LOVELACE BIOMEDICAL

Collection and Detection Strategies for Inhaled Biologics

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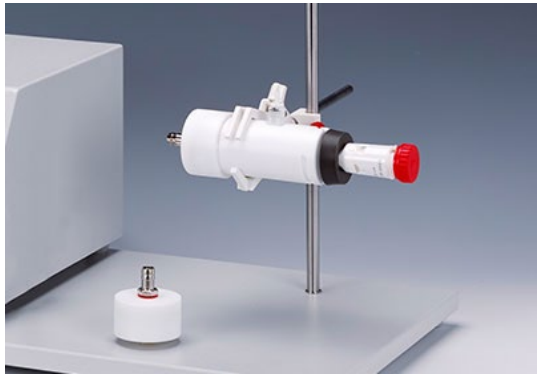
Senior Scientist

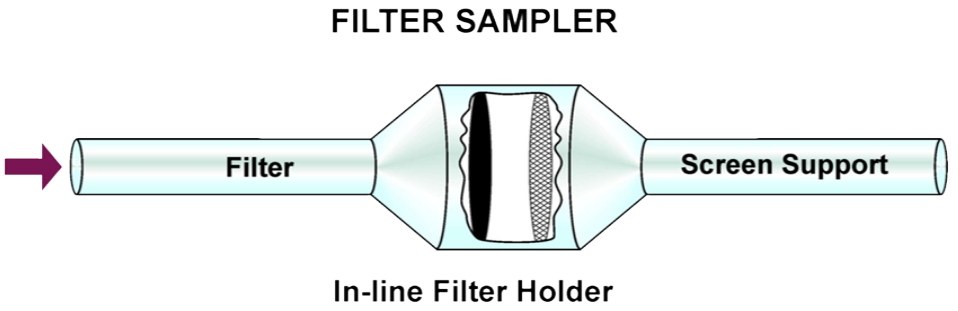
Collection and Detection Strategies

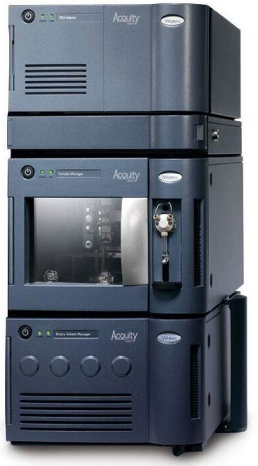
- Goal is to link between the preclinical toxicology presentation from Emily Resseguie and the analysis / quantification methods that Chris will present
- Give an overview of some of the things that have been evaluated
- Present data on some examples that span the range of inhaled biologic molecules

Classical Aerosol Concentration/Dose

- Focus on API collected
  - Extract filter in suitable solvent
  - Analysis is focused on amount of API / excipients collected
  - Often utilize HPLC/UPLC-UV analysis of a filter media
  - Fit for purpose is based on chemical stability and recovery of target compounds from the filter

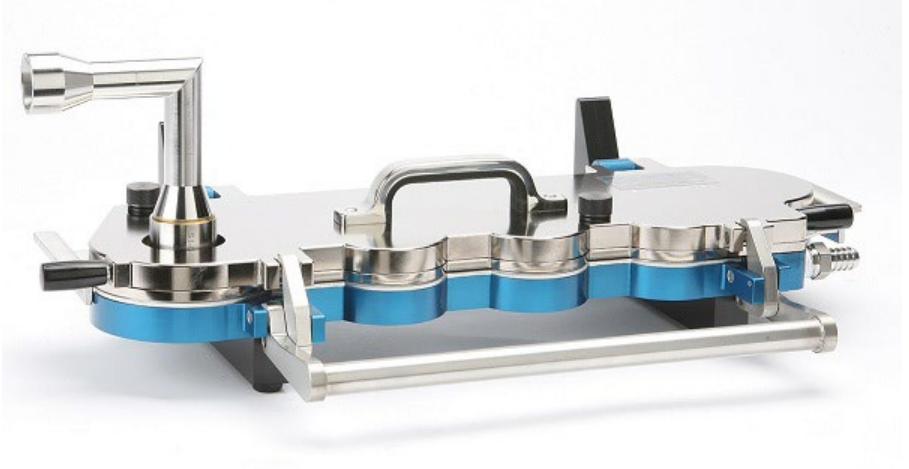






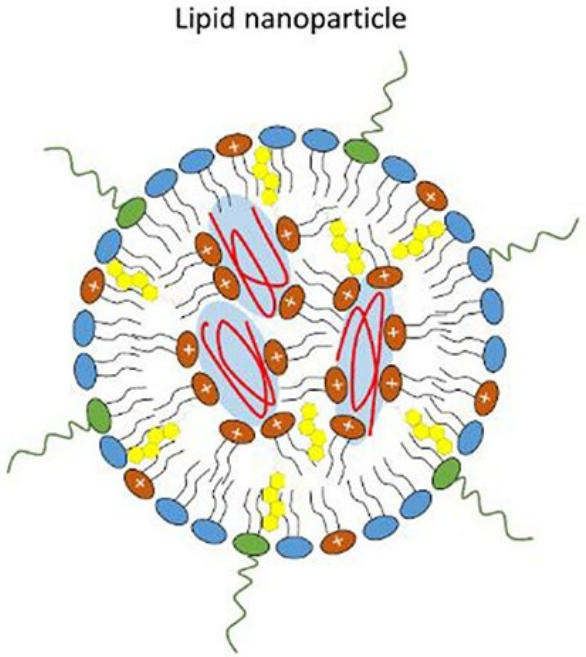
Classical Particle Size Distribution

- Typically focused on the API and sometimes includes mass
- Typically utilize inertial impaction, sometimes consider light scattering or other devices



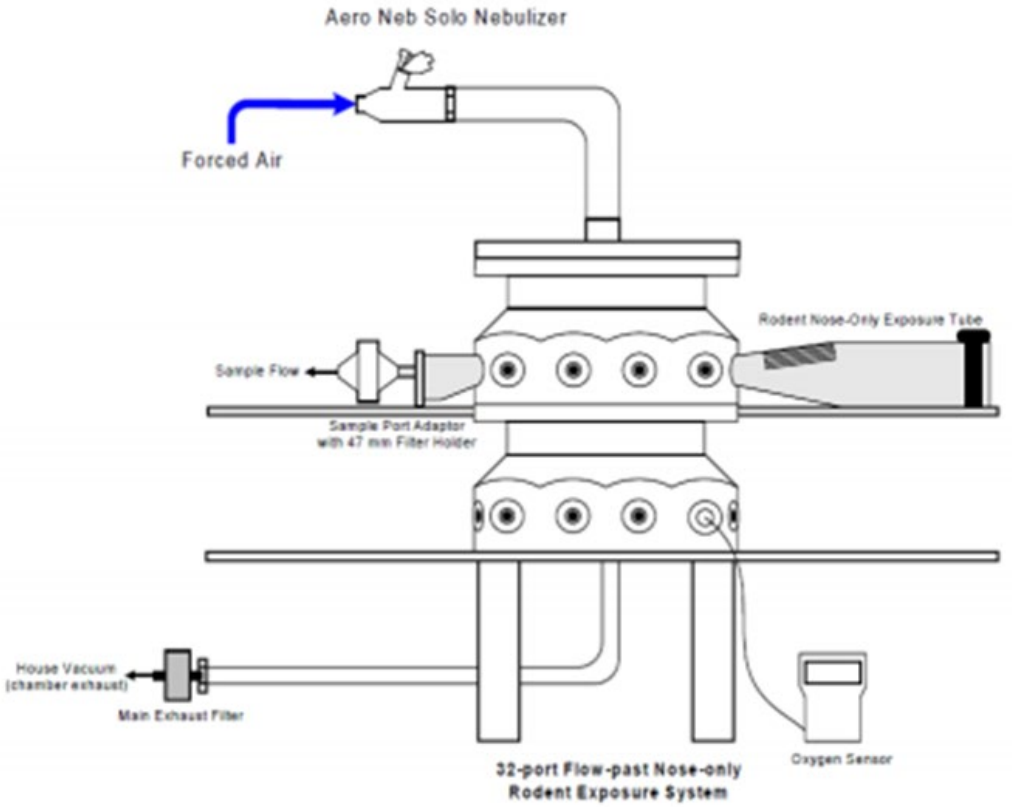
How does this change for a biologic?

