

Particle Identification

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April 2022



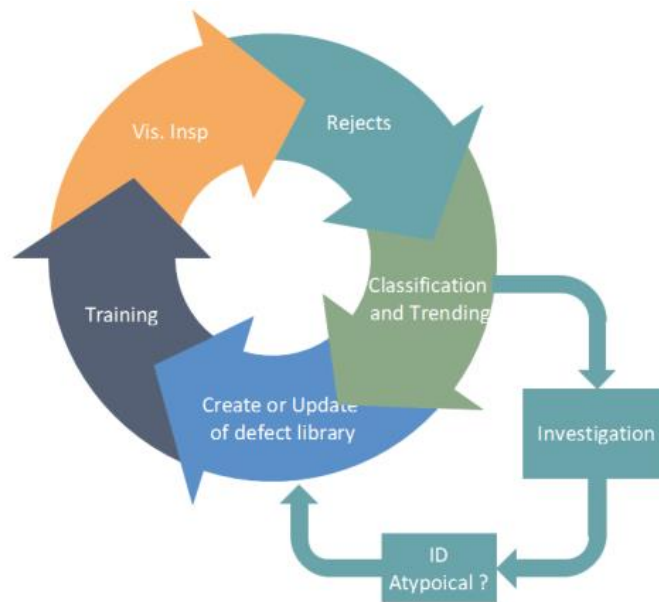
Root cause, particle characterization

*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*

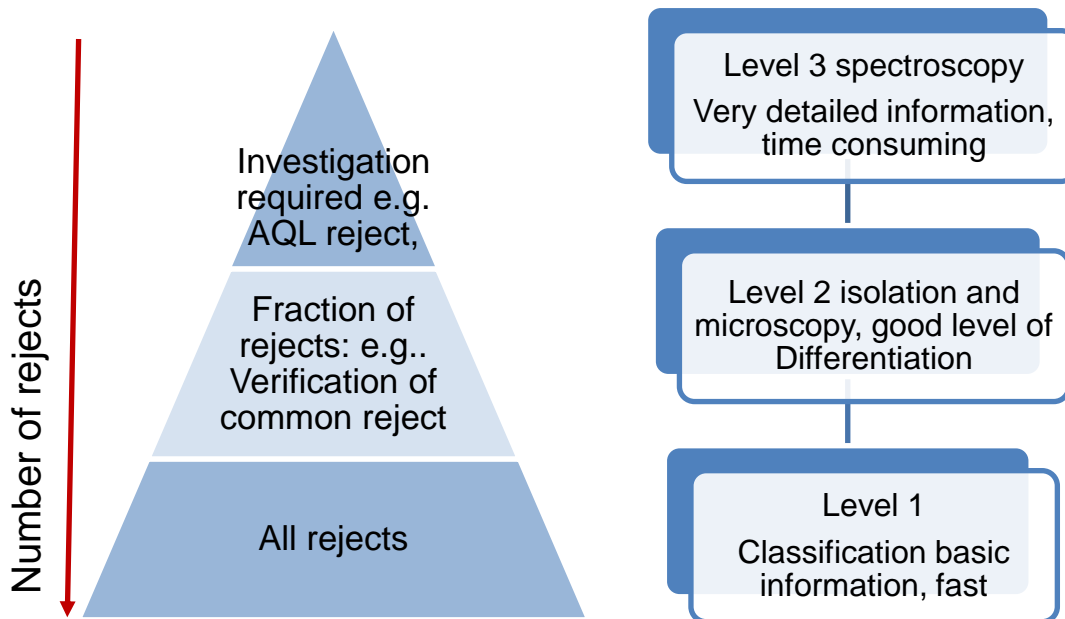
*“reported a particle identified in a vial during an AQL inspection. **There was no documentation on the identity of the particle and whether it was inherent or foreign (black debris, fiber, glass fragments, etc.).**”*

2015

- 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



Classification and Trending



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

| 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 |

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

| 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:

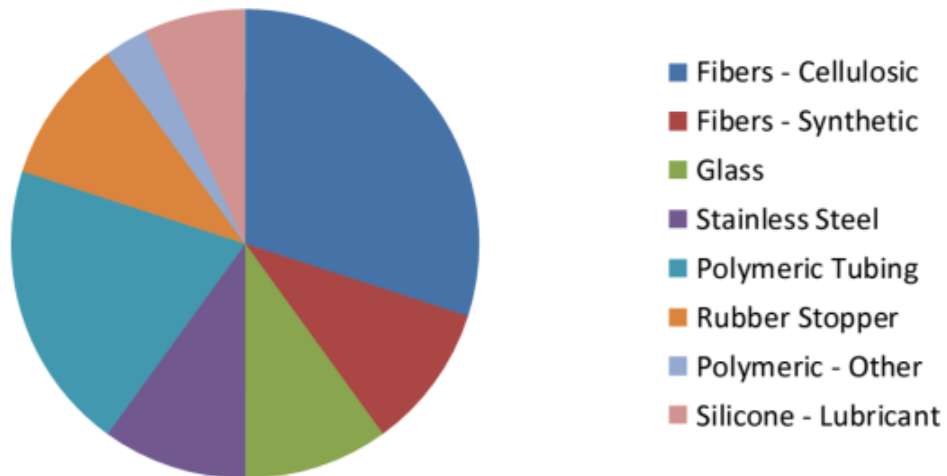
- Rubber, metal, synthetic vs natural fiber, crystal shape, color




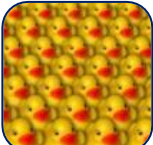



After isolation particle can be easily transferred to level three

| 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



| 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 |

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

| Category | Select |
|----------------|--------|
| Polymeric-like | [] |
| Dark Particle | [] |
| Light Particle | [x] |

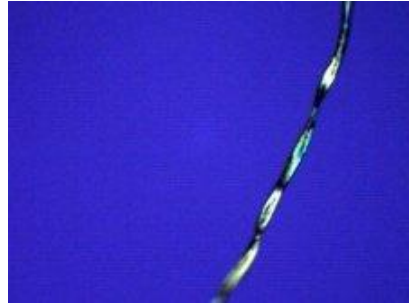


- Fibers can be easily classified. Might be sufficient for trending
- Further classification of fibers can be preformed in situ with an inverted microscope due to morphology and texture

- 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

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



Level 1

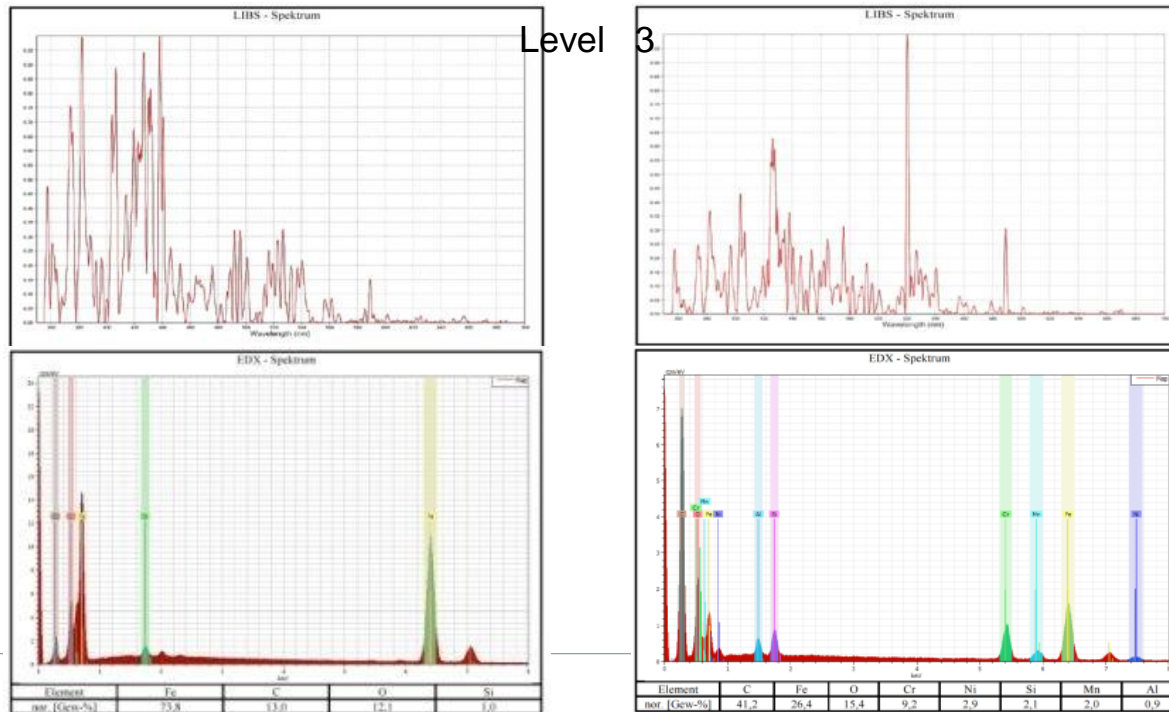


Level 2

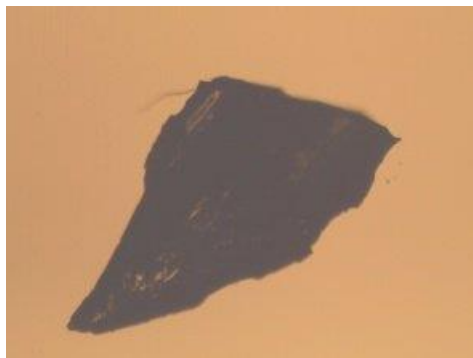


Level 2

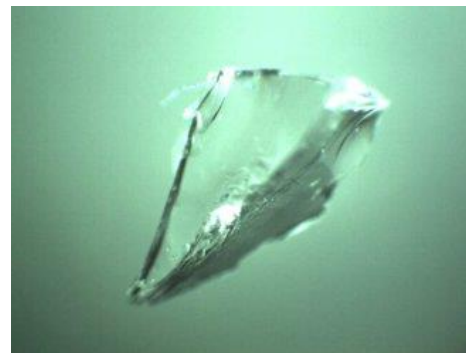
Spectroscopy gives more detailed information on the kind of steel e.g. low alloyed vs high alloyed steel which might be needed for root cause investigation



