Particle Identification

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Root cause, particle characterization
Investigation ….. regarding the metal particulate contamination in lots ….. was inadequate …..The atypical contamination found in these lots was metal, however, the batches were not rejected. Additionally, there was no investigation *conducted to determine the cause of the black metal particulates found in these lots*

"reported a particle identified in a vial during an AQL inspection. There was no documentation on the identity of the particle and whether it was inherent or foreign (black debris, fiber, glass fragments, etc.)."
2015

Visual Inspection Lifecycle

- Create baseline
- Use the Trending Data from Reject Characterization and Monitoring
- Review the various particulate sources for Process Improvement opportunities
- Focus on the most predominant particle types
- Repeat the Cycle of Monitoring, Trending, Corrective actions and follow-up Monitoring
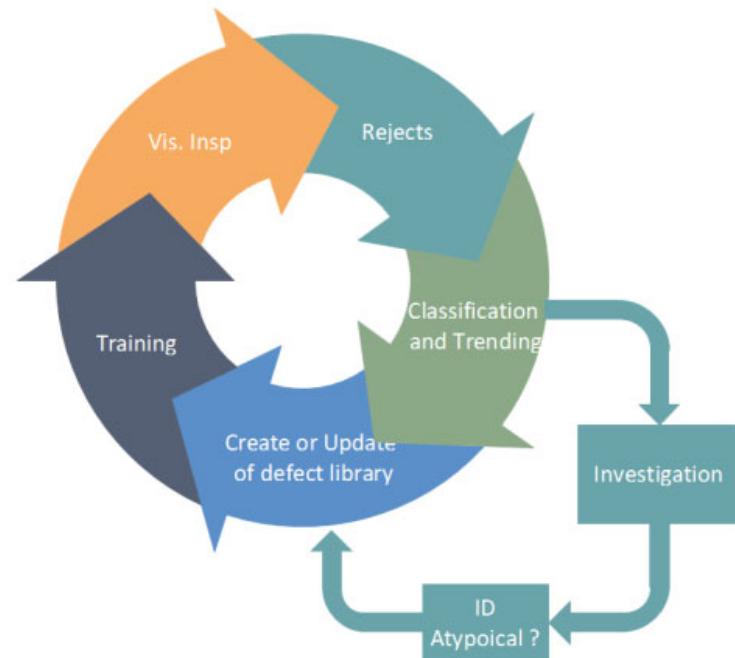
GUIDANCE DOCUMENT
Inspection of Injectable Products for Visible
Particulates *Draft Guidance for Industry*
DECEMBER 2021

…. manufacturers should conduct a risk assessment during <mark>product development</mark>. During this risk assessment, manufacturers **should identify the typical visible particulates** that could contaminate the injectable product and **characterize their size ranges, quantity, and composition**; <mark>Level 2/ Level 3</mark> determine risks for each type; and provide a **visual description (e.g., photographs or drawings of typical defects**) to be used for training purposes.

Manufacturers should also consider the potential sources of particulates, **appropriate analytical methods to monitor them**,…<mark>Level 1/ Level 2</mark>

Intrinsic particles

 … Manufacturers should also evaluate trends in reject data at designated manufacturing facilities and use a life cycle management approach <mark>to monitor and control process-related</mark> intrinsic particulates in their final products. <mark>Level 1</mark> ….In addition, an analytical method suitable for characterizing and monitoring product-specific particulates should be developed….
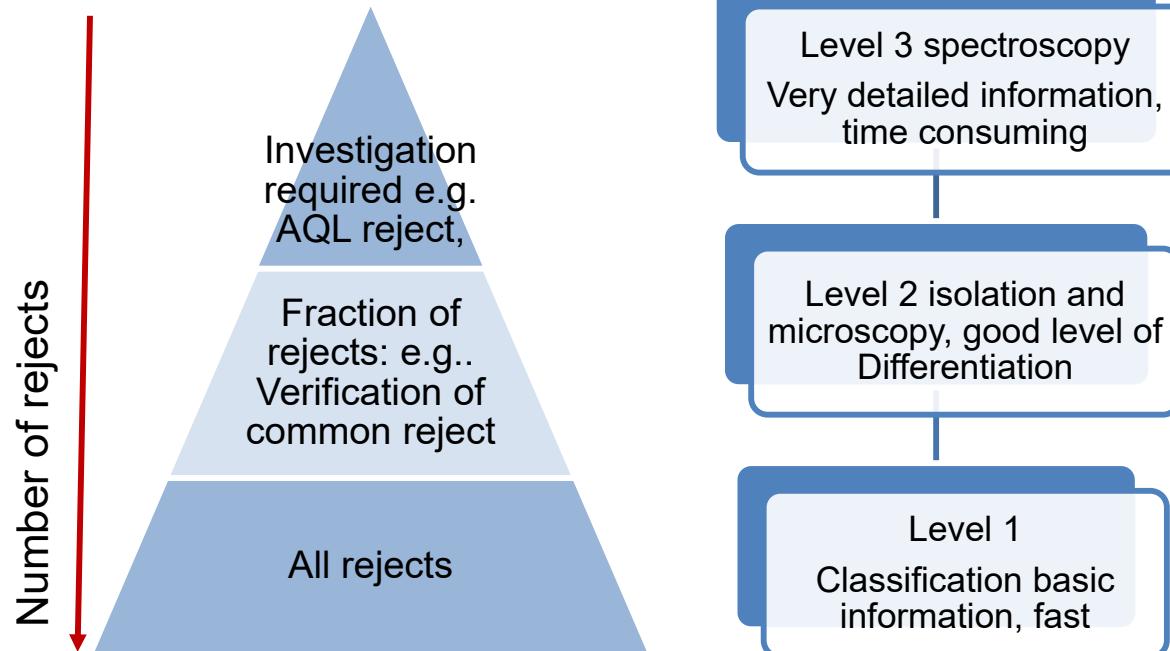
Knowledge of intrinsic particles is important in order to distinguish from extrinsic particles

Classification and Trending

Number of rejects

Investigation required e.g. AQL reject,

Fraction of rejects: e.g.. Verification of common reject

All rejects

Level 3 spectroscopy
Very detailed information, time consuming

Level 2 isolation and microscopy, good level of Differentiation

Level 1
Classification basic information, fast

Particulate Characterization/ID Levels

Level One: Visual classification (in-Situ)
• Nondestructive, as seen during manual inspection
• Light, dark, sinking, floating, color, shape, etc.

Level Two: Macroscopic and Microscopic
• Rapid characterization to specific material categories
• Metallic, glass, rubber, plastic, fiber (natural or synthetic), silicone lubricant, inherent particles, etc.

Level Three: Spectroscopic or other fingerprint ID
• FTIR, Raman, Elemental, Mass Spec, etc.

Roy Cherris Visual Inspection Forum 2013, Bethesda

Comparison of Characterization Level

Level		Cost	time/particle
1	light microscopy	Invest: 2T€ €	15 min
2	Isolation, Polarized Light microscopy	Invest: 60 T€	30 min
3	SEM / Raman/ IR	Invest: 70 T€ (IR), 150 T€ (Raman), 180 T€ (SEM)	30 min

Classification Level 1

1. Classification is based on basic observations
   Defined by trajectory, shape, density

2. Classification could be done by a experienced operator probably trained for special tools

3. Reason to go on with level 2 characterization could be statistics, uncertainty about nature of the particle

Categories / Attributes

Categories

Category		Category	
Glass-Like	[ ]	Polymeric-like	[ ]
Metallic-like	[ ]	Dark Particle	[ ]
Fiber-like	[ ]	Light Particle	[ ]

Attributes for further description

Shape	Colour	Location	Density	Size
Spherical	Light	Body	Floater	
Irregular	Dark	Bottom	Fixed	
Elongated	Transparent	Shoulder		

Level 1 characterization groups e.g. dark particle, light particles, fiber-like might be sampled by a basic universal sampling plan like $\sqrt{N}+1$

Isolation is required for further investigation
Clean area mandatory:
- clean room, clean bench, ultra cleaned glassware, requires trained personnel

Various tools for isolation:
- Capillary, tungsten needles, filtration

Microscope helps to give further details:
- Rubber, metal, synthetic vs natural fiber, crystal shape, color

After isolation particle can be easily transferred to level three

Microscopic information – Level 2

Incident Light	Select	Transmitted Light	Select	Level II Category	Select	Level II Category	Select
Clear	[　]	Transparent	[　]	**Glass**	[　]	**Polymeric**	[　]
Opaque	[　]	Opaque	[　]	**Metallic**	[　]	**Rubber Stopper**	[　]
Reflective	[　]	Crystalline	[　]	**Fiber**	[　]	**Semi-Solid - Silicone**	[　]
Physical	**Select**	**Crossed Polars**	**Select**	**Fiber - Natural**	[　]	**Possible Inherent API**	[　]
Crystalline	[　]	Isotropic	[　]	**Fiber - Synthetic**	[　]	**Possible Extrinsic**	[　]
Shaving	[　]	Anisotropic	[　]				
Resilient	[　]	Pseudo-Birefringence	[　]				
Shard	[　]	Isotropic Rod	[　]				
Size Length (um)		Uniform fiber	[　]				
Size Width (um)		Irregular frayed fiber	[　]				

Level Two Visible Particle ID Trending

- Fibers - Cellulosic
- Fibers - Synthetic
- Glass
- Stainless Steel
- Polymeric Tubing
- Rubber Stopper
- Polymeric - Other
- Silicone - Lubricant
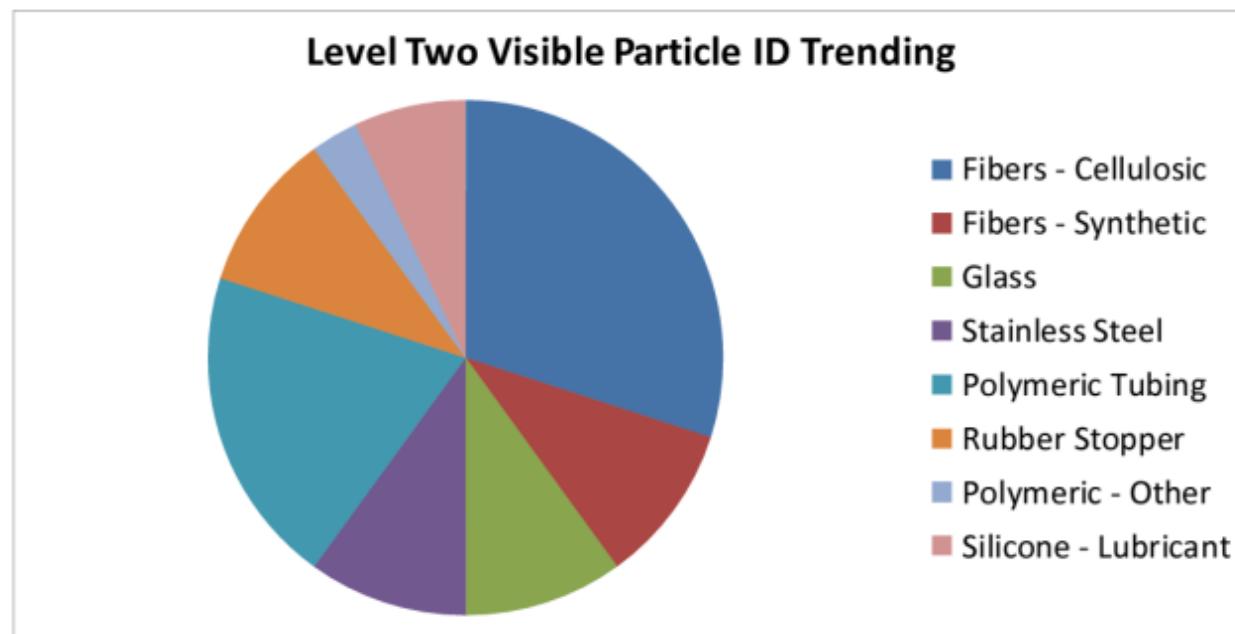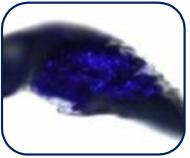
Spectroscopy Level 3

method	meaning		time/particle
PLM (polarized light microscopy)	color + shape e.g.: black fibres		1-5 min
SEM/EDS analysis	> 5µm Elements		20-180 min
IR – microscopy	> 50 µm Structure		20-180 min
RAMAN - microscopy	> 0.5 µm Structure		20-180 min

