Measurement Science and Standards to Support the Development of Novel Protein Therapeutics and Biosimilars

IBBR 2014 Biomanufacturing Technology Summit

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Michael J. Tarlov
Program Coordinator of NIST Biomanufacturing Program
Chief, Biomolecular Measurement Division
Material Measurement Laboratory
Talk Overview

• How measurements and standards support development of biosimilars (and new therapeutics)
• Overview of NIST Biomanufacturing Program
• Selected Activities
  - Protein particle measurements
  - Development of NIST monoclonal antibody (mAb) reference material
  - NMR fingerprint-like characterization of protein therapeutics
• Conclusions
Systematic engineering of biosimilar to match reference product: Importance of measurements

1. Target directed development
   - Recombinant cell line development
   - Bioprocess development
   - Purification process development
   - Drug product development
   - Target range
   - Process development

2. Confirmation of biosimilarity
   - Clinical
   - PK/PD
   - Preclinical
   - Biological characterization
   - Physicochemical characterization

Leveraging biological variability

Courtesy of Mark McCamish, Sandoz
Fingerprinting

• It may be useful to compare products using a meaningful fingerprint-like analysis algorithm – that covers a large number of additional product attributes and their combinations with high sensitivity using orthogonal methods.
  • Primary Structure
  • Higher order structure
  • Impurities (if similar source material and excipients)

• Advances in manufacturing science and Quality-by-Design approaches may allow for better matching a reference product’s fingerprint.

From S. Kozlowski, FDA
Improved Measurement Science, Standards, Technologies Support Key Aspects of Biosimilars Development

• Measurement science and standards can help:
  - Account for bias between different analytical methods or instruments
  - Determine variability of characterization and test methods and setting of product specifications
  - Determine test methods are in control
  - Assess performance of new analytical technologies

• Sound measurement science is essential for QbD, product understanding, development, & manufacturing
NIST Program in Biomanufacturing

Measurement science, standards, and data to support development, manufacturing & regulatory approval of biologic drugs

Developed from Over 5 Years of Stakeholder Input:

NIST Criteria for Priority Setting:

1. Magnitude/urgency of industrial need
2. Correspondence between need and NIST mission to develop infrastructural technologies
3. Potential impact of NIST involvement
4. Can NIST respond with a timely, high quality product
NIST Biomanufacturing Program Areas and Projects

**Protein Stability**
- Methods & Reference Materials for the Measurement of Protein Particles
- Broadband-CARS imaging for characterizing individual protein particles
- Bench Top Optical Method to Estimate Protein Stability in Solid Forms
- Microfluidic electrical sensing – optical imaging instrument for characterizing protein particles
- Microfluidic measurement of viscosity & rheology of protein drug products

**Protein Structure**
- Development of NIST mAb Reference Material
- MS Library of Peptides, Glycans and Glycopeptides for Therapeutic Antibodies
- NMR Measurements
- Neutron Measurements
- HDX-MS Multi-Lab Inter-comparison
- Raman spectroscopy and MVA for ID of protein therapeutics

**Understanding Production Cells**
- Optical microscopy of CHO cultures to assess clonal stability
- MS library of CHO and E. Coli bioreactor metabolites and compounds
- Determination of concentration of host cell DNA standards using dPCR
Protein Particulates in Biotherapeutics  
PI: Dean Ripple

- Proteins in solution partially denature and subsequently agglomerate
- Highly hydrated ($\approx 95\%$ water)
- Evidence of immunogenic properties
- Particulate size from 10s of nm to 100 $\mu$m

**Current state-of-the-art**
- Differing optical methods disagree by 10X
- No means of standardizing instruments for response to protein particulates

**Limitations of existing standards:**
- No particles of similar morphology or shape
- No particles with low optical contrast
- Existing standards have high density
Protein Particle Measurements and Standards
Activities

Goals:
• Reduce risks to safety and efficacy of biotherapeutics by supporting accurate counting and characterization of particles
• Support industry in understanding involvement of particles in biological pathways, e.g., immunogenicity

Activities:
1. Measurement science: appropriate models for instrument response
   - Identify and characterize physical properties of protein particles relevant to counting method considered
2. Standards: reference materials that mimic protein particles
3. Measurement tools: new orthogonal particle measurement technologies
Optical Method 1: Light Obscuration

