CONTROLLING PARTICULATE MATTER IN OPHTHALMIC BIOLOGICS

ESTABLISHING A RISK-BASED ANALYTICAL APPROACH TO SUB-VISIBLE PARTICULATES

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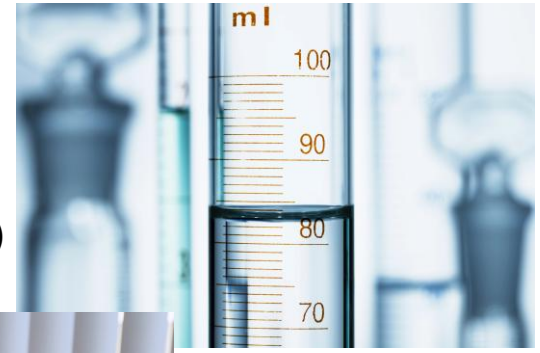
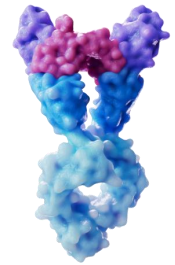
A Case Study in Risk-Based Qualitative and Quantitative Identification Analysis

- The control and characterization of Particulate Matter, specifically those <10µm, have historically presented challenges based on the limitations of quantitative analytical methods.
- Quantitative enumeration alone can be misleading for sub-visible particulates, specifically those 2 µm ≤ X ≤ 10 µm, resulting in misrepresentation of particle size distributions due to accumulation
  - Particle size distribution when determined using microscopic methods alone may not account for accumulation and should be limited to individual particulate enumeration.
- Qualitative identification and quantitative enumeration afford a holistic analytical approach to characterization of a sub-visible particulate matter profile
  - Qualitative Characterization Methods
    - Attenuated Total Reflection (ATR) Fourier Transform Infrared Spectroscopy (FTIR)
    - Scanning Electron Microscopy (SEM) with Energy-Dispersive X-ray Spectroscopy (EDS)
    - Inductively Coupled Plasma (ICP) Mass Spectroscopy (MS)
    - Digital microscopy
  - Quantitative Enumeration Methods
    - Malvern analysis
    - Microflow Imaging (MFI)





Qualitative vs Quantitative Analysis

Benefits and Drawbacks

Qualitative Analysis

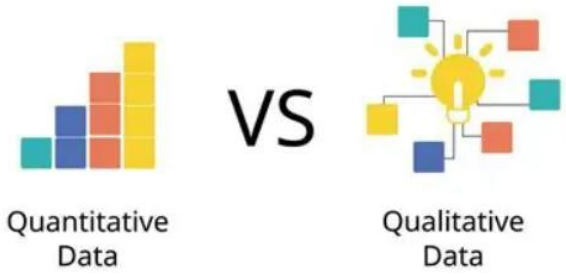
- Provides characteristics on chemical constituents but no enumeration

Quantitative Analysis

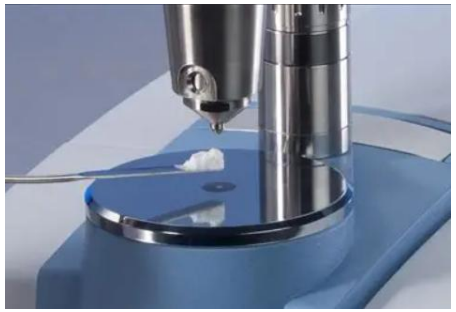
- Affords enumeration but may be limited due to limit of quantification and or misrepresentation of particle size distribution

A ***balance*** of both is needed for a holistic assessment and thorough

sub-visible particulate characterization profile

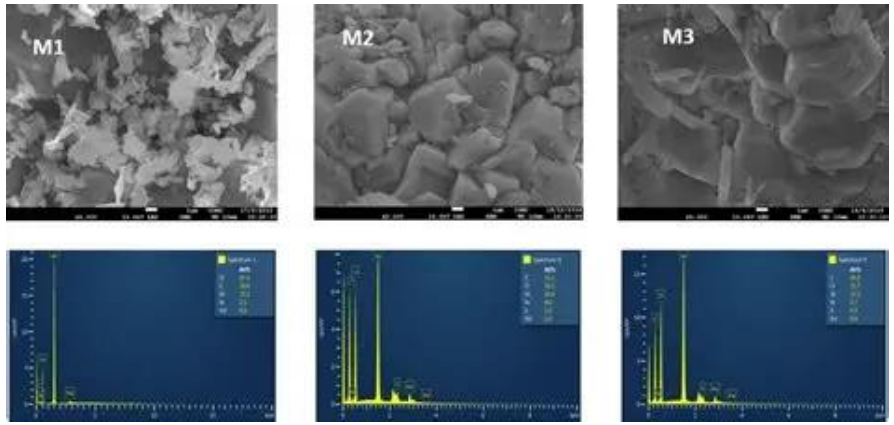


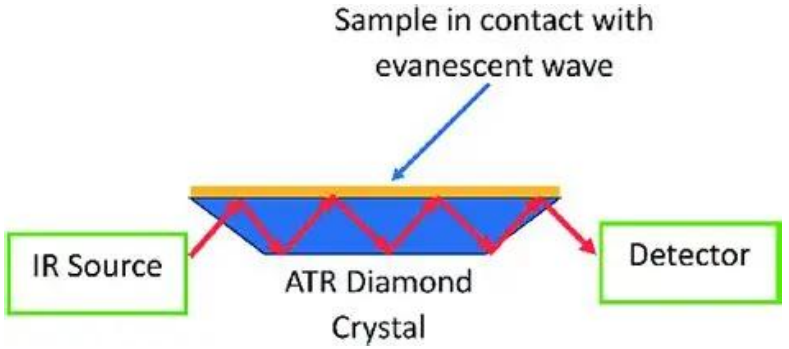
A Case Study –Benefits of Qualitative Analysis



Qualitative Characterization Methods

- **Attenuated Total Reflection (ATR) Fourier Transform Infrared Spectroscopy (FTIR)**
  - Affords direct sampling technique for a more convenient analysis
  - Useful for analyzing thin films, coatings, and surface layers for rapid high-level identification
  - Ideal for weak absorptions due to enhanced interaction of light with the sample at the ATR crystal interface
- **Scanning Electron Microscopy (SEM) with Energy-Dispersive X-ray Spectroscopy (EDS)**
  - Provides a mechanism for physical characterization (morphology) and chemical identification
  - Rapid and accurate elemental analysis which can be applied to a wide range of materials
  - Produces high-resolution images allowing for the examination of fine surface features of the sample's topography and morphology with elemental composition
  - An essential technique for insight into the structure and composition of materials at the microscopic level

