Methodology for Assessing Product Inactivation During Cleaning and Setting Cleaning Verification Limits

Experimental Approach, Analytical Methods, and Setting Acceptance Criteria

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Outline

- Literature and Regulatory
- Limitations of the MAC Calculations
- Experimental Approach
- Analytical Methods
- Setting Acceptance Criteria

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Peer Reviewed Papers

Methodology for Assessing Product Inactivation During Cleaning Part I: Experimental Approach and Analytical Methods

- Authors: Sharnez, Rizwan; Spencer, Abby; Romero, Jonathan; Runkle, Scott; Carolan, Carolina; Hayes, Ronan; Mott, Adam; Clark, Mary Ellen; Wyman, Edward; Rasmi, Moha; Donat, Stephanie; Bellorado, Kathleen
- *Journal of Validation Technology*, Vol. 18, Issue 4, November 2012.

Methodology for Assessing Product Inactivation During Cleaning Part II: Setting Acceptance Limits of Biopharmaceutical Product Carryover for Equipment Cleaning

- Authors: Mott, Adam; Henry, Bill; Wyman, Edward; Randall Greg; Bellorado, Kathleen; Blumel Markus; Clark, Mary Ellen, Parks, Michael; Hayes, Ronan; Runkle, Scott; Luo, Wendy
- *Journal of Validation Technology*, Vol. 19, Issue 4, December 2013.

Involved Companies

- Amgen
- Baxter
- Biogen Idec
- BMS
- GSK
- Human Genome Sciences
- Janssen
- Lonza
- MedImmune/AstraZeneca
- Novartis
- Pfizer
- BPOG

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Regulatory Expectations

- Regulatory Expectations
  - Demonstrate that potential carryover of the previously manufactured product/API (Product A) into the subsequently manufactured product (Product B) is below an acceptable (safe) level.
- Traditional Industry Approach
  - Often assessed using the Maximum Allowable Carryover (MAC or MACO) Calculation
  - Typically based on:
    - Minimum therapeutic dose
    - Acceptable Daily Exposure (ADE) of the previously manufactured API
    - Dose volume
    - Equipment Train Surface Area

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Limitations of the MAC Approach

- Based on the assumption that the product remains active after cleaning.
  - In Biopharma, API is often inactivated after the cleaning process
- Calculated acceptance limits are often below the limit of quantitation (LOQ) of non-specific methods
  - Large Surface Areas, small batch sizes and low concentrations in Biotech while using non-product specific testing (e.g., TOC) to determine MAC, the AC could be below the LOQ for TOC
  - LOQ for TOC is typically 0.05 and 0.2ppm
  - TOC specification for WFI is ≤ 500 ppm (≤ 0.500 ppm)
- Use of product specific immunoassays (PSIA)
  - Recognizes epitopes; however, epitopes are known to degrade/denature. Therefore, the results can be misleading.

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Product Inactivation Approach

- Sets acceptance criteria based on the knowledge that the product is not active.
  - Reflective of the phenomenological aspects of the cleaning process
  - Alleviates the limitations of the MAC approach.
    - Residual material after cleaning is not active, therefore limit based on dose/activity is not necessary.
  - Acceptance limits would not be calculated to be below the LOQ of TOC or WFI specification.

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Proposed Methodology

- Demonstrate that product becomes pharmacologically inactive during cleaning
  - Extreme pH (<2 and >13)
  - Temperature (60 - 80°C)
  - Known degradation/denaturation
- Demonstrate that the inactivated product has been removed below a predefined acceptance limit.
  - Consistent with the expectation that the carryover of an extrinsic impurity into a subsequent batch should be justified from the standpoint of the safety and efficacy of the product.
  - Obviates the need to develop Product Specific Immunoassays (PSIAs) for cleaning validation

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Guidance for Designing Inactivation Studies

- Design bench scale experiments to simulate full scale conditions
  - Typically in vials or within dialysis cassettes
- Conditions should be the least conducive (worst-case) for inactivation
  - Example: for a cleaning wash, the lowest applicable concentration of cleaning agent, temperature, duration, and ratio of cleaning solution to residual process soil
  - Other possible contributing factors: dirty hold time (DHT), drying conditions, sheer rate due to impingement and turbulence
- Simplify the bench scale studies
  - Eliminate operating parameters or steps if its elimination represents a worst case scenario from the standpoint of inactivation.
    - o If product inactivation increases with shear rate, then it can be eliminated
    - o Ratio of cleaning solution to process soil can be reduced
    - o Acid wash and rinse steps can be eliminated to minimize dilution of the process soil
- Ensure modifications do not result in experimental artifacts.

