Robert's Rules for Contaminant Analysis

Robert A. Carlton, Ph.D.

Contaminant Identification

Rapid and accurate contaminant identification is a crucial part of development and manufacturing operations in all industries including pharmaceuticals. Microscopy plays a key role in the isolation and identification of contaminants.

This is one of the most important ways for a microscopist/analyst to directly affect the performance of the company.

Common Examples

- Fibers, glass flakes, silicone oil, cleaning residue, etc. in parenterals
- Lubricating oil
- Environmental Particles (i.e. pollen, plant parts, insect parts)
- Tablet colorants
- Polymers (gasket material, bag parts, etc.)
- Metal (aluminum, steel, etc.)

Robert's Rules for Contaminant Identification

RULE #1 THINK BEFORE YOU ACT

RULE #2 GET ALL THE INFORMATION BEFORE YOU START

RULE #3 CLEARLY ESTABLISH THE GOAL OF THE INVESTIGATION

RULE #4 EVERY EXPERIMENT SHOULD TEST A HYPOTHESIS

Corollary: Do no experiment that does not test a hypothesis

RULE #5 START SIMPLE AND PROGRESS TO COMPLEX

RULE #6 KEEP CLEAR DISTINCTION BETWEEN FACT AND HYPOTHESIS

RULE #7 VERIFY CONCLUSIONS

RULE #8 DOCUMENT AS YOU WORK

RULE #9 HAVE A CLEAR EXIT STRATEGY

Rule #10 THE JOB'S NOT DONE TILL THE REPORT IS DONE

RULE #1 THINK BEFORE YOU ACT

A few moments reflection at each stage of the examination is beneficial. Given the nature of the work, you can be under tremendous pressure to work quickly. Just be sure you don't give in to the sense of panic and act rashly.

RULE #2 GET ALL THE INFORMATION BEFORE YOU START

There are two good reasons for this rule.

First, context can make all the difference in analysis. If you know that a gasket has failed just prior to the appearance of the contaminant, that can guide you quickly to the right conclusion.

Second, you need all the information for your report. It frequently is easier to get it before you do the analysis than after.

Contaminant ID Form

I like to have an electronic form which also includes the report. Here are the things I like to collect.

I keep a physical copy of the form with the samples in a basket in order to keep multiple analyses separate and organized.

Contaminant Identification and Report Form

Title:
Date Submitted:
Quality Investigation Number:
Date QI Due:

Submitted By:
Product Issue Observed By:
Date Issue First Observed:
Location:

Product (Number):
Lot Identifier:
Incidental Observations:
List of Submitted Samples:
Reference Samples Submitted:

Analyst Name:
Analysis Start Date:
Completion Date:

Analysis Summary:

Analysis Details

Visual Observations:
Stereomicroscopy:
Polarized Light Microscopy:
Vibrational Spectroscopy:
SEM/EDS:
Other Tests:
[Include instrument details (serial number etc.), sampling procedure, and data for each.]

Discussion:

Conclusion:

Reference Materials

Reference Number	Material	Condition*	New (Yes, No)

*Slide, bulk, spectrum database, etc.

RULE #3 CLEARLY ESTABLISH THE GOAL OF THE INVESTIGATION

Clearly, the immediate goal is to identify the particles.

The goal of the investigation though may be solely informational or there may be customer, legal, or other impacts.

The quality of your work should be the same – it is the endpoint that may be different. Knowing the fiber is nylon may be sufficient for the informational work, but you may need to know more if there are additional implications. The sequence of testing may be different.

Basic Contaminant Classification

USP 1787 (Parenterals and Ophthalmics)

Can be loosely applied to all contaminants

- Extrinsic (outside the process)
  Environmental
- Intrinsic (within the process)
  - Internal, additive
  i.e. rubber, silicone oil
  - Instability, change
  i.e. glass flakes from vial corrosion
- Inherent (formulation component)
  i.e. protein aggregate, buffer, excipient, drug substance

Classification of this type can typically be done quickly. This initial information can be exceedingly helpful for those working to identify the problem and fix it.

1
2

RULE #4 EVERY EXPERIMENT SHOULD TEST A HYPOTHESIS

Corollary: Do no experiment that does not test a hypothesis

It is very tempting to just start testing and use every tool in the lab. This approach inevitably leads to confusion and is inefficient.

Your visual examination with stereomicroscopy should lead to the first hypothesis. For example, the contaminant is a polymeric fiber.

