INTERNATIONAL CONSORTIUM for INNOVATION & QUALITY in PHARMACEUTICAL DEVELOPMENT

A Multi-company Assessment of Submicron Particle Levels in Biotechnology-derived Protein Products

Mario Hubert

Bristol Myers Squibb (Celgene)

CMC Strategy Forum – WCBP, Jan-27-2020

Acknowledgements

Current members of IQ SbVP WG:

Mario Hubert (chair, Celgene)

Linda Narhi (co-chair, Amgen)

Dennis Yang (Eli Lilly)

Ankit Patel (previously with Genentech)

Stan Kwok (Seattle Genetics)

Anacelia Rios (Roche)

Klaus Wuchner (J&J, Jansen)

Valentyn Antochshuk (Merck)

Nataliya Afonina

George Bou-Assaf (Biogen)

Tapan Das (BMS)

Vakhtang Loladze (GSK)

Luis Montrond (Takeda)

Parag Kolhe (Pfizer)

Friederike Junge (Abbvie)

Shawn Cao (Amgen)

Miguel Saggu (Genentech)

Past members:

Satish Singh (representing Pfizer, now at Moderna)

Teresa Horwitz (Pfizer)

Atanas Koulov (representing Roche, now at Lonza)

Jun Liu (Genentech)

Stephanie Fischmann (Abbvie)

Stefan Esswein (Abbvie)

Will Weiss (Eli Lilly)

Haihong Fan (representing GSK now at Janssen)

Andrew Weiskopf (Biogen)

Mark Brader (representing Biogen, now at Moderna)

Tetsuo Torisu (representing Takeda, now at Osaka Univ.)

John-Bruce Green (Baxter)

Jane Kline (Teva)

- IQ Consortium Secretariat
- IQ Consortium Analytical Leadership group
- IQ Consortium Biologics Leadership group

- Help with data collection:
  - Wenhua Wang (representing BMS, now at Regeneron)
  - Liling Zhang (Merck)
  - Frankie Petoskey, William Jackson (Seattle Genetics)
  - Mark Pollo, Kate Torres, Anjali Mehta (Pfizer)
  - Pavel Landsman (Biogen)
  - Adam Rauk, Dawn Norris (Eli Lilly)
  - Harri Rahn (Abbvie)
  - Fabian Stump (Roche)
  - Nancy Jiao (Amgen)

Agenda

- **Background on IQ Consortium**
- **Introduction and objectives**
- **NTA and RMM techniques**
- **Experimental results**
- **Conclusions**

Who is the IQ Consortium?

This work was developed with the support of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ). The IQ Consortium is a not-for-profit organization of pharmaceutical and biotechnology companies with a mission of advancing science and technology to augment the capability of member companies to develop transformational solutions that benefit patients, regulators and the broader research and development community.

www.iqconsortium.org

Biologics Leadership Group

- The IQ Biologics Leadership Group has defined the following mission:
  - To identify challenges that are impeding the progress of biologic development, including mAbs, other protein therapeutics and vaccines, and share information on cross-industry best practices to proactively advance innovative, science and risk-based phase-appropriate strategies for process and testing controls, and justify approaches to enable alignment with regulatory bodies.

- Members include representatives from 21 companies
- The Biologics LG members have varied expertise
  - Drug Substance, Drug Product, and Analytical Development
  - Vaccines, mAbs, ADCs, and other biological products

Working Groups

- Phase-Appropriate Specifications
- Comparability and Biosimilarity
- Linkage of CQAs and Process Parameters
- Clonality and Use of Cell Pools
- Subvisible Particles
- Analytical QbD
- Biologics on NIOSH and use of CSTD
- Temperature Excursion Stability

Introduction

- Overlooking subvisible particles in therapeutic protein products. Gaps that may compromise product quality
  - Carpenter JF, Randolph TW, Jiskoot W, Crommelin DJ, Middaugh CR, Winter G, Fan YX, Kirshner S, Verthelyi D, Kozlowski S, Clouse KA, Swann PG, Rosenberg A, Cherney B. 2009. J Pharm Sci 98:1201–1205.

- An industry perspective on the monitoring of subvisible particles as a quality attribute for protein therapeutics
  - Singh S (Pfizer), Afonina N (BMS), Awwad M (Pfizer), Bechtold-Peters K (Boehringer), Blue JT (Merck), Chou D (Genzyme), Cromwell M (Genentech), Krause HJ (Abbott / AbbVie), Mahler HC (Hoffman-LaRoche), Meyer BK (Merck), Narhi L (Amgen), Nesta DP (GSK), Spitznagel T (Human Genome Sciences)., J Pharm Sci. 2010 Aug;99(8):3302-21

- Subvisible (2–100 µm) Particle Analysis During Biotherapeutic Drug Product Development: Part 1 Considerations and Strategy
  - L. Narhi, V. Corvari, D.C. Ripple, N. Afonina, I. Cechini, M. R. Defelippis, P. Garidel, A. Herre, A. V. Koulov, T. Lubinieckil, H. C. Mahler, P. Mangiagalli, D. Nesta, B. Perez-Ramirez, A. Polozova, M. Rossi, R. Schmidt, R. Simler, S. Singh, T. M. Spitznagel, A. Weiskopf, K. Wuchner. Journal of Pharmaceutical Sciences, Vol. 104, 1899–1908 (2015)