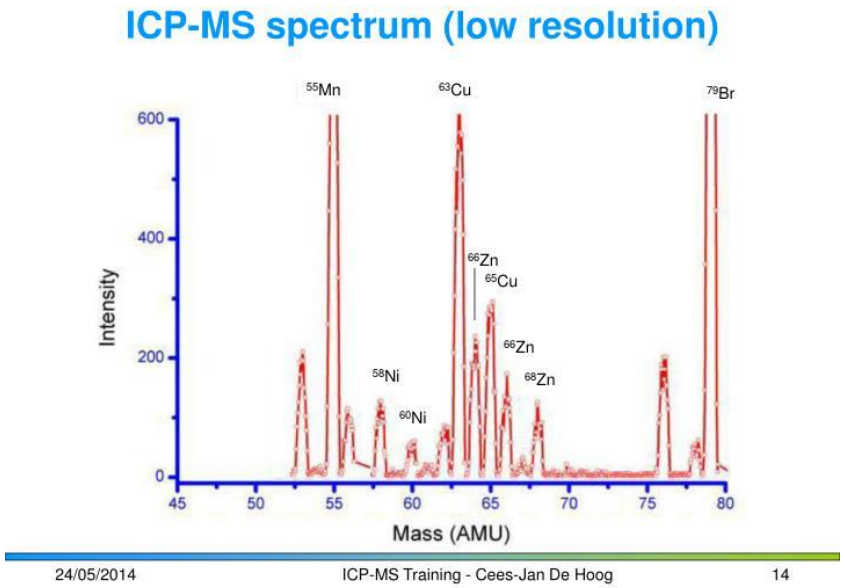


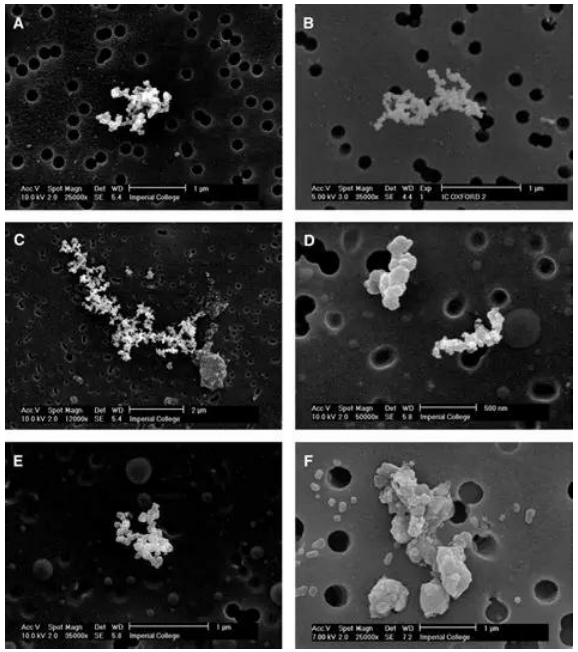


A Case Study –Benefits of Qualitative Analysis

Qualitative Characterization Methods

- **Inductively Coupled Plasma (ICP) Mass Spectroscopy (MS)**
  - Analytical technique used to detect trace metals and non-metals at ultralow concentrations via ionization, allowing for the detection of atomic and small polyatomic ions.
  - Capable of measuring elements at concentrations as low as parts per billion essential for applications requiring high sensitivity.
- **Digital Microscopy**
  - Digital Microscopy such as Keyence utilizes cameras and magnified optics to capture high-resolution images for inspection and analysis for immediate measurements of profile, roughness, flatness, level of wear or comparison of three-dimensional characteristics.