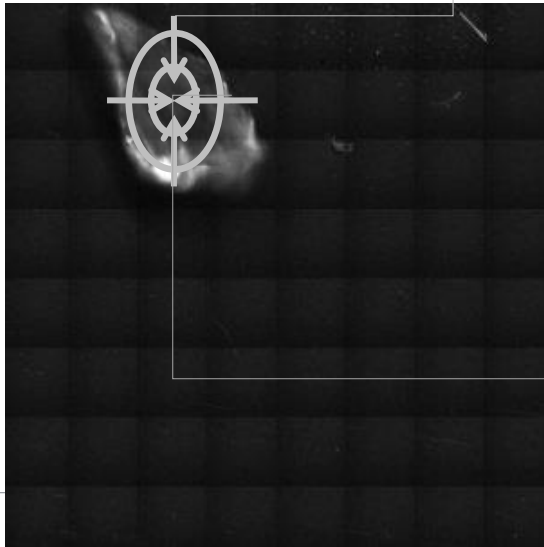
- Glass has a very characteristic shape which is sufficient for classification
- Further characterization for root cause investigation: element specific methods e.g. SEM or LIBS favorable



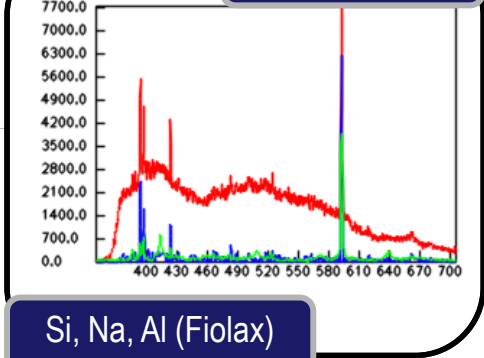
Level 1



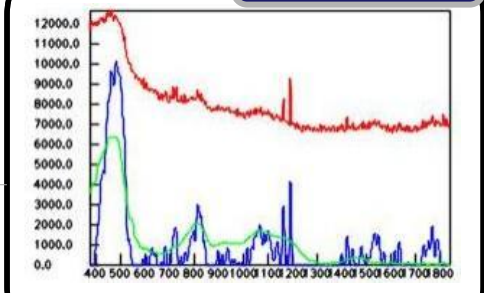
Level 2



LIBS



Raman



Particle Isolation



Isolation

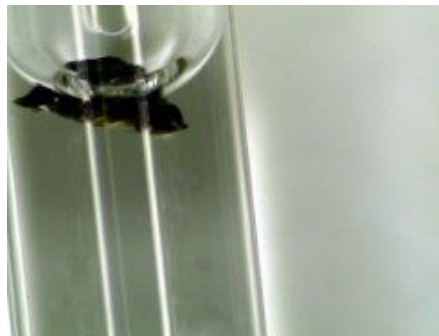
- Class 100 clean bench is essential
- „Ball-park“ clean rooms would be beneficial
- Cleaning is essential and system suitability tests (blanks) have to be taken
- Training and control is essential
- Benches, coats, sleeves, microscopes, equipment and water should be clean and non-shedding



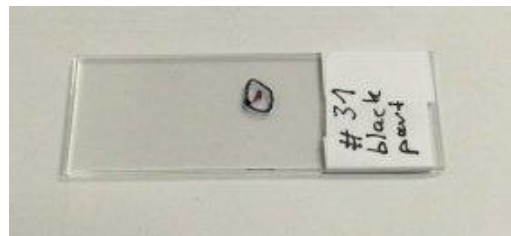
Tungsten needles for particle picking



Capillary trapping



Sending particles to a lab between 2 slides



Particle Sources

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

- personnel



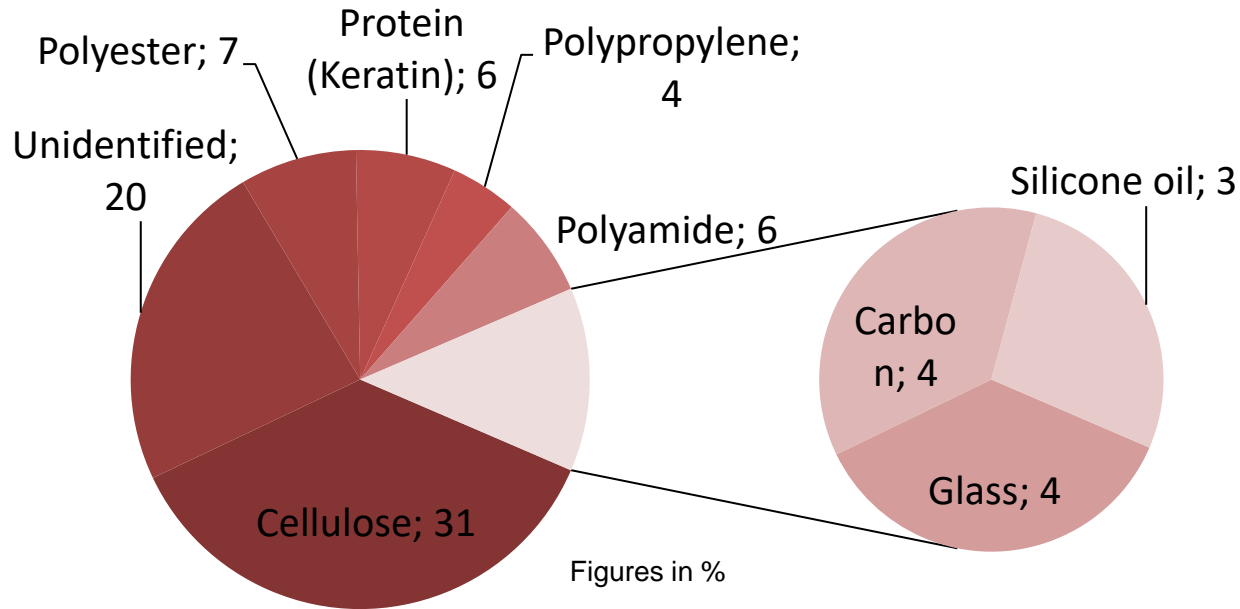
- Garnement
- Water
- container



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

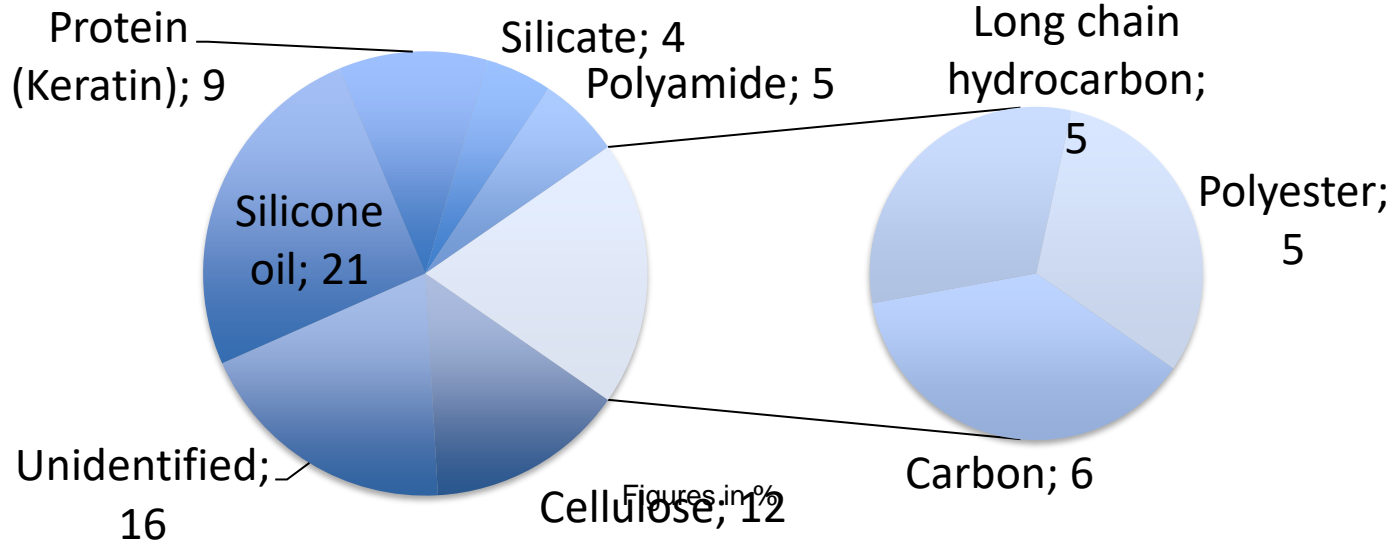


Visible



Cellulose, Polyester and Protein/Polyamide particles

Sub-visible



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

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

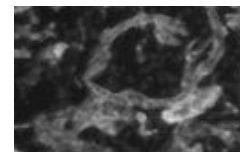


- Longchain hydrocarbon
 - source: rubber (stopper), PE (bottles)