- Biologics require
  - Aerosol concentration / dose
  - Particle size
  - Activity
  - Integrity
  - Encapsulation
- Often the assay(s) often change
  - Listen to Chris' talk!



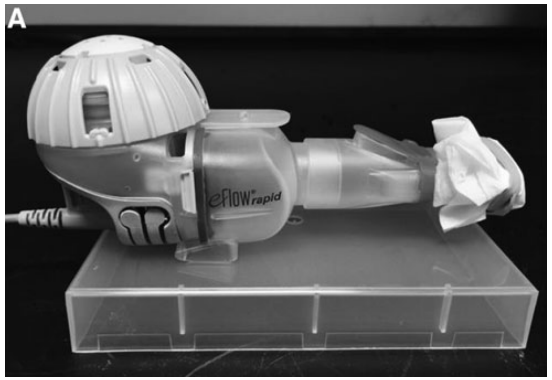
Aerosol Characterization

- Standard inhalation exposure system
- Highlight collection and analysis for each endpoint
- Each of these parallels collection for general aerosol characterization



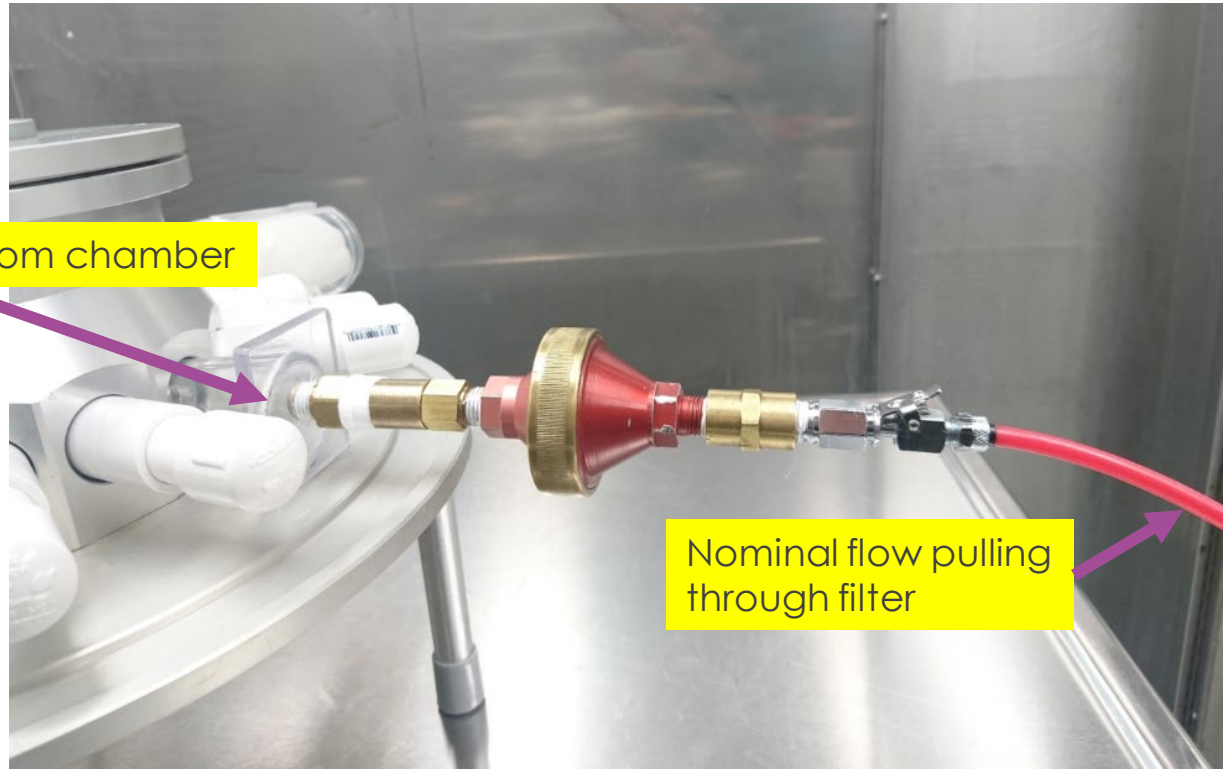
Aerosol Concentration/Dose

- Inhaled biologics may require different collection methods based o extraction / assay / artifacts that impact analysis
- Collection methods require modification/adaption to ensure no artifacts of collection in final results
- Things to consider:
  - Liquid collection – impinger, biosampler, etc.
  - Condensation – Cipolla et. al. 2015 or similar methods
  - Others – don't be afraid to try something new!



