

# PROCESS RESIDUALS

## Impurity & Residuals in Biotechnology Products

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# Impurities and Residuals

- Most impurities have been broadly categorized into two based upon their nature
  - Chemical or inorganic (e.g. heavy metals)
  - Biological or organics (e.g. protein, lipids, carbohydrates, Nucleic acids)

Type of impurity defines the analytical method to be used.



## Three major categories of process related impurities

- **Cell substrate derived:**
  - *Host Cell Protein (HCP)*
  - *Nucleic Acid* (host cell genomic, vector or total DNA)
- **Cell culture derived:**
  - *Inducers, antibiotics, serum, other media components*
- **Downstream derived:**
  - *Enzymes*
  - *Chemical and biochemical processing reagents,*
  - *Inorganic salts, solvents, carriers, ligands and other leachables*



## Process residuals of interest to regulators

<b>Media Components</b>	Transferrin, Insulin, Albumin, Bovine Immunoglobulins, tropolone, Soy proteins
<b>Cell Components</b>	HCP, DNA
<b>Chemical Additives</b>	Antibiotics, Methotrexate, Dithiothreitol, Glycols, Protease inhibitors, Antifoam agents
<b>Leachables</b>	Protein A, Heavy Metals, Plastics, Resin decompositions, Beta-glucans, Preservatives from filters