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Guidance for Designing Inactivation Studies

- New or Modified Cleaning Cycles
  - Design inactivation studies to evaluate the effect of key operating parameters
  - Couple with data collected from cleanability studies to identify cycle parameters that impact inactivating the API.

- For Existing Cleaning Cycles
  - Cleaning conditions should be based on worst-case operating parameters for all systems involved
    - Example: If different systems are cleaned with different cleaning solutions and at different temperatures, the study should be performed with the mildest cleaning solution, a the lowest cleaning agent concentration, at the lowest temperature, for the shortest duration of time, if these are the least conducive for inactivation.

- Post Exposure
  - Minimize further fragmentation and inactivation of the API
    - Titrate the samples to a neutral pH, if necessary.
  - Prepare the samples for analytical testing

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Inactivation Assays

- Methods that measure loss of biological activity or function (binding sites that are functionally intact).
- Measure the relative amount of biologically active product.
- Evaluate the degree of inactivation of the API during cleaning.

- Potency/Activity may be the only analysis performed to evaluate impact of cleaning process on the product.

- If further degradation information is desired or if the product does not have a Potency or Activity method, additional analysis may be performed.

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Analytical Methods for Further Evaluation

- Methods should
  - Evaluate fragmentation and inactivation of the API at bench scale
    - Understanding of cleaning conditions on the API
    - Set rational safety-based acceptance limits for target impurities
  - Detect impurities in the cleaning validation sample
    - Verify that the concentrations of target impurities in the samples are below their respective acceptance limits
- Additional Method Considerations
  - Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS-PAGE)
    - 4 to 20% gradient corresponds to a molecular weight range of 4 – 250 kDa, sufficient for most biological APIs.
  - Capillary Electrophoresis (CE)
    - Greater sensitivity, lower variability
    - Higher throughput capability
  - SDS-PAGE and CE
    - Adequate for demonstrating distinct, size-based separation of protein fragments
  - Size Exclusion High Pressure Liquid Chromatography (SE-HPLC)
    - Can also be used for size based separation
    - Difficult to obtain a distinct size based separation across a wide range of fragment sizes.

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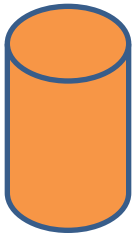
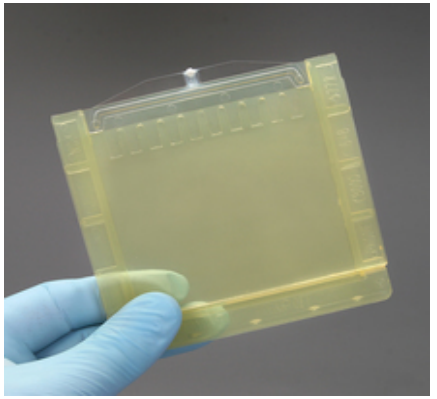
Example SDS-PAGE Experimental Design

1. Expose protein dilutions to different conditions



2. Load samples in gel wells

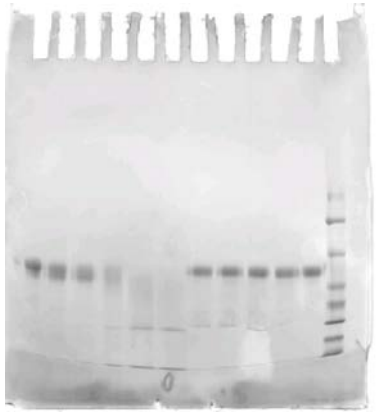
3. Apply voltage to electrophoresis



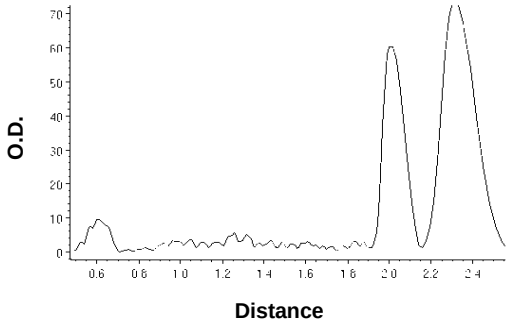
4. Lanes

Lane 1 – Blank
Lane 2 – 5 min
Lane 3 – Blank
Lane 4 – 30 min
Lane 5 – Blank
Lane 6 – 60 min
Lane 7 – Blank
Lane 8 – Protein X (Control)
Lane 9 – Blank
Lane 10 – Protein X (Control)
Lane 11 – Blank
Lane 12 – Molecular Weight Standard

5. Stain



6. Digitally Densitometry



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Analytical Method Results

- API is Inactivated
  - The acceptance criteria limit for the inactivated product can be set based on the approaches described later in this presentation.
- API is Not Inactivated
  - The acceptance criteria limit should be set based on the acceptable carryover of API.
- API is Partially Inactivated
  - The acceptance criteria limit should be determined for the API as well as for the inactivated product with the lower of the two limits being used.
  - Modify the cleaning parameters to ensure inactivation of the API

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Cleaning Verification Limits in Biologics

- If the cleaning process is shown to remove the product; inactivate and degrade/denature the product; and remove the product fragments then, product carryover is not a significant risk.

