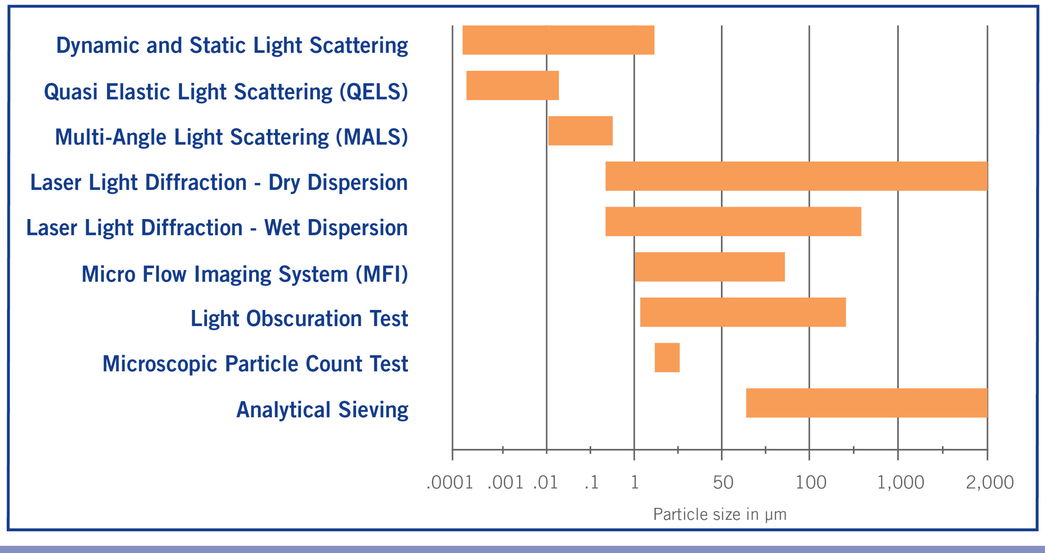
eurofins | Lancaster Laboratories

What are Therapeutic Protein Injections?

Therapeutic proteins are proteins that are engineered in a laboratory for pharmaceutical use. They are biotechnology-derived products of protein or peptides. They can be grouped based on their molecular types including antibody-based drugs, fusion proteins, anticoagulants, blood factors, bone morphogenetic proteins, engineered protein scaffolds, enzymes, growth factors, hormones, interferons, interleukins, and thrombolytics.

Why worry about particulate matter?

Particle Control is a small, but important, part of drug product development, as the presence of particles poses both safety and efficacy concerns. Particulate matter can be monitored at a number of sizes using different analytical methods, all of which can be performed at Eurofins Lancaster Laboratories.

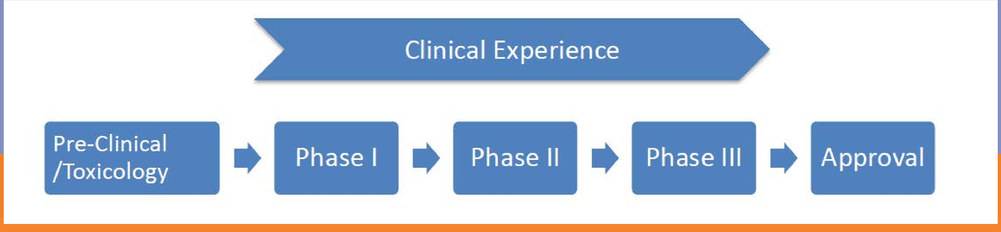


Sources of Particulate Matter

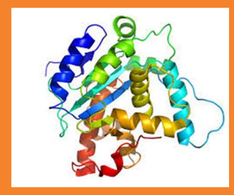
- **Extrinsic Particulate**
  - Truly foreign or unexpected material (examples: metal or fibers)
- **Intrinsic Particulate**
  - Due to degradation of formulation components or manufacturing and packaging components (examples: metals, lubricants (silicone oil), rubber, glass)
- **Inherent Particulate**
  - Particles of the protein, drug substance, or formulation components (examples: protein aggregates)

Monitoring Particulate Matter

Particulate matter can change over time. The best thing to do is monitor particulate matter throughout the clinical experience to ensure the presence of particulate is known and how it may change.