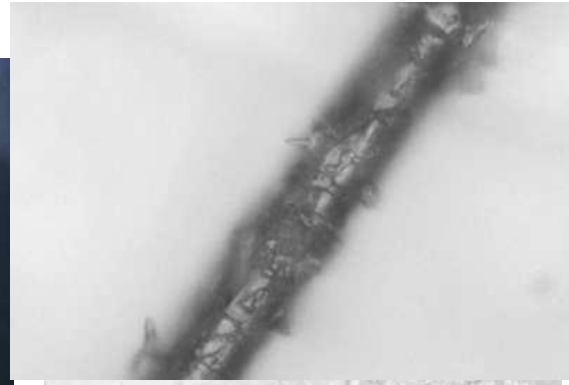
- Glass: fibres and particles
 - Source: Primary packaging
 - But also glassfibers and hollow glass fibres (filter material)
- Carbon: particles
 - Usually black particles contain high content of carbon:
 - Sealings rubber material filled with carbon
 - Burned material

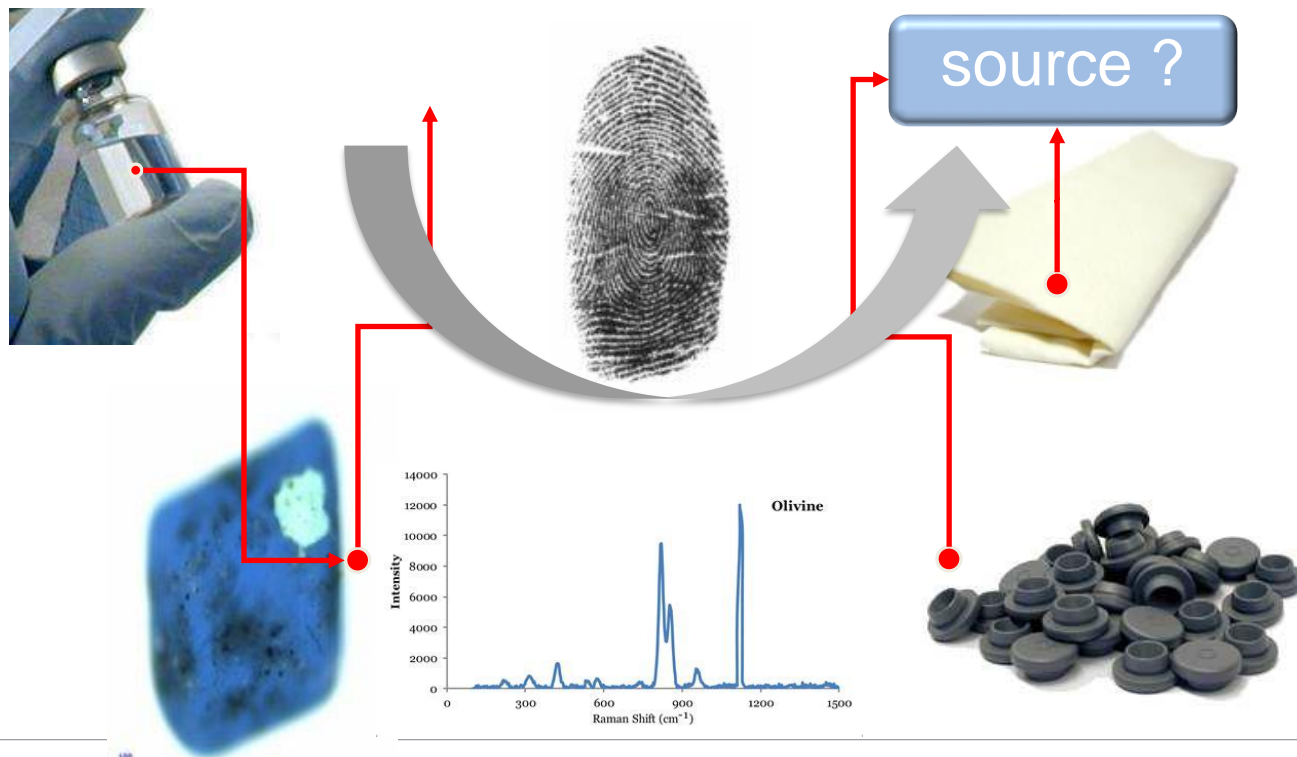


- Polyester: fibres and particles
 - Source: Cleanroom clothes and defect filter
- Protein: mostly flakes
 - Source human dust, protein particles from protein solution
- Silicone oil: compact particles
 - Source: sealings, siliconisation



- White or black spots on/between lips
- Foreign material trapped between plunger and glass wall Glass bits
- Rubber chunks
- Fibres
- Hair



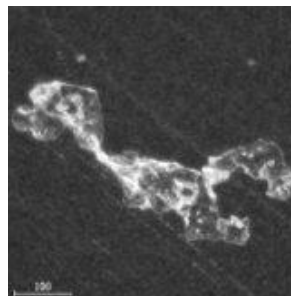
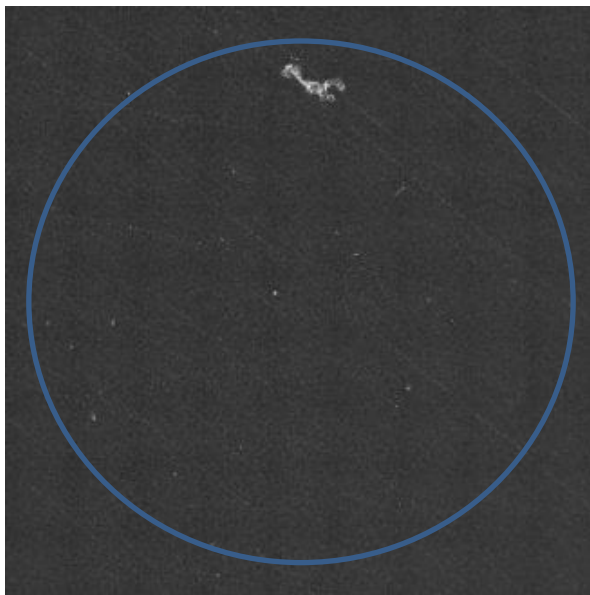




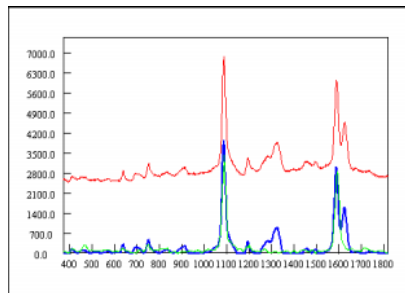
1. Documentation of the defect → in-situ (in the closed container)
2. Filtration and documentation of the sample on the membrane filter
3. Documentation of the analysis and the identification of the reject by Raman spectroscopy
4. Identification of sub-visible to gather further information
5. Verification of the findings (particle observed by visible inspection) with FT-IR or LIBS, EDX

Particle in a vial



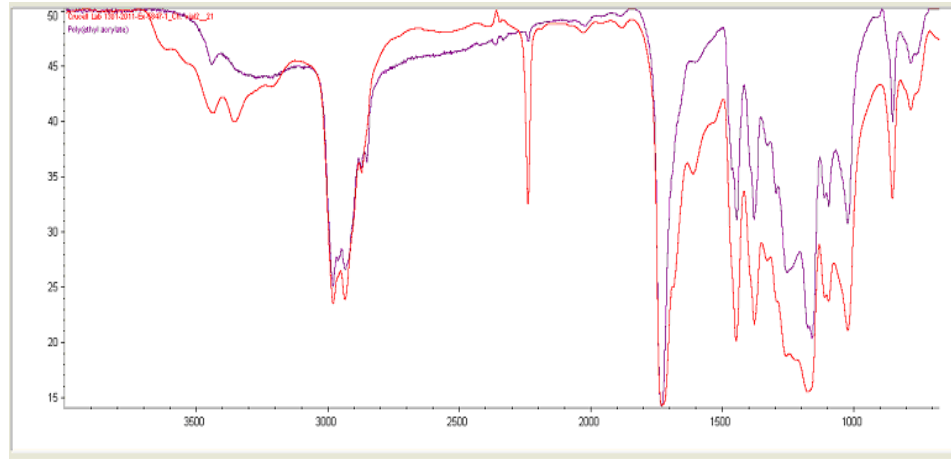
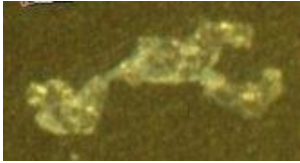