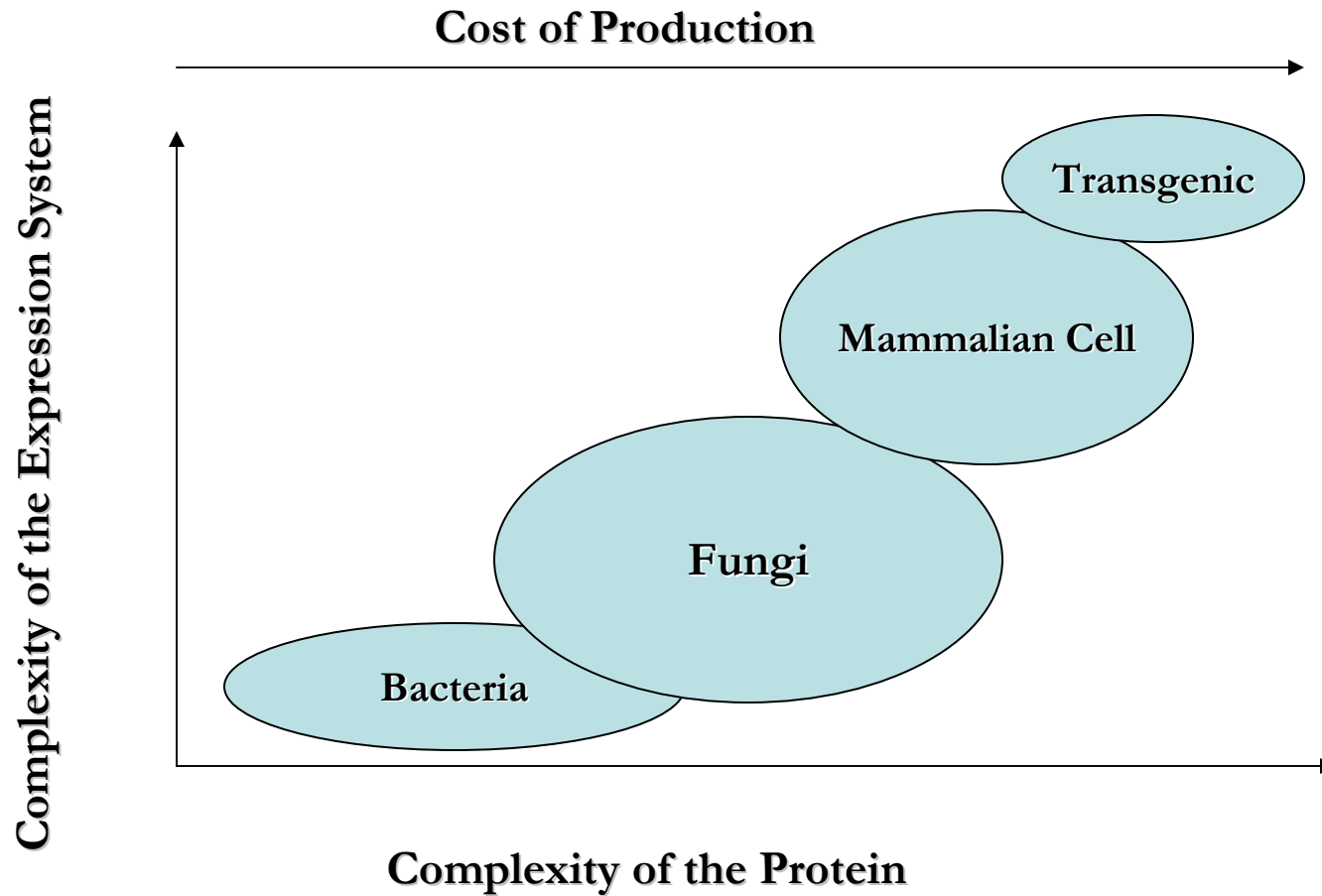


## Range of Biopharmaceuticals

<b>Hormones</b>	GH, insulins, insulin analogues
<b>Growth Factors</b>	PDGF, NGF, IGF-1
<b>Cytokines</b>	Interferon, interleukins, G-CSF
<b>Mabs</b>	Murine, chimeric, humanized, rDNA derived
<b>Antibody related products</b>	Single chain, fragments, fusion products
<b>Vaccines</b>	Conventional, recombinant protein antigen, modified bacteria or viruses, Polysaccharides, Polysaccharide-protein conjugates etc.
<b>Nucleic acid based products</b>	Gene therapy, DNA vaccines, ribozymes
<b>Cells, tissues and organs</b>	Autologous, allogenic, xenogeneic



## Expression System comparison



# Biological Products

Biotechnology products for therapeutic use include a very diverse range of products

## Therapeutic Proteins:

- *Naturally occurring human proteins.*
- *Recombinant copies of naturally occurring proteins.*
- *Mutated or modified version of a naturally occurring protein.*
- *Monoclonal antibodies.*



# Biological Products

## Polysaccharides:

- *Naturally occurring polysaccharides*

## Polysaccharide-Protein Conjugate:

- *Naturally occurring polysaccharides conjugated with natural and / or recombinant proteins*



# Current status

There are no internationally recognized specifications for process-related impurities. This has led to disparities among regulatory agencies in various global regions.

FDA has not issued a guidance dealing specifically with biopharmaceutical impurities. ***The International Conference on Harmonization*** (ICH) Q3 series of guidance – addressing impurities *for drug substances* (Q3A), drug products (Q3B) and residual solvents (Q3C) – *do not apply to proteins or other biological products*. ICH's Q6B on specification setting for biotechnology products does address impurities briefly, but the material is relatively basic.

One of the unique aspects of biopharmaceutical products is that the purification process for the target active protein can co-purify from the host cell itself.

The assessment of process residuals is one of the key considerations in the development of both innovator and follow-on biological products. Purification steps are incorporated to clear, or minimize, process residuals in the final drug substance. But the data depends upon the ability of several different analytical test methods to accurately, precisely and reliably measure specific process residuals.



# ICH Q6B

- **ICH Q6B defines product related substances and impurities and discusses the setting of specifications. But it does not describe how to approach individual situation.**
- **Acceptance criteria and analytical methods should be developed and justified, based upon previous experience with the drug products to measure changes in the drug substance during manufacture and/or storage of the drug products.**
- **The choice of analytical methods should be focus on the separation of the desired product and product-related substance from impurities including degradation products, and from excipients.**



# Overall ICH Q6B Requirement

Molecular Weight Information

Amino Acid Analysis

Isoelectric Focusing

SDS-PAGE

Size Exclusion Chromatography

Peptide Mapping by Mass Spectrometry

N-/C-terminal Sequencing

S-S Bridge Analysis

Glycosylation Analysis



# Why Do We Have Assays?

**Analytical tests/Assays are central and critical to all components of product development and manufacturing, assuring continued quality, safety and efficacy of the products. Especially post approval.**



# Why Validate Assays

“Method validation is the *process* of demonstrating that analytical procedures are *suitable for their intended use*”

## Making sure the analytical procedure:

- *Does what it is intended to do*
- *Yields data to answer a question*
- *Provides confidence in the result*



# Assay Development and Optimization

Assay development and optimization should involve the initial decisions over assay format, selection of appropriate reagent and procedure etc.