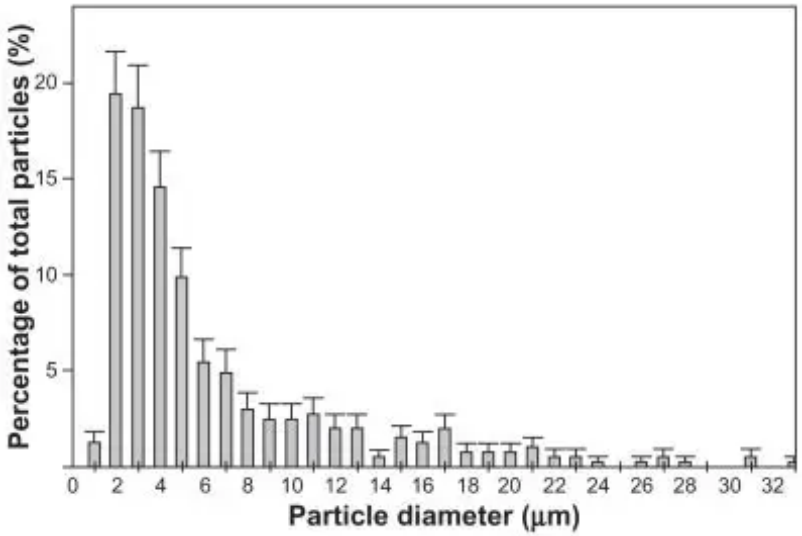


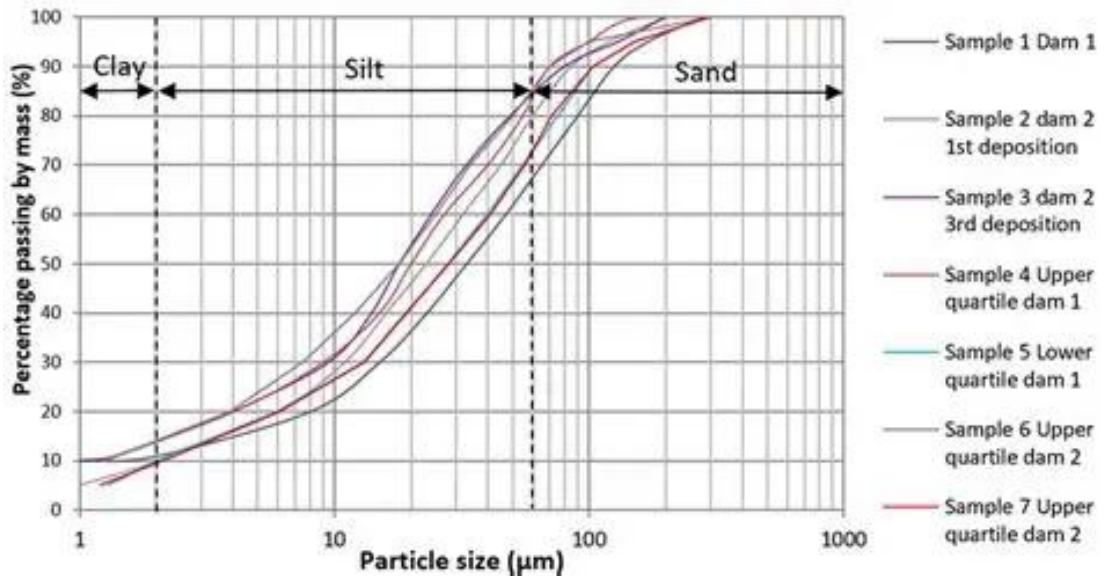


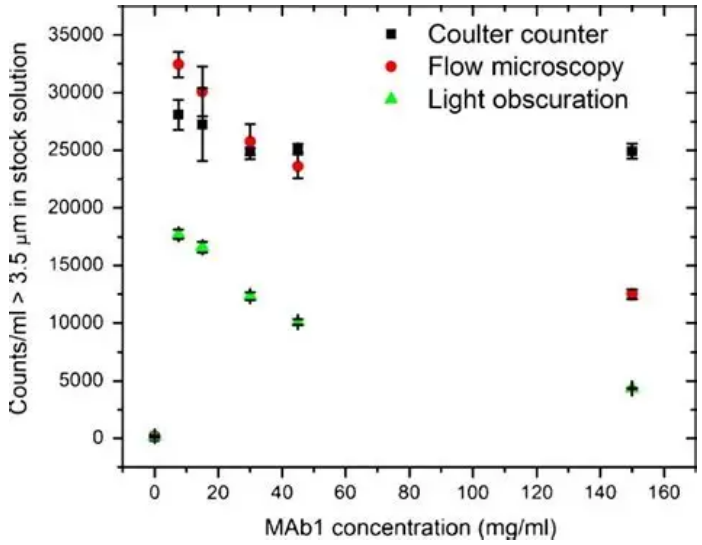
A Case Study –Benefits of Quantitative Analysis

Quantitative Enumeration Methods

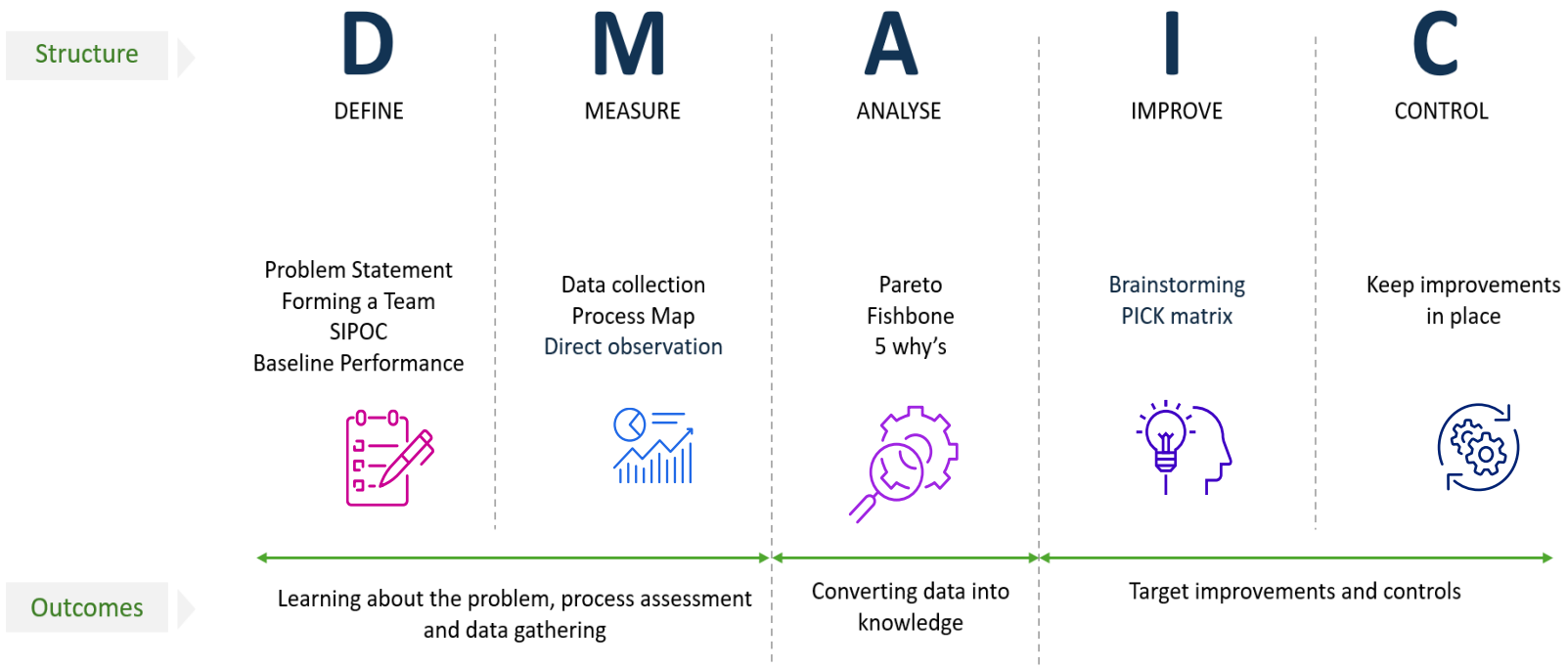
- **Malvern Analysis**
  - Dynamic Light Scattering (DLS) is used to determine particle size distribution
  - Benefits include rapid results and small sample size (~ 0.25g) but may be limited in characterization due to the distribution being based on diameter which does not account for accumulation of smaller sub-visible particulates (<2µm)
- **Microflow Imaging (MFI)**
- Micro-Flow Imaging (MFI), Flow Imaging Microscopy (FIM), or Dynamic Imaging Analysis (DIA) is a technique used for sizing, quantifying, visualization, for identifying (sub-) visible particles.