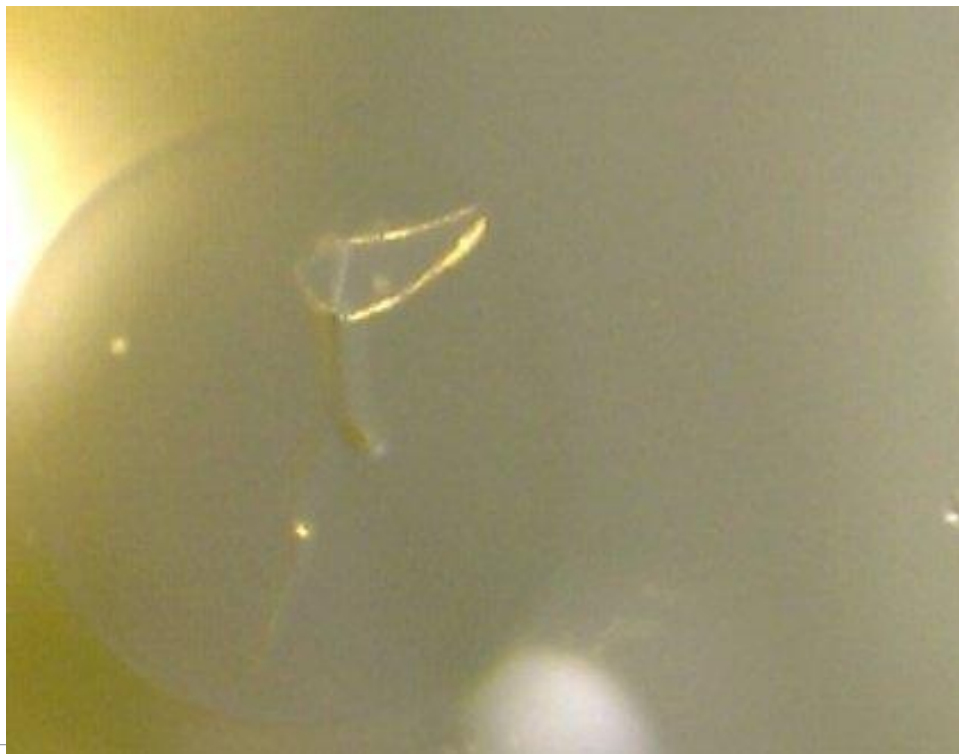
L=505.0 μm
w=202.6 μm
E=2.49
R=0.3071

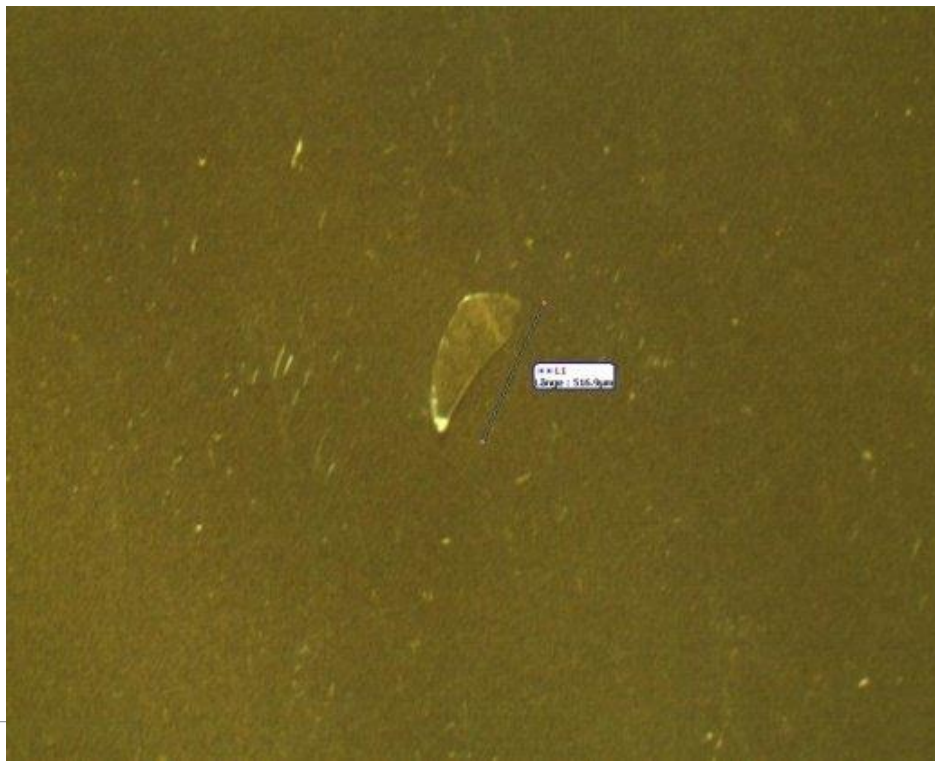


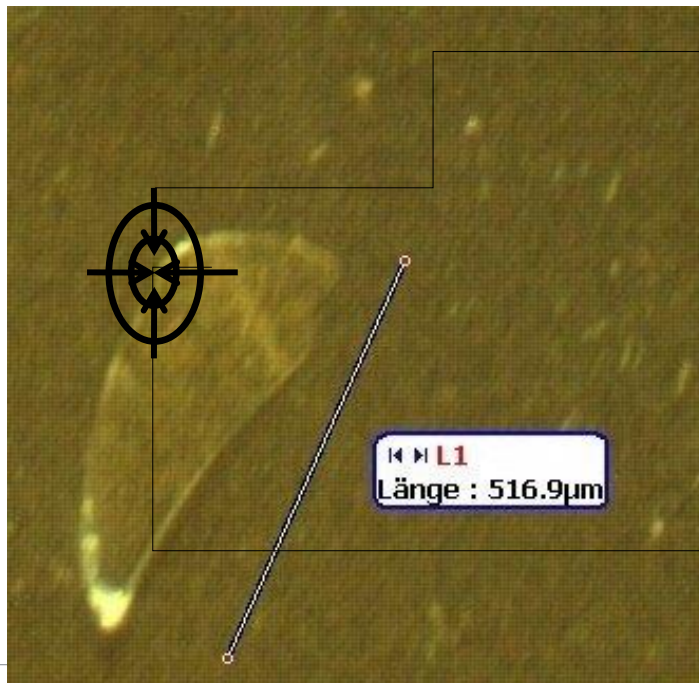
Raman.ID: Polyethylene-terephthalate, PET
Rank: 887

Verification by FTIR

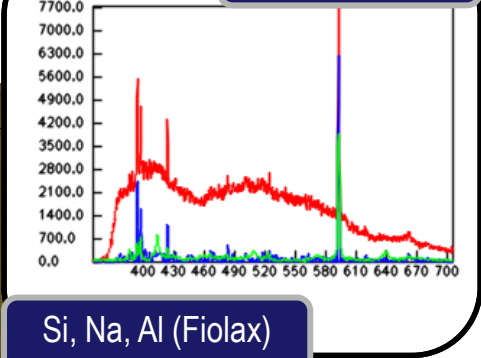




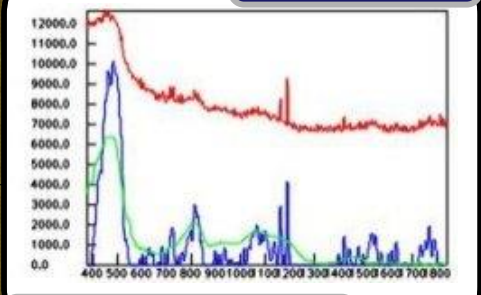




LIBS



Raman



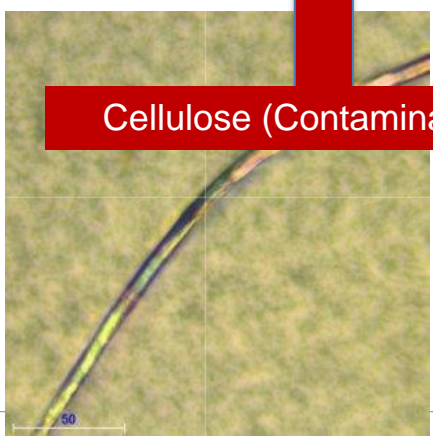
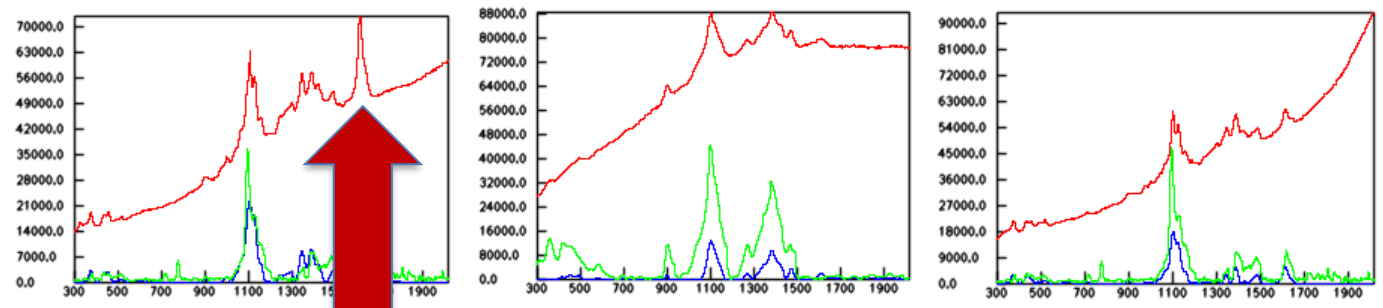
Glass