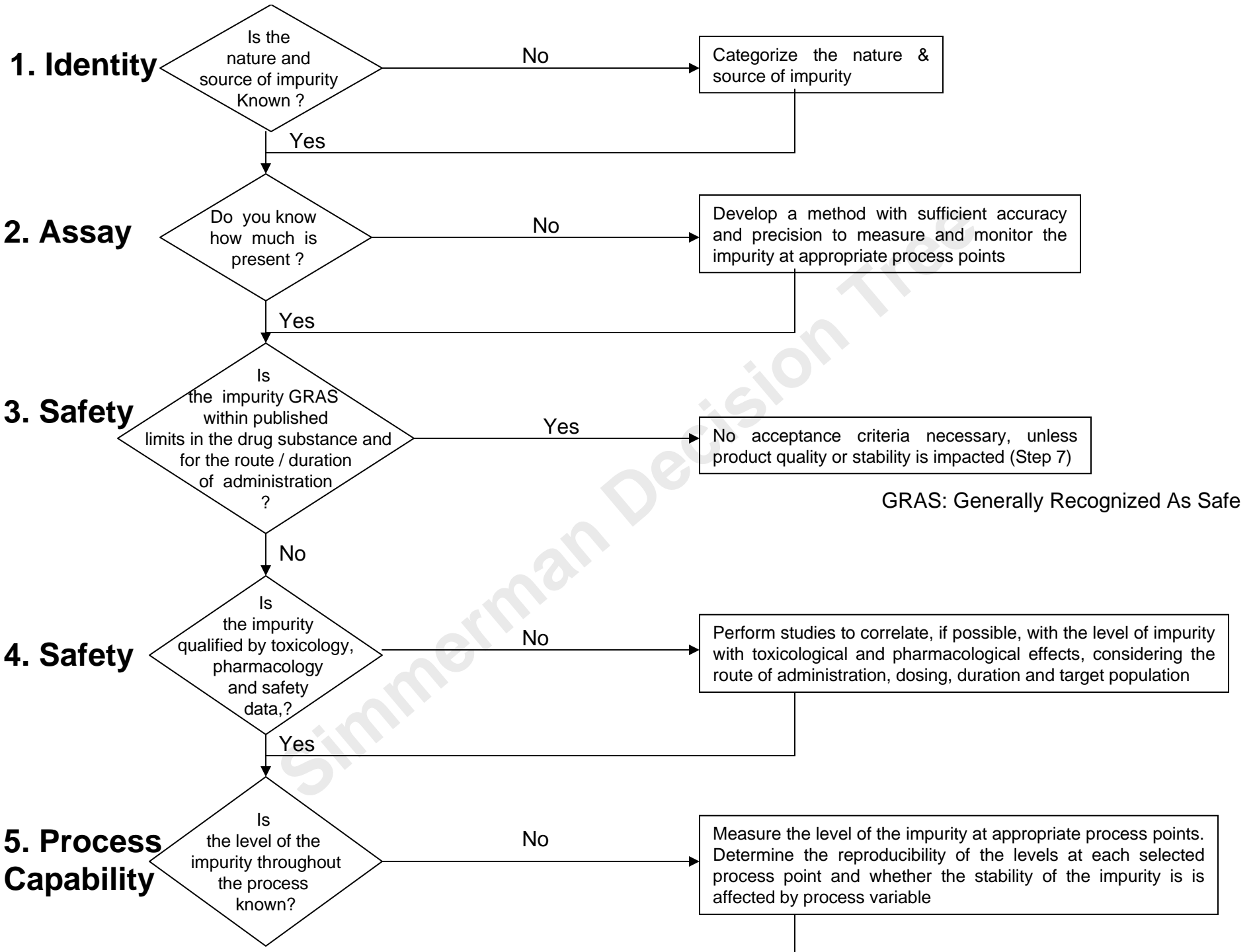
## Example for Immunoassay

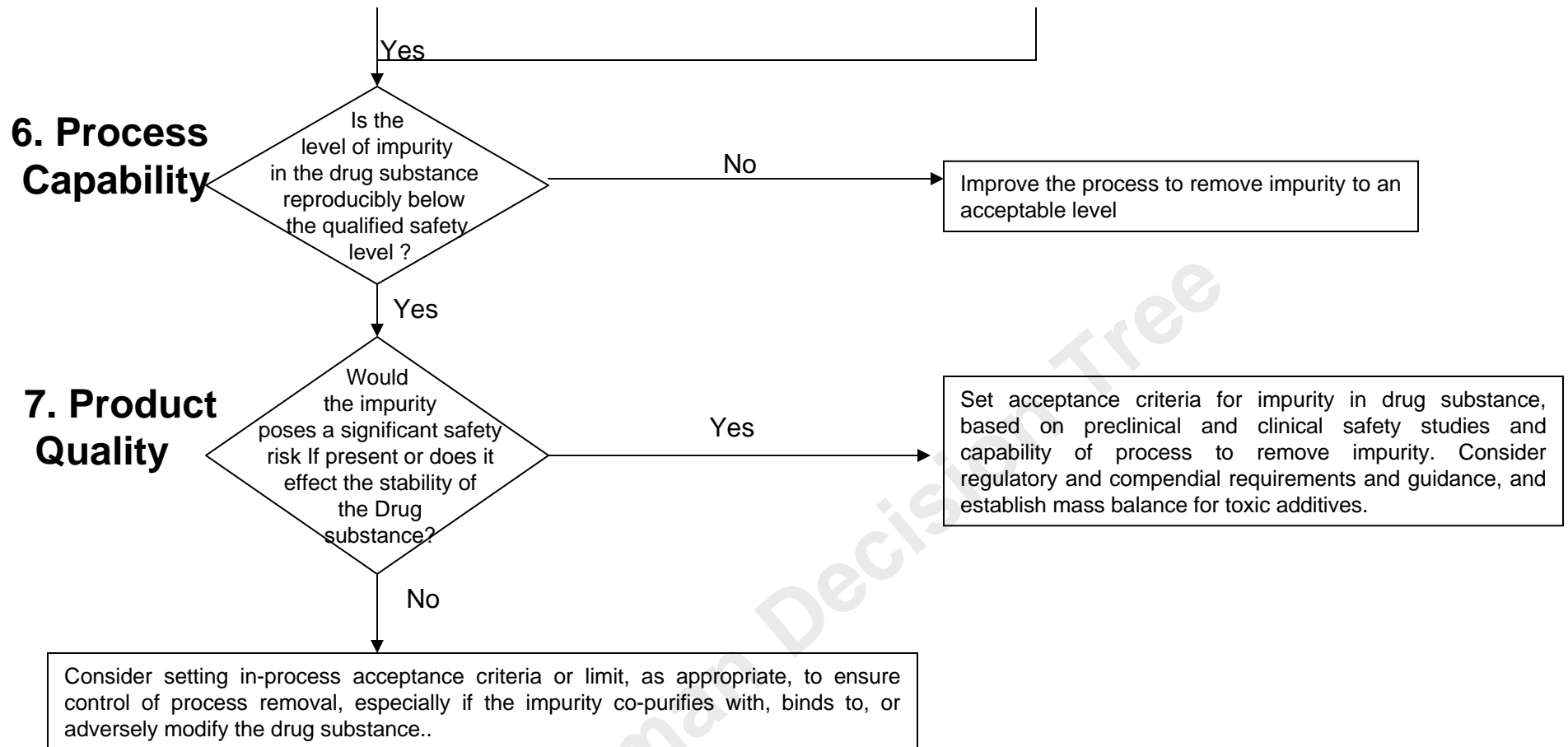
- *Capture reagent (Polyclonal/monoclonal/lectin)*
- *Coating concentration*
- *Buffer constituents*
- *Detection reagent concentration*
- *Wash procedure*
- *Reagent stability*



***Amgen's Heather Simmerman presented the following decision tree, intended to help biologics manufacturers with when and how to set acceptance criteria for process-related impurities.***







steps in the decision tree do not necessarily need to be followed sequentially but are considerations that manufacturers may address “concurrently.”



# Example!

## Product-Related Impurities: *Tackling Aggregates*

Protein can self-associate. For some protein, self-association is natural. However, protein isolated from their native environment can self-associate into non-native oligomers, both covalent and non covalent.

This has been proven problematic for biopharmaceuticals development, because it may negatively affect process efficacy and/or subsequent clinical use.

### ***Regulatory consideration:***

Regulatory agencies are concerned about aggregate levels. Although “5%” may be a common acceptance criteria, but it should not be regarded as a magic Number.

### ***Detection of Aggregates:***

SEC-HPLC and PAGE (SDS-PAGE) are considered robust analytical methods for QC.



# Minimum Requirement For Biological Products

National Institute of Infectious Diseases  
JAPAN



# Thank you



Respond  
Research  
Reach