Fiber – Level 1

Category	Select
Glass-Like	[ ]
Metallic-like	[ ]
Fiber-like	[x]

Category	Select
Polymeric-like	[ ]
Dark Particle	[ ]
Light Particle	[x]
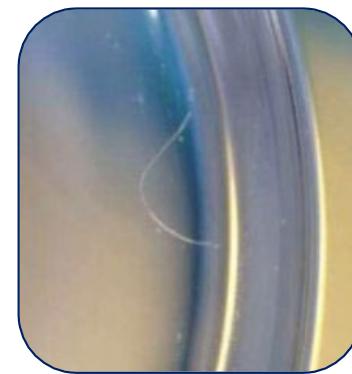
- Fibers can be easily classified. Might be sufficient for trending
- Further classification of fibers can be preformed in situ with an inverted microscope due to morphology and texture

Fiber – Level 2

- Microscopy of isolated fiber gives further information (cotton, protein based fiber, synthetic)
- Spectroscopy can give a very specific fingerprint for root cause or kind if synthetic fiber

Level 2
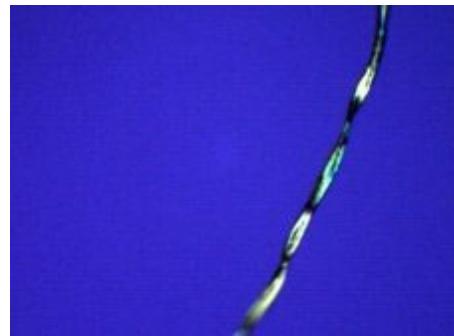
Level 2

Level 1

Metal particle Level 1 and Level 2

- Characterized density and reflectivity
- Sufficient for trending
- Hard to observe while swirling
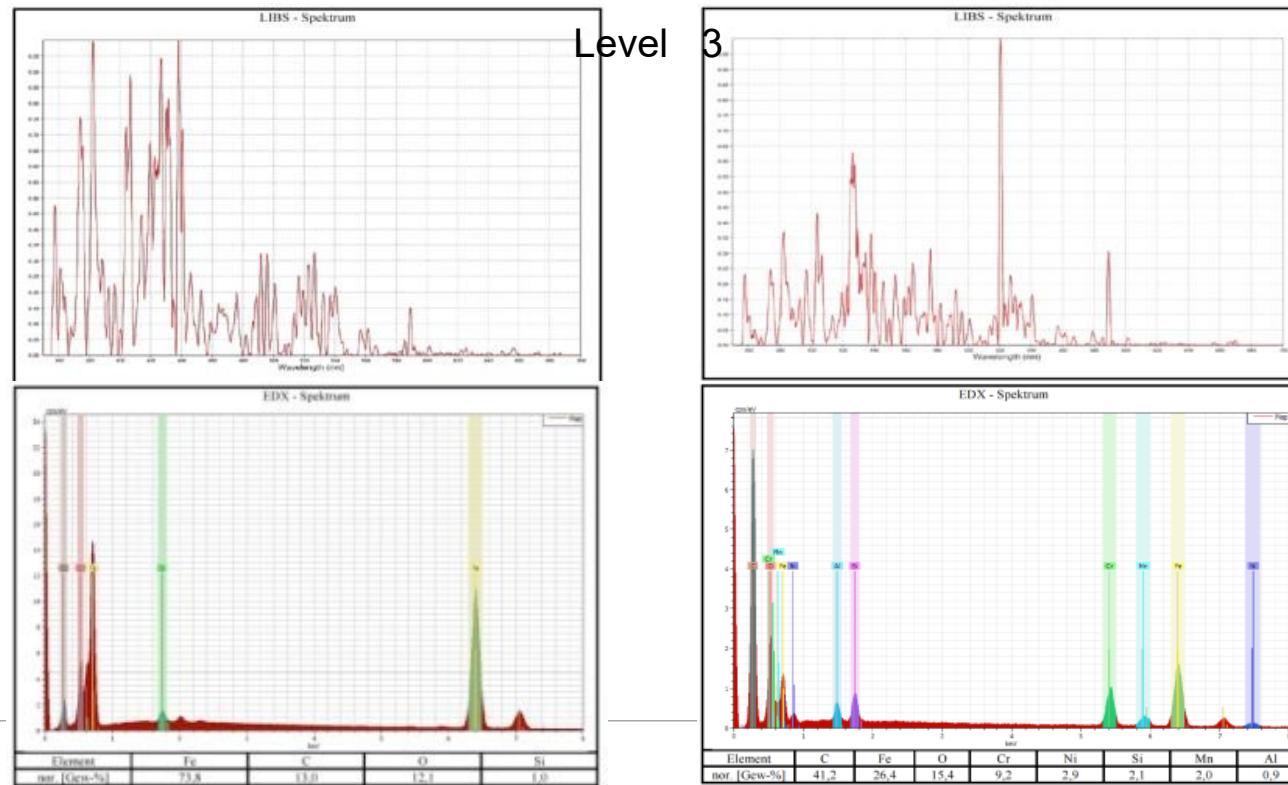- Usually easy to find at the bottom

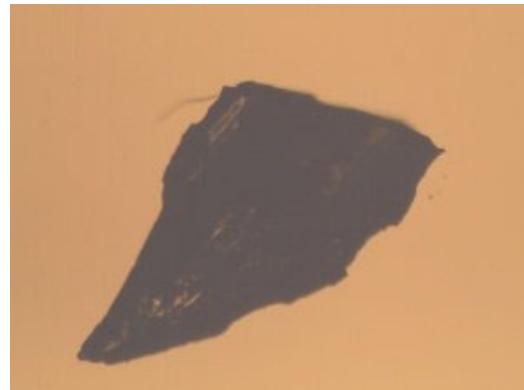Level 1   Level 2   Level 2

Spectroscopy on metals – Level 3

Spectroscopy gives more detailed information on the kind of steel e.g. low alloyed vs high alloyed steel which might be needed for root cause investigation

Level   3

Element	Fe	C	O	Si
nor. [Gew-%]	73,8	13,0	12,1	1,0

Element	C	Fe	O	Cr	Ni	Si	Mn	Al
nor. [Gew-%]	41,2	26,4	15,4	9,2	2,9	2,1	2,0	0,9

Glass particle Level 1 and Level 2

- Glass has a very characteristic shape which is sufficient for classification
- Further characterization for root cause investigation: element specific methods e.g. SEM or LIBS favorable
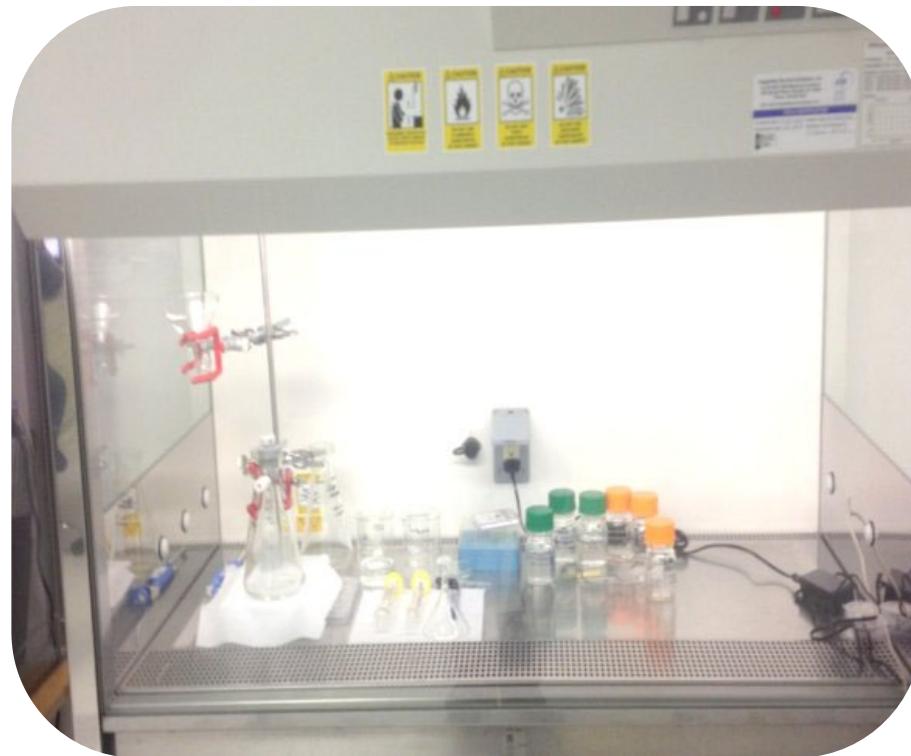

Level 1

Level 2

Glass particle Level 3

LIBS

Si, Na, Al (Fiolax)

Raman

Glass
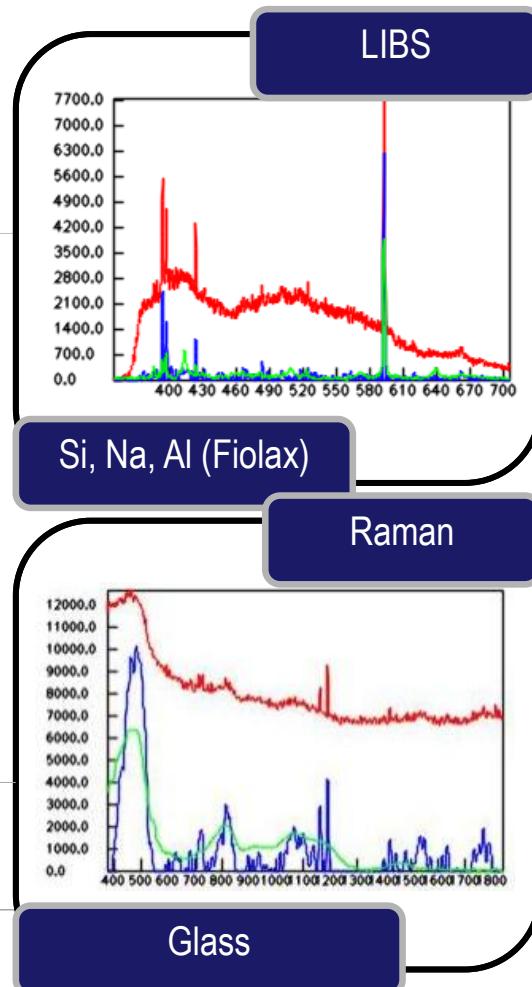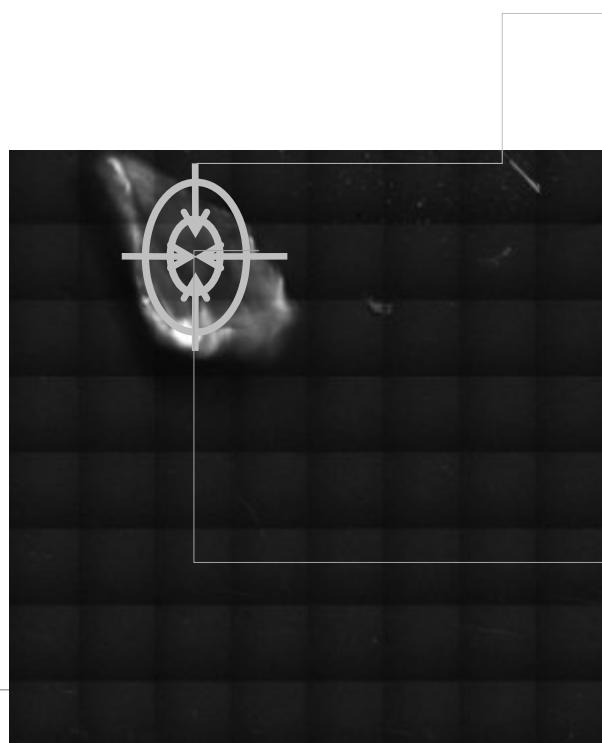
Particle Isolation