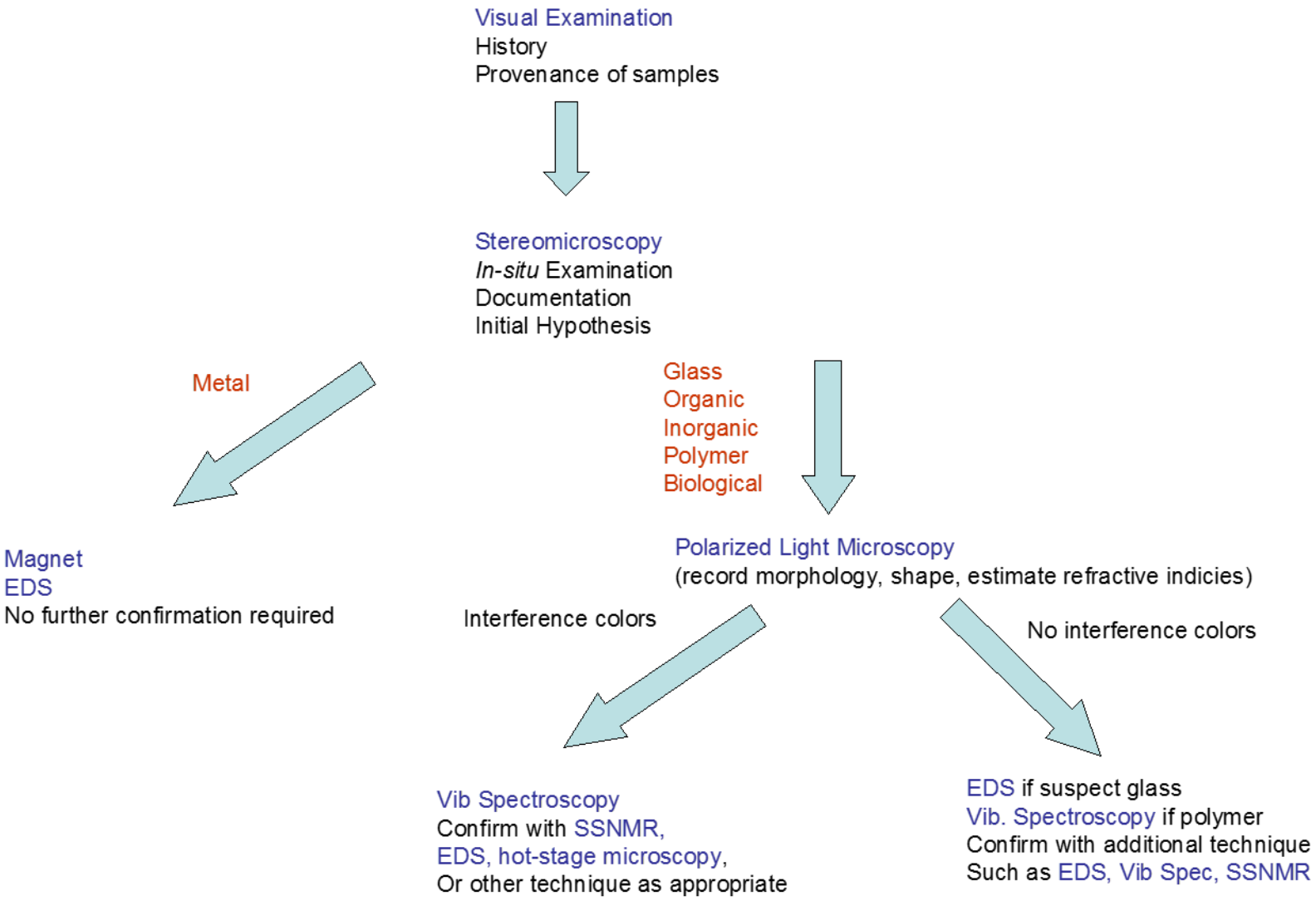
PLM may indicate that instead you may have a glass fiber or a plant trichome. Now you can revise your hypothesis and go the next test – SEM/EDS or vibrational spectroscopy.

RULE #5 START SIMPLE AND PROGRESS TO COMPLEX

This rule is mainly a caution against doing destructive testing before you the non-destructive ones.

Always begin with visual examination and stereomicroscopy. Don’t do any destructive test until you have all of your other data available.