Improved cleaning process understanding and its impact on the product changes how the cleaning limit may be determined and justified.

- The surface swab limit based on *active product* and health-based limits related to *active product* carryover are not necessary.

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Product Removal Verification

- All cleaning processes are qualified to confirm water rinses and cleaning agents contact all equipment surfaces
- All products are easily rinsed from surfaces by water...

  ...first step of all cleaning is a water rinse.
- All products significantly degrade/denature when exposed to “worst-case” cleaning cycle...

  ...any remaining residual product is inactive and not intact.
- Products after exposure to cleaning agents are easily rinsed from surfaces by water...

  ...any remaining product or product variants are removed by final water rinse of the cleaning cycle.

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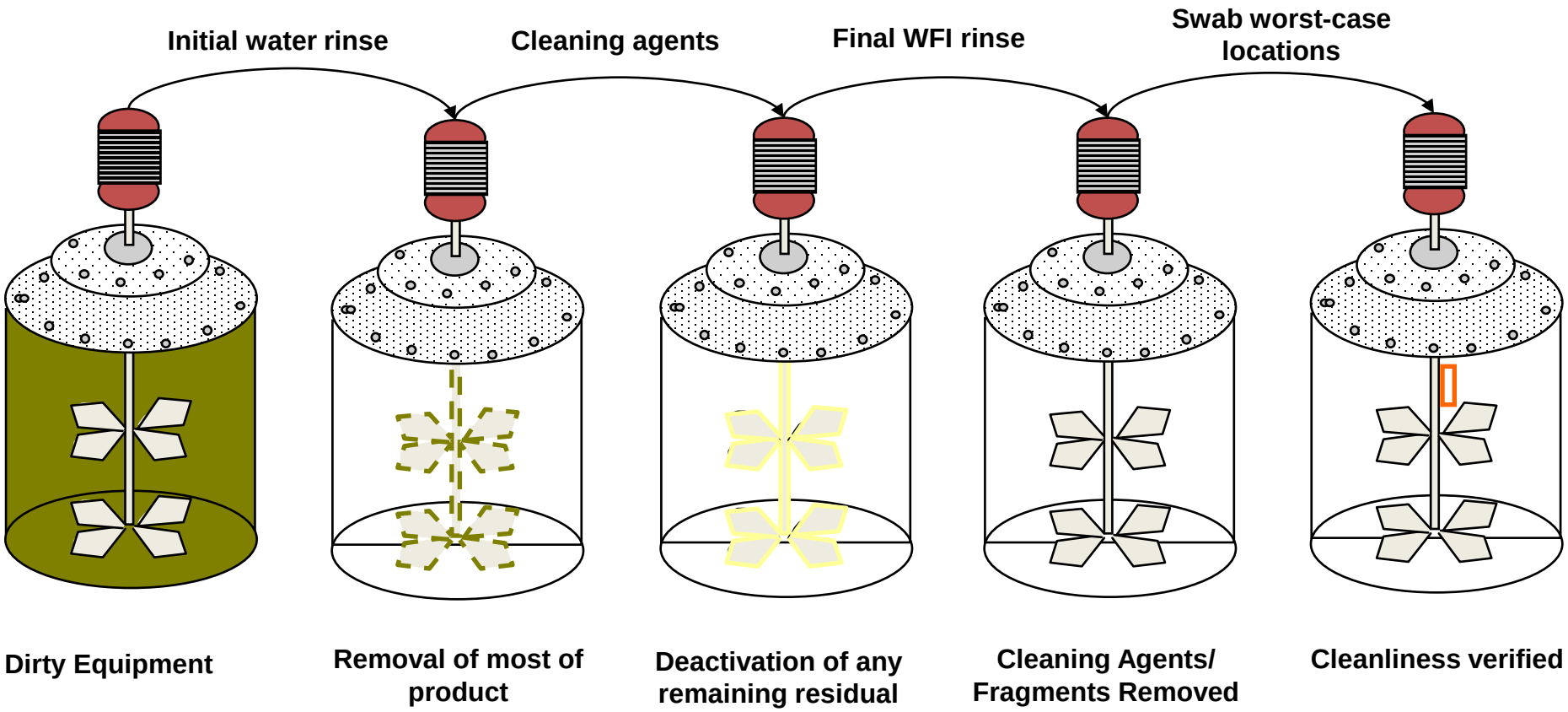
Cleaning Process: Rinse, Clean, Rinse, Verify



- Studies show product is easily rinsed away by water.
- Studies show active product is not present after exposure to cleaning agents.
- Studies show exposed/degraded product is easily rinsed away by water.