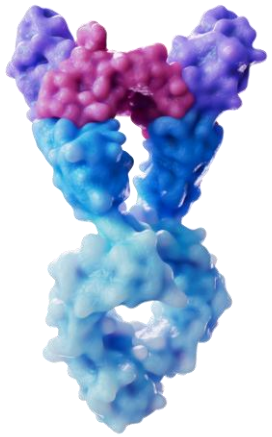






A Case Study in Risk-Based Qualitative and Quantitative Identification Analysis

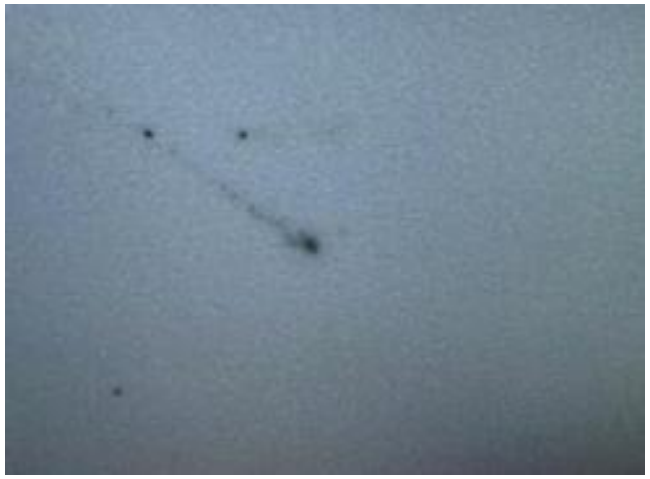


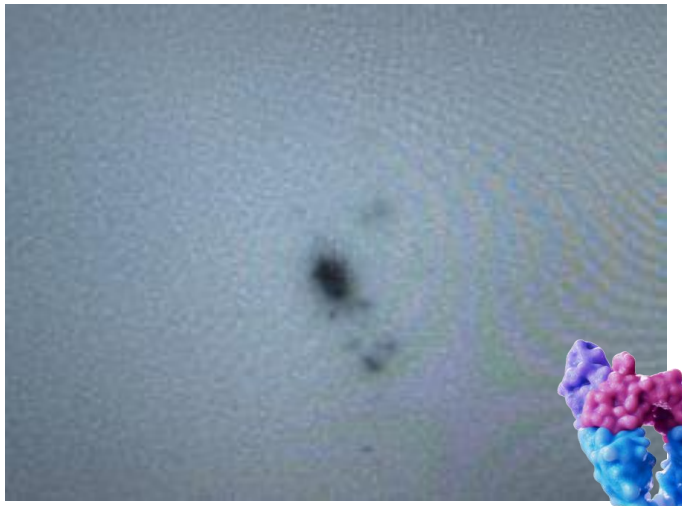


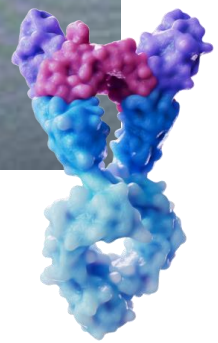
Suppliers	Inputs	Process	Outputs	Customers
Internal and external providers of resources, materials, knowledge and service	The resources, materials, services, and knowledge that feed into the process.	The step or sequence of steps that is being investigated, analyzed and improved.	The products and services produced by the process.	Organizations or users that receive the final outputs of the process.

Case Study Count of Sub-Visible Particulate Matter using Microscopic Analysis by Malvern

Sample	Results [Particles/mL]		
Acceptance Criteria USP <789>	*≥ 10µm*	*≥ 25µm*	*≥ 50µm*
	50 particles/mL	*5 particles/mL*	*2 particles/mL*
Acceptance Criteria System Suitability (Control)	*≥ 10µm*	*≥ 25µm*	*≥ 50µm*
	≤ 10 particles/mL	*≤ 1 particles/mL*	*≤ 0 particles/mL*
In-Process Test Article A	5	1	0
In-Process Test Article B	5	2	0
Stability Study Test Article A T_0	80	20	8
Initial re-test Article A	13	0	0
Expanded Test Article- A1	7	1	0
Expanded Test Article- A2	5	0	0
Expanded Test Article- A3	9	1	0
Expanded Test Article- A4	5	0	0
Expanded Test Article- A5	50	7	3
Expanded Test Article- A6	11	1	0