Identification Strategy



Microscopy Techniques for Contaminant Identification

Includes:

- Optical microscopy (OM)
- Polarized light microscopy (PLM)
- Infrared and Raman Microspectroscopy (Vib Spec)
- Scanning electron microscopy with energy dispersive x-ray spectrometry (SEM/EDS)
- Thermal microscopy (TM)
- Microchemical Tests
- Solid State NMR (SSNMR)
- Solubility

RULE #6 KEEP CLEAR DISTINCTION BETWEEN FACT AND HYPOTHESIS

It is only human to jump to conclusions. Be careful.

For example, most crystalline particles display interference colors between crossed polars. But there are two circumstances (cubic crystals, special crystal orientation) where the particle will not display birefringence but will be crystalline.

The fact is the particle displays no birefringence. The hypothesis is that the particle is not crystalline. We must keep that type of distinction clear in our own mind.

RULE #7 VERIFY CONCLUSIONS

Verifying conclusions can be quite challenging in contaminant identification.

Ideally, we can apply two or, even better, three tests using different physical and chemical principles on the contaminant. For instance, with a fiber we'd like to have optical crystallography, refractive index, and vibrational spectroscopy.

If we have only 1 very tiny particle, then we may only be able to do one or two tests.

Also, some particles will only be sensitive to one practical test – such as metals with SEM/EDS or biological parts with OM.

For these types of materials and with small specimens, you may need novel, creative approaches, i.e. stains with biological samples.

RULE #8 DOCUMENT AS YOU WORK

This is a particular, personal weakness. I tend too much to get on the trail of the particle and think I'll write stuff down later. The weakness has led to the need to duplicate work which is really irritating.

RULE #9 HAVE A CLEAR EXIT STRATEGY

Or, know your limits and how far you can take an analysis.

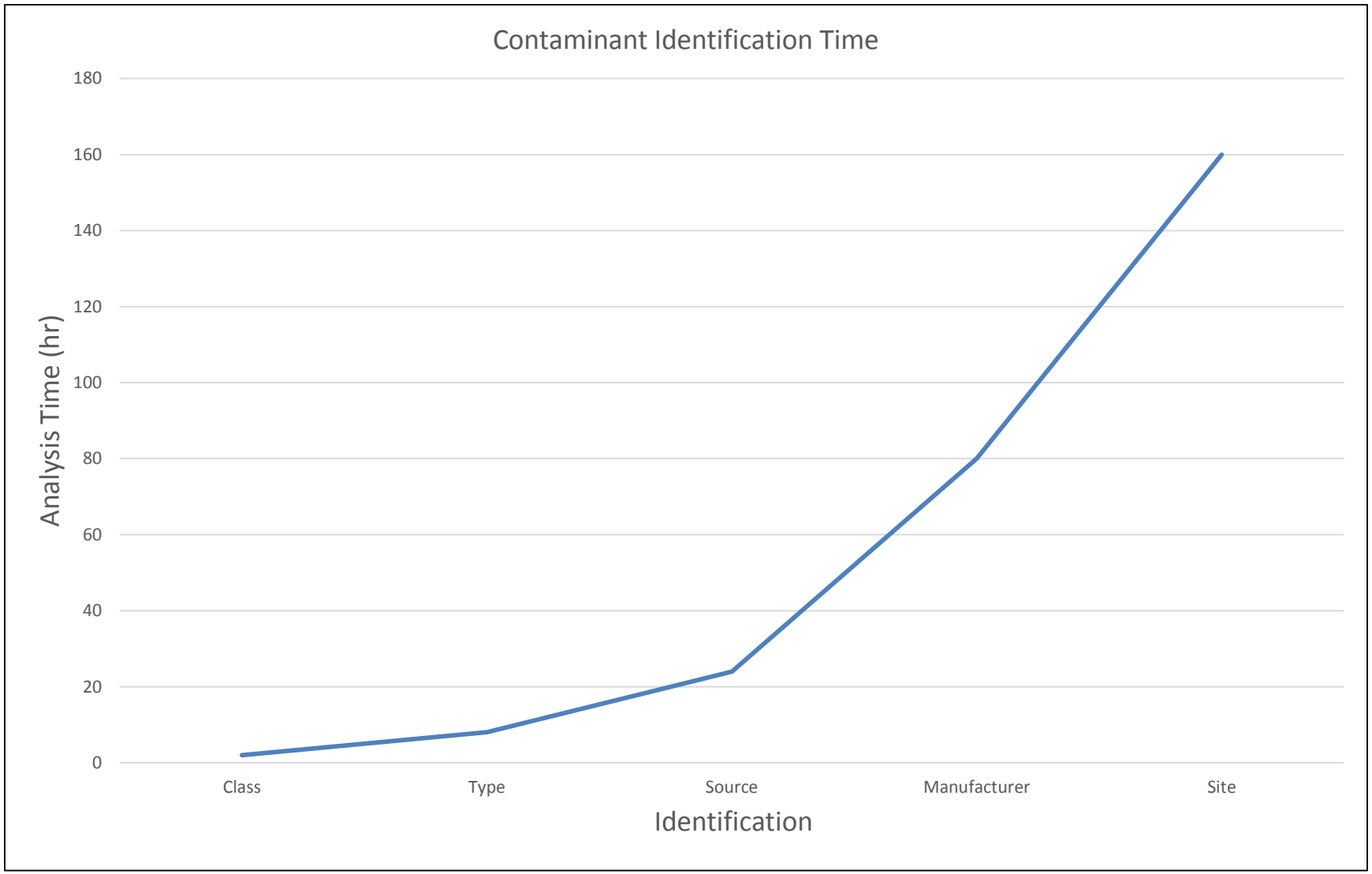
I have been asked on numerous occasions about the clinical relevance of this or that contaminant.