CELLULOSE SOURCE

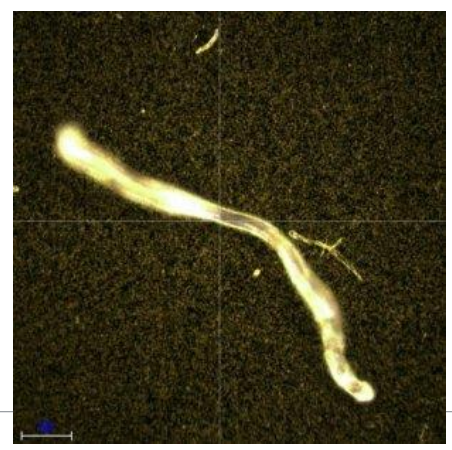
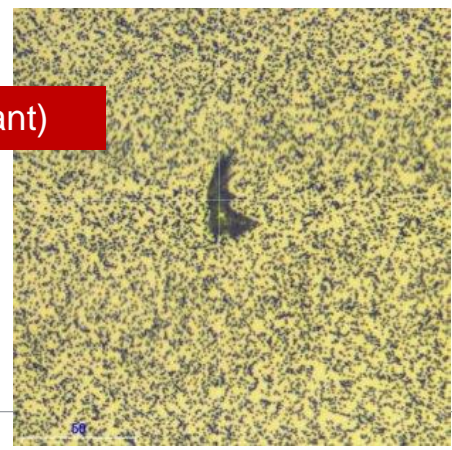


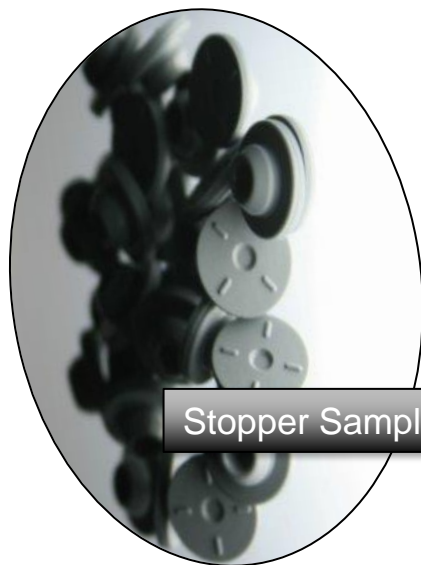
- 4 batches failed in a row
- 3 samples of each of the failed batches and one of the good batches were investigated
- Soon it became clear that the problem was cellulose related....

Several cellulose fibers were found



Cellulose (Contaminant)

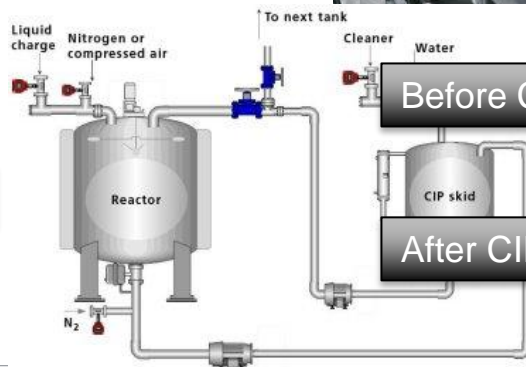




Stopper Sample

Before Tubing Rinse Sample

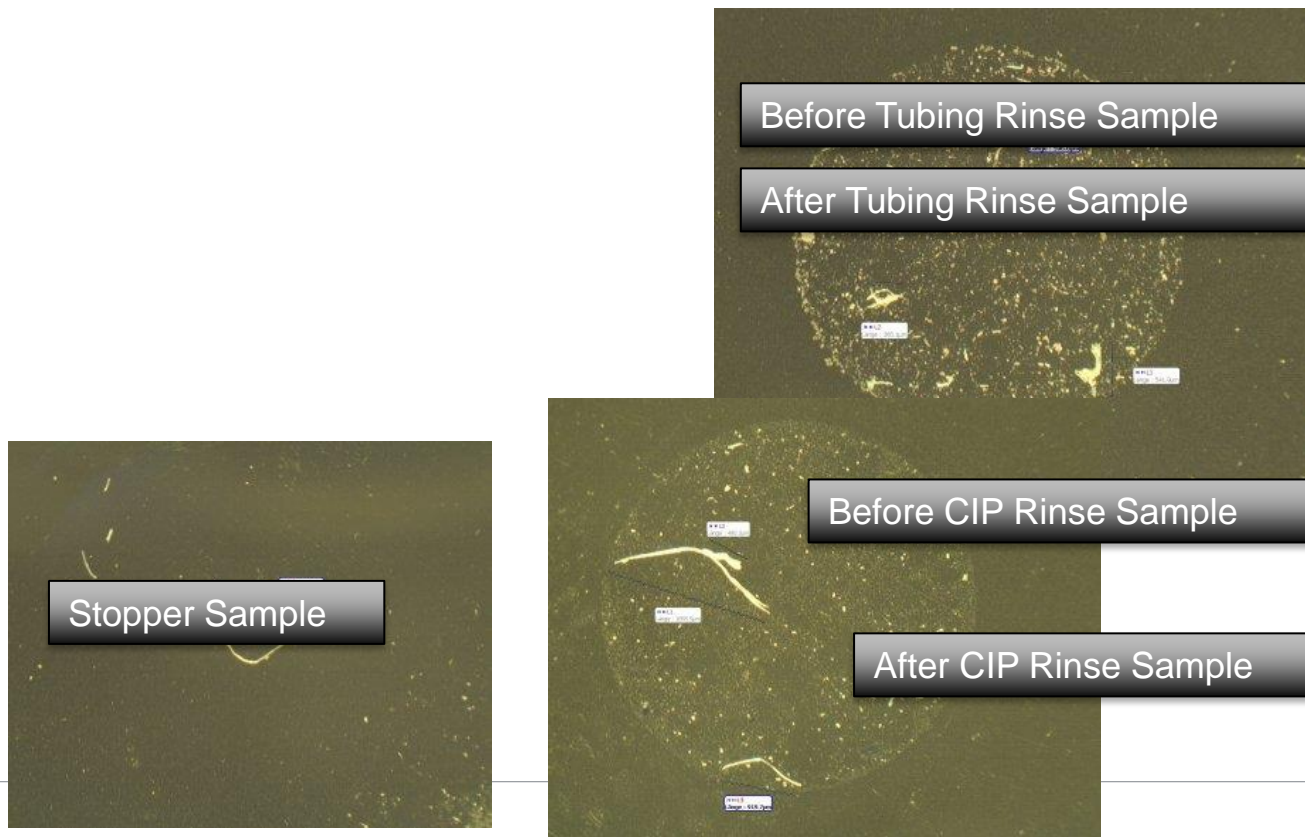
After Tubing Rinse Sample



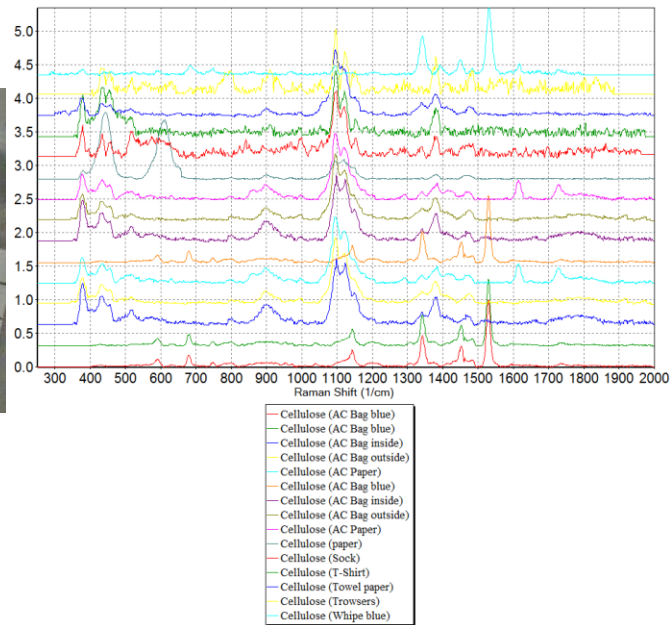
Before CIP Rinse Sample

After CIP Rinse Sample

Samples from the process were taken⁴



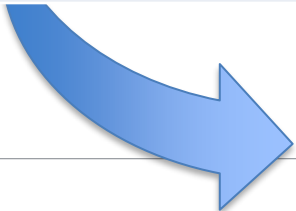
Database with filling line related materials was built



No Cellulose (Contaminant) !