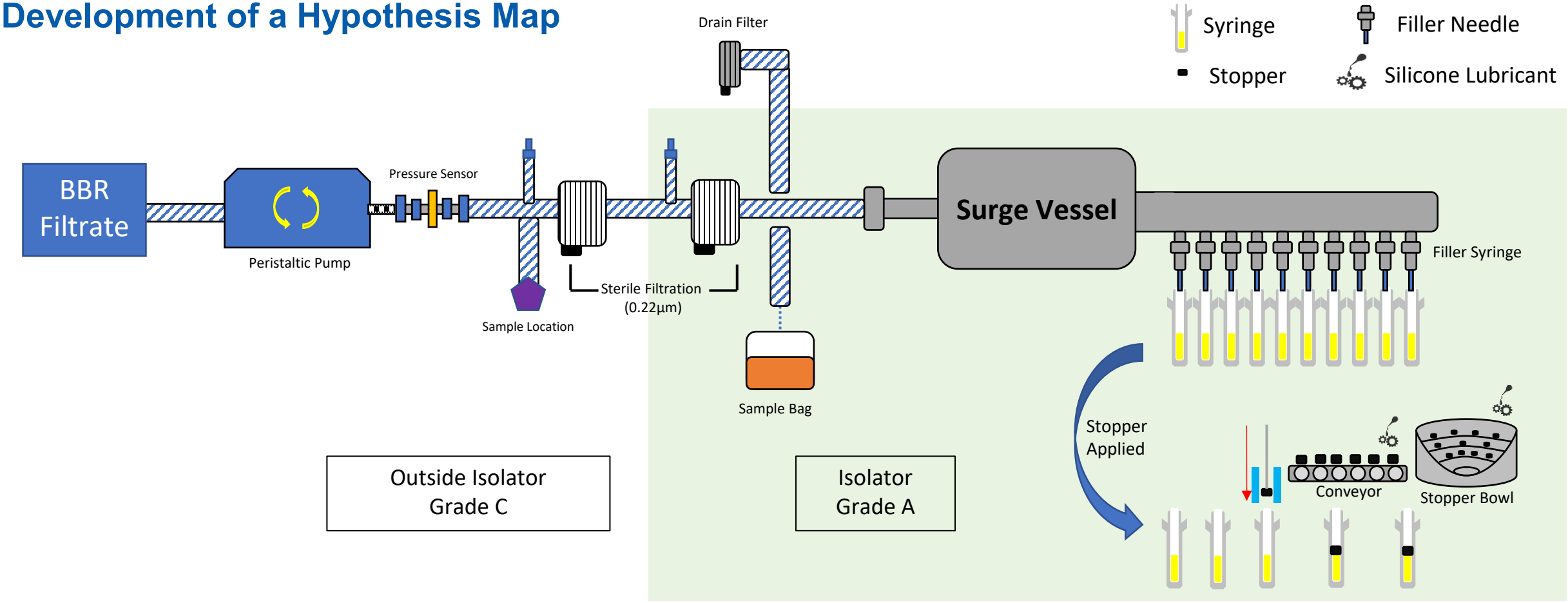




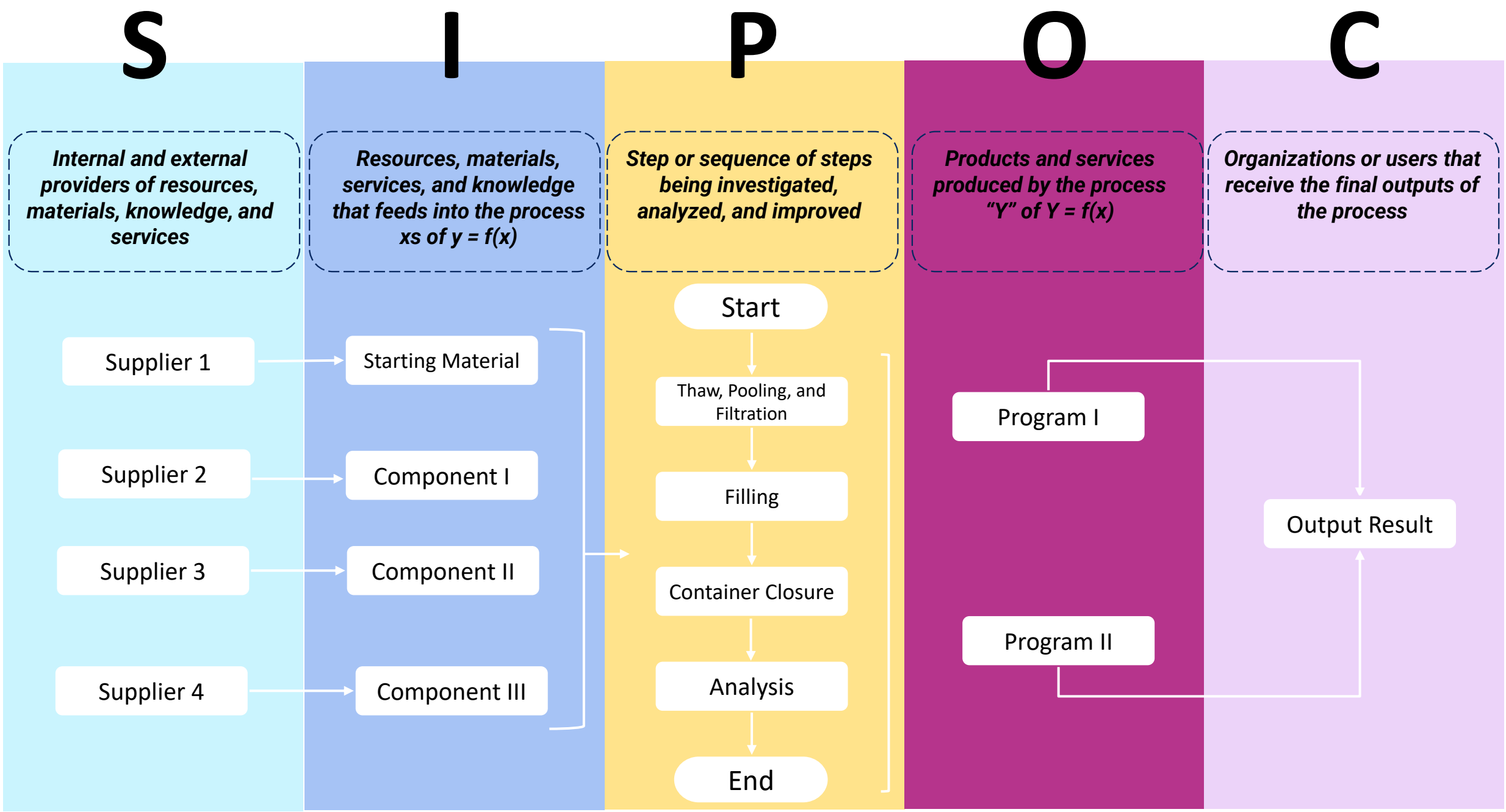
An accumulation of sub-visible particulates were observed and characterized to determine root cause

- Attenuated Total Reflection (ATR) Fourier Transform Infrared Spectroscopy (FTIR)
- Scanning Electron Microscopy (SEM) with Energy-Dispersive X-ray Spectroscopy (EDS)
- Inductively Coupled Plasma (ICP)
- Mass Spectroscopy (MS), and Keyence microscopy

Development of a Hypothesis Map

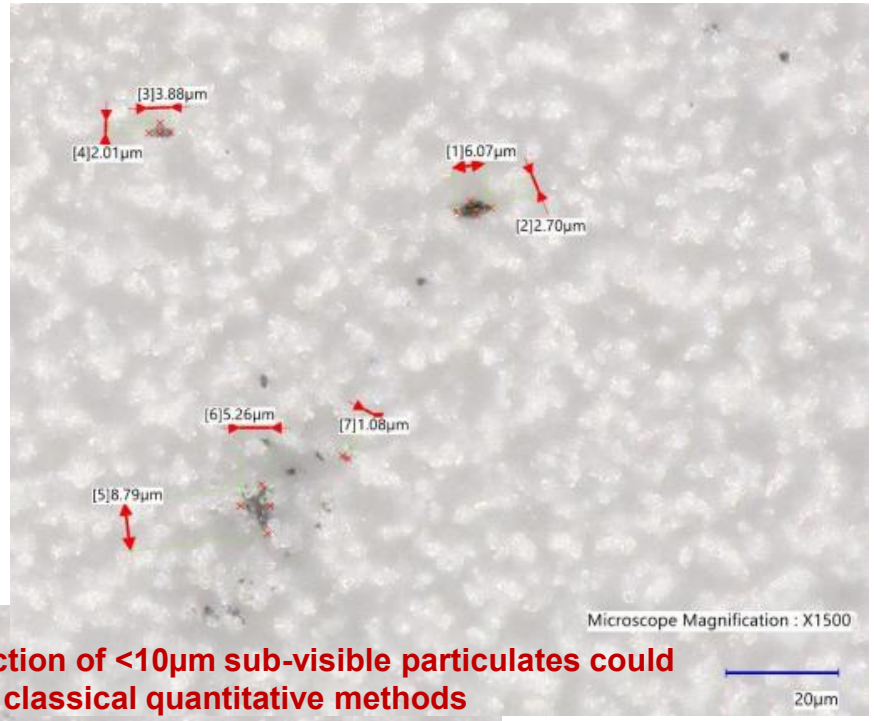


- Root Cause Analysis Tools and Identification of Isolates were used to develop a Hypothesis Map for potential points of ingress
  - SIPOC
  - Is/Is Not
- Historical analysis of sub-visible particulate matter across multiple programs was utilized to focus the investigation