Obviously, that is not my area of expertise. More frequently, I am asked to identify the exact source of the contaminant. Clearly, I'd like to help in this regard, but often I don't have access to enough information.

This rule is just a caution to know when you have exhausted the help you can give and when you need to move on.

Time Required for Identification

More specific ID requires more time



RULE #10 THE JOB ISN'T DONE UNTIL THE REPORT IS DONE.

Since contaminants come in swarms, you probably will have a time or two when you give people informal results once the analysis is complete. Don't forget the report!

Two Stories

My first attempt at contaminant identification was with a problem in a fiberglass insulation plant.

The liquid polymer filters (from holding tank to manufacturing line) were needing to be replaced every 2 days. Previously, the filters were only replaced at monthly preventative maintenance as a precaution.

I was sent the fouled filters.

Example of filter contents – but also with lots of fiberglass insulation. A little bit of everything.

Failing Filters

So I reported what looked to be …. well, *dirt*.

A significant amount of ridicule was heaped upon my head. It simply was not possible. The system was sealed from train to tank.

The plant management just decided to live with the problem.

Some months later, a plant supervisor happened to be walking by the room with the polymer tanks. There at the top of one of the tanks was the janitor dumping his waste bin into the tank!

As an aside, I was never given any sort of official acknowledgement that I was correct. BUT, I started receiving more requests for analysis.

2nd Story

X marks the spot

I received a metal piece like the hip ball on the previous page. It had a piece of tape on it with a bit of writing to explain the problem.

Being in a rush (as always), I jumped directly to the 'let's just put it in the SEM and do a quick test' solution.

EDS confirmed that I had some carbon rich material on the surface.

Thankfully, before I rushed off to report the result, it occurred to me that I had pulled the tape off the piece before I put it in the SEM.

Or does it?

Yes, you guessed it. I analyzed the tape glue residue not the offending contaminant.

Under the stereomicroscope, I could see the brown spot I was supposed to examine....and the glue just below it! Turned out to be rust.

No matter how tempting it is to skip a step, doing so will eventually bite you in the posterior.

Concluding Thoughts

There is an almost infinite variety of things that can contaminate our tablets, capsules, parenterals, topicals, inhalers, etc.

As an analyst this poses a continuing challenge…but also provides quite interesting work.

It does require patience, humility, and an ongoing dedication by the analyst to learning the behavior of materials.

Skill in contaminant identification comes mostly with experience and practice.

Key to Contaminant ID (and almost all microscopy)

"The skills possessed by the investigator are almost always the deciding factor in a successful analysis. Lack of advanced instrumentation is rarely a severe handicap. Consequently, an investigator with the most sophisticated instruments who lacks the basic skills and mental attitude stands almost no chance of success in this task."

Palenik SJ (1979) The Determination of Geographical Origin of Dust Samples. In: The Particle Atlas. 2nd Edition. Ann Arbor Science Publishers, Ann Arbor USA

Questions