- Regulators have also expressed interest in submicron particle (SMP) characterization: “As more methods become available, sponsors should strive to characterize particles in smaller (0.1–2 microns) size ranges. Sponsors should conduct a risk assessment of the impact of these particles on the clinical performance …”
  - FDA Guidance for Industry (2014). Immunogenicity Assessment for Therapeutic Protein Products, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/immunogenicity-assessment-therapeutic-protein-products

Problem statement and WG Objectives

Problem statement:

- Limited information available on the level of SMP in clinical and commercial products.
- Although SMP instruments are available, the robustness and performance of these detection methods and their proper use for routine characterization of clinical and commercial products still needs to be explored in more detail.

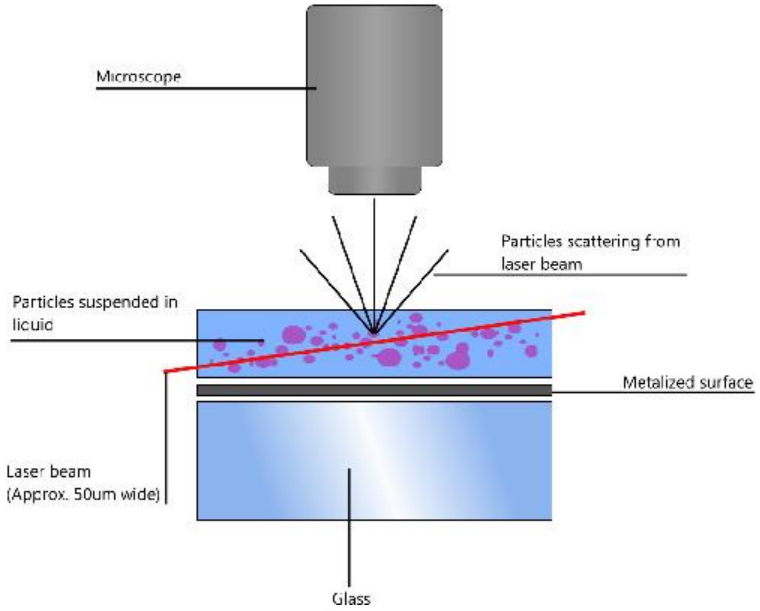
Objectives

- Determine the amount of SMP in currently marketed and clinical late-stage products
- Evaluate robustness of two most mature SMP characterization techniques, nanoparticle tracking analysis (NTA) and resonant mass measurement (RMM).

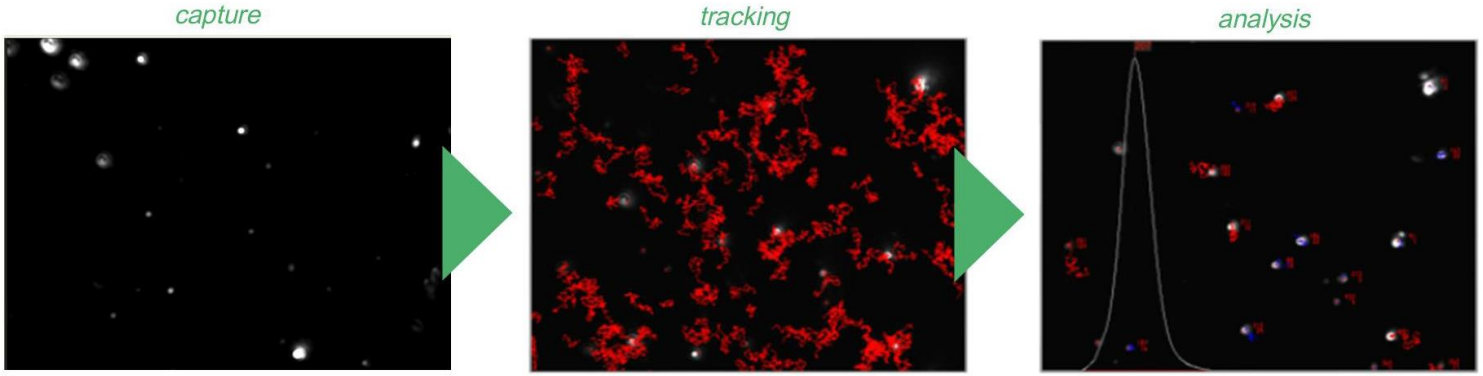
Objectives – plan

- Collect particle concentration data in 0.1-2 µm range for late stage clinical and marketed drug products
  - Each sample to be measured in triplicates and ideally multiple lots
  - Capture sample metadata (sample type, package, protein concentration …)
  - Regularly verify instruments performance
- Harmonize methods for the data collection
- Perform “Round Robin” study with NIST size standards and protein standard if available
- Identify and implement anonymous data storage solution (data is double-blinded)