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Setting Cleaning Acceptance Limits

- Once product inactivation has been demonstrated from the cleaning process, each of the following approaches to setting cleaning verification limits are scientifically appropriate to ensure product and patient safety is maintained.
  - Cleaning Process Capability
  - Safety Factor Limit
  - Toxicology Threshold Limit
  - Performance Control Limit
- As each facility has unique characteristics and products manufactured, variables to consider at each facility are also unique.
- These approaches are not intended to be inclusive of all acceptable methods to determine cleaning limits.

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Cleaning Process Capability Limit

- The TOC limit of the rinse solution represents possible TOC contribution that could be left on production surfaces.
- The surface TOC limit is where the residual TOC could have been from the WFI rinse solution alone.
- The surface TOC limit may also be stated as the maximum amount of TOC on surfaces that would not result in a subsequent process solution that would be ≥ 500 ppb TOC as discussed on the next slide.

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Cleaning Process Capability Limit

- Equipment can not be cleaner than the last solution to contact their surfaces.
- The last solution of the cleaning process is Purified Water (typically WFI).
- Purified Water contains an allowable and known/monitored amount of organic carbon (≤ 500 ppb TOC).
- Most process components contain organic carbon.
- TOC is a sensitive measurement capable of detecting active product, inactive/degraded product, product variants, cleaning agents, media and some buffers.

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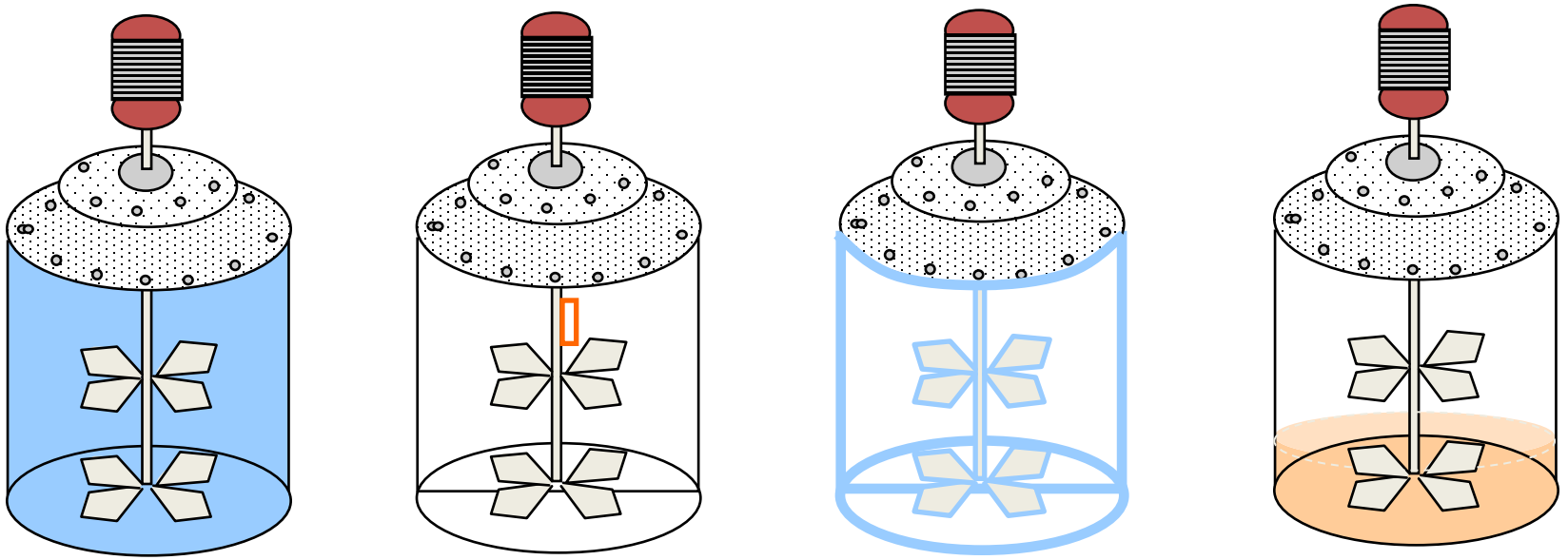
Cleaning Process Capability Limit Determination