Isolation

- Class 100 clean bench is essential
- „Ball-park" clean rooms would be beneficial
- Cleaning is essential and system suitability tests (blanks) have to be taken
- Training and control is essential
- Benches, coats, sleeves, microscopes, equipment and water should be clean and non-shedding
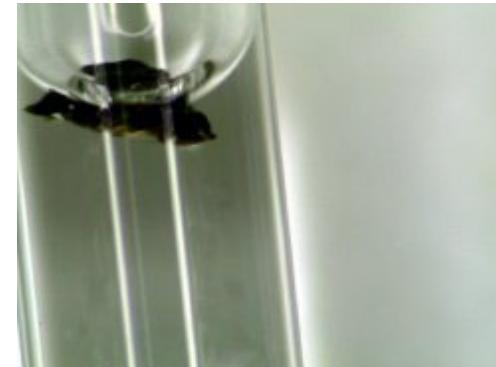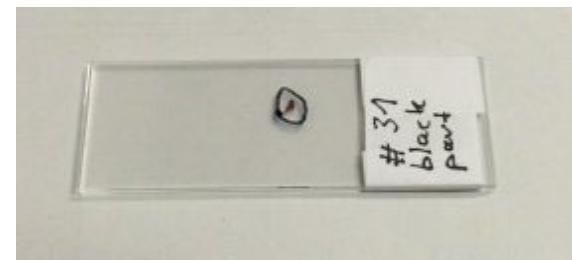
Isolation and transportation

Capillary trapping

Tungsten needles for
particle picking

Sending particles to a lab
between 2 slides

Particle Sources

Origin makes the difference

Inherent

Particulate made entirely of components of the formulated product, arising from the product itself. These particulates are related to the product formulation: API

Intrinsic

Particulate related to the production process of components of the formulated product, arising from the product itself. Processing Equipment, Primary Package, Active and other ingredients

Extrinsic (Foreign)
Environmental Contaminants
insect parts, hair, fibers, paint, rust

Sources for particulate matter ?
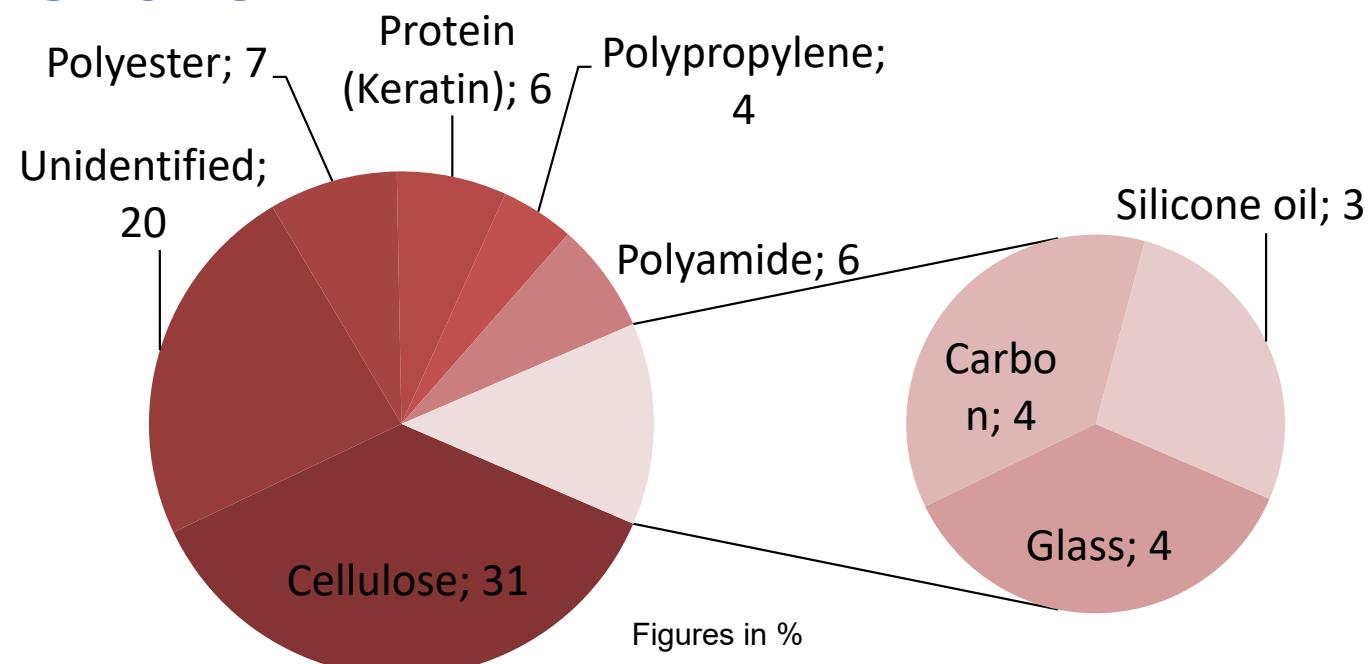

- personnel

- Garnement
- Water
- container

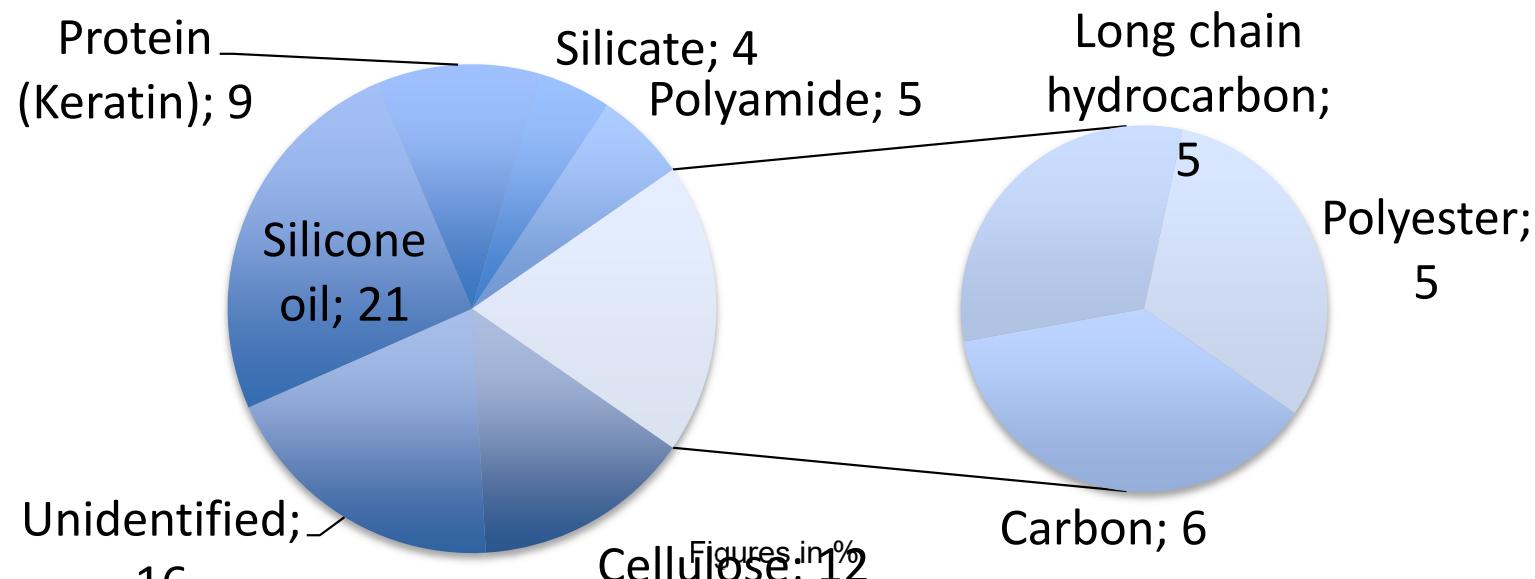- Process / Production Equipment e.g.: rubber
- Cleaning process

	2023	2014	2008
Lint/Fiber	1	1	1
Product Related	2	3	3
Glass	3	2	2
Rubber/Elastomer	4	4	4
Metal	5	5	5

Visible

Polyester; 7

Protein (Keratin); 6

Polypropylene; 4

Unidentified; 20

Polyamide; 6

Silicone oil; 3

Carbon; 4

Glass; 4

Cellulose; 31

Figures in %

Cellulose, Polyester and Protein/Polyamide particles

Sub-visible

Protein (Keratin); 9

Silicate; 4

Polyamide; 5

Long chain hydrocarbon; 5

Silicone oil; 21

Polyester; 5

Unidentified; 16

Figures in %

Cellulose; 12

Carbon; 6

Silicone oil, Protein, Cellulose particles are the most often found contaminants

- Cellulose: mostly fibres
  - source: clothes, **towels**, wipers, autoclave paper

- Longchain hydrocarbon
  - source: rubber (stopper), PE (bottles)

Top Ten in more detail

- Glass: fibres and particles
  - Source: Primary packaging
  - But also glassfibers and hollow glass fibres (filter material)
- Carbon: particles
  - Usally black particles contain high content of carbon:
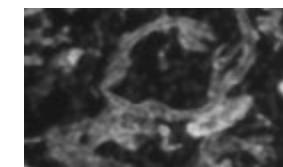 - Sealings rubber material filled with carbon
 - Burned material

Top Ten in more detail

35

- **Polyester: fibres and particles**
  - Source: Cleanroom clothes and defect filter

- **Protein: mostly flakes**
  - Source human dust, protein particles from protein solution