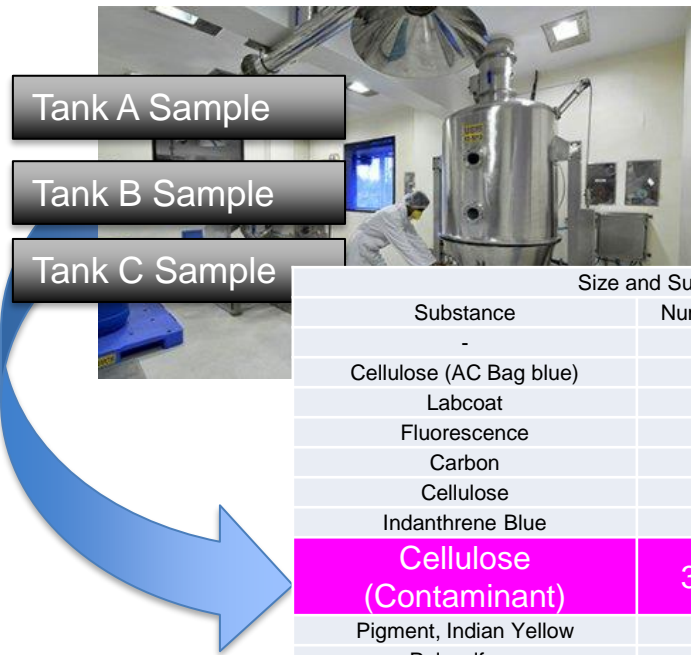
Tube rinse result

| Size and Substance Distribution of Measured Particles | | | | | | |
|---|--------|-------------------------------------|-----------|-----------|-----------|------------|
| Substance | Number | Size Distribution [μm] | | | | |
| | | ≥ 5 | ≥ 10 | ≥ 25 | ≥ 50 | ≥ 100 |
| - | - | ≥ 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)⁴⁸



Size and Substance Distribution of Measured Particles

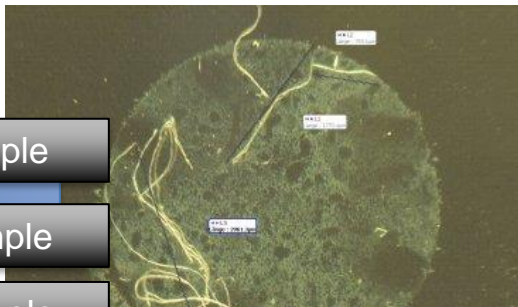
| Substance | Number | Size Distribution [µm] | | | | |
|--------------------------------|-----------|------------------------|----------|----------|-----------|----------|
| | | >=5 | >=10 | >=25 | >=50 | >=100 |
| - | - | >=5 | >=10 | >=25 | >=50 | >=100 |
| Cellulose (AC Bag blue) | 1 | 0 | 0 | 0 | 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

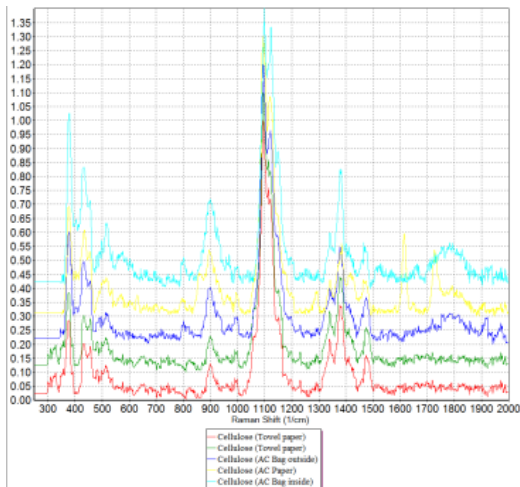
Tank C Sample



Size and Substance Distribution of Measured Particles

| Substance | Number | Size Distribution [µm] | | | | |
|--------------------------------|-----------|------------------------|----------|----------|-----------|----------|
| | | >=5 | >=10 | >=25 | >=50 | >=100 |
| - | - | >=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

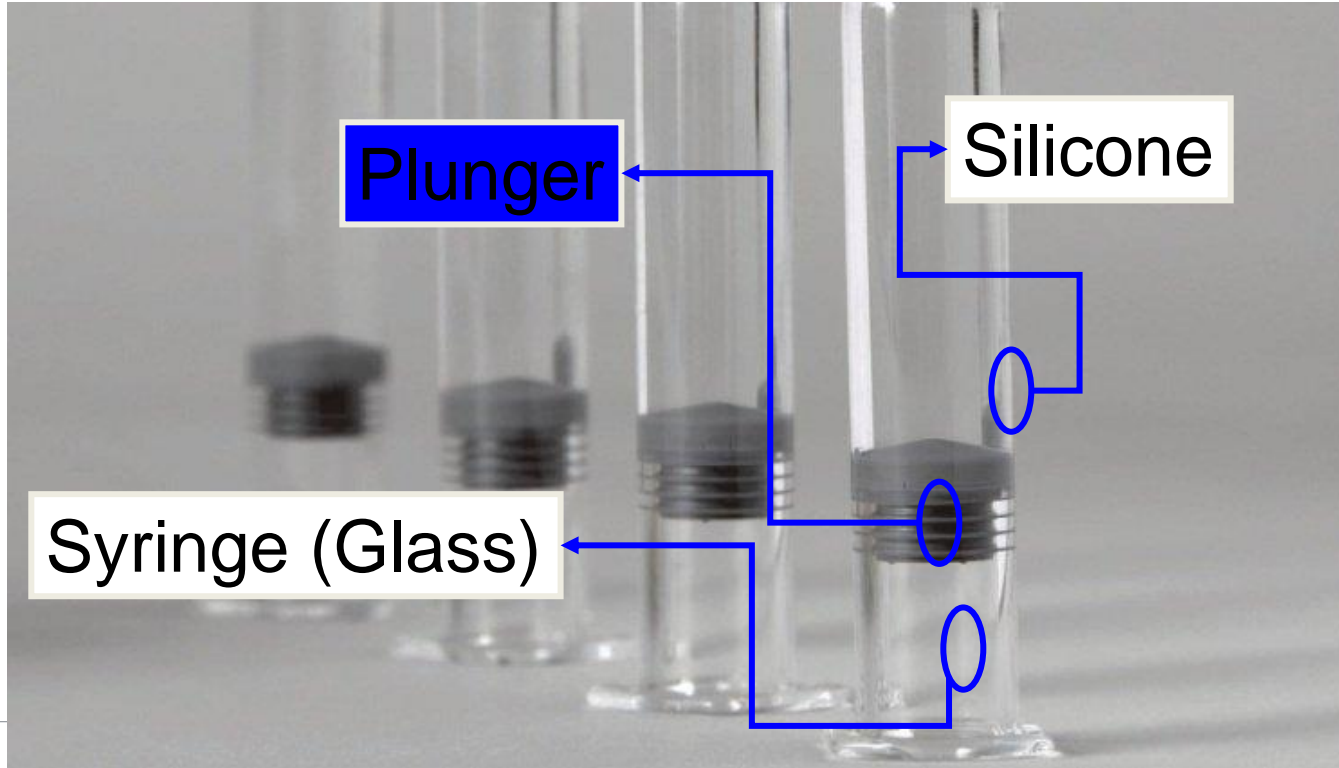


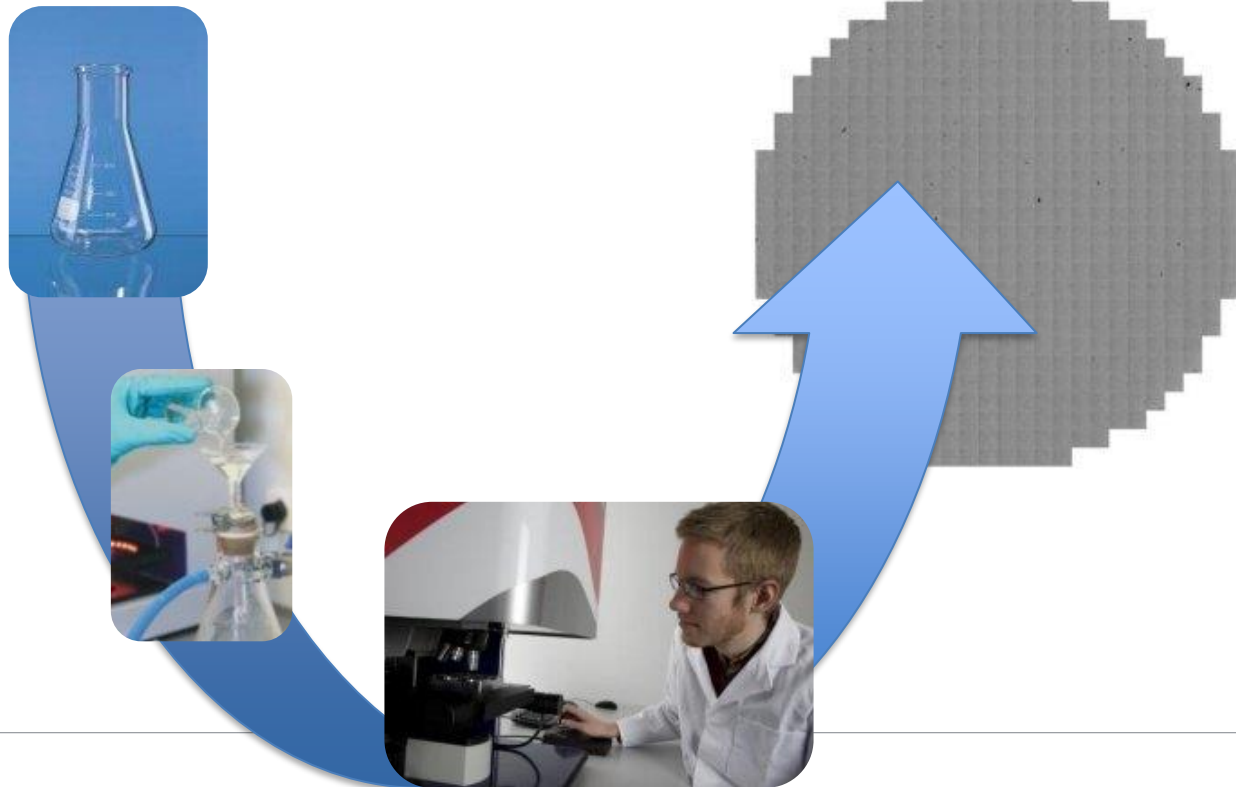
Cellulose (Contaminant)

- 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

Control Your Packaging Material 52





Technical Report No. 85

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

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 |

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**.