Nanoparticle Tracking Analysis (NTA)



General	Specification
Size	10 nm – 2 µm
Concentration (particles/ml)	10^6 - 10^9
Sample requirement	~ 500 µL



Images: https://www.malvernpanalytical.com/

Resonant Mass Measurement (RMM)

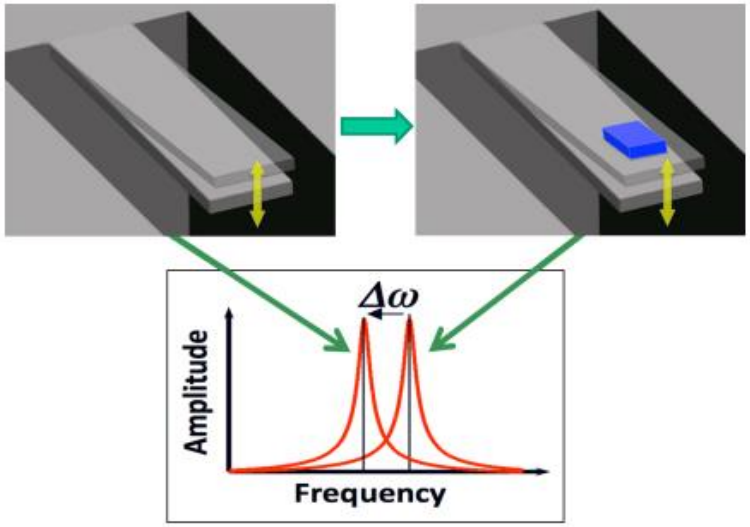


Image: https://www.malvernpanalytical.com/

General	Specification
Size (Micro/Nano sensor)	150 nm – 5 µm / 50 nm – 1 µm
Concentration (particles/mL)	10^5 - 10^9
Drawn / Measured volume	100 µL / 10 nL – 10 µL

NOTE: only the micro-sensor was used in the study

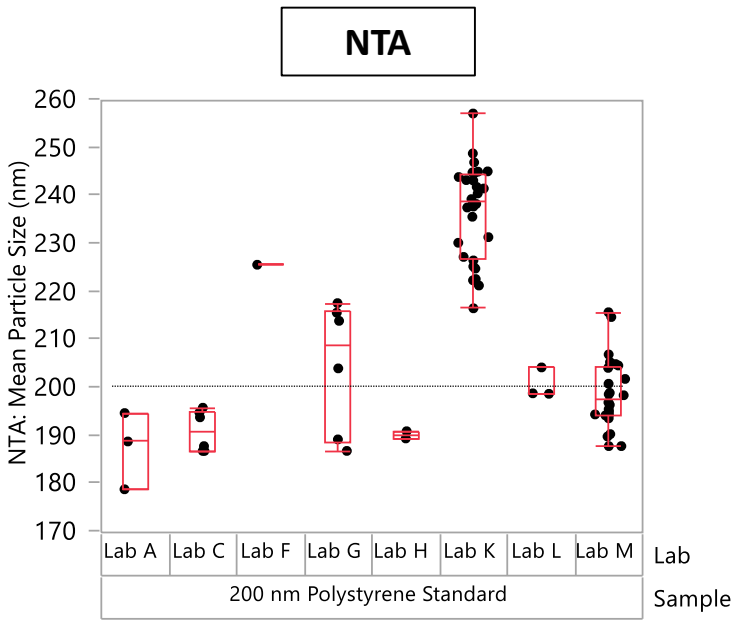
A cantilever suspended at one end, resonates at a specific frequency. When a mass is added to the beam, the frequency decreases; by measuring the shift in frequency, the mass can be determined to a very high precision.

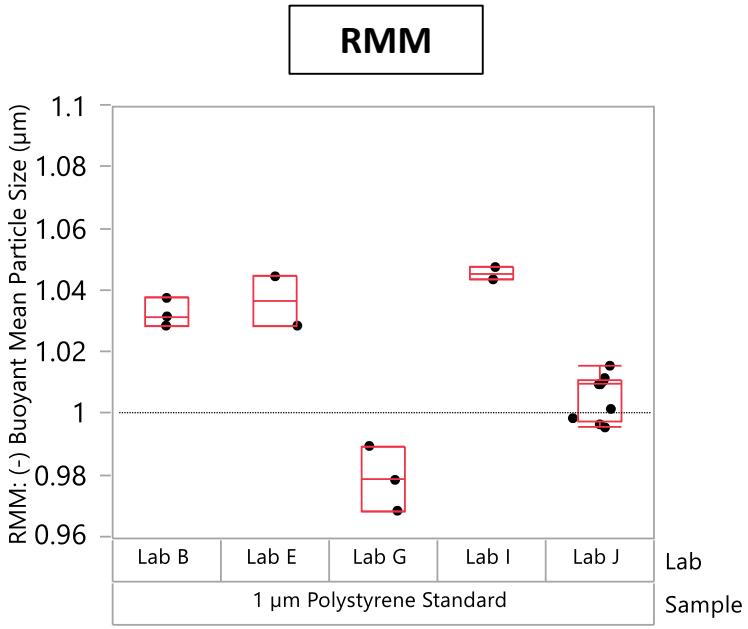
RMM can differentiate between positively buoyant particles (e.g., silicone oil) and negatively buoyant particles (e.g., protein particles).