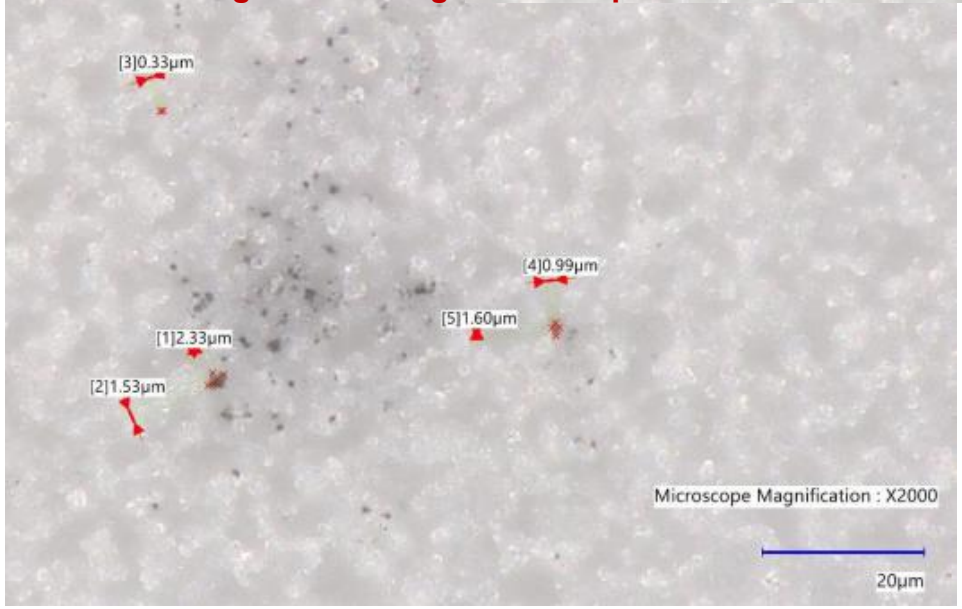

S
I
P
O
C
Internal and external providers of resources, materials, knowledge, and services
Resources, materials, services, and knowledge that feeds into the process xs of y = f(x)
Step or sequence of steps being investigated, analyzed, and improved
Products and services produced by the process "Y" of Y = f(x)
Organizations or users that receive the final outputs of the process
Supplier 1
Supplier 2
Supplier 3
Supplier 4
Starting Material
Component I
Component II
Component III
Start
Thaw, Pooling, and Filtration
Filling
Container Closure
Analysis
End
Program I
Program II
Output Result

Characterization Analysis

- Qualitative analysis was used in conjunction with RCA tools to identify potential points of ingress
- Characterization of the sub-visible particulate profile was then established using qualitative and quantitative analytical methods for equipment and consumable raw material components
- Design of Experiment included analysis of Drug Product and raw material components with 0.03% polysorbate PS80 and Milli-Q Water with comparison to negative and positive control
  - Test articles prepared in triplicate with concentration of 30 samples on one filter under the following conditions
    - Analysis performed at T_0
    - Sonication for 15min
    - Incubation at 2-8°C after 2 weeks



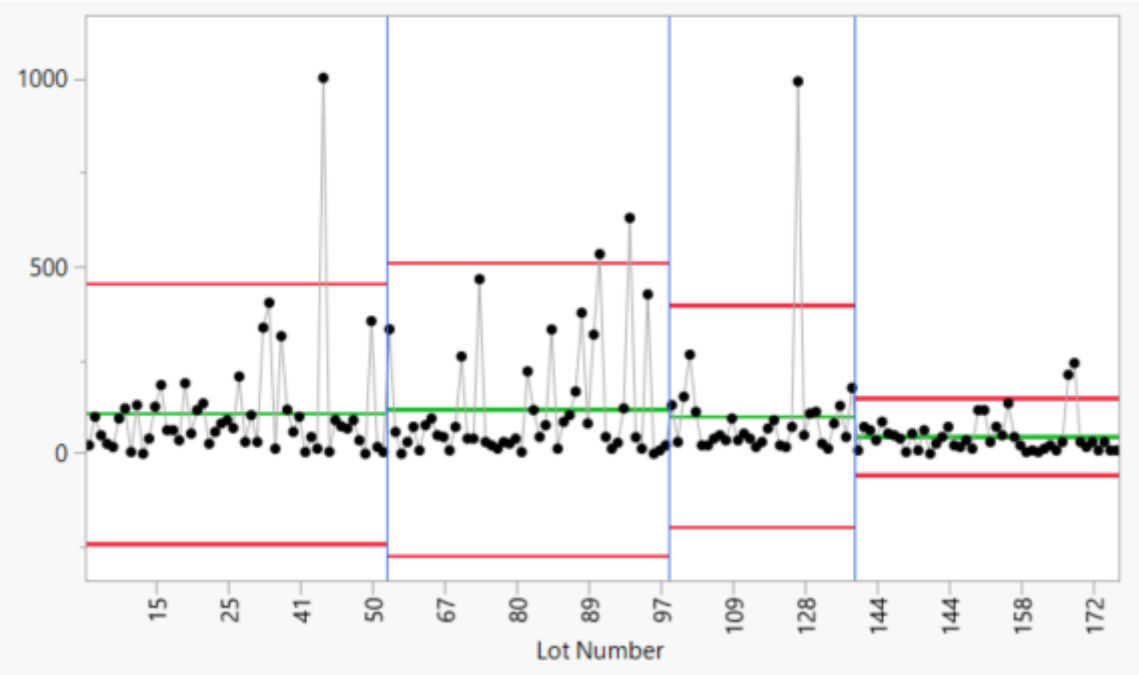
***Analysis confirmed a collection of <10µm sub-visible particulates could not be distinguished using classical quantitative methods**