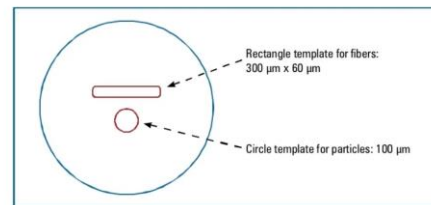
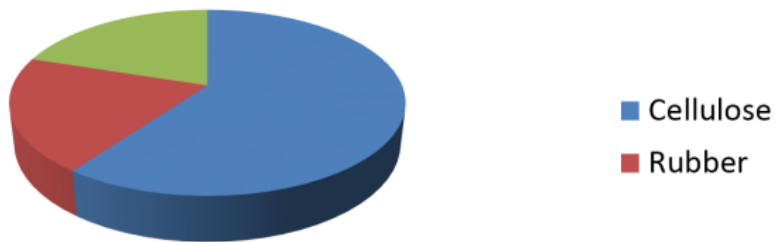


Figure 4.1.2-1 Microscope Reticle Design For Elastomer Component Particle Analysis

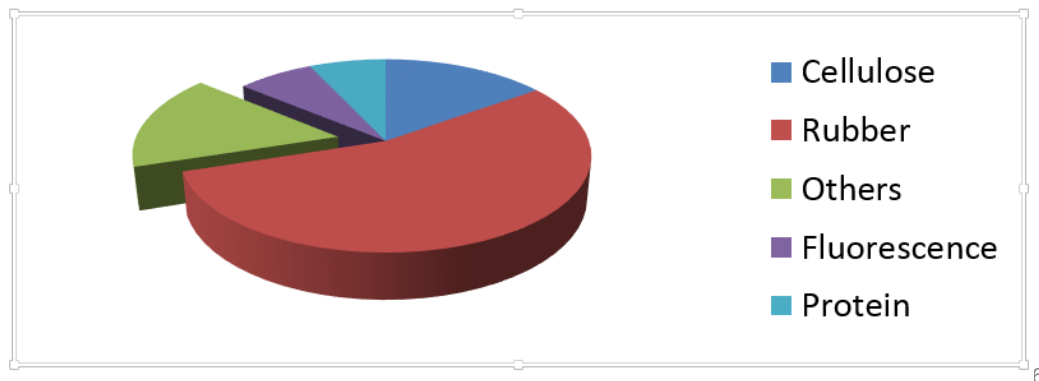
- A number of parts (normalized for surface area) are placed in an Erlenmeyer Flask,
- Add 50 ml of surfactant solution is dispensed and added to the flask.
- Agitate on an orbital shaker for 20 sto remove visible particles from the surface.
- Filter immediately through a membrane filter (a gray filter 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**

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



Large scattering in particle number and composition can be observed in one batch and different bags

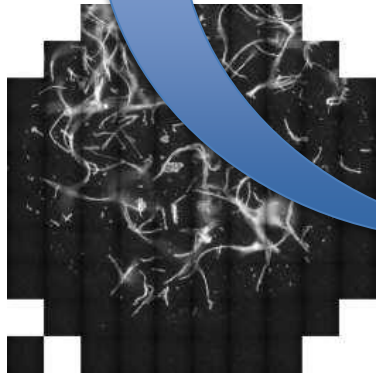
- 10 stoppers contaminated with particles Cleaning following ISO 8871
- 122 particles found > 100 μm



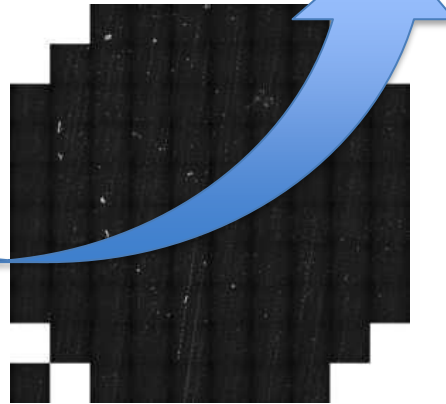
6



Stopper Bags have an impact
or reflect stopper quality

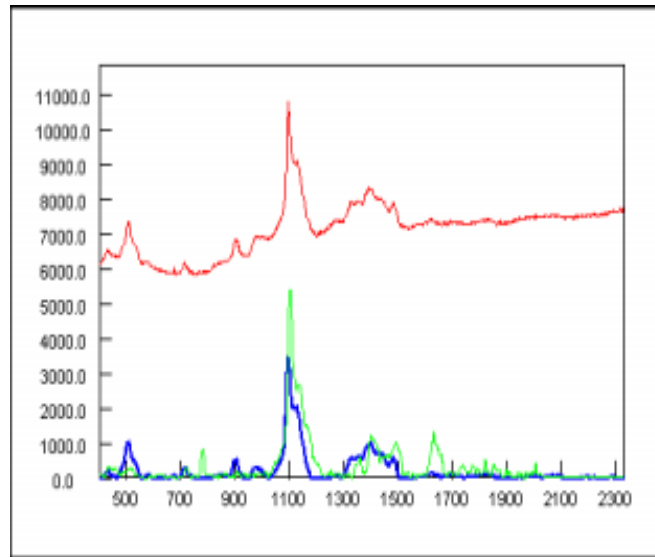


Fibres collected from one bag;
375 particles > 25 μ m

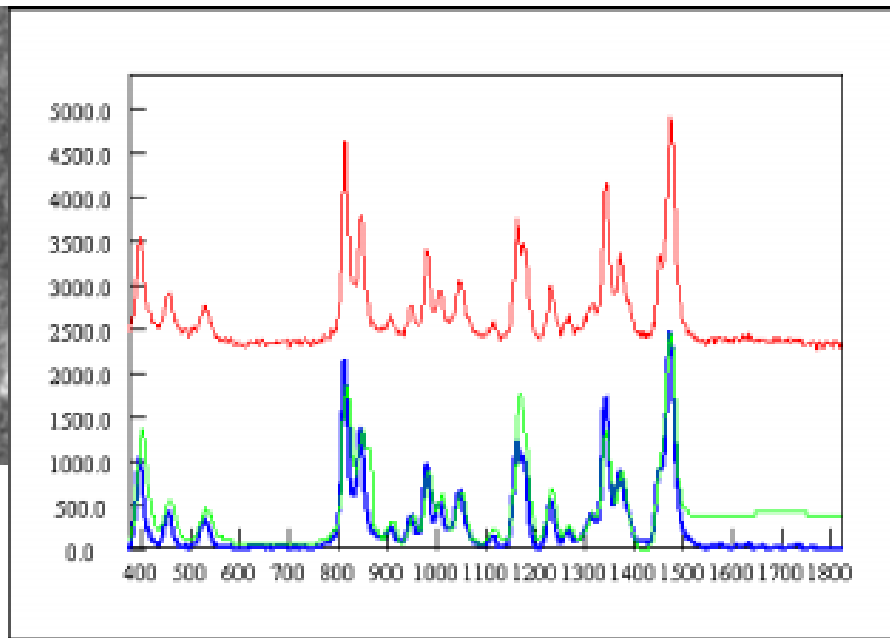
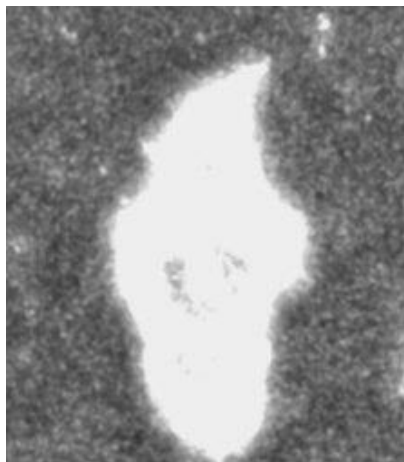


Fibres collected from one bag;
45 particles > 25 μ m

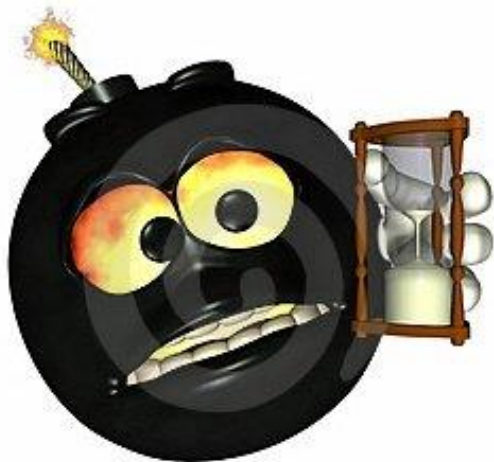
Test Procedure:
Bag rinsed with
250 ml water /
SDS, filtration,
counting