- **Silicone oil: compact particles**
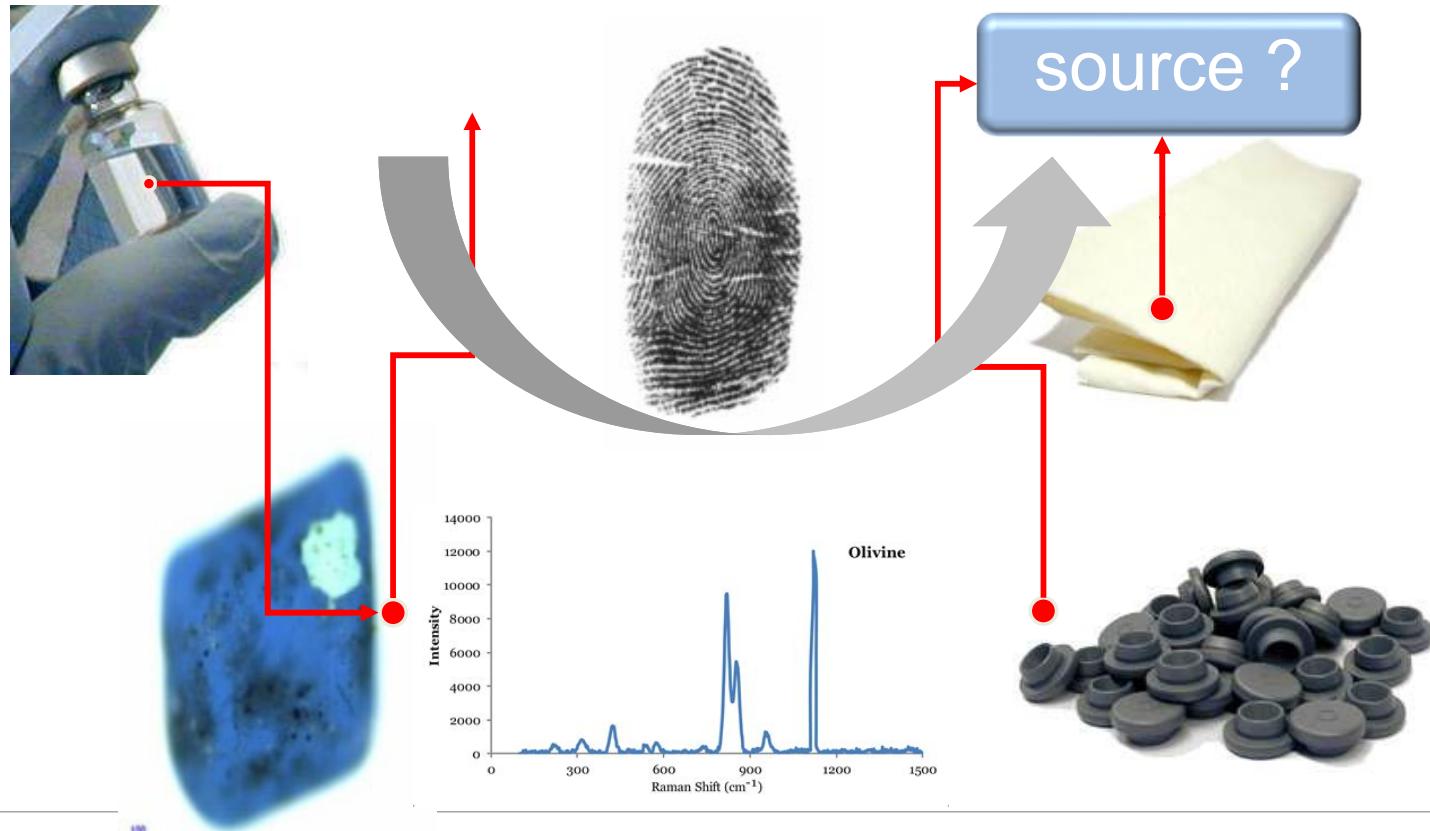 - Source: sealings, siliconisation

Rubber related defects

– White or black spots on/between lips

– Foreign material trapped between plunger and glass wall Glass bits

– Rubber chunks

– Fibres

– Hair

Root cause

source ?
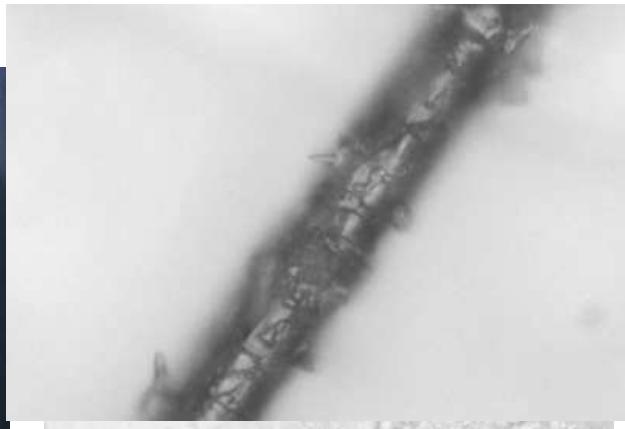
Olivine

Root cause

1. Documentation of the defect →in-situ (in the closed container)

2. Filtration and documentation of the sample on the membrane filter

3. Documentation of the analysis and the identification of the reject by Raman spectroscopy

4. Identification of sub-visible to gather further information

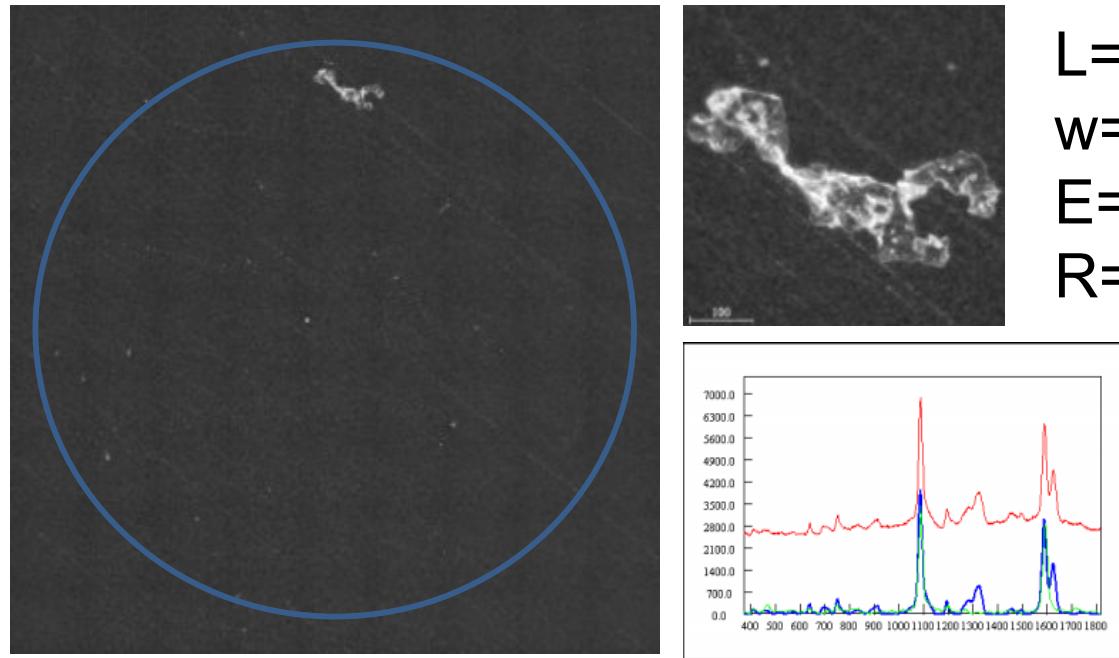5. Verification of the findings (particle observed by visible inspection) with FT-IR or LIBS, EDX

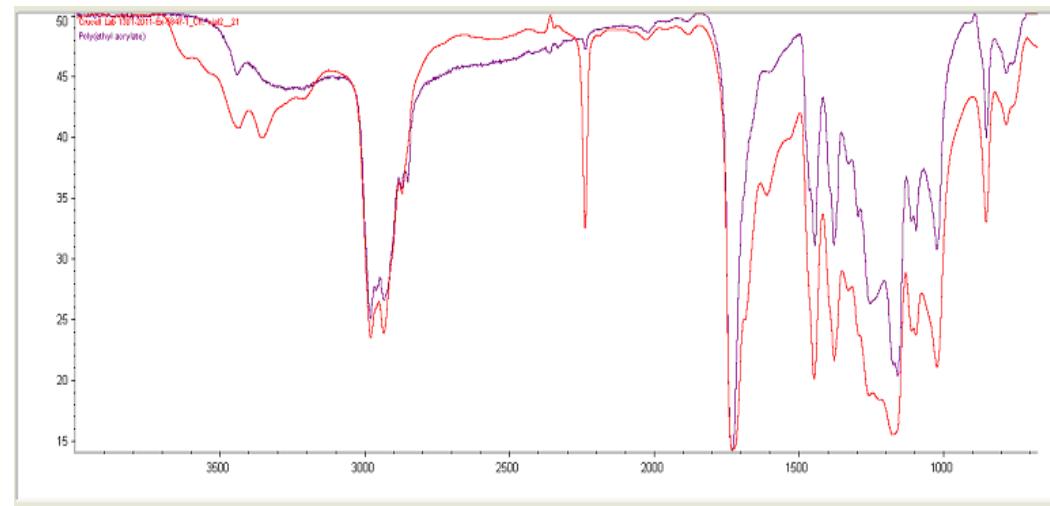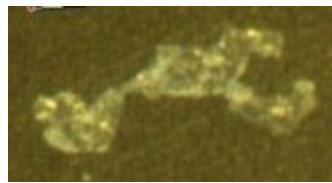# Particle in a vial

Particle Imaging + raman.ID

L=505.0 µm
w=202.6 µm
E=2.49
R=0.3071

Raman.ID: Polyethylene-terephtalate, PET
Rank: 887

Verification by FTIR
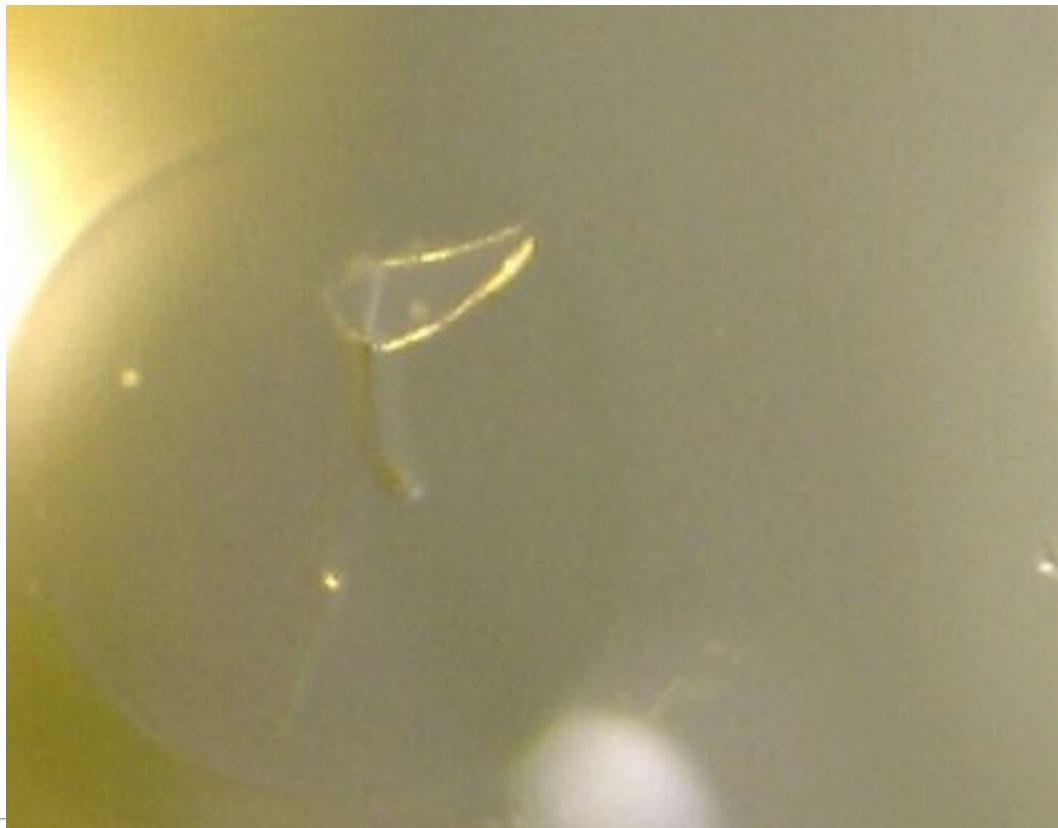
Sample prep. + Documentation

Level 3 ID

LIBS

Si, Na, Al (Fiolax)