Particle passing through light beam reduces optical transmission

- Scattering cross section depends on refractive index and particle morphology
- Other factors: optical configuration/alignment, acceptance angle of detector
- Low scattering intensity of protein particles can lead to undersizing
Optical Method 2: Flow Imaging

Digital imaging of particulates 1 to 100 µm, 2 instrument vendors

- Flat flow cells
- Contrast & spatial resolution depend on proprietary particle id algorithm, light source, objectives, aperatures
- Diffraction effects can result in oversizing of particles
Light Obscuration Scaling: Instrument Model

**Approach:**

1. Model small particles as spheroids; use scaling approximations for large particles.
2. Obtain the average refractive index of the particles from Quantitative Phase Imaging.
4. Transform the LO data using the instrument response curve to estimate the actual particle diameter corresponding to the measured diameters.

*Transformation scales particle diameter, not count*
Results of Adjusting Diameters

- Two flow imaging instruments, one light obscuration instrument
- Particles formed by agitation of polyclonal human IgG
Candidate Particle Reference Material: Abraded Fluoropolymer

**ETFE polymer** (tetrafluoroethylyene/ethylene copolymer) has desirable properties:

1. Rugged, with refractive index of 1.40—close to that of protein
2. Appears like protein with mechanical abrasion process—oscillatory motion pulls off irregular, tangled particles
3. Producing polydisperse suspension as reference material, 1 to 25 µm
Interlaboratory Comparison of ETFE Particles

- 24 participants: biopharma, instrument vendors, academia, FDA
- Diameter range 1 to 25 µm
- Paper submitted to J. Pharm. Sci.

![Light Obscuration Diagram](image1)

![Flow Imaging Diagram](image2)
NIST mAb Standard Reference Material + Data (SRM/D)
PI: John Schiel

A mAb (IgG1) reference material could be useful for:
- System suitability material or cross-checking test methods
- Testing new measurement technologies
- Comparing changing analytical test methods
- Will not replace reference product or in-house reference std.

NIST mAb attributes:
- Humanized mAb (IgG1κ) expressed in murine culture
- Frozen bulk “Drug-like substance”
  - 100 and 10 mg/mL, ≥ 98% purity
  - 12.5 mM L-His, 12.5 mM L-His HCl (pH 6.0)

“Crowd-Sourcing” approach for IgG characterization:
- Complete extensive interlaboratory characterization
- 65+ Biopharma, Academic, FDA participants
  - Results used for ACS book “State-of-the-Art and Emerging Technologies for the Analysis of Monoclonal Antibodies”
- NIST will certify concentration traceable to the kg
- Compile reference data (MS library), methods, etc.
  - Publically available: http://igg.nist.gov/

Possible uses for IgG SRM:
- Amino Acid Sequencing
- Amino Acid Analysis
- N-terminal Sequencing
- C-terminal Sequencing
- Peptide Mapping by MS
- S-S Bridge Analysis
- Glycosylation Analysis
- Molecular Weight Information
- Isoelectric Focusing
- SDS-PAGE
- Extinction Coefficient
- Post-Translational Modifications
- Spectroscopic Profiles: CD, NMR
- LC: SEC, RP, IEX
NIST CHARACTERIZATION

• Separation Science
  • SEC, RP, HIC, CEX, WAX

• Mass spectrometry and LC-MS
  • Peptide mapping, middle down, and intact
  • PTM analysis
  • Sequence Variant
  • Glycoanalysis
  • HCP’s

• Mass spectral database
  • Peptide MS/MS
  • Glycan MS/MS

• Certification of Total Protein Concentration
  • AAA
  • Peptide IDMS

• Future potential certified values
  • Extinction coefficient
  • Monosaccharide content

• Higher Order Structure
  • NMR
  • XRD
  • HDX
  • Small angle neutron scattering (SANS)
  • Small angle x-ray scattering (SAXS)

• Biophysical Measurements
  • AUC
  • SEC-MALS/DLS
  • CD
  • FTIR
  • Fc binding assays
  • Rheology
Building a Comprehensive MS Reference Library of Peptides, Glycans, & Glycopeptides of the NIST mAb