Clean Equipment
Last rinse = WFI

Swab *worst-case locations* for residual carbon

Assume worst-case residual is uniformly distributed over *Total Surface Area*

Total Surface Area residual in the *Minimum Volume* of the subsequent process is *< Final WFI Rinse TOC Action Limit*

Swab Limit

$$\text{Swab Limit} = \frac{\text{Minimum Volume of Subsequent Process} \times \text{Final WFI TOC Action Limit} \times \text{Swab Area}}{\text{Total Surface Area}}$$

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Cleaning Process Capability: Example

Maximum Surface Residual TOC (ng TOC/cm^2) =

$$\frac{\text{Equipment Volume (mL) x Purified Water Alert Limit (ng TOC/mL)}}{\text{Equipment Surface Area } (cm^2)}$$

$$= \frac{25{,}842 \text{ mL} \times 250 \text{ ng TOC/mL}}{3{,}916.45 \text{ } cm^2}$$

$$= 1649.58 \text{ ng TOC/}cm^2$$

Residual TOC Swab Limit =

Maximum Surface Residual TOC (ng TOC/cm^2) x SSA (cm^2/swab) x 1 µg /1000 ng

$$= <1649.58 \text{ ng TOC/}cm^2 \times 25 \text{ } cm^2\text{/swab} \times 1 \text{ µg} / 1000 \text{ ng}$$

$$= <41.2 \text{ µg TOC/swab}$$

$$= <41 \text{ µg TOC/swab}$$

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Safety Factor Limit

- The cleaning verification limit determined by the Safety Factor Approach calculates the reduction of the inactivated product at the acceptance criteria level as an organic impurity in the Drug Substance.

- This organic impurity limit is 0.10% which is the equivalent to a Safety Factor of 1,000, which may be considered worst-case since TOC is not a product specific assay.

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Safety Factor Limit

- The Safety Factor is calculated as follows:

Safety Factor =

$$\text{Concentration (mg/mL)} \times \frac{1\ \text{ppm}}{1\ \mu\text{g/mL}} \times \frac{1000\ \mu\text{g}}{1\ \text{mg}} \times \frac{1}{\text{TOC Acceptance Limit (ppm)}} \times 50\%$$

Where,

- Concentration is the amount of active ingredient in the drug substance/drug product.
- 50% represents the approximate amount of carbon in protein. This may also be calculated specifically for the applicable protein product.

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Safety Factor Limit - Example 1

- In the example below a 2 ppm TOC limit for a product with a concentration of 100 mg/mL is considered. ingredient.

Safety Factor =

$$= 100 \text{ mg/mL} \times \frac{1 \text{ ppm}}{1 \text{ µg/mL}} \times \frac{1000 \text{ µg}}{1 \text{ mg}} \times \frac{1}{2 \text{ ppm}} \times 50\%$$

$$= 25{,}000$$

- A Safety Factor of 25,000 is calculated. Since this is greater than the 1,000 Safety Factor considered acceptable, the 2 ppm acceptance limit would be appropriate.

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Safety Factor Limit Example 2

- The equation can be rearranged to determine the appropriate limit for a desired Safety Factor.
- In this case, the targeted Safety Factor is 10,000, for a product with a concentration of 100 mg/mL and consisting of 53% carbon.

TOC Acceptance Criteria =

$$= \text{Concentration (mg/mL)} \times \frac{1\ \text{ppm}}{1\ \mu\text{g/mL}} \times \frac{1000\ \mu\text{g}}{1\ \text{mg}} \times \frac{1}{\text{Safety Factor}} \times 50\%$$

$$= 100\ \text{mg/mL} \times \frac{1\ \text{ppm}}{1\ \mu\text{g/mL}} \times \frac{1000\ \mu\text{g}}{1\ \text{mg}} \times \frac{1}{10{,}000} \times 53\%$$

= 5.3 ppm (round down to 5 ppm TOC)

- Once the initial acceptance limit has been set based on the safety factor, the surface area limit can be calculated.

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Safety Factor Surface Area Limit

- The residual TOC Swab Limit is calculated as follows:

Residual TOC Swab Limit

$$\leq \text{TOC ppm Limit} \times \frac{\text{Volume (mL)}}{\text{Surface Area (cm}^2\text{)}} \times \frac{1\ \mu\text{g/mL}}{\text{ppm}}$$

Where,

- The TOC acceptance limit in ppm.
- Volume is the amount of desorption solution used in mL.
- The surface area swabbed in cm^2

- Using a 5 ppm limit and a 30 mL desorption volume and a 25 cm^2 area swabbed, the swab limit would be ppm TOC.

