor.	RMM	SV-AUC	CG-MALS	DSC	DSF
# of replicates	1	3	2	3	3	3	3	1	1	1	1	3	3	3	1	1	3	1	1	1	1
Participating Labs																					
AstraZeneca	1 vial	1500	150	180	180	750	75		1000								75			500	100
Amgen	1 vial	1500	150			750	75	500	1000	300		1500								500	100
BMS	1 vial	1500	150	180		750		500	1000	300								1500		1000	
Compassion					180								3000								100
Coriolis	1 vial	1500	150	180	180	750	75	500	1000	300	600	900		1500			75	1000		400	100
FDA		1500			180								3000								
GSK		1500			180	750			1000					1500			75				
Lonza	1 vial	1500	150	180	180	750	75				600			1500							
Merck	1 vial	1500	150	180		750			1000			900			500					100	100
NIST	1 vial	1500	150	180	180	750	75	500		300	600	900		1500			75			500	
Novartis	1 vial	1500	150	180	180		75										75				
Pfizer			150	180						300											
Sanofi-Aventis	1 vial	1500	150	180		750	75	500			600	900									100
Leiden University		1500	150	180	180	750	75	500	1000					1500	500	500	75				
KBI Biopharma	1 vial	1500	150	180		750	75					600					75	1000		500	
Univ of Colorado		1500		180	180		75														
Univ of Copenhagen								500	1000	300						500				1000	
Wyatt		1500				750							1500	1500					5000		

Abbreviations: VI - visual inspection; LO – light obscuration; NTA – nanoparticle particle tracking analysis

4. Samples

Participants will receive 2 separate shipments of samples for analysis; both shipments will be sent frozen. Please store at −70 °C or below upon receipt.

❖ Shipment # 1

Shipment 1 will contain approximately 14 vials (some labs might receive more vials depending on how much material they initially requested) containing unstressed and stressed NISTmAb IgG1 and Amgen IgG2 (see Table 2) along with corresponding buffers. Each sample type will have 2 vials, filled with nominally 0.2 mL to 4.5 mL of sample in a 5 mL cryovial. The 2 vials will be for Tier 1 methods (highest priority methods) or for Tier 2 and other methods (see Table 1 for the methods that fall under the Tier 1 or Tier 2 categories). The values in Table 1 represents the maximum amount of material allotted for each method. These vials will be labeled with the following information: protein, type of stress, Tier #, nominal volume, nominal concentration of 1 mg/mL, and lab name.

Table 2: Samples in Shipment 1

NISTmAb	Amgen mAb
Buffer	Buffer
Unstressed, Tier 1	Unstressed, Tier 1
Unstressed, Tier 2	Unstressed, Tier 2
Stir-Tier 1	Stir-Tier 1
Stir-Tier 2	Stir-Tier 2
Light-Tier 1	Light-Tier 1
Light-Tier 2	Light-Tier 2

❖ Shipment # 2

The second shipment will contain **a second, prediluted set of stir stressed samples only** for the Amgen mAb and NISTmAb along with 8 sterile 2 mL glass vials and stoppers. Ten 15 mL conical tubes will contain approximately 5 mL of prediluted (100 fold diluted) stir stressed samples (5 tubes for stirred NISTmAb and 5 tubes for stirred Amgen mAb). These samples are **intended to be used primarily for the Tier 1 particle methods** (visual inspection, light obscuration, 2 types of flow imaging, nanoparticle tracking analysis). They can also be used for RMM, if selected by labs (see Table 1 above). These vials will be labeled with the protein, type of stress, 100-fold dilution, methods it can be used for, preparation date, and nominal volume of 5 mL.

Participants may perform multiple assays from the same thawed vial (i.e. perform flow imaging simultaneously as light obscuration) or thaw multiple vials for multiple assays, but this needs to be coordinated based on analyst and instrument availability and the general practices of the lab. Regardless, because of the time sensitivity of the samples, analysts should consider time as an important factor when performing these particle measurements on these samples.

Analysts will need to prepare samples for visual inspection using the 2 mL vials provided.

5. Formulations

- Buffers supplied have been filtered through 0.22 µm PVDF filters prior to being filled in clean vials. The participants are encouraged to re-filter the buffers prior to use.
- The NISTmAb formulation buffer is composed of 25 mM histidine buffer, pH 6.
- The Amgen mAb dilution buffer is composed of 10 mM Acetate buffer, pH 5.

6. Aliquoting of Samples

Shipment 1 - After receiving the 1st shipment, thaw the Tier 1 and Tier 2 samples by placing the vials upright at room temperature for no more than 30 minutes, aliquot, and refreeze. Aliquot the samples in a laminar airflow hood according to the scheme shown in Figure 1 based on the assays being performed by your lab (see Table 1).

Important notes regarding Figure 1:

1) Some participants will receive vials that are filled with 4.5 mL sample in a 5 mL capacity vial. Quick introduction of the pipet into these filled vials will cause the sample to spill over so be careful when inserting the pipet into these vials for mixing. Be sure to depress the plunger while placing it into the vial so the sample can be pipetted up just as the pipet tip goes into the vial.
2) Remember to thaw the samples right before aliquoting or before analysis because of the tendency of the particles to reverse. Samples sitting in the formulation buffer for more than 2 hours reverse, resulting in a decrease in particle concentration.
3) A proper mixing of the samples prior to aliquoting or analyzing is important to eliminate particle clumping. To "mix" the sample, do the following: gently invert vial 10 times, pipet with 1 mL tip (or 200 uL tip if removing the sample from a 1 mL vial) near the bottom of the sample tube 10 times (see note #1) in different directions but not touching the bottom or creating bubbles. Remove sample from near the bottom of the vial, from about ¾ of the depth of the tube from the top of the liquid before dispensing the sample into another container.