- NIST MS reference libraries most widely used in world
- No comprehensive MS spectral library of mAbs exists
- Build integrated IgG MS library of:
  - Tryptic peptides – all modifications
  - Glycans – all forms
  - Glycopeptides
- Future mAb Standard Reference Material will include MS reference data (SRM/D)

High quality MS reference data
- Spectra for ion trap and collision cells (range of energies) for various precursor ions
- Consensus spectra: peak voting; intensity averaging; reject contaminants & noise
- Quality control: % explained peaks, proper energy dependence, precursor purity

NA2G1F from Rituximab Matches Library
From IgG to MS Library
Enzymatic Fragmentation – LC Separation - MS Identification

IgG Fragmentation:
• Break ‘hinge’ (papain,...)
• Break S-S (reduce)
• Stabilize S (alkylate)

Proteolysis:
• Trypsin
• Asp-N
• Glu-C
• Lys-C

Big Fragments  Deglycosylate  Peptides
Glycans
Separate by LC, identify by MS/MS

Photos courtesy of Matt DeLorme
LC-UV-MS/MS Peptide Map

- Peptide level coverage
  - 98/96 % coverage Trypsin
  - 100% coverage multi-enzyme

- Peptide specific peptide map optimization
  - Cleave after K and R
  - Digest, MS, LC critical
  - ETD, CID, HCD to be optimized for each peptide
  - Long gradient
  - Target 100% at amino acid level
  - Optimized DD settings

- Heavy Chain
  - Trypsin = 98.67%
  - Chymotrypsin = 91.33%
  - GluC = 64.44%

- N: N-glycosylation site
- K: Lysine-clipping
- *: pyro-glutamination

- Consistent with Expected Primary Sequence

Peptide sequence:

```
QVLTXXXXXXLVKPTQTLTLTCTFSGFSLSTAGMSVGWIRQPPGKALEWLAXXXXXXXXXXXXXXXXRLTXKDTSKXXXXXKXXXXXXX
```

- N: N-glycosylation site
- K: Lysine-clipping
- *: pyro-glutamination

Peptide coverage:

- Trypsin: 98/96%
- Multi-enzyme: 100%

Peptide specific optimization:

- Peptide fragmentation
- ETD, CID, HCD to be optimized for each peptide
- Target 100% at amino acid level
- Optimized DD settings

Time (min)
18 hr - NIST Standard IgG
Guanidine/HT: HCD fragmentation

"Proteomics"$10^{11}$

Retention/min

Minor Constituents, Digest Quality

Glycopeptide
Peptide
Sampled/No ID
Unsampled
Single Protein Digest Data Analysis Pipeline
For MS Library Construction

Raw Data

Translate, Annotate

XTandem, OMSSA, Prospector, Comet

Integrate, Class FDR

Consensus Spectrum

MS1, MS2, RT

Six Filters

Build Library

Library
# NIST mAb HCD Tryptic Peptide Library

## Peptide Classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Common or Uncommon</th>
<th>Peptide Class</th>
<th>Spectra</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>C</td>
<td>Simple Tryptic</td>
<td>567</td>
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<tr>
<td>2</td>
<td>C</td>
<td>Tryptic with Expected Missed-Cleavage</td>
<td>461</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Common Modifications</td>
<td>369</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>In-Source Semitryptic</td>
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<td>5</td>
<td>U</td>
<td>In-Solution Semitryptic</td>
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<td>6</td>
<td>U</td>
<td>Artifacts and PTMs</td>
<td>372</td>
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<tr>
<td>7</td>
<td>U</td>
<td>Unexpected Missed-Cleavage</td>
<td>689</td>
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<td>8</td>
<td>U</td>
<td>Under/Over Alkylation</td>
<td>42</td>
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<tr>
<td></td>
<td></td>
<td><strong>Total</strong></td>
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## Peptide Modifications