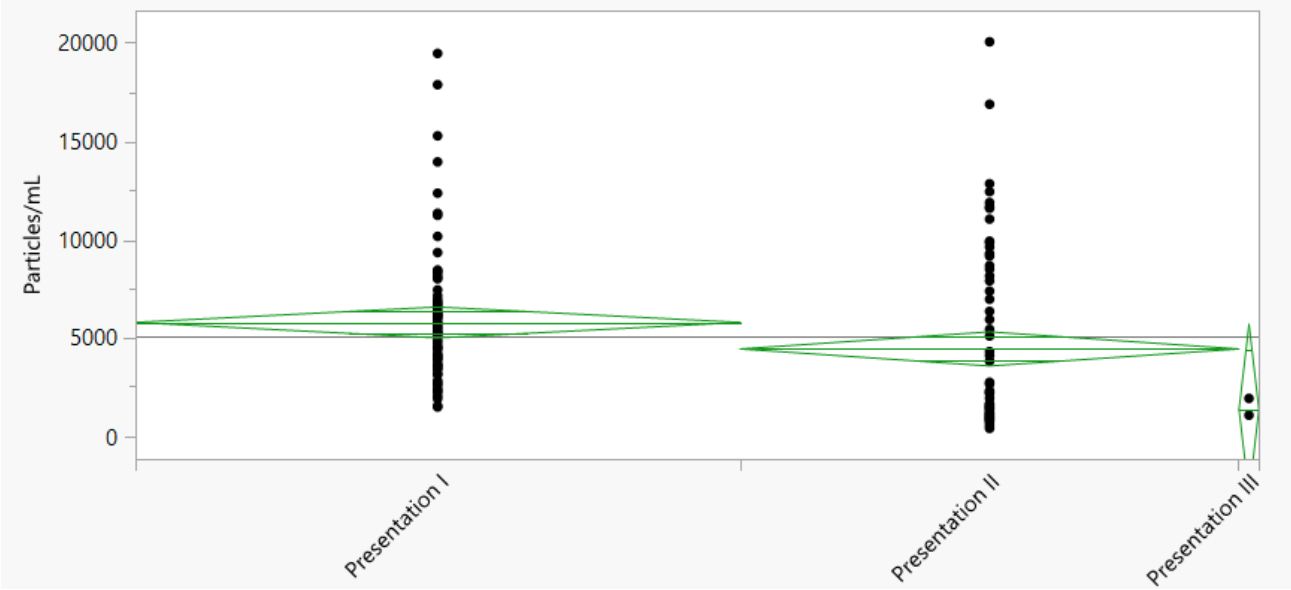


Sample Description	Component Evaluated	
	Component I Source lot	Component II Source lot
Control In-Process Materials to Produce Test Article A	N/A	N/A
Components used to Produce Test Article A	CI-1	CII-1
		CII-2
	CI-2	CII-1
		CII-2
	CI-3	CII-1
		CII-2
Components used to Produce Test Article B	CI-1	CII-2
	CI-3	CII-1
Components used to Produce Test Article C	CI-1	CII-2
	CI-3	CII-1

Historical Review of Quantitative Analysis of Sub-Visible Particulate Matter

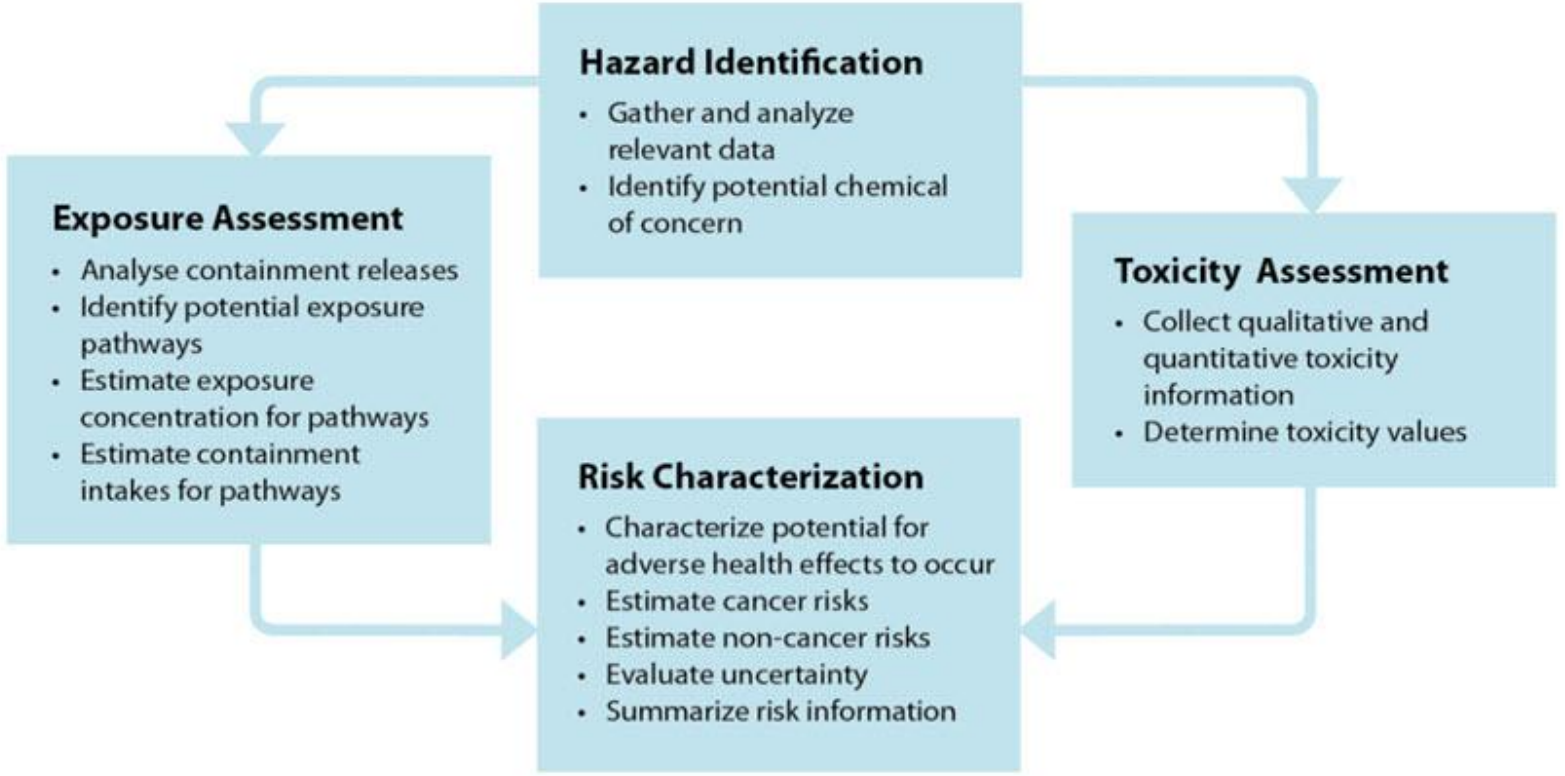
- Historical review of the subvisible particulate profile for multiple program presentations was performed using Microflow Imaging (MFI)
  - MFI provides a method by which to identify and enumerate sub-visible particulates ranging in size from 2 µm ≤ X ≤ 10 µm
- Determination of sub-visible particulate by microscopic methods should account for individual particulate enumeration reported as ≥10µm, ≥25µm, and ≥50µm, and not combined size due to accumulation