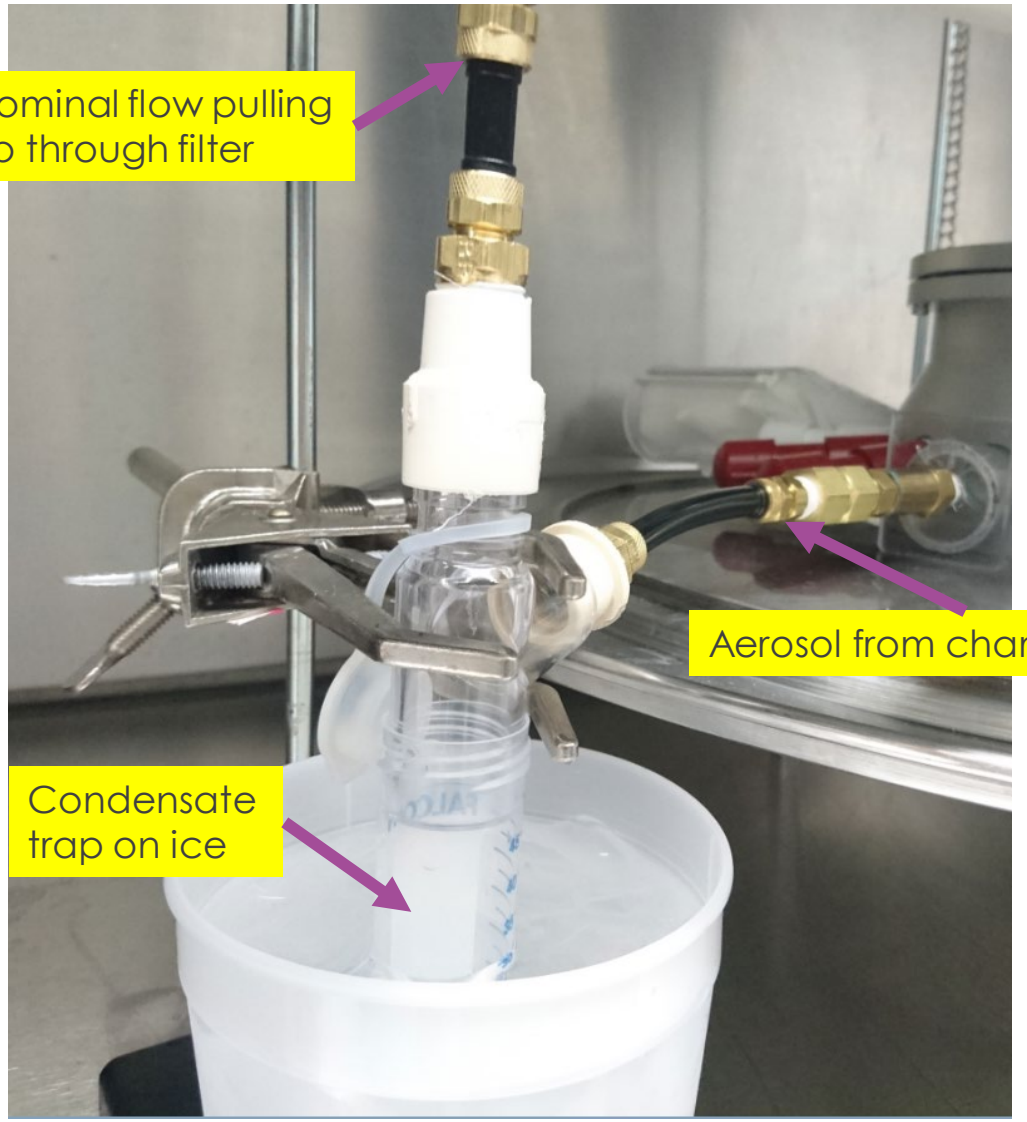
Aerosol Concentration

- Traditional method to collect at breathing zone
- Aerosol on filter



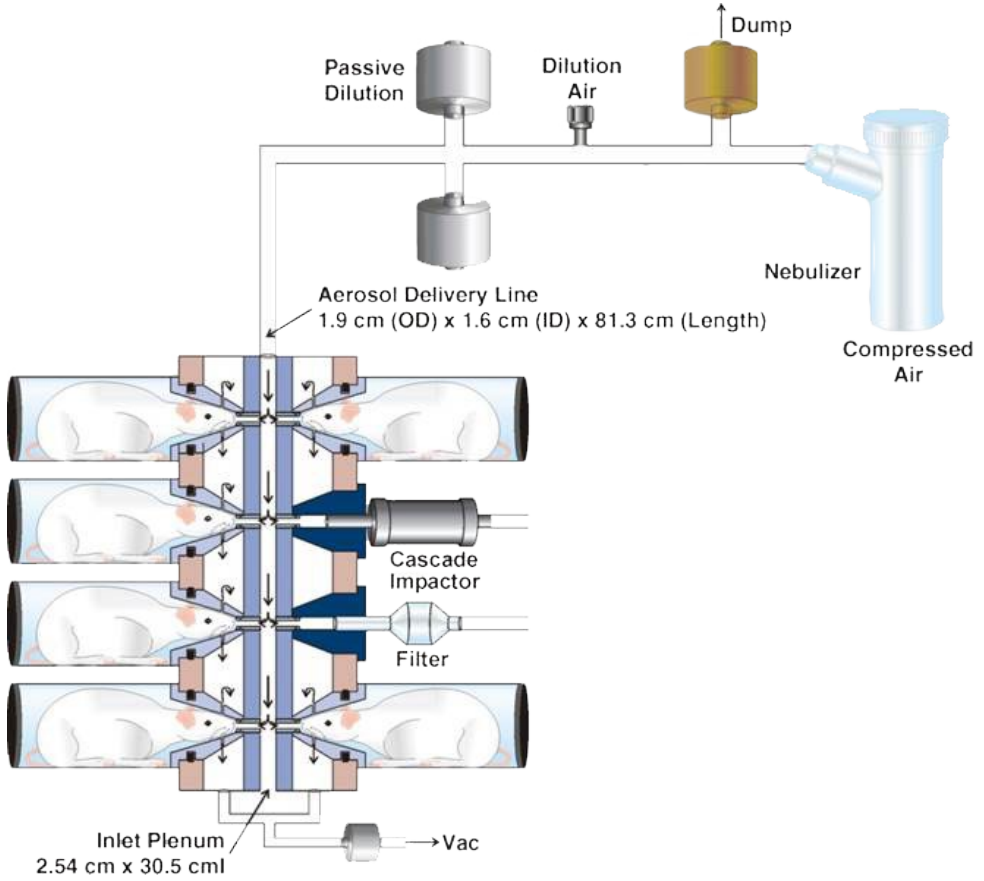
Aerosol Concentration

- Similar collection at the breathing zone
- Collection via a liquid collection system



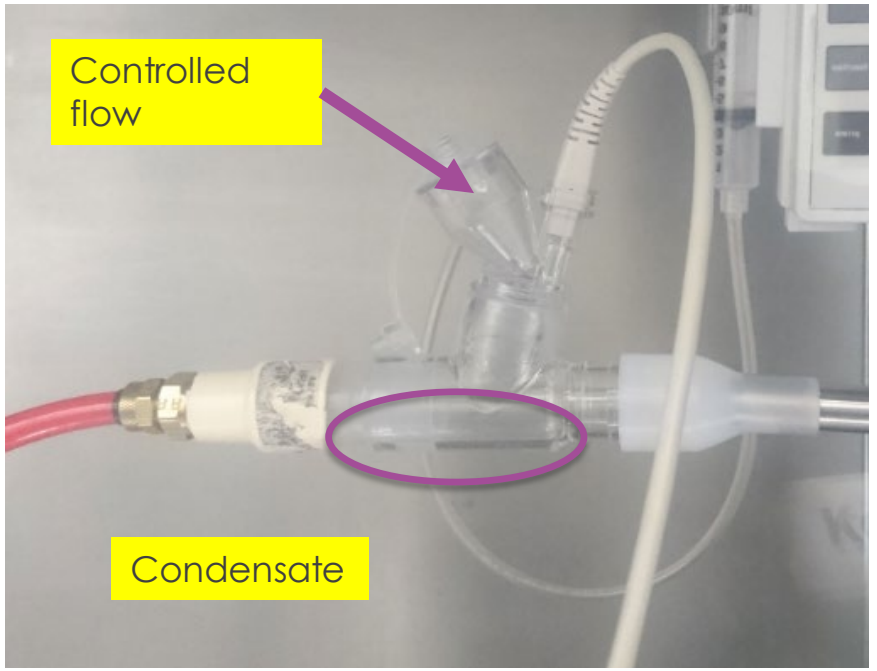
Particle Size

- Similar collection at the breathing zone
- Collect with appropriate device (impactor)
  - Assay by chemical analysis and/or differential mass



Integrity/Activity/Encapulation

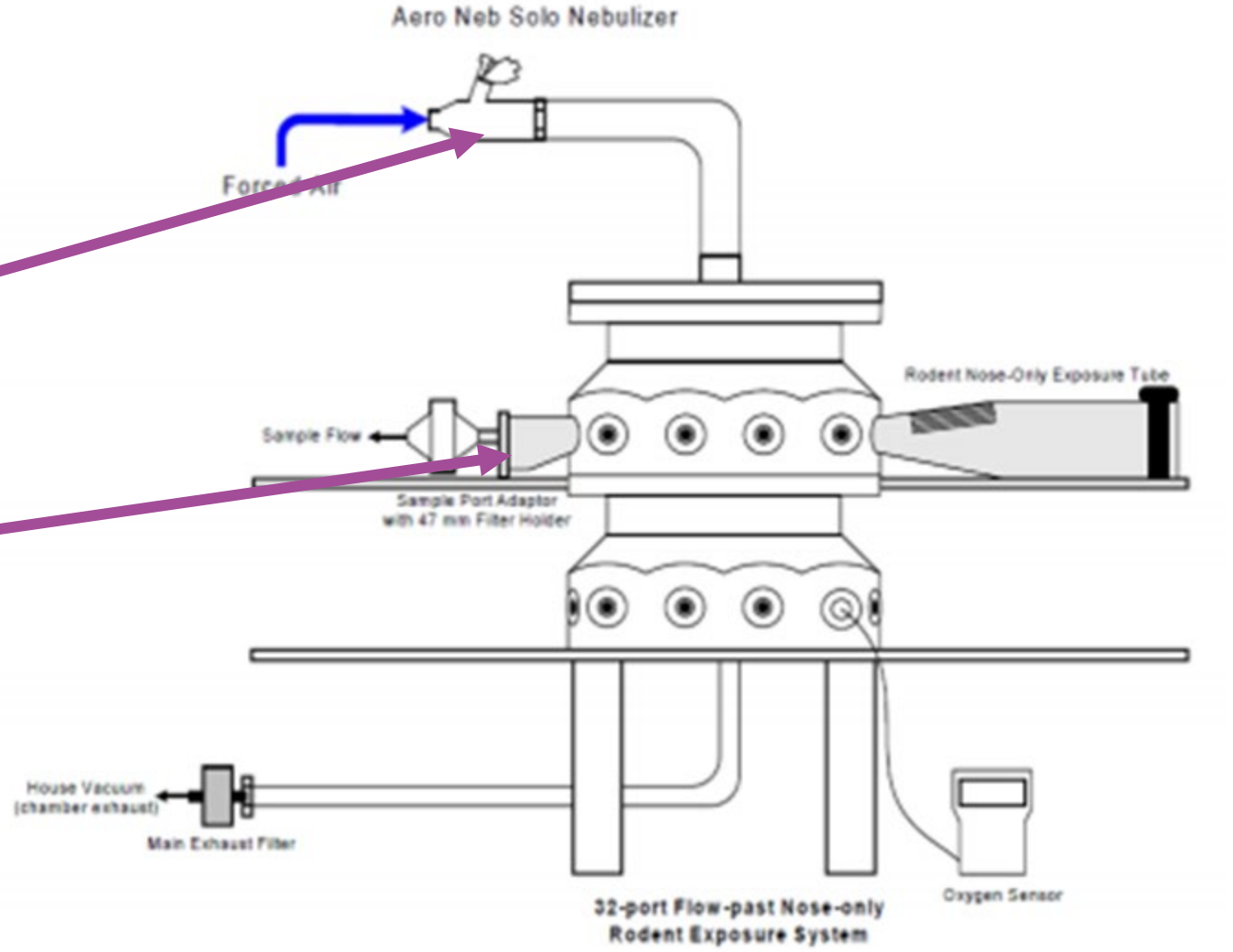
- Don't be afraid to try something!
  - This was a real struggle on initial programs
- Condensate in the generation line for aerosol entrainment