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<tr>
<th>Modification</th>
<th>Delta mass</th>
<th>Modified site</th>
<th>Spectra</th>
</tr>
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<tbody>
<tr>
<td>Oxidation</td>
<td>+15.9949</td>
<td>M, H, W</td>
<td>778</td>
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<tr>
<td>Deamidation</td>
<td>+0.9840</td>
<td>N, Q</td>
<td>96</td>
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<tr>
<td>Cation:Na</td>
<td>+21.9819</td>
<td>D, E</td>
<td>84</td>
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<tr>
<td>Formyl</td>
<td>+27.9949</td>
<td>N-terminus, K, S, T</td>
<td>50</td>
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<tr>
<td>Pyro-carbamidomethyl</td>
<td>+39.9949</td>
<td>C at N-terminus</td>
<td>24</td>
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<tr>
<td>Gln-&gt;pyro-Glu</td>
<td>-17.0265</td>
<td>Q at N-terminus</td>
<td>21</td>
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<tr>
<td>Dehydrated</td>
<td>-18.0106</td>
<td>D, S, T</td>
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<tr>
<td>Glu-&gt;pyro-Glu</td>
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<td>E at N-terminus</td>
<td>14</td>
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<tr>
<td>Cation:Ca[II]</td>
<td>+37.9469</td>
<td>I/L, P, S, T, G…</td>
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<tr>
<td>Methyl</td>
<td>+14.0157</td>
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<td>Dioxidation</td>
<td>+31.989829</td>
<td>M, W</td>
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<td>Carbamyl</td>
<td>+43.0058</td>
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<tr>
<td>Trioxidation</td>
<td>+47.984744</td>
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Higher-Order Structure is a Distinguishing Feature of Protein Therapeutics

Structure → Function

“Our current ability to **predict the potency of biologics** would be enhanced if we had improved ability to **measure and quantify** the correct (major) three-dimensional structure, aberrant three dimensional structures (**misfolding**), and the **distribution** of different three-dimensional structures”.

Steven Kozlowski, M.D. CDER, FDA (Congressional Testimony, 2009)

**NMR can assess higher order structure of protein therapeutics at atomic resolution**
Inter-laboratory Comparison: Harmonization and Validation of High-Resolution NMR as a Metric for Structure Comparability of NMR spectral 'finger prints' assessed using standardized NMR experiments & $^{15}\text{N}$-Labelled Filgrastim sample

- **4 Sites in North America and Europe**
  - FDA; Health-Canada; MPA-Sweden; NIST
- **4 Fields**
  - 500, 600, 700 and 900 MHz
- **Different Instrument vintages**
- **2 Vendors**
  - Bruker Biospin, Varian/Agilent

$^{15}\text{N}$-Labelled Filgrastim ($^{15}\text{N}$-GCSF)

Met-G-CSF (19 kDa) – used in cancer patents with neutropenia.
900 MHz NMR at IBBR
Inter-laboratory Comparison: Establish the robustness and confidence using a $^{15}$N-GCSF “system suitability” sample

- Determined performance variation across different instrument manufacturers, laboratories, field strengths and pulsed experiments.

- Agreed upon protocols for data acquisition, processing and analysis for comparability applications.

Spectra from 4 labs and platforms of $^{15}$N-GCSF suitability sample

Purple: NIST
Blue: MPA
Green: HC
Red: FDA
Precision across labs is comparable to measurement precision of instrument!
Comparison of 4 Filgrastim Products: $^1$H-$^{15}$N HSQC NMR Spectra at 4 sites

Nearly identical ‘finger print’ map between the 4 samples/instruments/magnetic fields using comparable acquisition and processing parameters
Multivariate Statistical Analysis for Comparability: 4 Filgrastim Products and System Suitability Sample

FDA500 (dark blue), NIST900 (light blue), NIST600 (green/yellow), and MPA600 (orange). $^{15}\text{N-G-CSF}$ are colored red.
Application of High-Resolution NMR to Structure Characterization of Monoclonal Antibodies: NIST mAb

Feasibility of NMR Spectral Mapping of mAb Domains (Fab, Fc)

The NIST mAb Fab Domain Spectral Fingerprints

1H-15N

1H-13C
Conclusions

• NIST Biomanufacturing Program is developing improved measurements, standards, and technologies to support development of biosimilars and new therapeutics

• Particle measurement science and standards help in obtaining more accurate sizing and counting of protein particles

• NIST monoclonal antibody reference material will find use in:
  - Assessing method variability, utility, etc.
  - Determining performance of new technologies
  - System suitability testing/method qualification
  - Publicly available source of historical data

• NMR shows promise as a robust, highly specific method for characterization of therapeutic proteins
Acknowledgements

- NIST
  - John Schiel (NIST mAb PI)
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  - Karen Phinney

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  - Darryl Davis, Janssen
  - Oleg Borisov, Novavax
  - Other participants