Raman

Glass

L1
Länge : 516.9µm
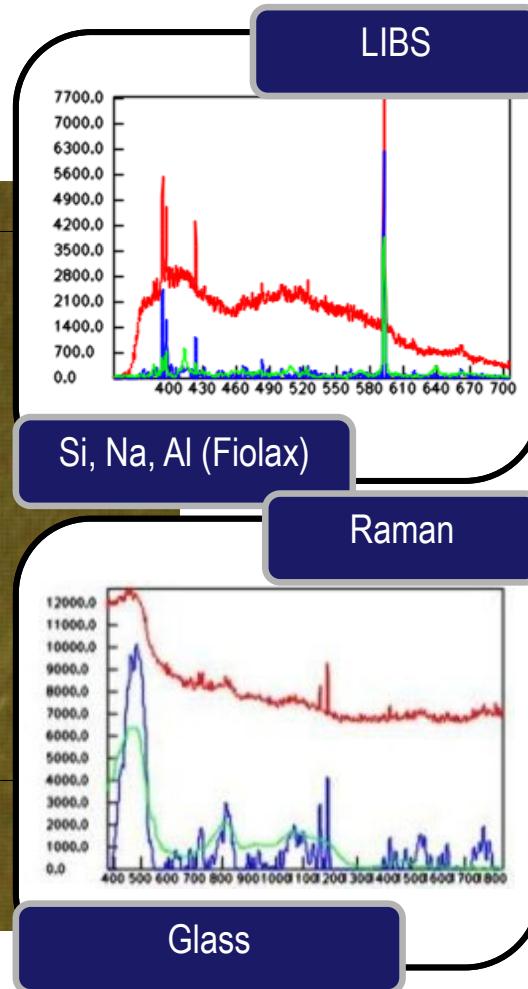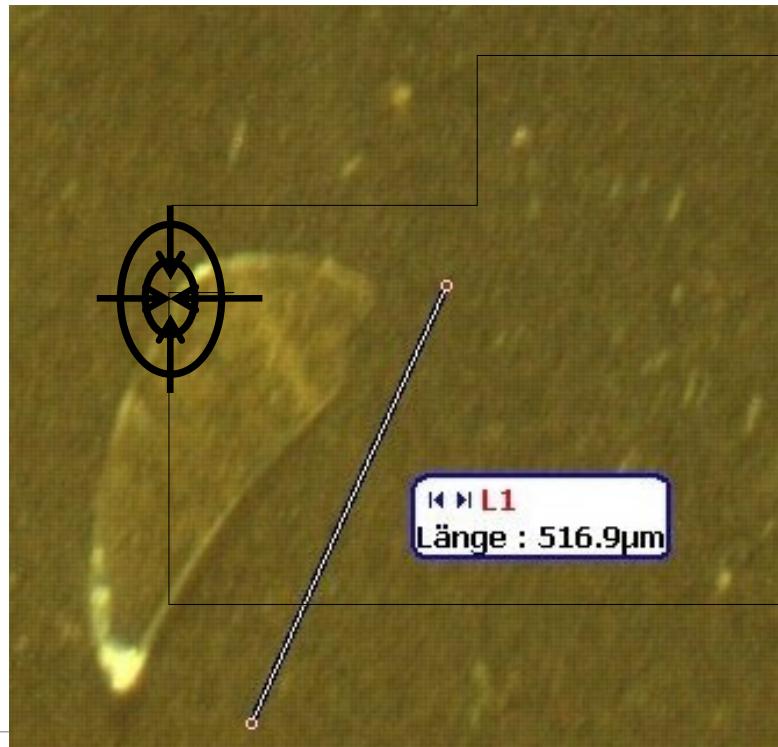
CELLULOSE SOURCE

- 4 batches failed in a row

- 3 samples of each of the failed batches and one of the good batches were investigated

- Soon it became clear that the problem was cellulose related….

Several cellulose fibers were found

Cellulose (Contaminant)
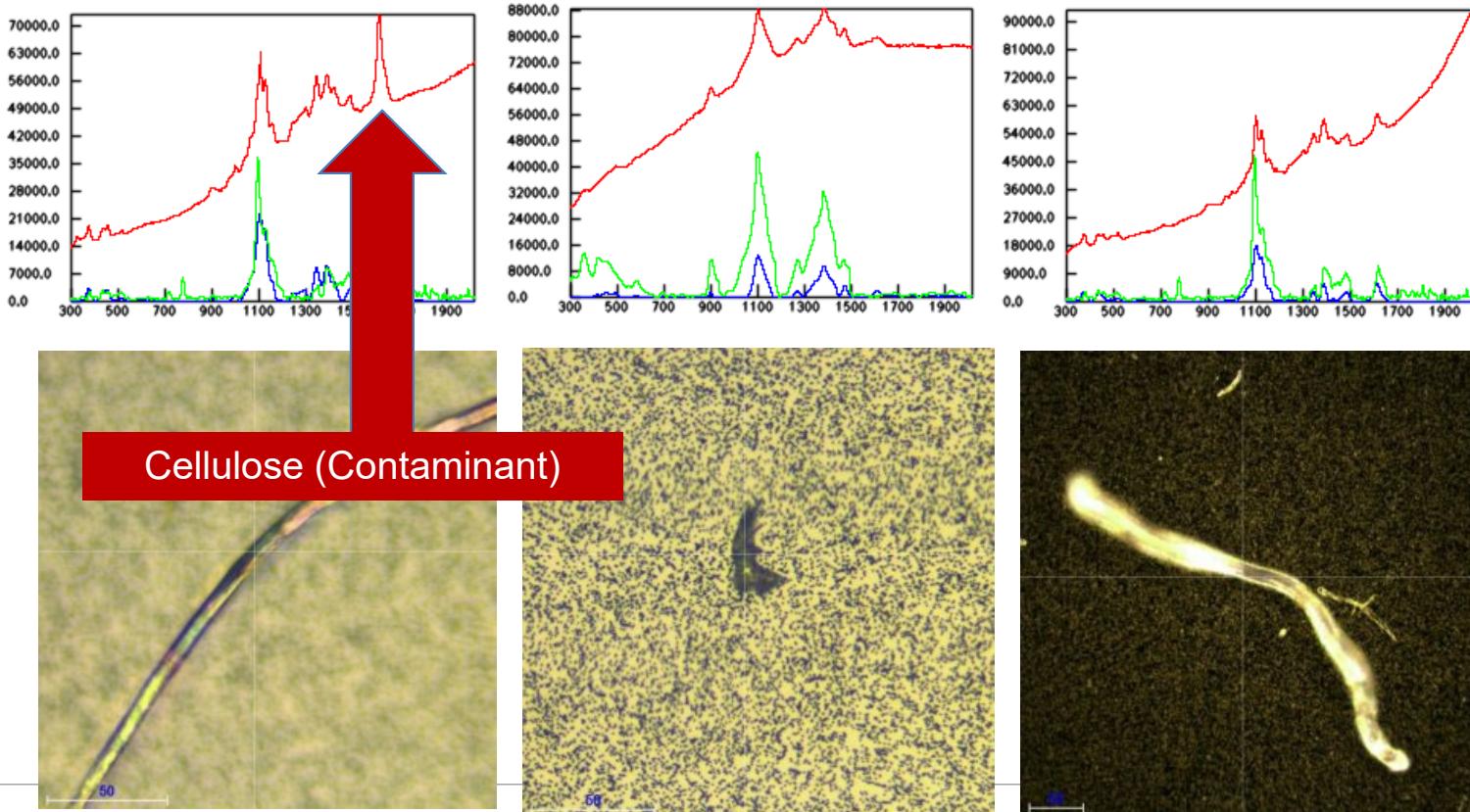
Samples from the filling were taken
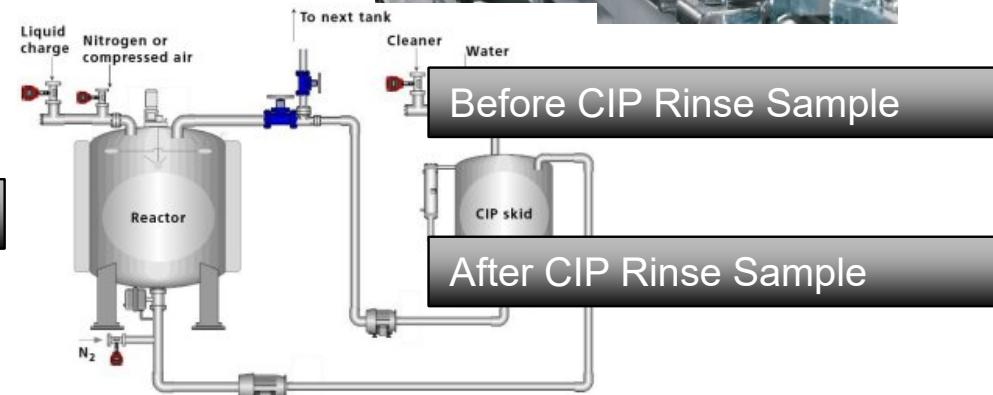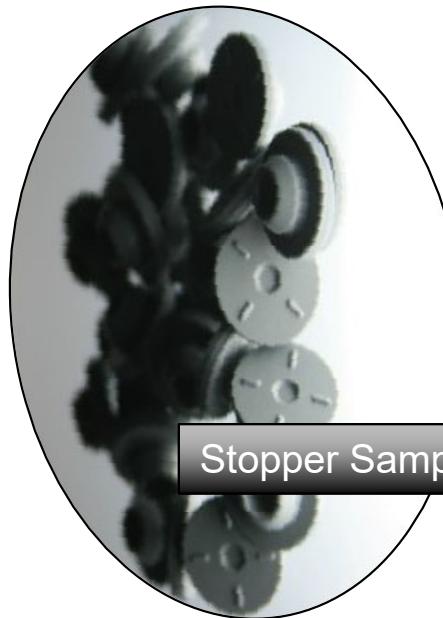
Before Tubing Rinse Sample

After Tubing Rinse Sample

Stopper Sample

Before CIP Rinse Sample

After CIP Rinse Sample

Before Tubing Rinse Sample

After Tubing Rinse Sample

Stopper Sample

Before CIP Rinse Sample

After CIP Rinse Sample
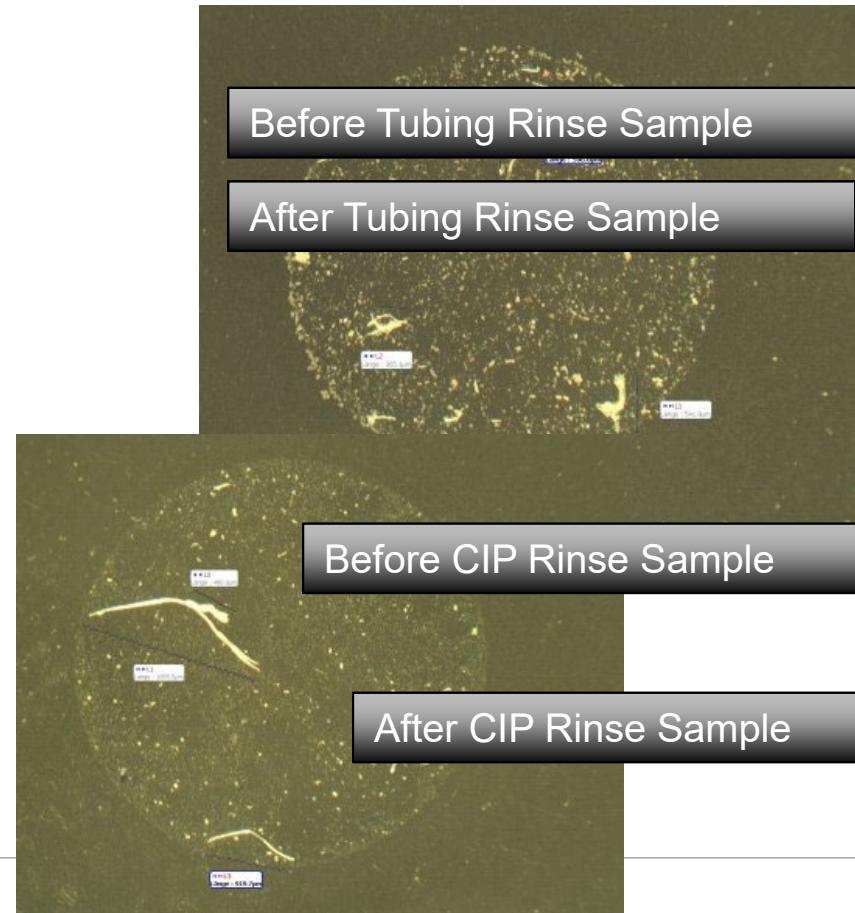
Database with filling line related materials was built

No Cellulose (Contaminant) !