Collection Approach

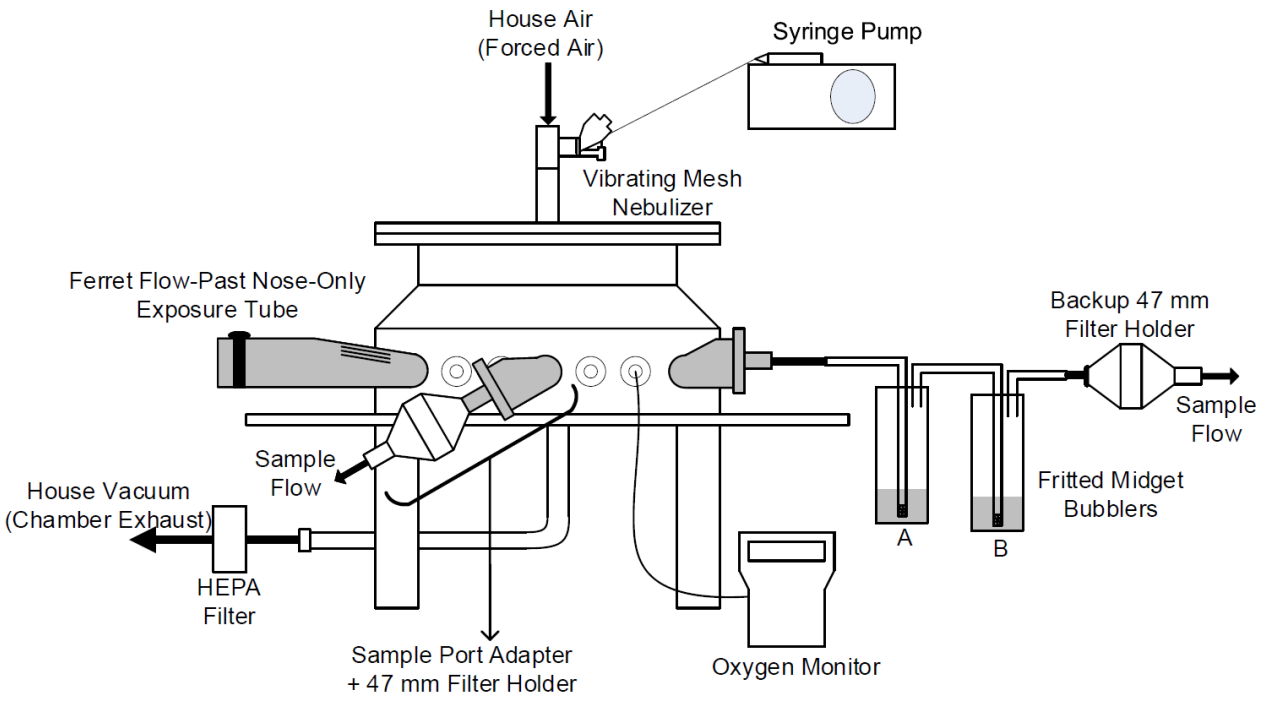
Condensate post-nebulizer

Aerosol concentration at breathing zone



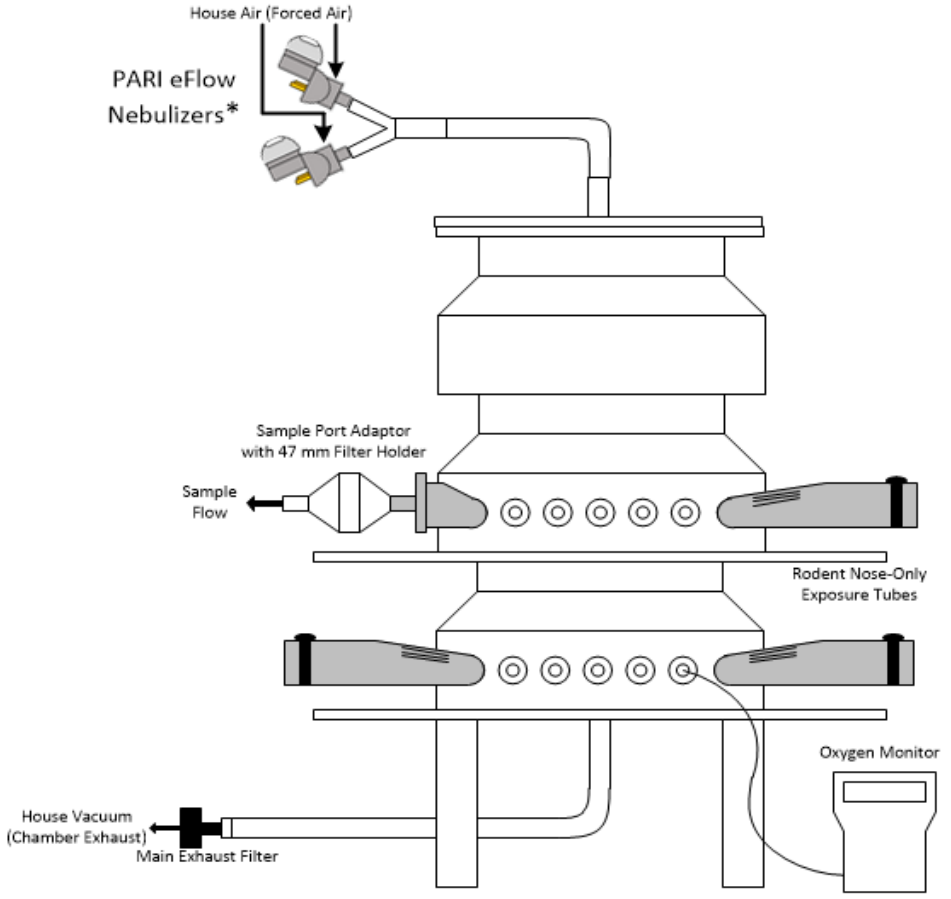
Example Novel AAV with mRNA

- Vibrating mesh nebulizer
- Mass aerosol concentration via filter sample
  - Total aerosol concentration
- Genomic titers via glass frit impingers with ddPCR analysis
  - ddPCR and PCR analysis
- Integrity, aggregation and infectivity via condensate
  - Cell based activity
  - Light scattering for aggregation