- FDA
  - Kurt Brorson
  - Erik Reed
  - Michael Boyne
  - Cyrus Agarabi
  - Scott Lute
Intact $m/z$

- Support primary sequence
- High abundance variants
  - PTM's, truncation, etc.
  - Intact analysis shows major glycoforms
- Deglycosylated verifies
  - C-terminal truncation
  - N-terminal pyro-glu
  - Glycation

Consistent with Expected Primary Sequence

Intact

- Low abundance
- -GlcNAc
- +Hex

Deglycosylated

Proteoform
- Deglycosylated
- 1. – Gly
- 2. + Lys
- 3. + glycation
- 4. +2 Lys
- 5. +2 glycations
Guidance for Industry Quality Considerations in Demonstrating Biosimilarity to a Reference Protein Product

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the Federal Register of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5650 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the Federal Register.

For questions regarding this draft document contact (CDER) Sandra Benton at 301-796-2500.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

February 2012
Biosimilarity

The three dimensional conformation of a protein is an important factor in its biological function. Proteins generally exhibit complex three-dimensional conformations (tertiary structure and, in some cases, quaternary structure) due to their large size and the rotational characteristics of protein alpha carbons. The resulting flexibility enables dynamic, but subtle, changes in protein conformation over time, some of which may be absolutely required for functional activity. These rotations are often dependent on low-energy interactions, such as hydrogen bonds and van der Waals forces, which may be very sensitive to environmental conditions. Current analytical technology is capable of evaluating the three-dimensional structure of many proteins. Methods such as X-ray crystallography and multi-dimensional nuclear magnetic resonance (NMR) spectroscopy can help define tertiary protein structure and, to varying extents, quaternary structure, and can add to the body of information supporting biosimilarity. At the same time, a protein’s three-dimensional conformation can often be difficult to define precisely using current physicochemical analytical technology. Any differences in higher order structure between a proposed biosimilar and a reference product should be evaluated in terms of a potential effect on protein function. Thus, functional assays are also critical tools for evaluating the integrity of the higher order structures.
Keys to Acquisition and Processing for Comparability

**Spectral Resolution:**
- Data acquired with comparable resolution calibrated to instrument
- Data processed using the same functions & parameters
- Cross-peaks picked with a common method

How well can peak positions be determined sets the precision of the spectral comparison

**Signal to Noise**
- Experiments are acquired across labs and platforms using comparable S/N in acquisition

Determines the threshold of detection and lower limit of peak detection.
Inter-laboratory Round Robin Study: 2nd Round

Establish recommendations for NMR data acquisition, processing and analysis for testing structure comparability of formulated protein drugs

Biosimilarity Assessment

Compare results using commercially available Filgrastim products:

FDA Approved Innovator: Neupogen® (Amgen)

Follow-ons: Neukine® (Intas Biopharmaceuticals), Nufil Safe™ (Biocon), Grafeel™ (Dr. Reddy's Laboratories)

formulate drugs concentrated to 1 mM drug substance by one lab (FDA) and distributed to the participating sites.
Inter-laboratory Comparability Study – Initiated in 2012: Establish Measurement Standards and Confidence

Round robin study on the comparability of NMR spectral 'finger prints' obtained using standardized NMR experiments

- **4 Sites in North America and Europe**
  - FDA; Health-Canada; MPA-Sweden; NIST

- **4 Fields**
  - 500, 600, 700 and 900 MHz

- **Different Instrument vintages**

- **2 Vendors**
  - Bruker Biospin, Varian/Agilent

(Filgrastim; Neupogen®)

Met-G-CSF (19 kDa) – used in cancer patents with neutropenia.
Inter-laboratory Round Robin Study: 1st Round

Establish the robustness and confidence in NMR spectral ‘fingerprints’ using $^{15}$N-labeled recombinant, hG-CSF

- Determined performance variation across different instrument manufacturers, laboratories, field strengths and pulsed experiments.