Residual TOC Swab Limit

$$\leq 5 \text{ ppm TOC} \times \frac{30\ \text{mL}}{25\ \text{cm}^2} \times \frac{1\ \mu\text{g/mL}}{\text{ppm}}$$

$$\leq 150\ \mu g/25\ cm^2 \text{ (swab)}$$

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Toxicology Threshold Limit

- If it can be demonstrated that the biological products becomes degraded and inactivated, application of a toxicological threshold of concern (TTC) may be applied in order to mitigate the risk of process residuals affecting the next biopharmaceutical produced.
- Once an appropriate TTC has been determined based on structural class of process residuals, a calculation such as the one below can be applied.

$$\textbf{Acceptable Residual Limit (ARL) (µg/cm}^2\textbf{)} = \frac{\text{TTC (µg/day) x MBS (µg)}}{\text{MDD (µg/day) x SA (cm}^2\text{)}}$$

Where,

- ARL = Acceptable Residual Limit = µg/day
- TTC = Toxicological Threshold of Concern = µg/day
- MBS = Minimum Batch Size for Subsequently Manufactured Product = µg
- MDD = Maximum Daily Dose for Subsequently Manufactured Product = µg/day
- SA = Surface Area (SSA) = cm^2

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Toxicology Threshold Limit Example

- Degraded biopharmaceutical product fragments may be considered to be Class I chemicals with a residual soil threshold of 100 µg /day.
- A 200L Final Product Vessel may have a surface area of 28,573cm^2; minimum batch size is 400 g; and a maximum daily dose is 50,000 µg/day.

Acceptable Residual Limit (ARL) (µg/cm^2)

$$= \frac{100\ \mu g/day \times 400{,}000{,}000\ \mu g}{50{,}000\ \mu g/day \times 28{,}573\ cm^2}$$

$$= 28\ \mu g/cm^2$$

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Toxicology Threshold Example

- To calculate the TOC limit of a swab sample using the ARL determined in the previous slide, the following equation would be used.

Residual TOC Swab Limit (µg TOC/swab)

= Acceptable Residual Limit (µg/cm^2) x SSA (cm^2/swab) x 50%

Where,

- SSA (cm^2): Swabbed Surface Area
- 50%: Represents the approximate amount of carbon in protein/protein fragments.

- Continuing with example where ARL is 28 µg/cm^2 and swabbed surface area is 25 cm^2 the following limit would be calculated.

Residual TOC Swab Limit (µg TOC/swab)

= 28 µg/cm^2 x 25 cm^2 x 50% TOC

= 350 µg TOC/swab

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Performance Control Limit

- Performance Control Limits may be considered once cleaning validation studies have been completed and routine cleaning consistently meets established acceptance limits.
- Performance Control Limit approach does not change the scientific rationale for acceptable cleaning verification limits.
  - The Performance Control Limit may be considered an Alert Limit.
- Performance Control Limit establishes a limit that may be more reflective of the performance of the cleaning process.
  - Enables detection of a change in the performance of the cleaning process
  - Enables proactive investigation into a potential cleaning process issue.

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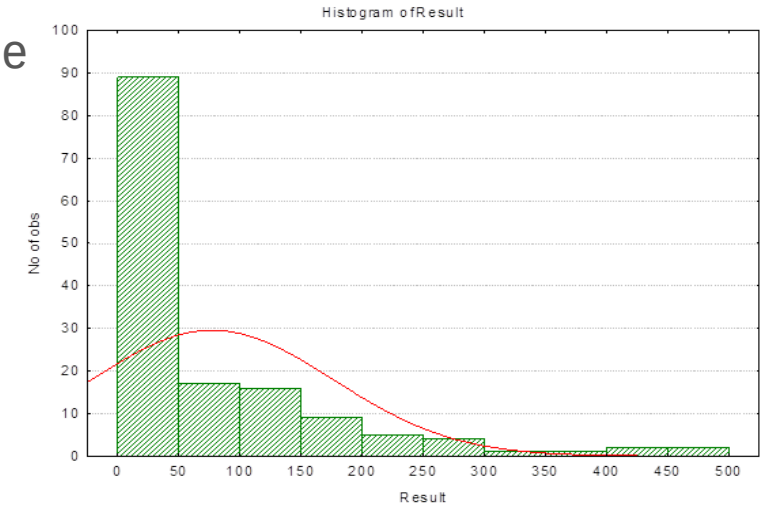
Performance Control Limit: Statistical Data Evaluation