Example LNP with mRNA

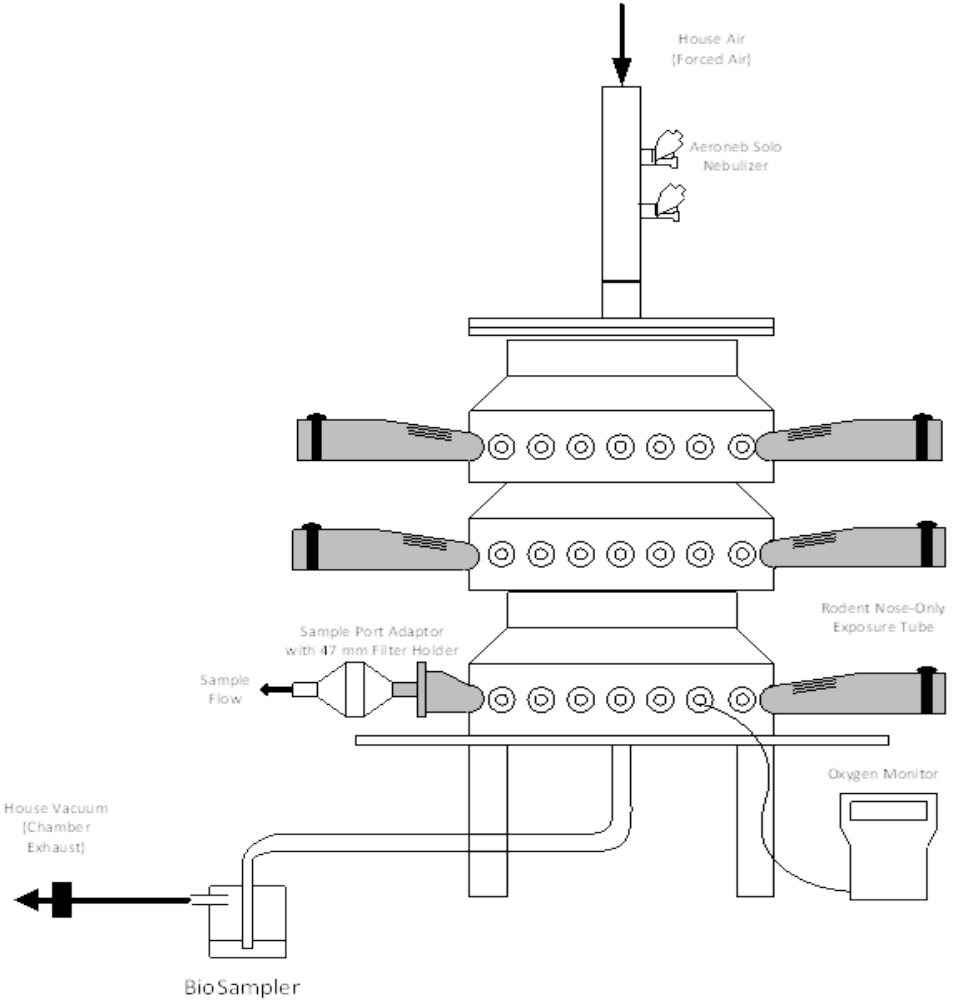
- Vibrating mesh nebulizer
- Standard filters collected for mass aerosol concentration
- Novel filters collected for analysis of mRNA and lipid analysis
- Condensate collected in generation line for activity and encapsulation efficiency



* Nebulizers are in same horizontal plane and are parallel to delivery line.

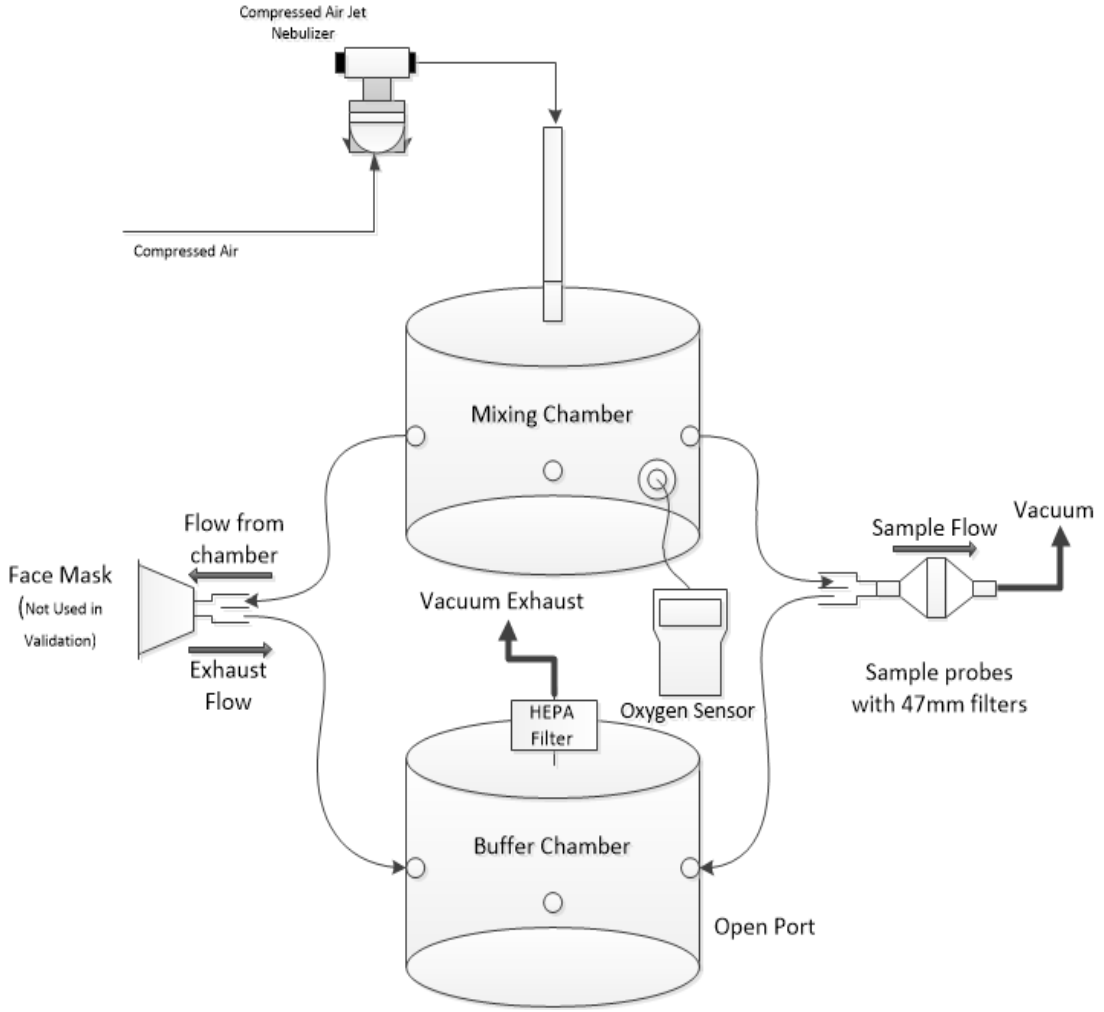
Example Novel Viral Vector

- Vibrating mesh nebulizer
- Standard filters collected for mass aerosol concentration
- Biosampler collected in exhaust for PCR analysis of viral genomes



Example Oligo

- Compressed air jet nebulizer
- Mass aerosol concentration via filter sample
- Oligo aerosol concentration from filter with HPLC-UV assay



FDA Expectations

- Novel AAV based payload for CFTR
- Vibrating mesh nebulizer
- Mass aerosol concentration via filter sample
- Genomic titers via glass frit impingers with ddPCR analysis
- Integrity, aggregation and infectivity via condensate

- Validated dose formulation analysis method – ddPCR
- Validated concentration collection and quantification methods – PCR, SEC-HPLC, ddPCR
- Validated particle size methods
- Scientifically sound integrity, aggregation and infectivity assays