Results from standards – size accuracy

- Size accuracy of techniques determined using polystyrene beads
  - NTA – 200 nm polystyrene beads
  - RMM – 1 µm polystyrene beads

- Majority of instruments used in the study appeared to be properly calibrated for size. Minor offset in the size is not expected to compromise the particle counting data.

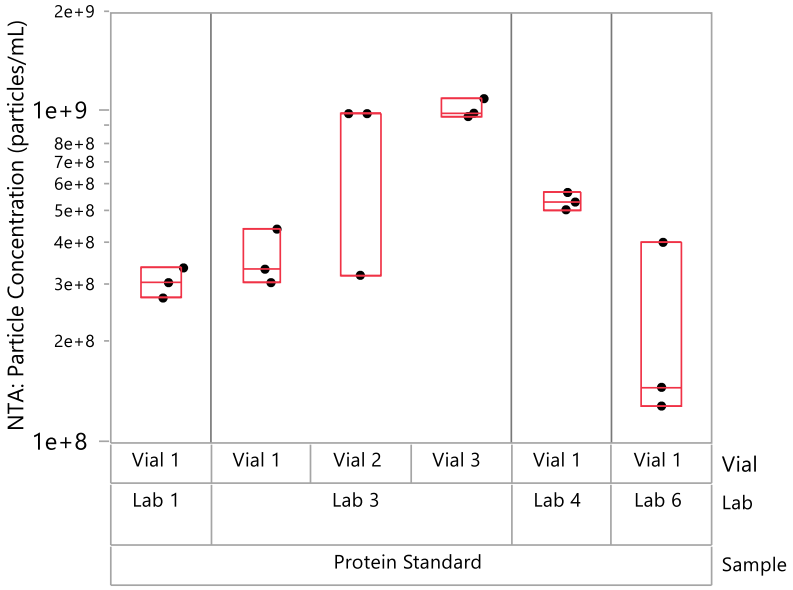




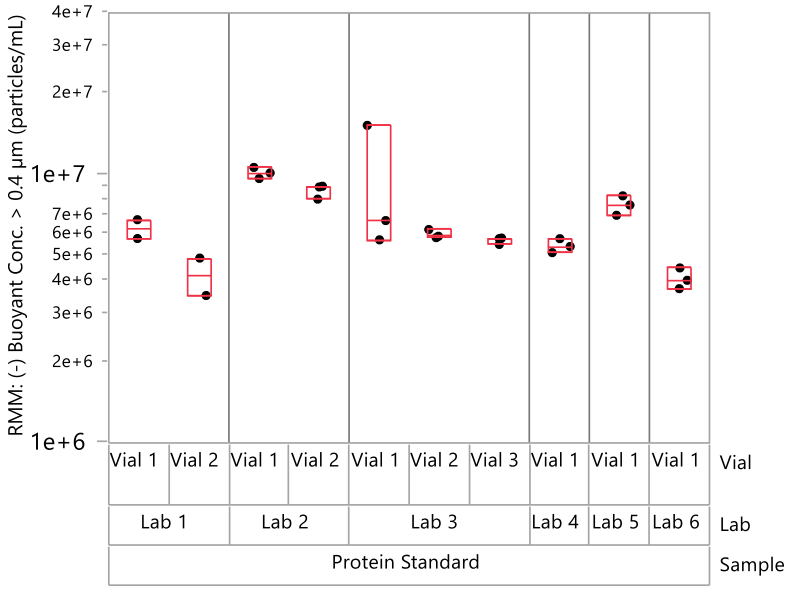
Results from standards – count reproducibility

- Count reproducibility determined using protein control standard (stressed BSA), which was originally made specifically for RMM