Tube rinse result

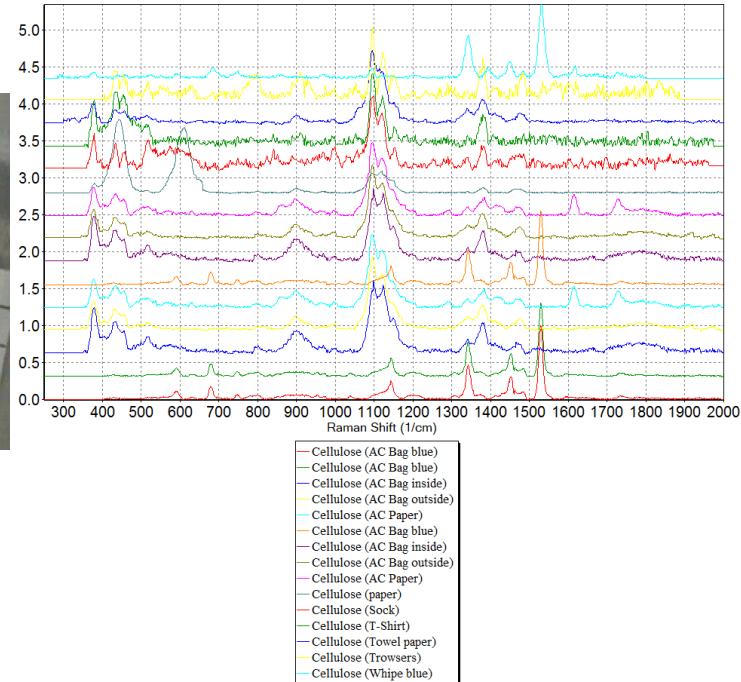| Size and Substance Distribution of Measured Particles							
Substance	Number	Size Distribution [µm]					
-	-	>=5	>=10	>=25	>=50	>=100	
Cellulose (AC Bag blue)	5	0	0	0	1	4	
Cellulose w. Polyester (Papertowel II)	1	0	0	0	1	0	
Ethyl Cellulose	1	0	0	0	0	1	
Cellulose (AC Bag inside)	19	0	0	0	6	13	
Pigment, Indian Yellow	1	0	0	0	0	1	
Other Particles	143	0	0	5	38	100	
beta-Carotene	50	0	0	3	19	28	
Skipped particles	2283	889	808	432	137	17	
All particles	2503	889	808	440	202	164	
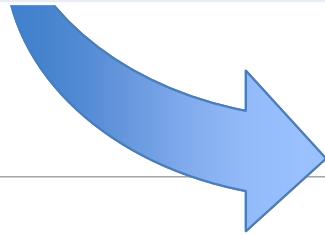
No Cellulose (Contaminant) !

Closer look into the API production (site in Italy)

Tank A Sample

Tank B Sample

Tank C Sample

Size and Substance Distribution of Measured Particles						
Substance	Number	Size Distribution [µm]				
-	-	>=5	>=10	>=25	>=50	>=100
Cellulose (AC Bag blue)	1	0	0	0	1	0
Labcoat	1	0	0	1	0	0
Fluorescence	1	0	0	1	0	0
Carbon	4	0	0	3	1	0
Cellulose	1	0	0	0	1	0
Indanthrene Blue	1	0	0	1	0	0
Cellulose (Contaminant)	31	0	0	8	18	5
Pigment, Indian Yellow	3	0	0	3	0	0
Polysulfone	5	0	0	1	2	2
Cellulose (Towel paper)	5	0	0	5	0	0
Other Particles	28	0	0	22	1	5
Skipped particles	1716	1353	362	1	0	0
All particles	1797	1353	362	46	24	12

Samples from API tanks and tubings showed this type of fiber.

Tank A Sample

Tank B Sample

Tank C Sample

Size and Substance Distribution of Measured Particles						
Substance	Number	Size Distribution [µm]				
-	-	>=5	>=10	>=25	>=50	>=100
Cellulose (AC Bag blue)	1	0	0	0	1	0
Labcoat	1	0	0	1	0	0
Fluorescence	1	0	0	1	0	0
Carbon	4	0	0	3	1	0
Cellulose	1	0	0	0	1	0
Indanthrene Blue	1	0	0	1	0	0
Cellulose (Contaminant)	31	0	0	8	18	5
Pigment, Indian Yellow	3	0	0	3	0	0
Polysulfone	5	0	0	1	2	2
Cellulose (Towel paper)	5	0	0	5	0	0
Other Particles	28	0	0	22	1	5
Skipped particles	1716	1353	362	1	0	0
All particles	1797	1353	362	46	24	12

# Update of the library with towels used in API production
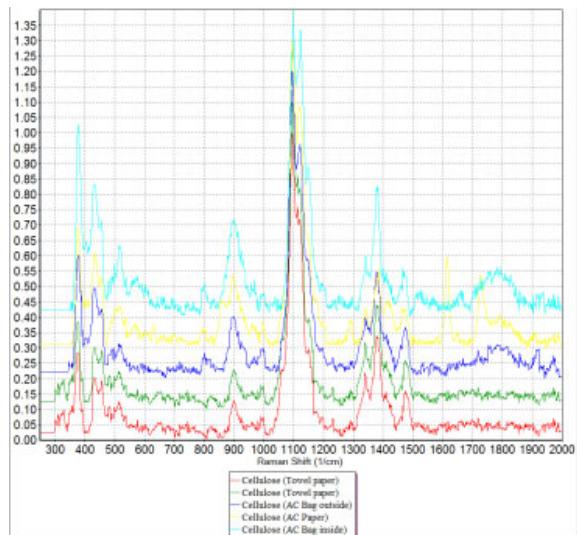
Cellulose (Contaminant)

Conclusion Cellulose Example

- One special type of cellulose could be identified by the typical peak @ 1600
- Database was built with suspect cellulose samples used in production
- These Cellulose (contamination) fibers were found in smaller concentration in CIP rinses no fibers …were found in the process prior to filling!
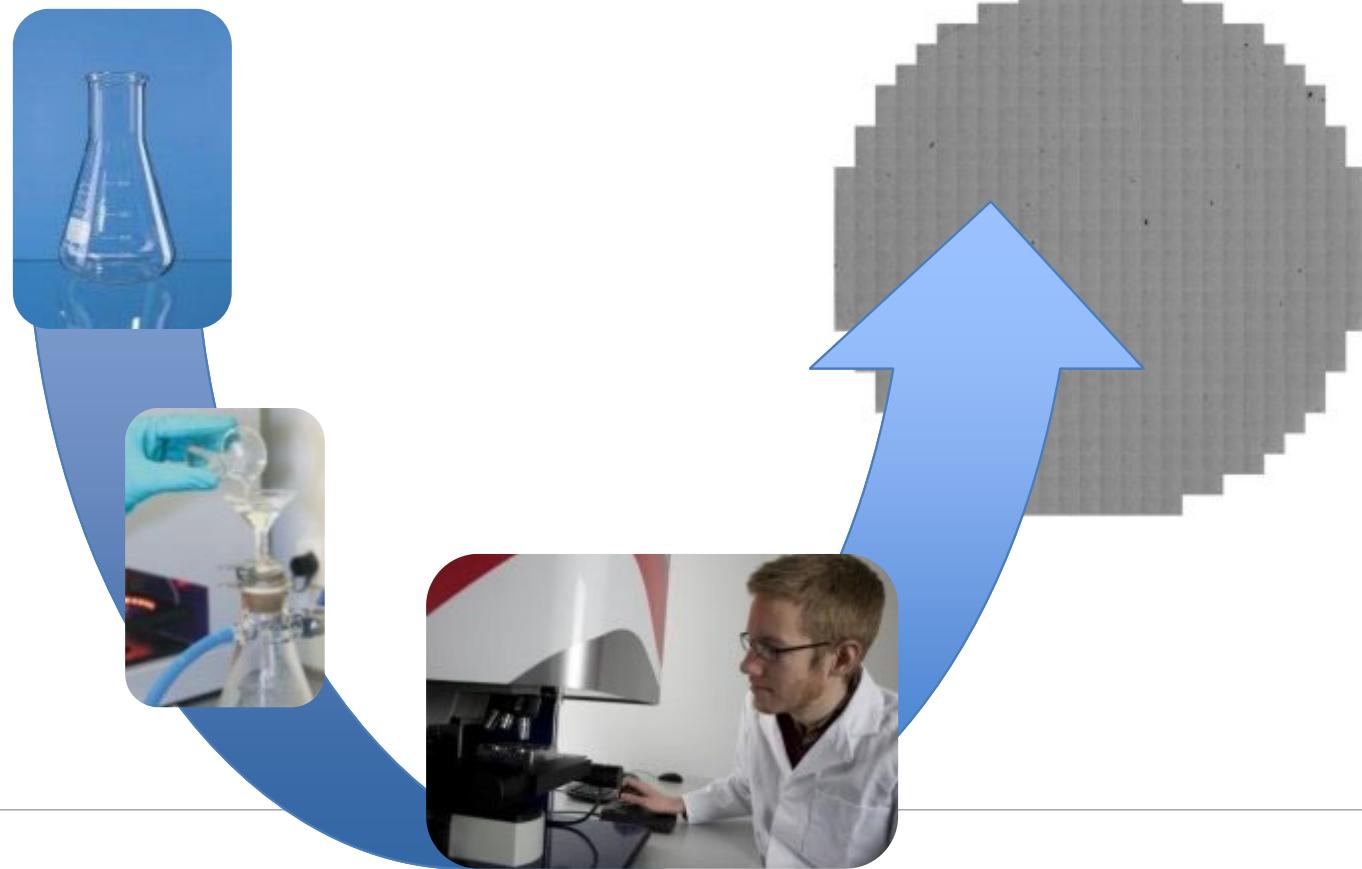- Samples from API tanks and tubings showed this type of fiber.