- Established recommendations for optimal data acquisition, processing and analysis for comparability applications.
HC Data shows sensitivity of temperature offset on $^{15}$N-GCSF shifts

Small lab-to-lab variations, Health Canada shows temperature variation.
Reference = AVERAGE (FDA500, NIST900, NIST600, MPA600)

HC Data shows sensitivity of temperature offset on $^{15}$N-GCSF shifts
Direction Forward: NMR Structural Fingerprint of mAb Glycans as a Complement to MS Analysis

Complex (i.e. IgG)

NIST mAb 1D 1H Fingerprint

900 MHz
100% D2O; 35°C

Anomeric

NIST mAb \(^1\text{H},^{13}\text{C}\) Fingerprint

Distinguishing Glycan Patterns

RNaseB

NIST mAb
Failure Modes and Effect Approach for Determining Overall Risk Level

\[
PQRA \text{(Overall Risk)} = f\left( \text{PQA Criticality Assessment (Severity)} , \text{Process Capability (Likelihood of Occurrence)} , \text{Testing Strategy (Detection)} \right)
\]

Detection scoring = \( f \) (method capability, control stringency)

- Detection scoring combines two concepts
  - **Method capability** considers limit of quantitation, precision, specificity and orthogonality
  - **Control stringency** accounts for frequency of testing and limits applied

Courtesy of Brent Kendrick, Amgen
Role of Measurement Science and Standards in Supporting QbD

• Testing controls are key element of overall control strategy to ensure process consistently delivers correct product QAs

• Measurement science and standards can help:
  - Determine variability of test methods and setting test limits
  - Determine test methods are in control
  - Assess performance of new analytical technologies
Protein Particles in Biotherapeutics

Particle characteristics
- High hydration (≈ 95% water); low optical contrast can lead to large discrepancies between methods
- Existing bead standards do not mimic properties of actual particles

Three step approach:
1. Develop appropriate models for the instrument response
2. Identify and characterize the physical properties of protein particles relevant to the counting method considered
3. Develop reference materials that mimic protein particles.
Example of Instrument Models: Light Obscuration and Flow Microscopy

Correct flow microscopy for diffraction effects

Model light obscuration with light scattering model
  • Need independent measurements of particle refractive index
  • Need to account for aperture angles of instrument

10x to 50x discrepancy in counts for raw data

1.5x to 12x discrepancy in counts for corrected data
Quality Considerations Draft Guidance

• Focuses on analytical studies that may be relevant to assessing the similarity between a proposed biosimilar protein product and a reference product

• General principles:
  – Importance of extensive analytical, physico-chemical and biological characterization
  – Identification of lots used in the various analyses for biosimilarity determination
Monoclonal antibodies are complex...

**Bacteria, Yeast**

- Peptide
- Protein (no sugars)

**Mammalian**

- Glycoprotein (with sugars)

**Calcitonin**, ~3.5 kDa
**filgrastim**, ~19 kDa
**somatropin**, ~22 kDa
**epoetin**, ~30 kDa

**Monoclonal Antibody**, ~150 kDa
... but can be thoroughly characterized using state-of-the-art analytical science

**Biological characteristics**

- **Antigen binding**
  - Fab
    - \( V_L \)
    - \( V_H \)
    - \( C_L \)
    - \( C_H \)

- **Effector functions**
  - Complement interaction
  - Fc Receptor interaction

**Physicochemical characteristics**

- **N-terminal heterogeneity**
  - Pyroglutamate formation
  - Other modifications

- **Amino acid modifications**
  - Deamidation, Oxidation, Glycation, Isomerization

- **Fragmentation**
  - Cleavage in hinge region, Asp-Pro

- **Oligosaccharides**
  - Fucosylation, Sialylation, Galactosylation,

- **Disulfide Bonds**
  - Free thiols, disulfide shuffling, thioether

- **C-terminal heterogeneity**
  - Lysine processing, Proline amidation
Storage and Vialing Plan

Multiple Production Runs
(12.5 mm l-HIS, 12.5 mm l-HIS HCl, pH 6.0, currently at -20°C)

Dilute to 10 mg/mL and vial at 1 mL

Performed as needed

- Initial Fill 16,000 vials (-80°C)
  - 10,000 as RM/SRM
  - 6,000 reserved as primary standard

- 1 L x 80
- 100 mg/mL
- (-80°C)
Engineered Particles I: Understanding Effects of Tumbling in Coulter Counters

Image and electrical signal for tumbling and non-tumbling discs in NIST Micro-Coulter Counter.