- The Performance Control Limit approach discussed here is based on the TOC data collected during on-going cleaning studies.
- Many standard statistical methods are based on the assumption of normality and independence of the data population.
- The setting of a control limit at three standard deviations from the mean is an appropriate approach for setting a Control (or Performance) Limit assumes a normally distributed dataset.
  - A control limit at three standard deviations from the mean ensures a false out of tolerance (OOT) rate of 0.27%.
  - This 0.27% value is referred to as the *alpha rate*.
- The problem with the data typically from effective cleaning processes is that the data are not normally distributed.



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Performance Control Limit

- Since the data are not normally distributed, data transformation techniques such as Box-Cox method should be used to normalize data.
  - If the dataset contains an excessive number of zero values (or <LOQ), the “0” values should be removed and the alpha rate (e.g., 0.27% or 0.0027) adjusted accordingly prior to transforming the data with the Box-Cox method.
- The Box-Cox method computes the lambda value to optimize normality using the following equation.

$$Y_{transformed} = \frac{Y_{original}^{\wedge\ lambda} - 1}{lambda}$$

Where,

$Y_{original}$ is each TOX value, which must be greater than 0 (e.g., LOQ)

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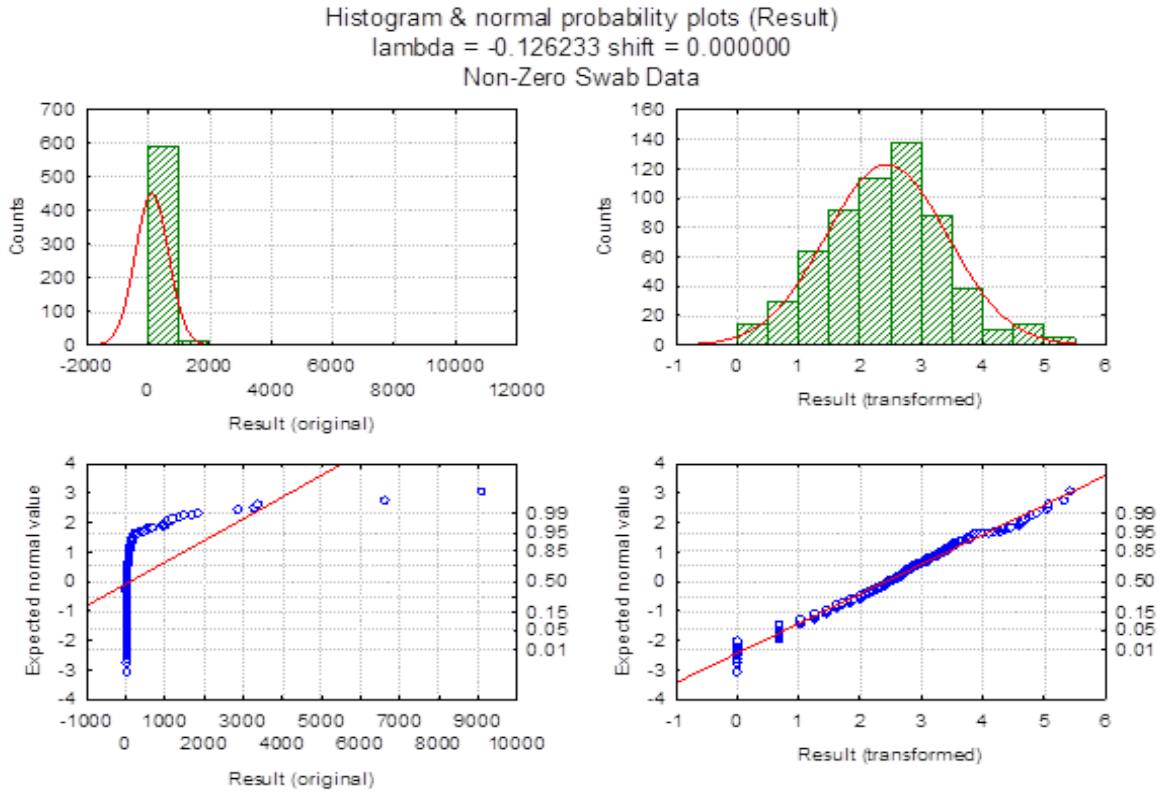
Performance Control Limit

- The top-left histogram describes the distribution of the original TOC dataset.
- The same non-normal phenomenon is displayed in the associated normal probability plot in the lower-left.
- The top-right histogram describes the same data after applying the Box-Cox transformation.
- In this case, the data are normally distributed as evidenced with the normal probability plot in the lower-right.