Determining Sub-visible Particulate Matter in Therapeutic Protein Injections



Formulation of Particulate Matter

Particulate matter can be formed in a multitude of ways throughout the clinical experience.

- Post-translation modification
  - Enzymatic modifications of proteins during or after biosynthesis
- Stressed conditions
  - Light, freeze-thaw, vortex, etc.
- Surface induced
  - Liquid-solid interface, air-water interface
  - Filling and packaging techniques
- Storage
  - Stability conditions: Time, temperature, closure system, configuration, etc.

Compendia Specifications

United States Pharmacopeia guidelines state the particle limits for therapeutic protein injections:

- Must be essentially free of visible particles
- Must contain low amounts of sub-visible particles, and USP <787> has the same requirements as USP <788>
  - Not more than 6000 particles/container ≥10µm
  - Not more than 600 particles/container ≥25µm

What is USP <788>?

This is the other particulate matter chapter that was used for testing prior to the development of USP <787>

- Testing for injections
  - Administered by the intramuscular or subcutaneous route
- Has two methods defined in its chapter:
  - Method 1: Light Obscuration
  - Method 2: Microscopic

ELLI Instrumentation:

HIAC 9703+ Sensor for Light Obscuration

- Measures the size of the particle as it passes between the light and the sensor

Benefits of USP <787>

	USP <788>	USP <787>
Minimum Containers	10	1
Minimum Volume	25mL	1mL
Sampling Size	5.0mL	0.1mL to 5.0mL
Sizes Analyzed	2.0-400µm	2.0-400µm

- This chapter allows for the use of smaller test product volumes and smaller test aliquots to determine particulate matter content
- USP <787> can be used as an alternative method to USP <788> when appropriate

System Controls

There are two types of system controls processed prior to testing:

- Blank Test
  - Environment is suitable for testing
  - Glassware used is properly cleaned
  - Water (or suitable solvent) is particle free
  - Result is NMT 1 particle/mL
  - Also processed between samples to ensure there is no contamination
- System Suitability Verification
  - Ran using 10µm and 25µm standards
  - Must meet calibrated concentration and range prior to testing

Testing of Samples

Samples are to be tested in the manner that most suitably represents administration of the product

- Testing directly from the sample container is the best practice.
  - Least amount of manipulation
- If testing directly is not possible, samples can be pooled into cleaned glassware.
  - Samples to be pooled in most appropriate manner to not introduce additional foreign particulate or air bubbles that could be counted as particulate

Testing of Solvents for Particulates

Solvents may be tested alone to ensure it is not a source of particles

- Allows for further evaluation in the case of an OOS result
- Particle free water is preferred as best practice when applicable.

- However, the solvent particle counts cannot be subtracted from the count of the sample
  - Solvent is considered part of the product

Special Sample Handling

- No use of needle
  - Can cleave proteins, creating inaccurate results, so a pipette and tips are used if pooling of a sample is needed.
- No inversion of sample
  - Minimizing bubbles and disruption of proteins.
- No sonication

Degassing

Proteinaceous products tend to retain gas, eliminating gas bubbles is key

- Two methods of degassing
  - Passively Degassing
  - Vacuum Degassing
- Sonication is not used
  - Sonication can disrupt proteins, thus creating more particles and higher counts

Sampling Sizes

Samples are tested by withdrawing four aliquots as well as a tare volume. Sampling sizes can vary based on volume of sample

Sampling Size	Total Volume Needed
0.1mL Samplings	1mL
0.2mL Samplings	1.5mL
0.3mL Samplings	2mL
0.5mL Samplings	3mL
1.0mL Samplings	5mL
2.0mL Samplings	9mL

Analyzing of Data

- First of the four aliquots is discarded, and the average of last three aliquots are calculated for a reportable result
- Particles ≥10µm and ≥25µm are only sizes with specifications
  - Agencies have requested particulates from sizes 2-10µm be studied and monitored
  - Particles smaller than 10µm could affect the safety, efficacy, and immunogenicity of products and should be evaluated.

Evaluation of Data

Evaluate the presence of particulate matter throughout the clinical experience, from pre-clinical trials to approval.