Histogram of measured resistance changes for rods. Red bar indicates tumbling region. Arrow indicates expected diameter.
Engineered Particles II: Understanding Biases in Flow Imaging

- Diffraction effects result in oversizing of fibrous particles
- Lithographic “rods” produced
- We developed a simple algorithm that uses the measured perimeter to give an improved measurement of particle area

\[ D_m - 6.7\mu m \]

<table>
<thead>
<tr>
<th>Method</th>
<th>Equiv. dia. (µm)</th>
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<tbody>
<tr>
<td>SEM</td>
<td>13.4</td>
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<tr>
<td>Instrument algorithm</td>
<td>17.9</td>
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<tr>
<td>NIST algorithm</td>
<td>13.1</td>
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Flow imaging (top) & SEM (bottom)
Flow Imaging: Instrument Model

Approach:
1. Measure diameters of silica beads suspended in water/glycerol mixtures
2. Adjust the measured diameter for protein particles by the bias measured for silica beads

Transformation scales particle diameter, not count
Direction Forward: NMR Fingerprinting of mAb Domains – a Divide and Conquer Approach

NIST mAb

L-Histidine

50 °C

37 °C

25 °C

900 MHz, 50 °C

1H (ppm)

15N (ppm)

Fab

Fc
Neutron Measurements of Protein Therapeutics

Why neutrons?
- Neutron spectroscopies provide information on geometry of motion and length scale (nm - µm)
- Simplicity of the interaction allows easy interpretation of intensities & comparison of theory and models
- H & D scatter differently, many materials transparent to neutrons
- Neutrons can probe high conc. liquid, solid, & frozen formulations, & interfaces

Protein therapeutic projects
- Antibody structure and interactions: what causes high viscosity?
- Dynamics in freeze-dried formulations
- Protein association & aggregation
- Adsorption of proteins at surfaces & interfaces: Air-water & ice-water interfaces

Why neutrons?
- Neutron spectroscopies provide information on geometry of motion and length scale (nm - µm)
- Simplicity of the interaction allows easy interpretation of intensities & comparison of theory and models
- H & D scatter differently, many materials transparent to neutrons
- Neutrons can probe high conc. liquid, solid, & frozen formulations, & interfaces

Protein therapeutic projects
- Antibody structure and interactions: what causes high viscosity?
- Dynamics in freeze-dried formulations
- Protein association & aggregation
- Adsorption of proteins at surfaces & interfaces: Air-water & ice-water interfaces

nSoft
- NIST consortium enabling access to neutron facilities for soft materials manufacturers
Utility of a NIST mAb Reference Material

- Used to distinguish analytical variability from product variability and cross-check analytical methods
- Publically available, certified material with historical characterization data representative of a large class of biotherapeutic
- Used to reconcile differences between orthogonal methods measuring same attribute
- Used in qualification or assessment of changing analytical test methods
- Used to assess performance of new analytical technologies
Biomanufacturing Timeline

- 2005: Internal discussions on NIST involvement in Biopharma
- 2008: Joint FDA/NIST Protein Therapeutic’s Measurement Needs Workshop
- 2009: Sept. Congressional Testimony
- 2010: NIST Pilot Program $800K
- 2012: ACA: BPCIA (Biosimilars)
- 2013: $2M Initiative
- 2014: First European Biosimilar Approved
- 2014: $4M Initiative

Year

- Initiative shaped from years of outreach, talking, planning, & doing ($$)
CEX-HPLC Methods are used but often Lack Specificity to Measure Single Modifications

Intrinsic Poor Separation of Large Proteins such as Mabs
- **Acidic Forms**: Deamidated species, glycated species, oxidized species, sialylated species
- **Basic Forms**: C-terminal variants (HC -Lys, HC-Pro Amide), N-terminal variants (Gln vs pyro Glu), oxidized forms
- **Main Peak**: Typically contains mixture of variants with different degrees of modifications that “cancel out”

Regulatory Concern due to the outlined Gaps in Charge Based Methods
Analytical Characterization Is Foundation of Similarity Assessment

Modified from S. Kozlowski, FDA
Optical Method 2: Flow Imaging

Digital imaging of particulates 1 to 100 µm, 2 instrument vendors

- Flat flow cells
- Contrast & spatial resolution depend on proprietary particle id algorithm, light source, objectives, apertures
- Diffraction effects can result in oversizing of particles

Flow imaging (top) & SEM (bottom)