NTA



RMM



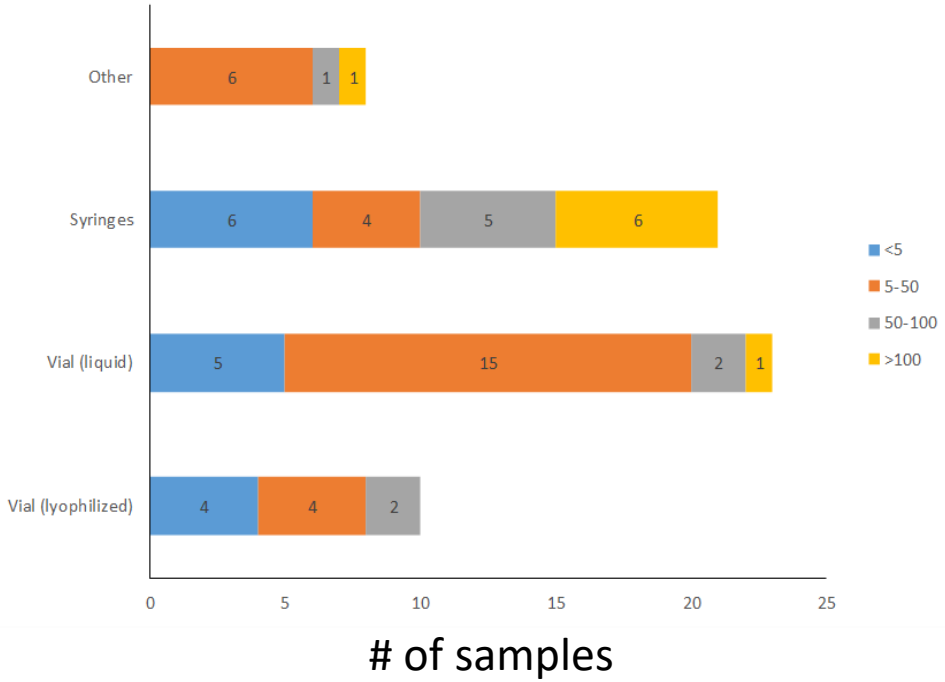
Points correspond to individual measurements.

Average %RSD = 36%

Average %RSD = 20 %

Distribution of Therapeutic Products

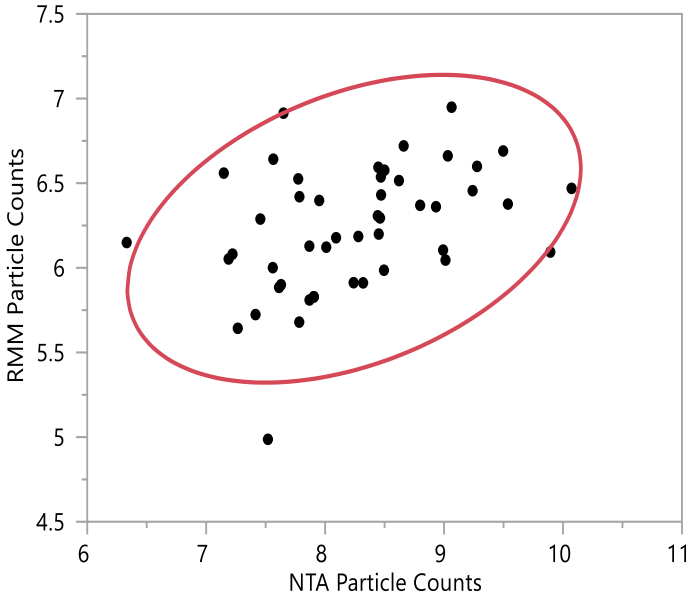
- 52 unique products (62 total samples)
- Additional product related information captured for further data analysis:
  - Sample type: liquid or lyophilized
  - Protein concentration: <5, 5 – 50, 50 – 100, or >100 mg/mL
  - Package: syringe, vial, or other
- >70% of samples were tested by both NTA and RMM



Significant amount of particle count and size data from NTA and RMM captured in database, however the focus of this presentation will be on total particle counts in clinical and commercial products.

NTA vs RMM

- A correlation between the two techniques observed
- However, differences in particle size and counts observed between two techniques:
  - NTA measures 2-3 orders magnitude higher particle counts compared to RMM
  - Mean particle size by RMM is approx. 200 nm higher than by NTA



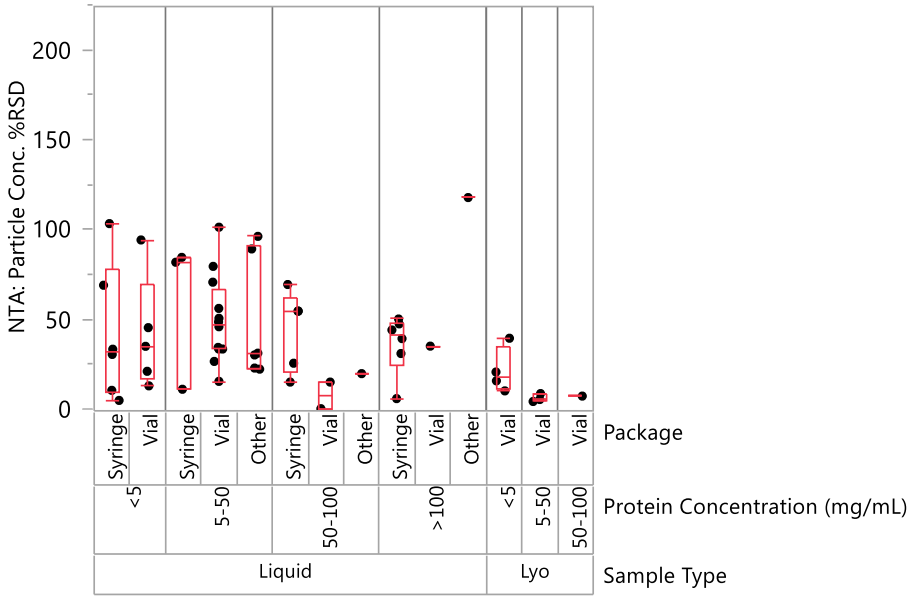
Bivariate normal density ellipse (P = 0.95) fit to the mean particle concentration (log-scale) by RMM versus NTA.