- A lower acceptance criteria may be warranted based on product
  - Further analyzation can determine this
  - Characterization of particulate
- Additional information in USP chapter <1787>

Determining Best Test Methodology

Agencies tend to agree that all testing should be designed for each product tested

- Feasibility Testing
  - Changing test factors to see how results are impacted
    - Amount of sample tested
    - Degassing Time
    - Sampling sizes
  - Maybe a dilution is needed
    - Smallest level for reproducible results
    - Useful for high concentration samples
- Verification Testing
  - Establish Acceptance Criteria based on the nature of the product tested
    - Set up limits more strict than compendia
    - Standard Deviation
    - Precision – Establish a desired number of replicates
    - Accuracy – The sample meeting established acceptance criteria

Microscopic Particle Count Test

Microscopic particle evaluation can be used for further evaluation, especially in cases of OOS results

Microscopic doesn't allow for much in way of more information

- Doesn't allow for evaluation of 2-10µm range that agencies look for
  - Only larger sizes can be evaluated (≥10µm, ≥25µm, and even ≥50µm)
- Microscopic can rule out some types of particles that are counted
  - Things such as silicone oil and other liquid contaminants will pass through the filter
  - Filter will catch all solid particulate and allows to see shape and size of these particles

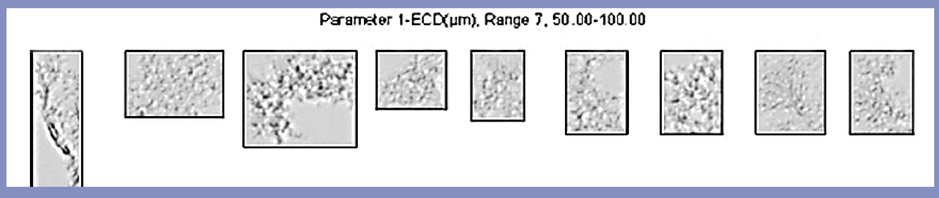
Author: Zachary Beck, B.S.

Acknowledgements: Jonathon Ribera, Daniel Peckman, Sue Williams, Tom Lehman

Further Evaluation

Micro-Flow Imaging (MFI) can be used as a complimentary test to Light Obscuration, and provides more in depth information on products. It is not a compendial test yet, but can be used as a compliment throughout the clinical process.

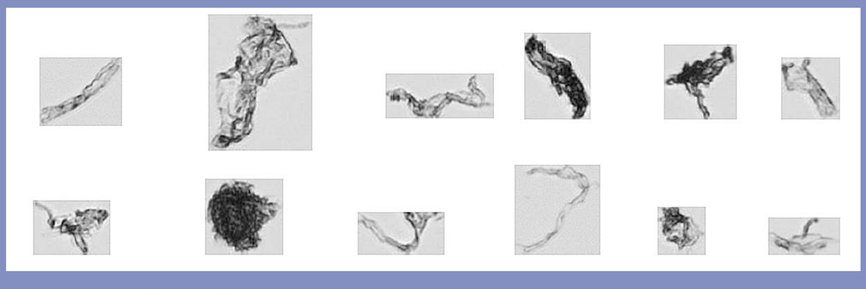
- **MFI is an image based analysis**
- **Analytical tool for evaluation of particles in solution**
- **MFI is an orthogonal method**
  - **Allows for evaluation of three-dimensional objects with a linear perspective**



The MFI has been proven to have the ability to screen for the presence of translucent particles (see image above) that may have been missed by light obscuration methods, due to light passing directly through the particles.

- **The MFI is able to better characterize the properties of the particulate present in a product**
  - **Particle Shape: circular, filamentous, etc.**
  - **Particle Type: protein, silicone oil, metal shavings, glass, or other production based particulate**
- **Allows for better evaluation of particulates in the 2-10µm range**
  - **Silicone oil droplets or proteins could conjoin and possibly result in larger particles later on in a stability study**
  - **Production based particles such as glass and metal shavings would more than likely stay consistent and not become larger**

Typical Protein Aggregates



Easy to Classify Particles

- **Silicone Oil**
- **Glass**
- **Air Bubbles**
- **Rubber**