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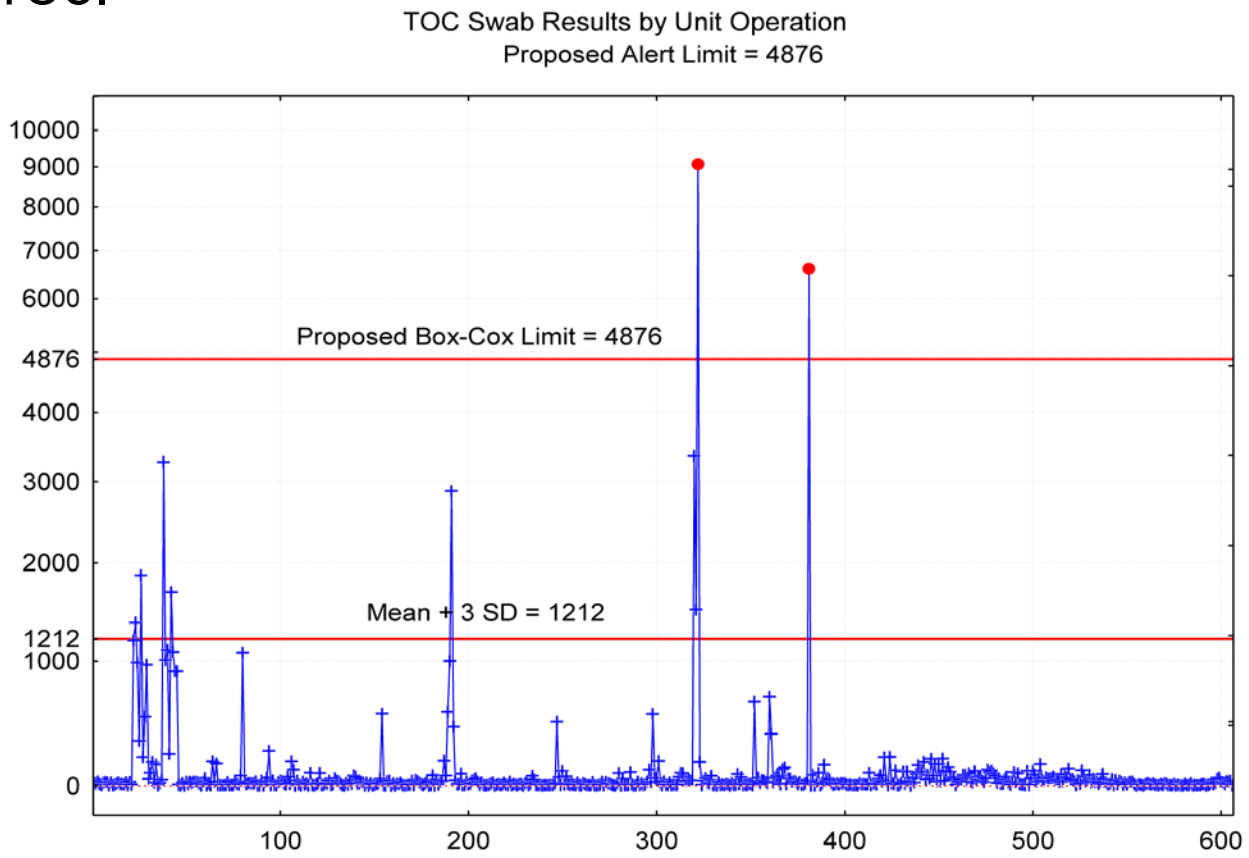
Performance Control Limit

- Performance Limits are then back-calculated to the original scale using the transformed dataset and the equation below.

$$Y_{original} = (Y_{transformed} * lambda + 1)^{(1/lambda)}$$

- In this example, the Performance Control Limit for this process would be 4876 ppb TOC.



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Summary

- Setting acceptable limits for process residue following equipment cleaning in multi-product biopharmaceutical facilities requires an understanding of each product's composition and the effects of the cleaning process on the API.
- The degrading and denaturing effects of chemical detergents should be studied for each product manufactured within the facility.
- Setting acceptance limits for product carryover based on TOC can be accomplished with the Cleaning Process Capability, Safety Factor, or Toxicology Threshold approaches.
- As on-going cleaning studies collect TOC data, these data can be evaluated with the Performance Control Limit approach to ensure control of the equipment cleaning process is maintained.

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