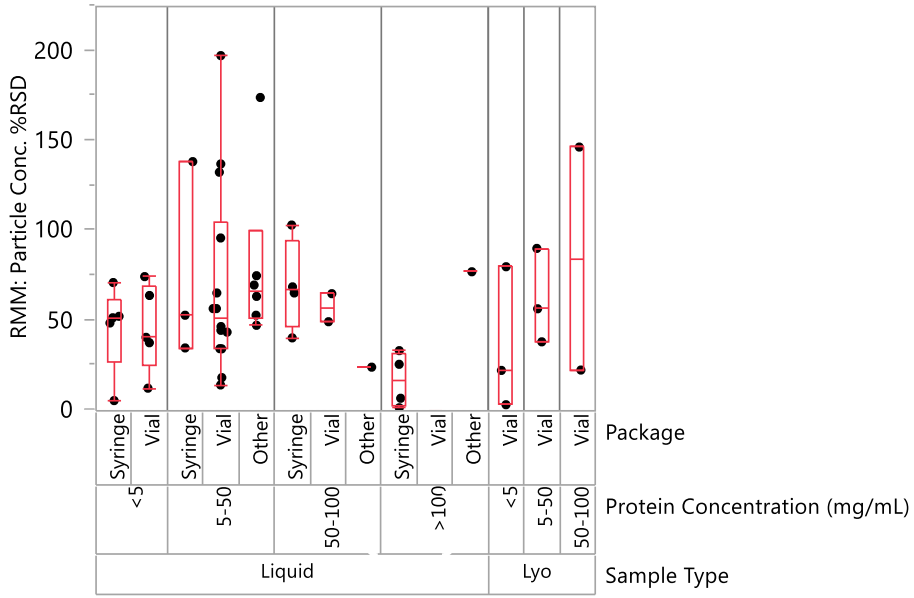
	Mean	Std Dev.	Correlation	Signif. Prob	Number
Log NTA	8.2	0.8	0.39	0.006	48
Log RMM	6.2	0.4			

	Mean Particle Size (nm)
NTA	197
RMM: (-) buoyant particles	421
RMM: (+) buoyant particles	628

Robustness of NTA and RMM with clinical and commercial products

- NTA and RMM are less robust (poor repeatability) than existing SbVP characterization techniques (MFI and HIAC)
  - Average %RSD for actual products was 51% and 73% for NTA and RMM, respectively.
  - %RSD on y-axis is determined from at least triplicate measurements





Note: No correlation observed between particle counts and %RSD for both NTA and RMM.

Robustness of NTA and RMM with clinical and commercial products

- Poor repeatability observed for NTA and RMM in this study is consistent with those reported in literature, and could be due to the small measurement volume and corresponding large extrapolation factors of these techniques.

- The poor repeatability could be an inherent limitation of the SMP particle counting techniques

Table V Sample volume and applied extrapolation factors to report final particle concentration normalized to 1 mL of the different instruments are summarized

Instrument	Measurement volume, V (mL)	Extrapolation factor, $1/V$ (mL^{-1})
HIAC	>1	1.0x
MFI	0.6	1.6x
CC	0.05	20x
RMM	0.01	100x
NTA	0.00000008	12500000x