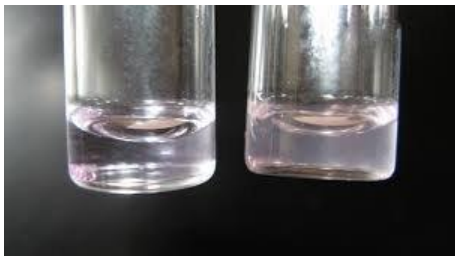
RESULT: Cellulose [Paper]
RANK: 882, S/N: 39.2



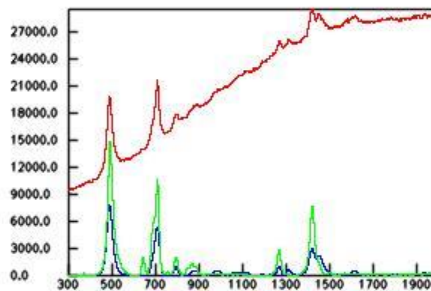
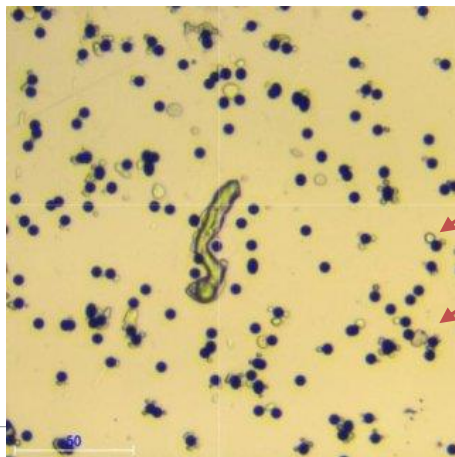
Rubber material and filler

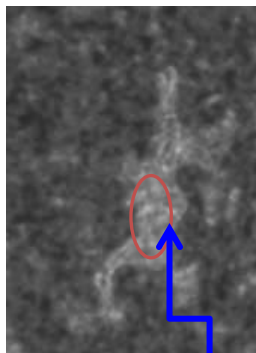


- Increase of rejects with time
- Chemical reactions taking some time
 - Silicone oil on stoppers:
Agglomeration of Proteins
 - Coatings
 - Glass delamination

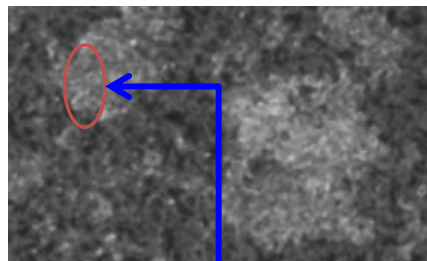


Observation of haziness and aggregates in a new a new batch after slight process change



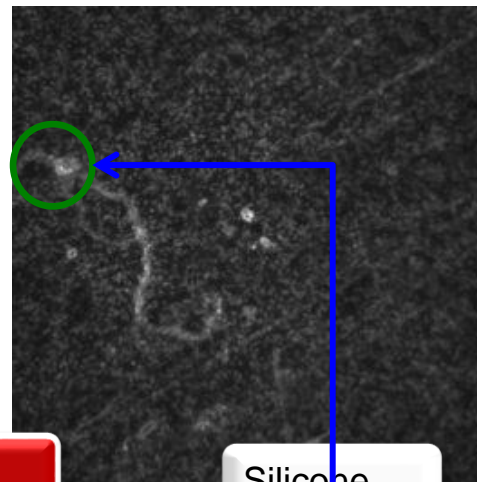


Protein

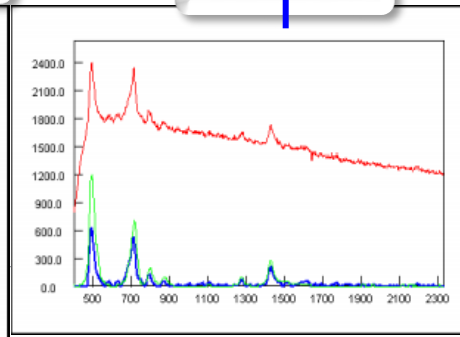
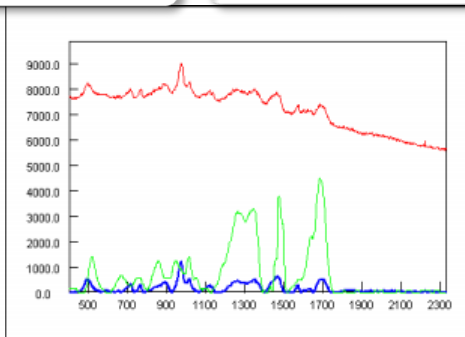
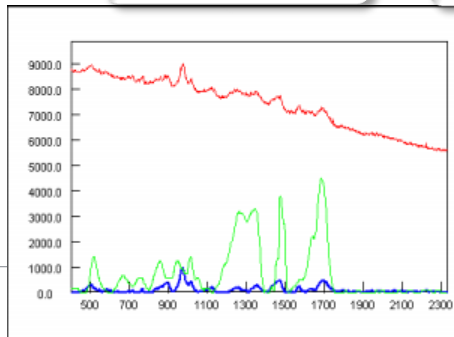


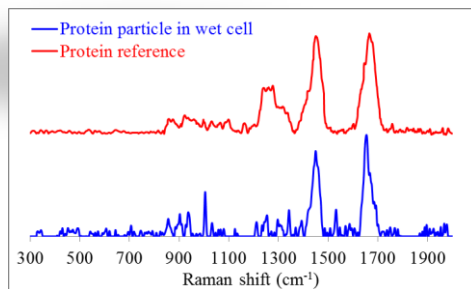
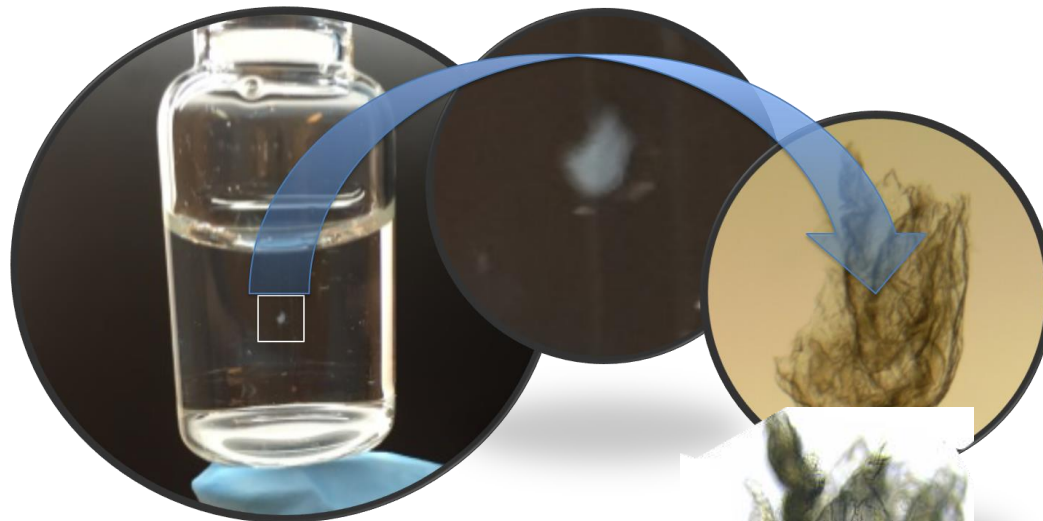
Silicone

Protein

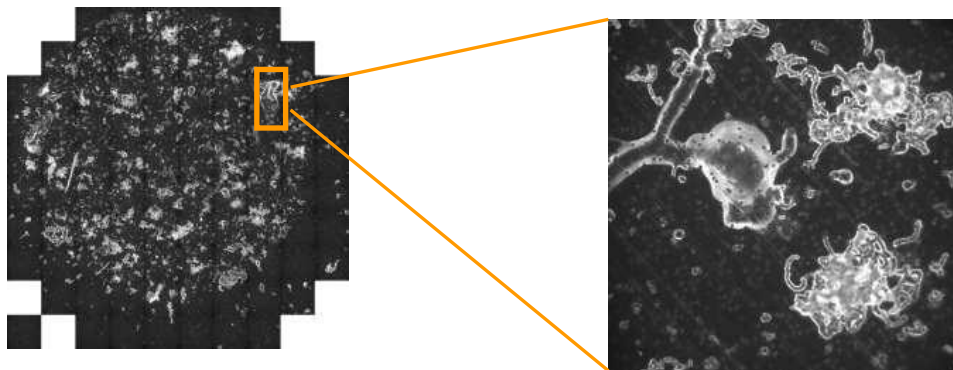


Silicone





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 |
| - | - | >=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 |

Destructive reconstitution, dilution, transfer, clearing, solubilizing, filtration, screening, or sieving that allows 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 |