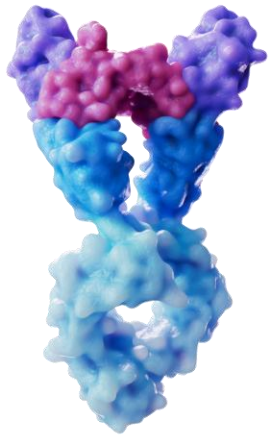




Assessment of Risk

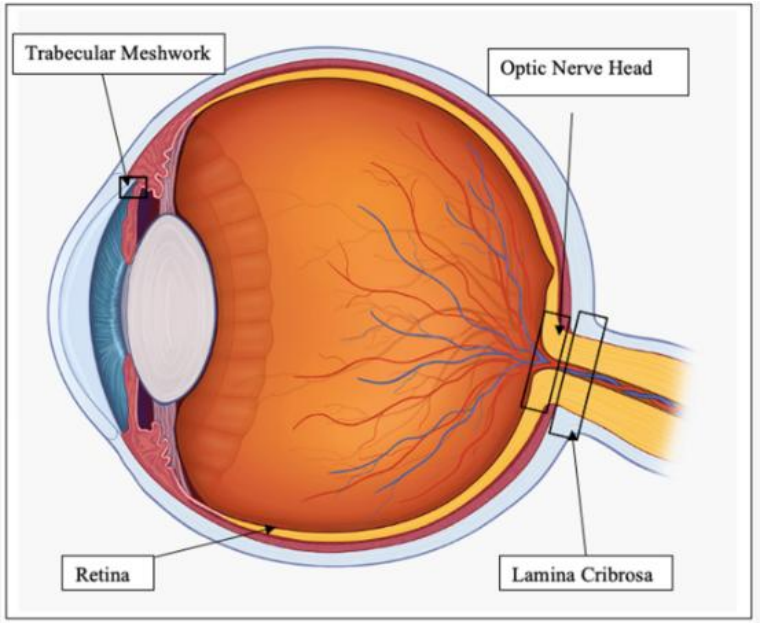
- **Evaluation of the potential risk to product, process, and quality system should be considered**
  - Health Hazard Evaluations and Health Risk Assessments may be conducted by a physician or committee of physicians, engineers and other scientists to determine the level of risk posed by an event.
- An assessment for potential Sub-Visible Particulates to be present requires characterization by qualitative and quantitative methods and should consider the following.
  - Route of Administration
  - Isolate Composition (toxicological effects)
  - Enumeration





- The harm of sub-visible particulates to be present within an ophthalmic indication is the potential for isolates to accumulate within the human eye
  - This presents an increased risk of intraocular inflammation (IOI) ranging from minor afflictions to severe ophthalmic conditions
- Aspects considered relate to the potential presence of sub-visible particulates (≤10µm)
  - Anatomical and physiological response to sub-visible particulates (≤10µm) within the human eye based on chemical properties associated with isolate composition
  - Mechanical affect of sub-visible particulates (≤10µm) to be present within the process
  - Evaluation of the Quality system based on historical experience

Mechanism of Action: An Evaluation of Sub-Visible Particulates



Aspects considered relative to the potential presence of sub-visible particulates (≤10µm) in ophthalmic indications

1. Anatomical and physiological response to sub-visible particulates (≤10µm) within the human eye based on chemical properties associated with isolate composition

- The anterior segment of the human eye includes the cornea, lens, iris, ciliary body, and ocular drainage tissues, primarily the trabecular meshwork (TM) and Schlemm's Canal (SC) responsible for regulation of intraocular fluid.
    - Trabecular Meshwork ~350µm by 50–150µm
    - Schlemm's Canal ~25mm
- Intraocular Inflammation (IOI) is a known risk factor of intraocular injections which can be associated with an increased Interocular Pressure (IOP) affecting either the optic nerve or regulation of fluid across the trabecular meshwork of the human eye.
- Based on the anatomical properties of the human eye, a presence of sub-visible particulates does not pose a potential for mechanical obstruction as particles ≤ 50 µm would be expected to pass freely across the interstitial space of the drainage tissues within the human eye.

2. Mechanical affect of sub-visible particulates (≤10µm) to be present within the process

- General preventative maintenance
- Cleaning regiment (cleaning agents and frequency)
- Operational limitations based on equipment design and functionality inclusive of device component considerations

3. Evaluation of the Quality system based on historical experience

Conclusions & Take Aways

- Particle size distribution using classical microscopic methods alone may not account for accumulation and should be limited to individual particulate enumeration.
- Qualitative identification and quantitative enumeration is required for characterization of a sub-visible particulate profile across a process and to focus investigational approach and inform risk assessment
  - Affords a holistic analytical approach to the control and characterization of Sub-visible Particulate Matter in accordance with USP <789>
  - Determination of sub-visible particulate by microscopic methods should account for individual particulate enumeration reported as ≥10µm, ≥25µm, and ≥50µm
  - MFI provides a method by which to identify and enumerate sub-visible particulates ranging in size from 2 µm ≤ X ≤ 10 µm for cases where HIAC may not be suitable
- Evaluation of the potential risk to product, process, and quality system should be considered using HHE or HRA tools

- These assessments should be inclusive of anatomical and physiological response to the potential presence of Sub-visible Particulate Matter based on
  - Route of Administration
  - Isolate Composition (toxicological effects)
  - Enumeration

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