→ API manufacturer used paper towels and introduced cellulose into the process

Plunger

Silicone

Syringe (Glass)
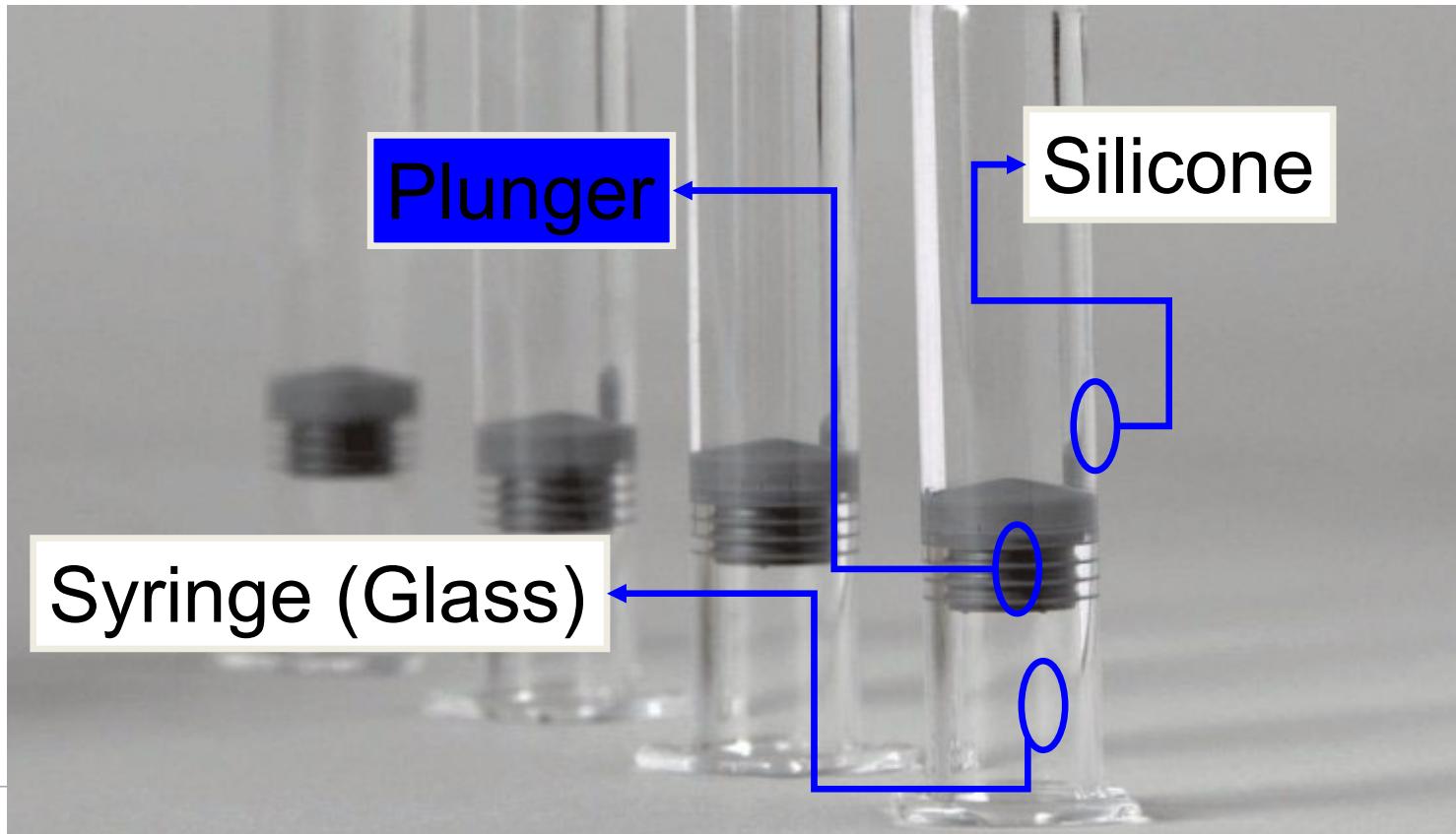
Technical Report No. 85

Enhanced Test Methods for Visible Particle Detection and Enumeration on Elastomeric Closures and Glass Containers

A thorough discussion of the sample preparation process and the counting methodology used to evaluate within and between operator replicate samples can also be found in **8.2 Appendix B**.

Table 4.1.1-1 Threshold Value to Use when Classifying Particles as Visible or Subvisible (by methods more sensitive as compared to the unaided eye)

Category	Aspect Ratio (length:width)	Visible Threshold	Subvisible
Particle	<5	100 μm	<100 μm
Fiber	≥5	300 μm	<300 μm

Rectangle template for fibers: 300 μm x 60 μm
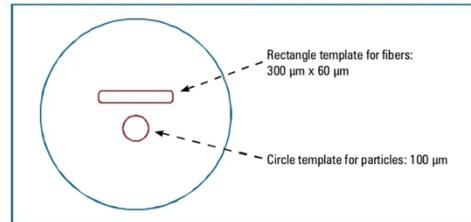
Circle template for particles: 100 μm

Figure 4.1.2-1 Microscope Reticle Design For Elastomer Component Particle Analysis

- A number of parts (normalized for surface area) are placed in an Erlenmeyer Flask,
- Add 50 ml of surfactant solution is dispensed and added to the flask.
- Agitate on an orbital shaker for 20 sto remove visible particles from the surface.
- Filter immediately through a membrane filter (a gray filter was used to enhance contrast for both light and dark colored particles and fibers)
- The rinsing process is repeated
- Once the filter is dry, any visible particles present are counted by using a specialized reticle and an optical stereomicroscope
- Appendix B. Method: Determination of Visible Partides and Fibers on Elastomeric Components by Membrane Filtration and Microscopic Examination

Fibers and particles on rubber

- 10 stoppers contaminated with fiber
  Cleaning following ISO 8871
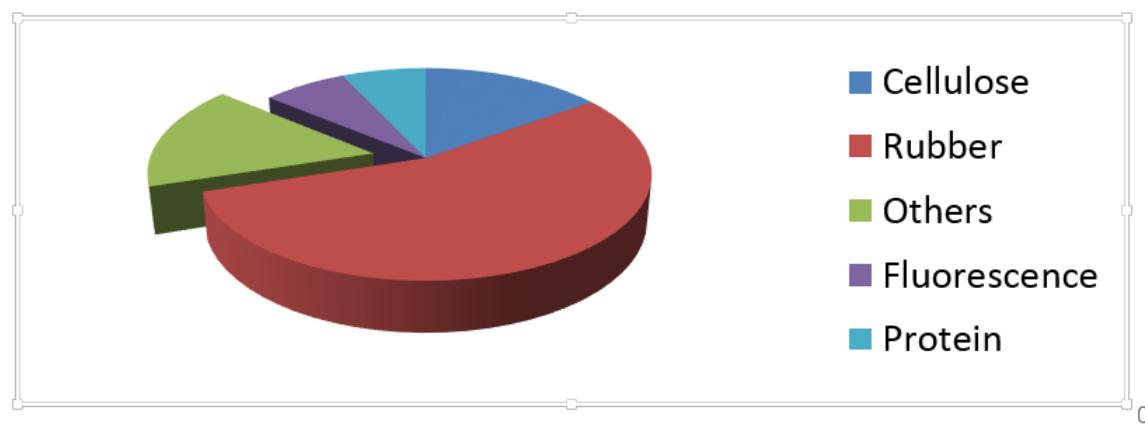
- 43 particles > 100 µm found

- Cellulose
- Rubber
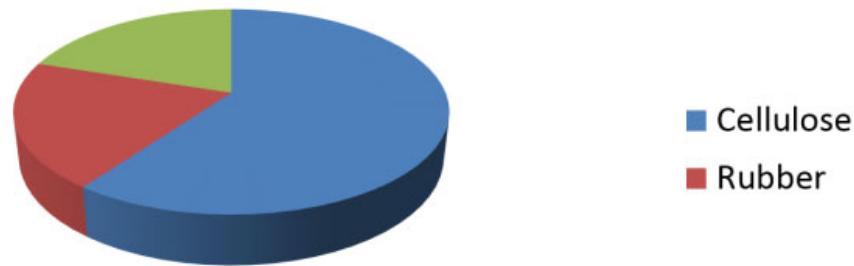
Large scattering in particle number and composition can be observed in one batch and different bags
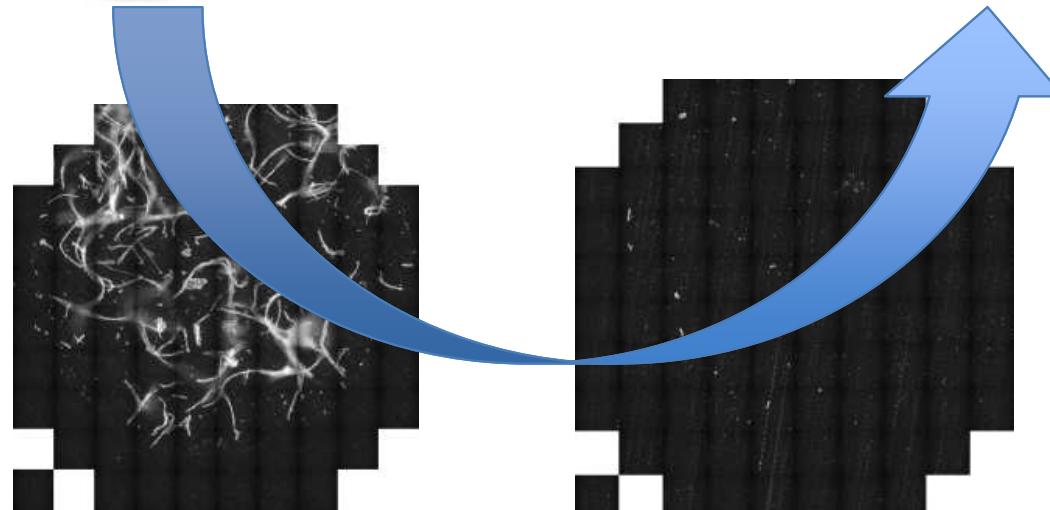
Fibers and particles on rubber

- 10 stoppers contaminated with particles Cleaning following ISO 8871

- 122 particles found > 100 µm

Legend: Cellulose, Rubber, Others, Fluorescence, Protein

Rubber related particles

Stopper Bags have an impact or reflect stopper quality

Test Procedure: Bag rinsed with 250 ml water / SDS, filtration, counting

Fibres collected from one bag; 375 particles > 25 µm

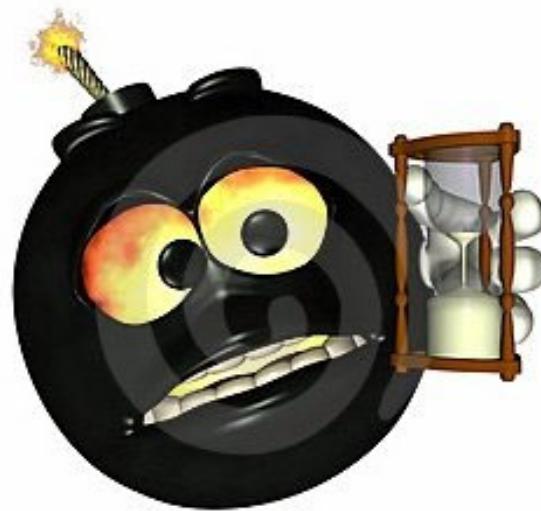Fibres collected from one bag; 45 particles > 25 µm

Rubber related particles
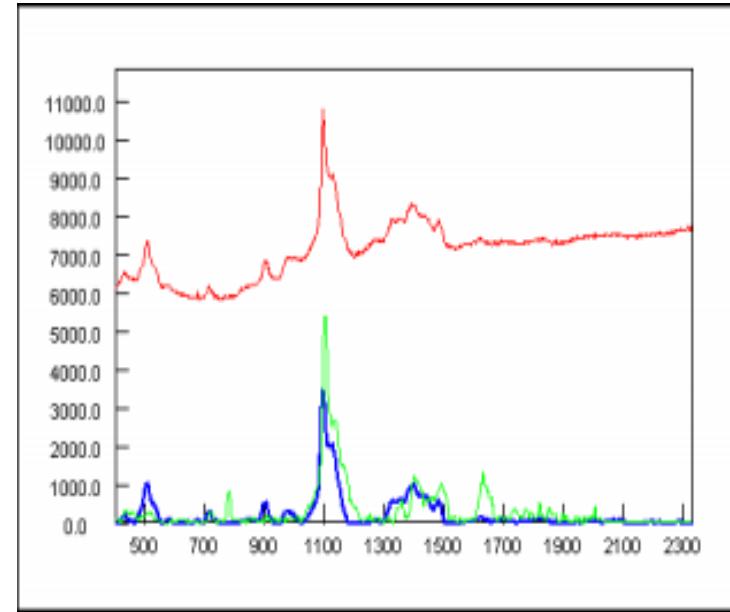
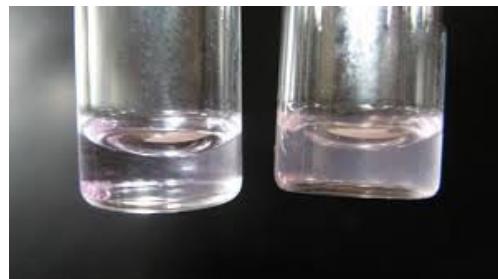RESULT: Cellulose [Paper]
RANK: 882, S/N: 39.2