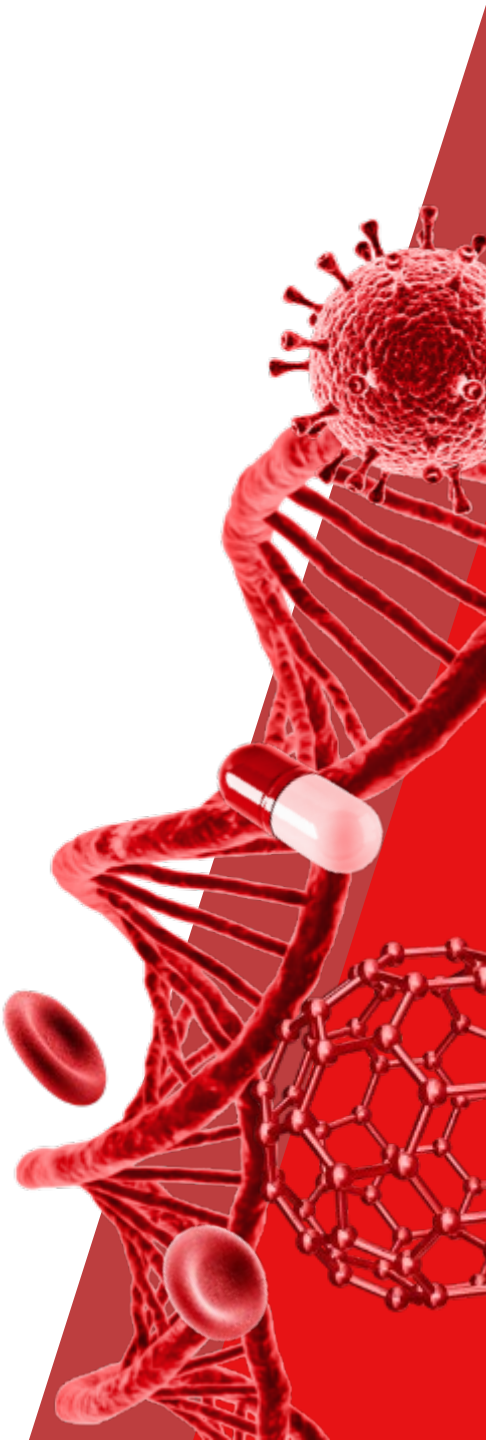
Inhaled Biologics – Opportunities

- The field of inhalation biologics will continue to expand:
  - Compound classes
  - Disease indications
  - Patient populations
- Define the best science
  - When possible publish/present the success stories and the 'lessons learned'
- Adapt and evolve methods, guidance documents and instrumentation to support new API's and formulations

ThermoFisher SCIENTIFIC

Detection Schemes for Inhaled Biologics

Christopher J. Gruenloh, Ph.D.
Research Fellow
September 5, 2024



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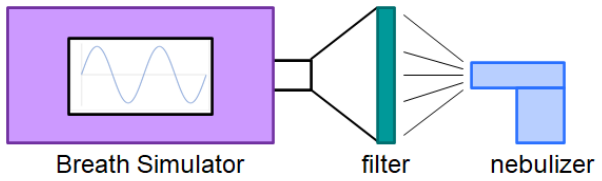
Starting point

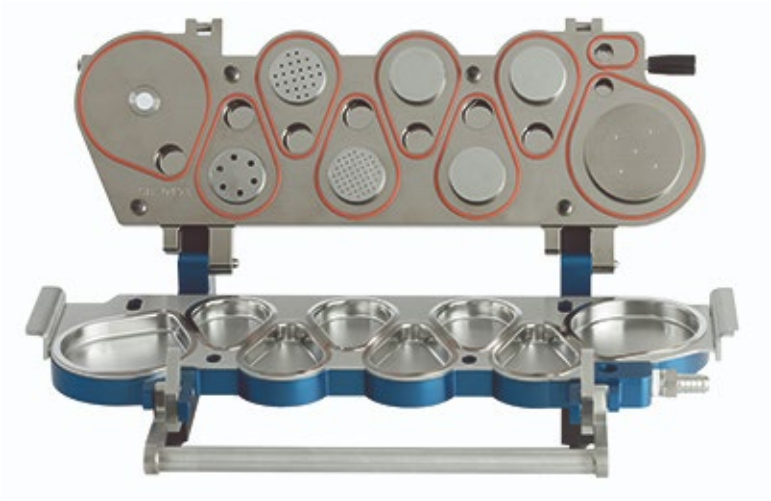
Pickup where Philip left off…focus on detection schemes for aerosol performance testing

Questions

- What is the “active” and how is it formulated?
- Is it a molecule, a particle, a living cell or some constituent thereof?
- What detection schemes can be used?
- What detection schemes should be used?

From sample to results in the GMP world





Sample Handling	2 parts: a) analyst safety and b) how not to change what you're trying to measure → sometimes competing factors
DDU/APSD Collections	Does the device or test apparatus impact what is being measured?
Recovery	Can you get the sample out of the DUSA, filter or sample cup/plate?
Detection	**What is the most appropriate measure? Content (of active or delivery system) or activity?**

Perceived Analytical Complexity

- synthetic peptides and oligonucleotides
- antibodies and fragments (e.g., Fabs)
- mRNA (often encapsulated in LNPs)
- viruses: bacteriophages, viral vectors
- cells (e.g., stem) and cellular mixtures

Attributes of an ideal detection method for DDU and APSD

Analytical Merits

- ❑ Specificity
- ❑ Dynamic range (esp. for APSD, ~3 orders of magnitude)
- ❑ Precision
- ❑ Fast / High Throughput *

Low Cost per Sample

- ❑ Instrumentation
- ❑ Reference Standards (if required)
- ❑ Reagents/kits
- ❑ Effort to develop/validate method
- ❑ Effort for routine sample preparation/analysis

High throughput detection: a critical attribute

Assume nebulized delivery of 8 mL sample

DDU

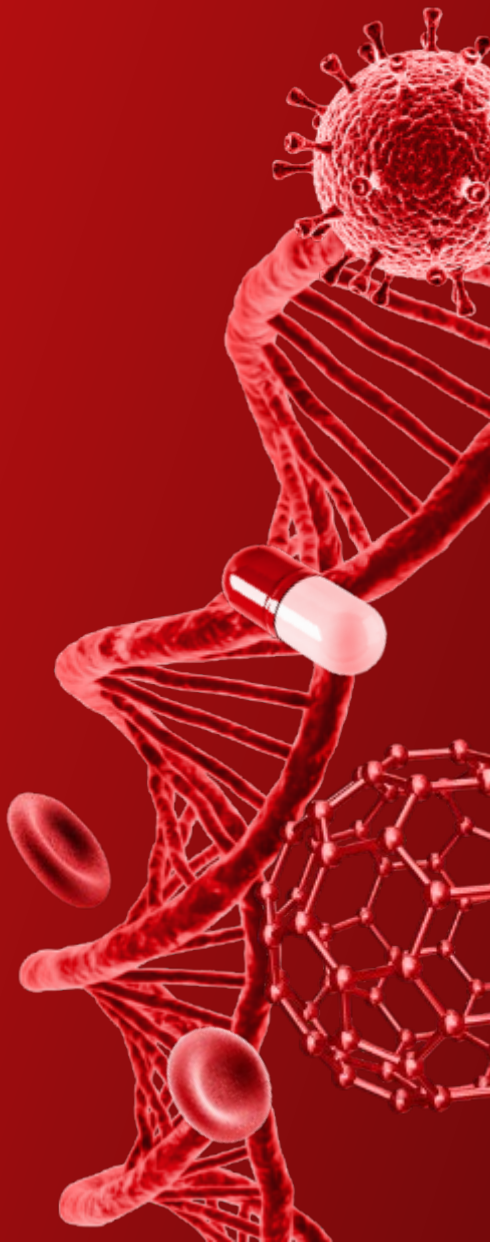
- 10 collections
- Device + 4-5 filters / collection
- 10 values to report but from 50-60 samples

APSD by NGI

- 5-6 replicate collections
- Device + IP + 7 stages + MOC filter
- 50-60 samples to analyze

*** HPLC typically uses 1 injection/sample + external standards. For other detection schemes (e.g., ELISA, PCR and plaque assays), need to consider the impact of additional 'instrument replicates' per sample**

Physical methods



Physical Method: UV Absorption & Optical Density

A = εbc Beer's Law

Protein Concentration : A280 measurements often used for pure samples

AAVs Cp and Vg Titers * : Optical Density

λmax (DNA) = 260 nm / λmax (capsid protein) = 280 nm

A260/280 Ratios

Full capsid:	~1.4
Empty capsid:	~0.6

Merits

- (+) fast, does not require ref standard, and uses common lab equipment
- (-) Need highly pure samples; potential interference from protein/nucleic acid imps, buffers, excipients and other contaminants from DUSA and NGI
- (-) indirect measure of genome; requires correlation via another technique (e.g. AUC) due to interference from DNA at 280 nm with titer error increasing with % light capsid
- (-) does not resolve partial genome species (insensitive to capsid content)

* Jürg M Summer, et al., Quantitation of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement, MOLECULAR THERAPY Vol. 7, No. 1, January 2003, 122-28