Rios Quiroz, A., et al. Pharm Res 33(2), 450-461

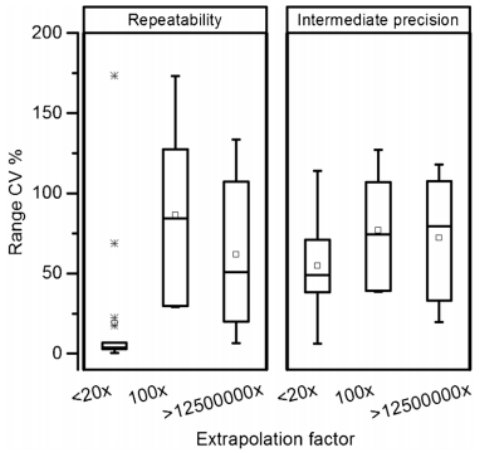
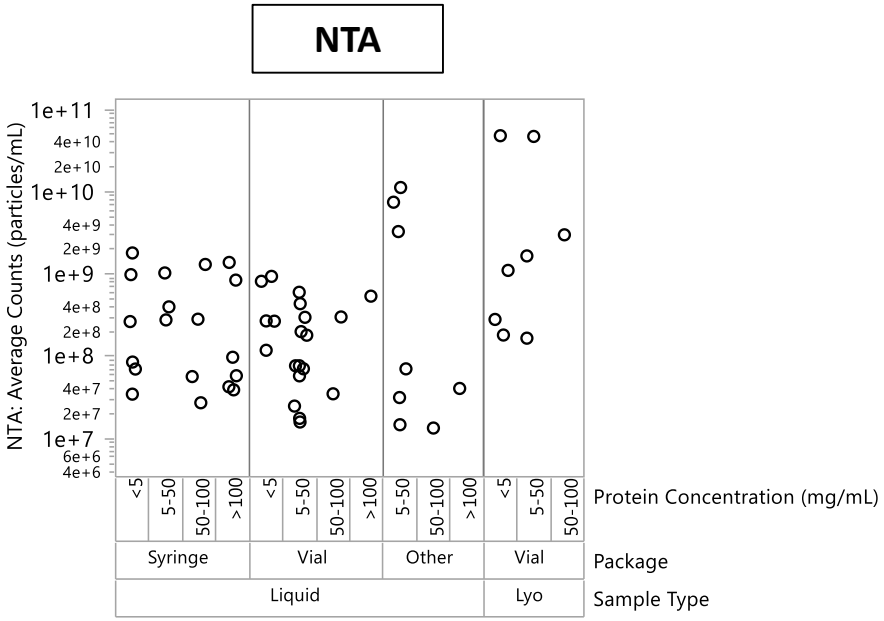
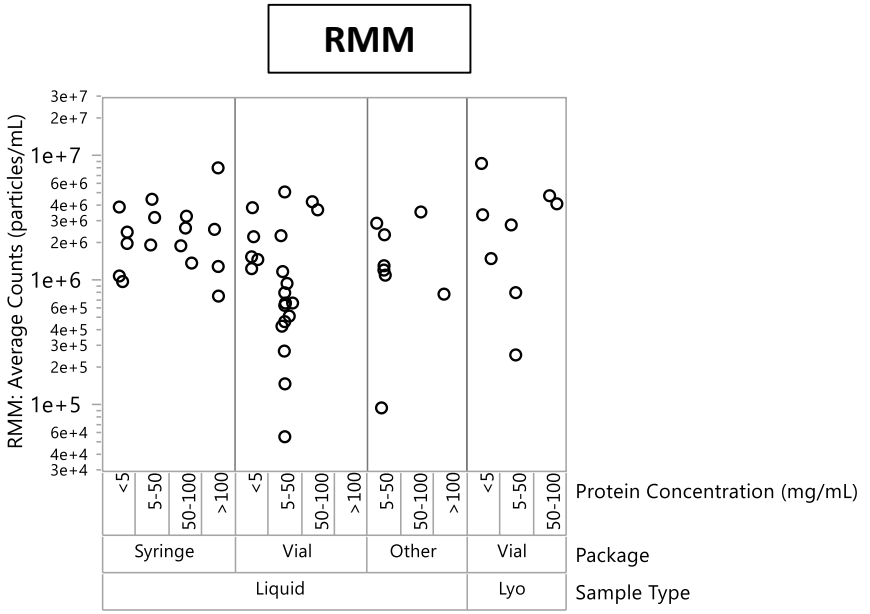


Fig. 1 Precision of subvisible particle methods in relation to the applied extrapolation factors. Syringes containing protein formulation stored for 2 months at 2–8°C were used for precision assessment. Results, reported as CV% were plotted against the corresponding extrapolation factors. Factors used were <20x for HIAC, MFI and CC. 100x for RMM. 12500000x for NTA.

Range of SMP in Clinical and Commercial Products

- Wide range of SMP counts observed in late-phase clinical and commercial products
  - 4 orders of magnitude by NTA ($1x10^7$ – $1x10^{11}$ particles/mL)
  - 3 orders of magnitude by RMM ($4x10^4$ – $4x10^7$ particles/mL)





NOTE: Certain samples were diluted (up to 200x) in order to fall within the linear range of the NTA instrument ($1x10^7$ – $1x10^9$ particles/mL). Particle concentrations shown are corrected for dilution and represents the average of 3 or more replicate measurement of the same sample .

Statistical Analysis of SMP in Clinical and Commercial Products

- Observations:
  - No statistically significant difference observed in positively buoyant particle counts for syringes vs vials
  - Increase in protein concentration does not lead to increase in SMP concentration
  - Although statistically significant differences were observed for sample type by NTA and protein concentration by RMM, these differences did not hold any practical significance

- Conclusion: None of the factors (sample type, package, or protein concentration) had any practically and statistically significant effect on particle counts by either techniques.