Time bombs

- Increase of rejects with time
- Chemical reactions taking some time
  - Silicone oil on stoppers: Agglomeration of Proteins
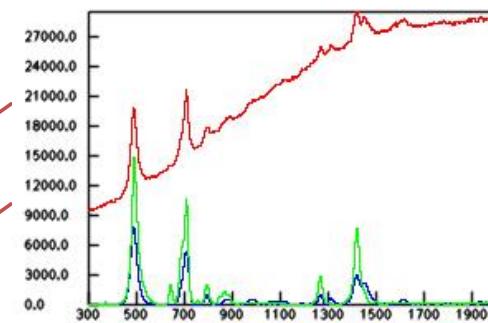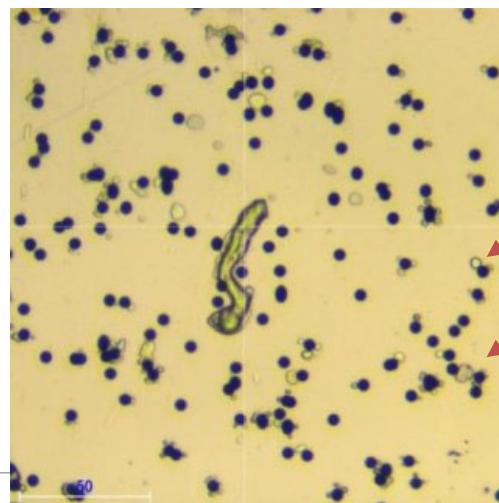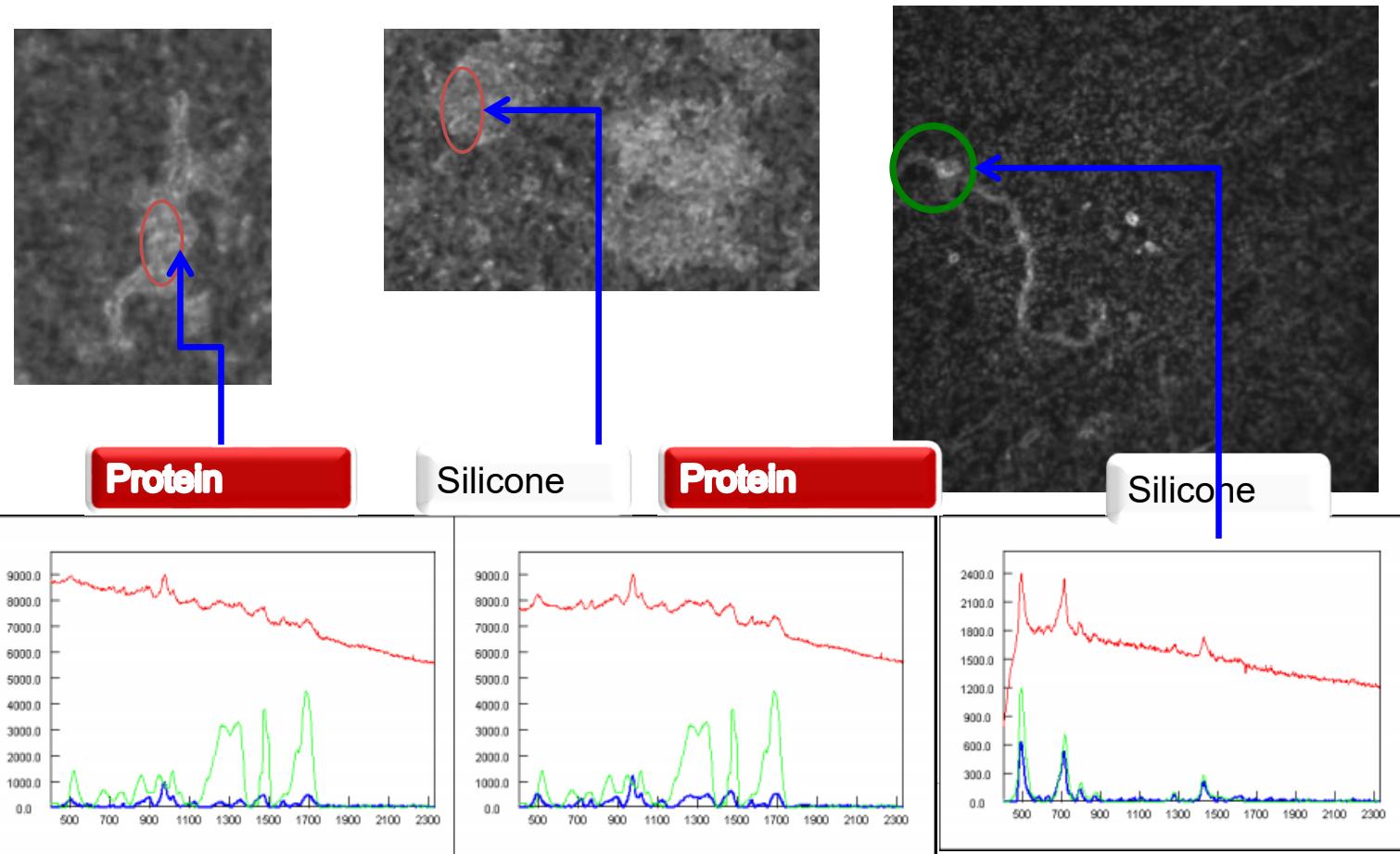 - Coatings
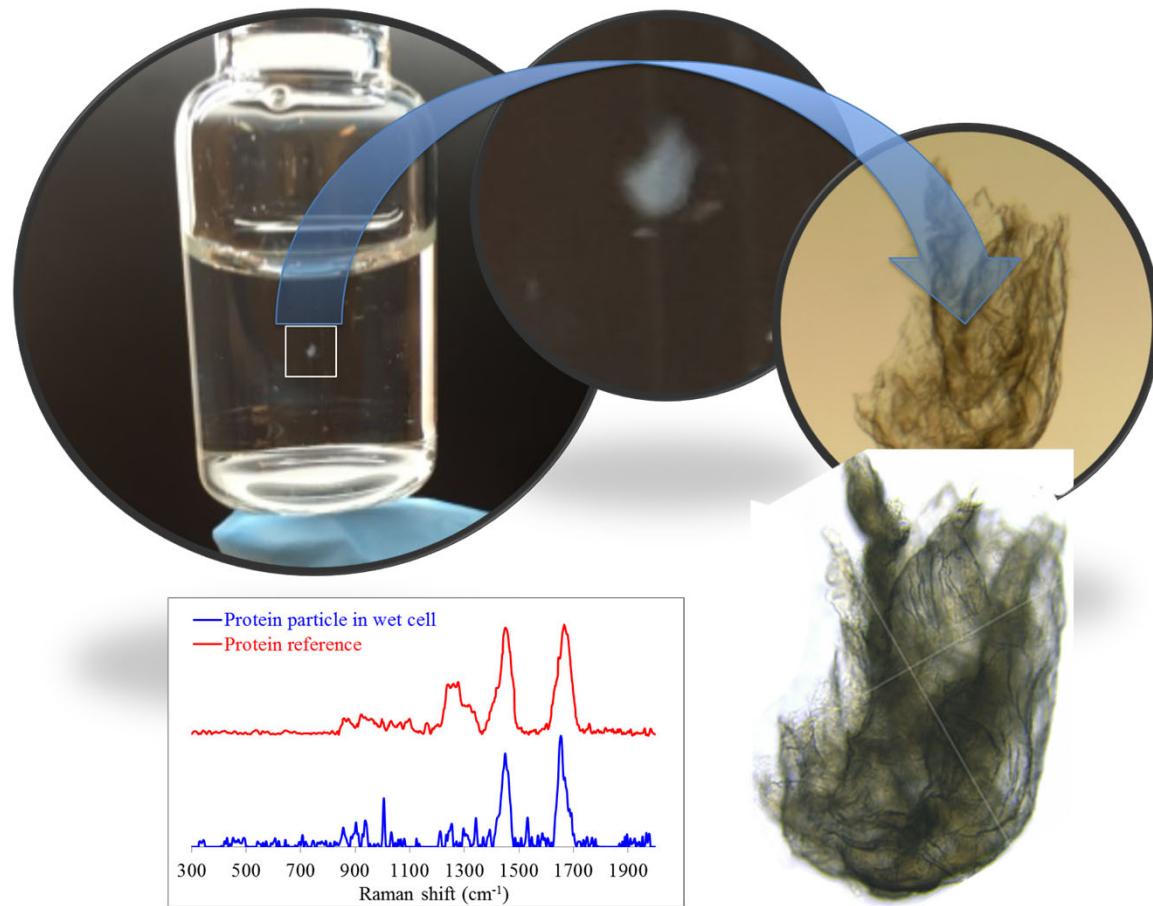 - Glass delamination

Turbidity / Haziness

Observation of haziness and aggregates in a new a new batch after slight process change

Silicone Protein Aggregation

Visible Inherent Particle

The spectra chart labels:
- Protein particle in wet cell
- Protein reference

Raman shift (cm^{-1}): 300 500 700 900 1100 1300 1500 1700 1900

Coating

Increasing number of rejects in visual inspection with time

Size and Substance Distribution of Measured Particles					
Substance	**Number**	**Size Distribution [µm]**			
-	-	>=10	>=25	>=50	>=100
Proteine	6	0	0	1	5
Fluorescence	18	0	0	1	17
Coating	185	23	44	32	86
Skipped particles	3058	2142	657	232	27
All particles	3267	**2165**	**701**	**266**	**135**

Supplemental Testing or Inspection

Destructive reconstitution, dilution, transfer, clearing, solubilizing, filtration, screening, or sieving that mallows a product to be visually examined or evaluated microscopically to determine the presence, type,and size of foreign particulate contamination present within the product, container, or device.

Destructive Inspection and Test Methods
- Reconstitution
- Filtration
- Clarification
- Transfer Dilution
- Sieve/Mesh
- Panning
- Rinse/Flush and Filtration

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5.3 DIP Product Formulations

Common inspection or testing approaches for DIP product formulations are listed in **Table 5.3-1.**

Table 5.3-1 Common Inspection or Testing Approaches for DIP Product Formulations

DIP Formulation Type	Common Destructive Methods Applied	Method #
Deeply colored solutions (opaque)	Filtration and microscopic exam in sub-visible and/or visible ranges	2
	Transfer and dilution (if required) in a verified clean transparent container followed by visual inspection	4
Emulsions	Clarification and visual Inspection	3
	Clarification → Filtration and microscopic exam in sub-visible and/or visible ranges	3
	Sieving	5
	Additional considerations: – Inspection of settled product with observation of bottom layer for dispersion of dense (sinking) metallic or glass particles	
Gels	Direct visual inspection (USP <790> with modifications, if needed, for increased illumination and dwell time)	USP790
	Dilution → Filtration and microscopic exam in sub-visible and/or visible ranges	4
Lyophilized (freeze-dried product)	Reconstitution and visual inspection	1
	Reconstitution → Filtration and microscopic exam in sub-visible and/or visible ranges	2
	<1 ml Small volumes reconstitution and pooling	4
Powders, API	Reconstitution and visual inspection	1