Shipment 2 - The samples in the 2nd shipment **do not need** to be aliquoted and should remain frozen until ready for analysis. Prior to analysis, these samples should be mixed in the same way as described above in #3.

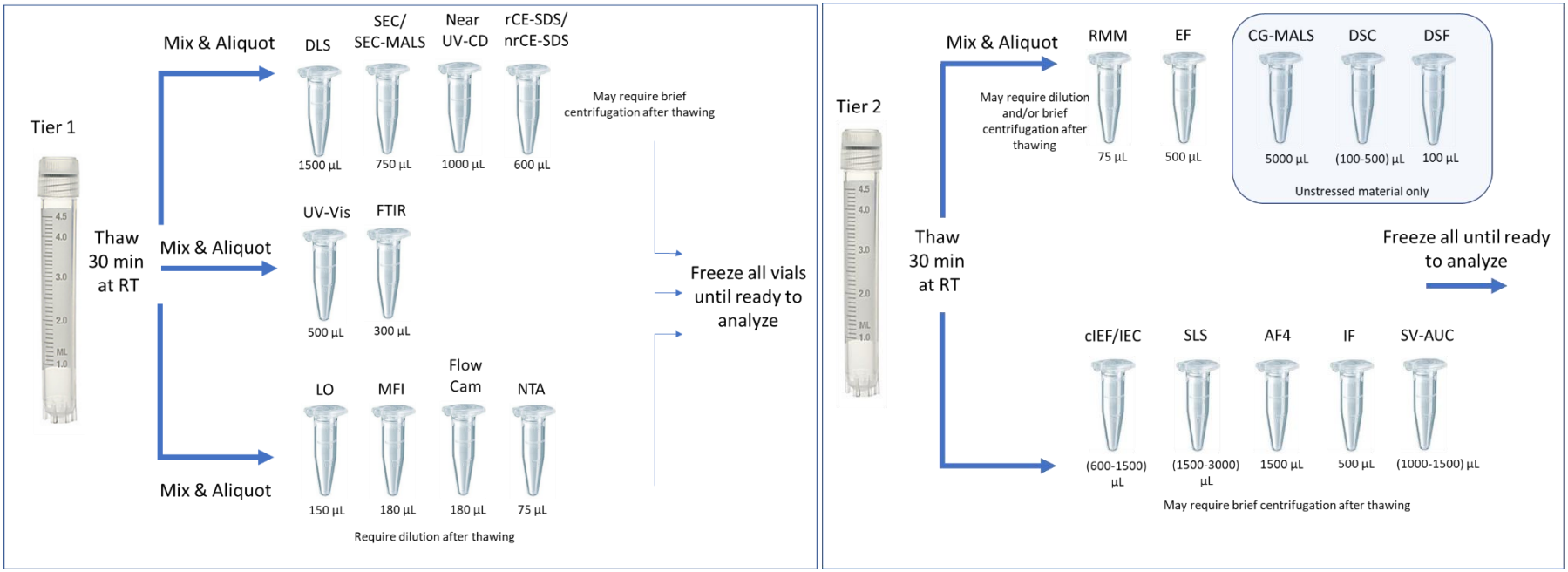

Figure 1. Aliquoting scheme for Tier 1 and Tier 2 vials from Shipment 1. The approximate volume of the sample to be aliquoted for each assay is shown below each method (the range shown for some methods are provided by the labs, see Table 1). CG-MALS, DSC, and DSF will only be performed on unstressed material. Based on the sample and method, some aliquots may require dilution (100 fold) or may require a brief centrifugation after thawing for analysis.

Abbreviations: LO – light obscuration; VI – visual inspection; IF – intrinsic fluorescence; EF – extrinsic fluorescence.

7. Data Collection

Follow the method protocols, if available, to collect the data. Perform Tier 1 methods first with the sample allotted for Tier 1, then follow with Tier 2 methods. Measure both aliquots from Shipment 1 and samples from Shipment 2. Shipment 2 samples should only be analyzed with the Tier 1 particle methods (light obscuration, Microflow Imaging, Flow Imaging, Nanoparticle Tracking Analysis, and RMM, if selected).

8. Data Reporting

Report all data in the appropriate template accompanying the method, if available. Include method parameters and any notes for future reference. If a template is not available, record the measurement parameters and the final, analyzed data on an Excel file.

In general, save all raw data files in the following format, for later retrieval, if necessary:

"Protein_Stress_Method_Replicate#_Date"
Ex: NISTmAb_Stir_DLS_3_21082023

Return all completed Excel files saved in the following format:

"Lab_Method_Date"
Ex: NIST_DLS_21082023

Perform all routine data analysis (i.e. averaging, standard deviation, etc.) and include the **final data** in the templates. Controls and standards should be run to ensure optimum performance of the instrument but there is no need to share the data. **Please retain all raw data for the duration of this study.** It is strongly recommended that a second analyst cross-check data entered into the templates before submitting any data.

Data is due **three months** after receipt of the material. Submit all data to Srivalli Telikepalli at aapsinterlab@nist.gov.