	Prob > F			
	Log NTA Particle Concentration	**Log RMM Particle Concentration**	**Log RMM (-) Buoyant Particle Concentration**	**Log RMM (+) Buoyant Particle Concentration**
Sample Type	0.0033	*NSS (0.1719)*	*NSS (0.3723)*	*NSS (0.0772)*
Package	*NSS (0.7762)*	*NSS (0.3625)*	*NSS (0.4676)*	*NSS (0.099)*
Protein Concentration (mg/mL)	*NSS (0.4704)*	0.0236	*NSS (0.1703)*	*NSS (0.1394)*

NOTE: Particle concentration is the average of 3 or more replicate measurements of the same sample; NSS = not statistically significant

Conclusions

- 52 unique clinical and commercial protein therapeutics covering 62 dosage forms were evaluated for submicron (≤2 µm) particle levels (SMP) by NTA and RMM
- Observed particle concentration in therapeutic products range from $1x10^7$ – $1x10^{11}$ particles/mL for NTA and $4x10^4$ – $4x10^7$ for RMM.
- No practically significant differences in SMP concentration was observed as a function of sample type (lyo vs. liquid), package type (vial, syringes or others), and protein concentration (<5 to >100mg/mL)
- The SMP levels should only be compared within a given technique and cannot be used to predict levels determined by other SMP counting techniques
- Results obtained by NTA and RMM exhibits higher variability than well-established subvisible particle characterization techniques such as light obscuration (e.g. HIAC) and flow imaging (e.g. MFI)
- **NTA and RMM characterization techniques may provide relevant SMP data during product development but are not appropriate for quality control-related testing**

Conclusions

- More results and details can be found in

Journal of Pharmaceutical Sciences

DOI: https://doi.org/10.1016/j.xphs.2019.10.025

Journal of Pharmaceutical Sciences, Jan-2020, Vol. 109, Issue 1, p830–844

Article in Press

A Multicompany Assessment of Submicron Particle Levels by NTA and RMM in a Wide Range of Late-phase Clinical and Commercial Biotechnology-Derived Protein Products

Mario Hubert[1,*], Dennis T. Yang[2], Stanley C. Kwok[3], Anacelia Rios[4], Tapan K. Das[5], Ankit Patel[6], Klaus Wuchner[7], Valentyn Antochshuk[8], Friederike Junge[9], George M. Bou-Assaf[10], Shawn Cao[11], Miguel Saggu[6], Luis Montrond[12], Nataliya Afonina[13], Parag Kolhe[14], Vakhtang Loladze[15], Linda Narhi[11]

Acknowledgement

This presentation was developed with the support of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ, www.iqconsortium.org). IQ is a not-for-profit organization of pharmaceutical and biotechnology companies with a mission of advancing science and technology to augment the capability of member companies to develop transformational solutions that benefit patients, regulators and the broader research and development community.

Questions

Learn more about IQ

For more information about the IQ Consortium's past work and current activities, we invite you to review the following resources.

IQ Website	https://iqconsortium.org
IQ Annual Report 2018	https://iqconsortium.org/annual-report-2018

To find out how your company can join the IQ Consortium or if your company is already a member and you would like to get involved, please email us at info@iqconsortium.org.

Select IQ Working Groups

(BIOLOGICS) COMPARABILITY AND BIOSIMILARITY WORKING GROUP

Establish consistent expectations and approaches across regulatory agencies and companies for comparability and biosimilarity.

ANTIBODY DRUG CONJUGATES WORKING GROUP

Collect data, formulate an industry position of risk-based bioanalytical and DDI strategies

SMALL MOLECULE IMPURITY FOR ADC WORKING GROUP

Develop and propose strategies for setting specifications for the Drug-Linker components of an ADC and Regulatory strategies for setting specifications and post-approval changes

PEDIATRIC WORKING GROUP

Become a catalyst for advancing science and technology related to pediatric formulation development, collaboratively advance pediatric formulation development and regulatory harmonization globally, share best practices and standardization, publish and consider new mechanisms for collaboration, and build holistic understanding of patient, caregiver and regulatory needs and challenges

FOOD EFFECTS PBPK MODELING WORKING GROUP

Position paper assessing the predictive performance of PBPK models with respect to mechanistic prediction of food effect of biopharmaceutics using consistent input data and modeling strategy. 20-25 compounds from various BCS classifications will be selected from literature. Modelers from all companies will develop and validate PBPK models for compounds using the same input data and modeling approach. Highlight cases where high vs. low confidence is expected in predicting food effect using PBPK and provide an industry perspective on best practice for mechanistic modeling of absorption and food effect.

EXTEMPORANEOUS FORMULATIONS WORKING GROUP

Provide a forum to share experiences and benchmarking for the use of compounding to prepare investigational materials. Seek to understand & influence topics such as extent of use across the industry, regulatory submission strategies and corresponding challenges, evolving facility inspection expectations, use of EU vs US standards, partnering between product and clinical development functions, experience in Asia.

(BIOLOGICS) TEMPERATURE EXCURSION WORKING GROUP

Provide industry with a common understanding on how to interpret and translate the current temperature excursion (TE) regulatory requirements from Brazil and Australia into real-life experiments and stability test plans.

(DRUSAFE) IMPURITY SAFETY WORKING GROUP

Develop harmonized, scientific strategies for assessing the safety of impurities, advocating 3R strategies, and educational / outreach activities for impurities.

(BIOLOGICS) ANALYTICAL QUALITY BY DESIGN

Develop and disseminate new approaches for analytical validation for large molecules and vaccines. Engage regulatory authorities around the world in the discussion of challenges and AQbD solutions.