Physical Method: UV Absorption & Optical Density

Specificity	Range	Precision	Throughput	Cost

A = εbc Beer's Law

Protein Concentration : A used for pure samples

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λmax (DNA) = 260 n = 280 nm

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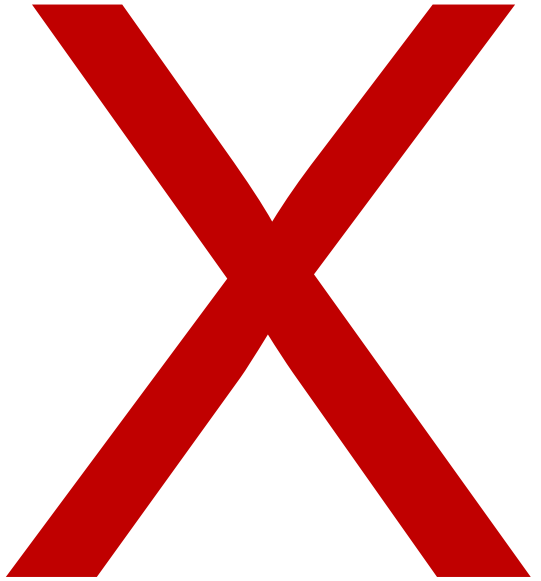

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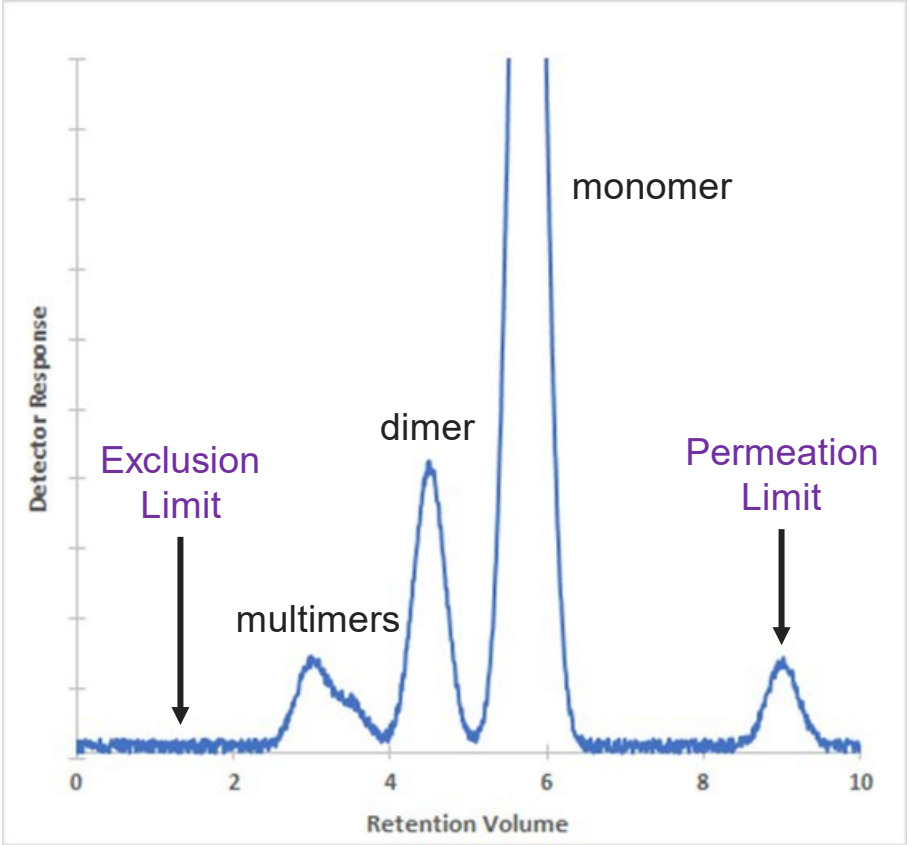
Not enough specificity to be used as a detection scheme for DDU and APSD!

* Jürg M Summer, et al., Quantitation of Adeno-Associated Virus Particles and Empty Capsids by Optical Density Measurement, MOLECULAR THERAPY Vol. 7, No. 1, January 2003, 122-28

Physical method: SEC-UV

Specificity	Range	Precision	Throughput	Cost

- Separation based on hydrodynamic volume
  - largest particles excluded from pores and hence elute first
  - smallest particles spend time partitioning to and from pores and so elute later
  - Specificity achieved depends on size difference
- UV Detection: 215 nm (std curve) and/or OD A260/280



Merits

(+) separation increases specificity vs UV Abs alone

(+) fragments + multimers

(-) requires external standardization

(-) limited to particles smaller than ~100 nm

(-) insensitive to payload

(+) RSDs < 3%

Go-to technique for DDU/APSD Detection if it is applicable to your Biologic (e.g, peptides, oligos, proteins, AAVs, LNPs)

Physical method: SEC-(UV-RI)-MALS

Specificity	Range	Precision	Throughput	Cost

MALS – multi-angle light scattering

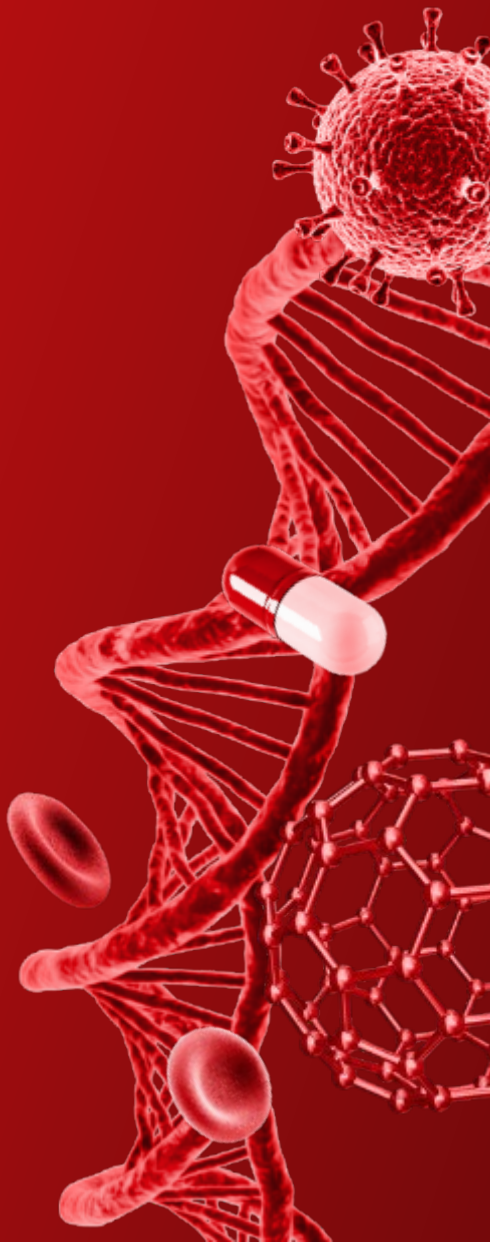
aggregation, psd, capsid content, capsid molar mass, genome molar mass, Cp and Vg titer *

- UV (280 nm) + MALS + RI → molar mass, size, number of light-scattering species
- (-) sensitivity of RI method limits sensitivity/range
- (-) complex methodology requiring specialized software to derive results
- (+) absolute method → no std curve required

Opportunity to assess use if SEC-UV does not provide the required information. Not as suitable for routine QC use given limited range and software derived results.

* McIntosh NL, et. al., Comprehensive characterization and quantification of adeno associated vectors by size exclusion chromatography and multi angle light scattering. Sci Rep 11, 3012 (2021). https://doi.org/10.1038/s41598-021-82599-1

Molecular methods

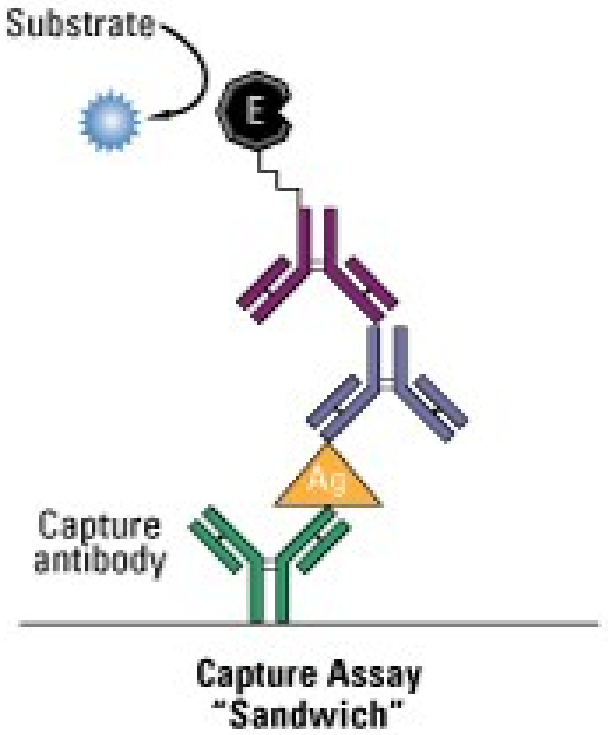


Molecular: ELISA

Specificity	Range	Precision	Throughput	Cost

"Sandwich" Assay for Proteins and Protein-containing biologics

- Key: linkage of sample to the plate that allows reagents to be washed away
- Capture antibody is pre-coated on plate in excess
- Stepwise addition of analytical sample and secondary antibody
- Use of blocking and wash steps
- Detection via colorimetric, fluorescence, chemiluminescence



Merits

(+) highly specific for capsid; requires interaction with 2 epitopes on capsid

(+) highly sensitive

(-) 10-20% RSD

(-) measurement does not reflect contents of capsid

Opportunity to assess use for protein and protein-containing biologics

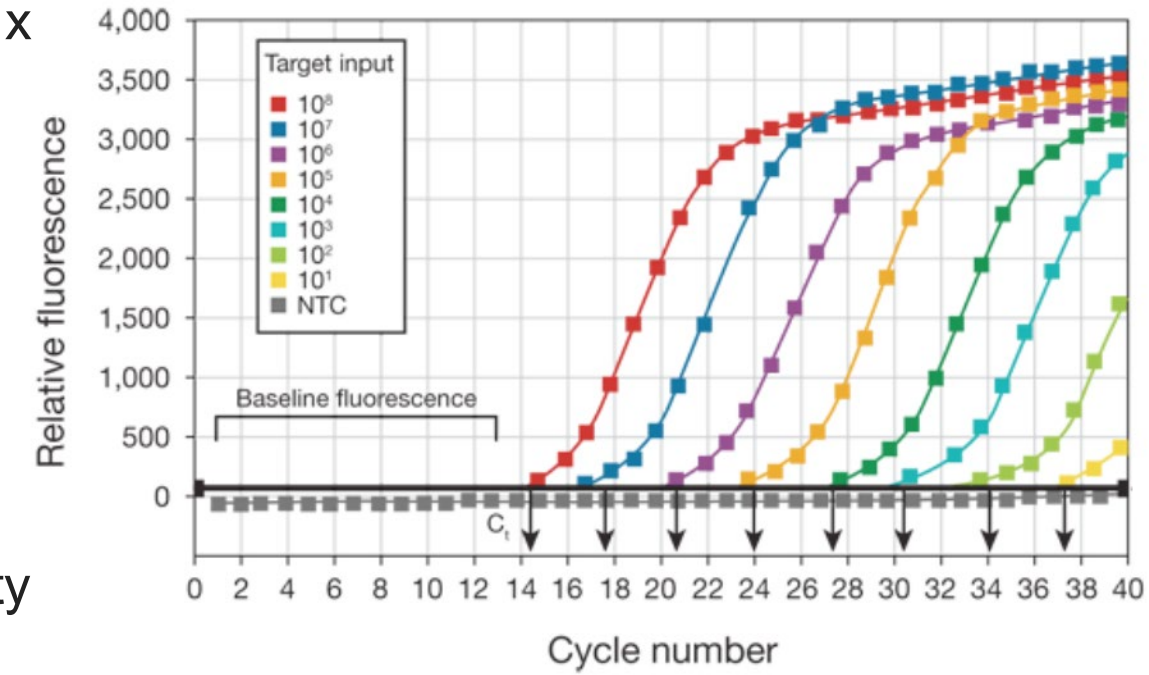
https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html

Molecular: qPCR

Specificity	Range	Precision	Throughput	Cost

RNA (via cDNA) or DNA payload (viral genomes, Vg/mL)

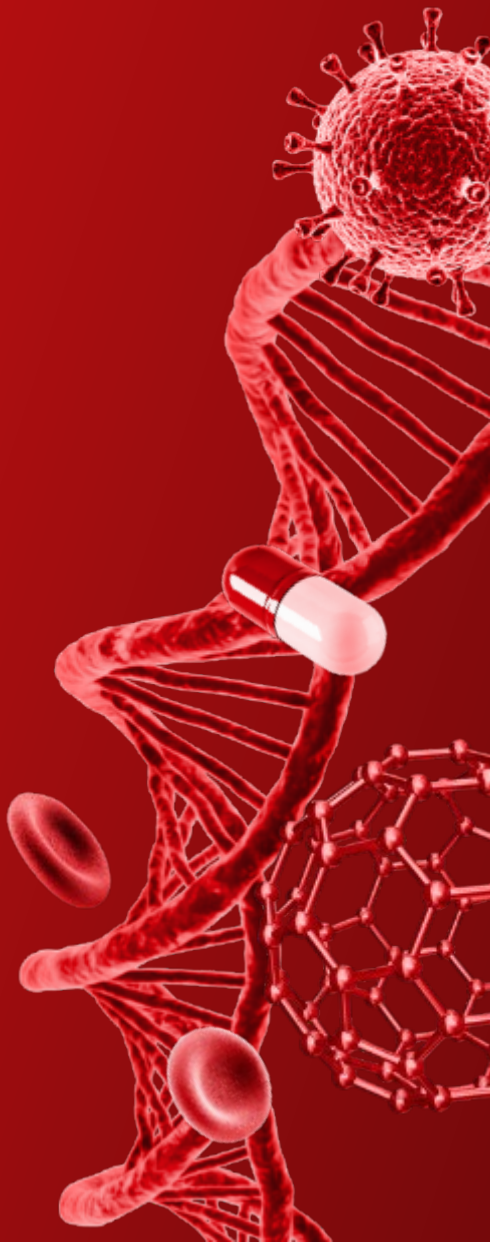
- Copy / ”Amplify” targeted genetic material 10^3 – 10^6 x via thermal cycling with fluorescence detection
- Amplicons double every cycle assuming 100% efficiency
- Cycle Threshold or C_t used with standard curve
- (+) direct detection of viral genome
- (-) 5-15 % RSD
- (-) genome titer does not necessarily relate to activity



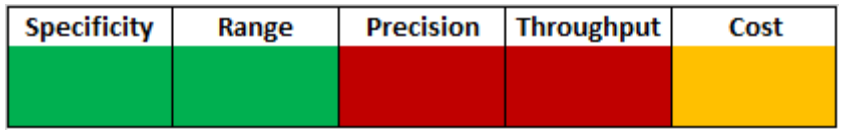
Opportunity to assess use for nucleic acid-based therapeutics
But costly and will need to address plate replicates and cross plate variability

https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/real-time-pcr-handbook.pdf?cid=rtpcrhandbook-rtpcr

Activity



Activity: plaque assay



Merits

Example: bacteriophage targeting pseudomonas aeruginosa infection

- Incubate *P. aeruginosa* cells on a series of plates to achieve monolayer coverage
- Dilute phage of known concentration (10^0, 10^{-1}, 10^{-2}…10^{-10})
- Apply diluted phage to no fewer than 3 replicate plates per each of the 4-5 dilutions required to cover the potential sample range
- Remove non-attached phage, apply nutrient medium and incubate
- Count the number of plaques per plate and calculate PFU/mL based on dilutions

(+) activity for drug delivery system

(+) valuable in assessing handling practices as well as impact to formulation and/or device type changes

(-) not all viruses cause plaques

(-) run time: 4-5 days

(-) not amenable to running 50 samples in parallel

(-) labor intensive: plating, dilutions, counting of plaques

(-) 30% RSD repeatability/intermediate precision

Not a suitable method for DDU / APSD detection

https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/real-time-pcr-handbook.pdf?cid=rtpcrhandbook-rtpcr

Conclusions

- Diversity of inhaled biologics continues to increase
- Need to define the “Active” → molecule, delivery system or payload
- Discussed a number of physical, molecular and activity-based methods
- Select methods for DDU and APSD detection that provide best precision while balancing needs for specificity, accuracy, throughput and cost

Thank You

and

Questions