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

Adam Mott, Bill Henry, Edward Wyman, Kathleen Bellorado, Markus Blümel, Michael Parks, Ronan Hayes, Scott Runkle, and Wendy Luo

Abstract

For multi-product biopharmaceutical facilities, setting the acceptable level of process residues following equipment cleaning is an important regulatory, business, product quality, and patient safety consideration. Conventional approaches for setting an acceptance limit for process residues have been based on the assumption that the active pharmaceutical ingredient (API) (depending on the process soil, API refers to the active pharmaceutical ingredient in the drug product, drug substance, or drug substance intermediate) is chemically or functionally intact following the cleaning process. These approaches include Maximum Allowable Carryover (MAC) Health Based Exposure Limits and other "dose" or Permissible Daily Exposure (PDE)-based limits. The concept for cleaning acceptance limits based on intact product originated from the manufacturing of small molecule pharmaceuticals (1). In contrast to pharmaceutical small molecules, biopharmaceutical products are large molecules that are likely to degrade and become inactive when exposed to cleaning conditions. Therefore, an alternative approach to setting cleaning acceptance limits for biopharmaceutical products based on the actual process residues that could potentially be present on production equipment should be considered.

Part I described the methodology to assess and verify API inactivation during cleaning (2). In Part II, alternative approaches for setting acceptable levels of process residue will be described building upon the basis that API inactivation by the cleaning process has been demonstrated.

Introduction

When multiple products are manufactured using the same equipment, it is important to ensure that potential product or process residues from the previously manufactured batch are removed to an acceptable level to ensure the subsequently manufactured product will not be impacted. The acceptable level of carryover has often been based on the active, intact API. However, for biopharmaceutical products, the API typically degrades and becomes pharmacologically inactive during cleaning, and therefore the cleaning acceptance criteria do not need to be based on the concept of intact and active product. Rather, the cleaning acceptance limit should be based on potential process residues that have a greater carryover potential founded on phenomenological aspects of the cleaning process. The scope of this paper targets biopharmaceutical APIs; nonetheless, the underlying concepts may be useful in setting acceptance limits for other types of pharmaceutical products where inactivation during the cleaning process can be demonstrated.

This paper will include a review of product inactivation, information on product detection using total organic carbon (TOC), and alternative approaches for setting acceptance limits for equipment cleaning. The intention of this paper is to propose acceptable approaches for setting cleaning limits for biopharmaceutical process equipment that may be considered. However, it should not be considered prescriptive for what approach is most appropriate or should be used since every production facility, processes, and products manufactured are unique.

Product Inactivation

Biopharmaceuticals are large molecule drug products (e.g., monoclonal antibodies, therapeutic proteins, etc.) that are made in processes using living organisms rather than extracted from a native source or by synthesizing compounds. The equipment cleaning

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cycles are designed to expose product contact areas to cleaning detergents that include alkaline and acidic chemicals. Under these exposure conditions, the high pH in alkaline chemicals (typically pH >11) and low pH in acidic chemicals (typically pH <2) are efficient in hydrolyzing biological peptide bonds, rendering biopharmaceutical products biologically inactive by degradation and denaturation. If it is demonstrated that the product becomes pharmacologically inactive during cleaning, there is no longer a risk of active product carryover and, furthermore, a limited value in verification of the removal of active product from equipment surfaces.

It should be noted that an antibody-drug-conjugate (ADC) is considered a biopharmaceutical product, but it contains an extremely toxic small molecule that attaches to a protein through organic linkers. Due to the functional and toxicological behavior of an ADC product, specifically the toxic small molecules attached to the large molecule, PDE limits should be established for ADC products based on the toxicity of the conjugate; therefore, they are not in the scope of this paper.

Part I discussed experimental approaches and analytical methods that can be used to evaluate product inactivation by the cleaning detergents. This important first step characterizes the biological activity of the API and may also be used to gain a further understanding of remaining product fragments.

## Inactivated Product Rinsibility/Removal
The inactivated product and/or product fragments may be further evaluated to better understand the effect of the cleaning process and the potential for carryover. The final step in most, if not all, cleaning procedures is a final rinse of higher grade water quality, typically Water for Injection (WFI). The volume and flow rate of this rinse are designed to be sufficient to remove all residual cleaning agent(s) to a conductivity level approaching the WFI source water. The inactivated product that results from exposure to the cleaning conditions is likely to be more water-soluble than the intact protein due to its decreased size (3) and, therefore, should be readily rinsed from equipment surfaces in the last step of the cleaning process. The "rinsibility" or ease of removal of inactivated product/product fragments may be evaluated in a rinsibility study, where the inactivated product material is spiked onto representative coupons and exposed to a worst-case (e.g., no impingement, lower flow rate, etc.) water rinse in comparison to full scale cleaning cycles. If the worst-case rinse removes the product spike from the coupon, it demonstrates that the inactivated product fragments are not a carryover concern.

The Product Inactivation Study demonstrates the product is not active after exposure to cleaning conditions. The rinsibility study demonstrates that the potential product fragments created from exposure of the product to cleaning conditions are not a carryover risk. Therefore, setting acceptance limits for equipment cleanliness based upon intact product activity or potential product fragments would not be reflective of the actual residuals that are most likely to be present on equipment after cleaning based upon the phenomenological effects of the cleaning process.

Detection of Product or Process Residues
Most biopharmaceutical process components (e.g., API, host cell proteins, media, and

Figure 1: Determination of Carryover Limit based on Cleaning Process Capability.
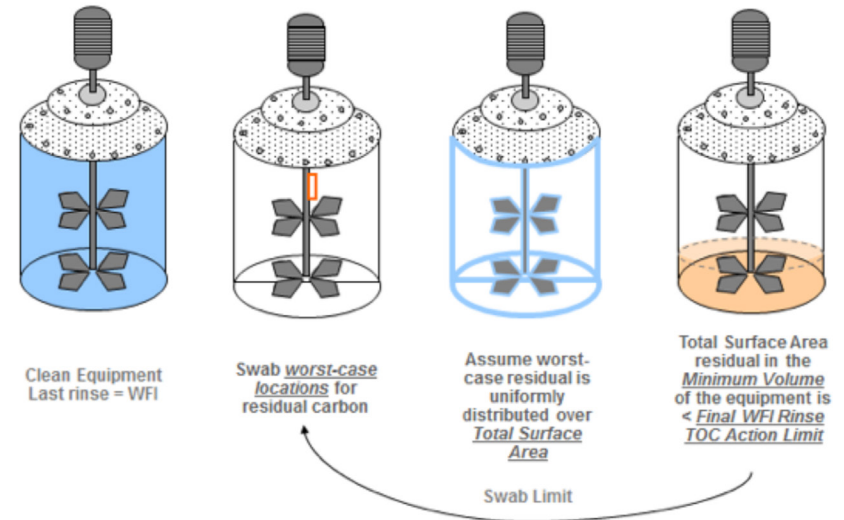
cleaning detergents) include organic carbon within their composition. The application of TOC as the post-cleaning detection method for product carryover is considered more stringent than a product-specific method as it would detect all process/cleaning residuals containing carbon, including potentially difficult to remove materials. The TOC analysis method is relatively sensitive (scale of ppb limits of detection and quantitation) that can be used for swab samples, rinse samples, and inline monitoring.

The approaches included in this paper for assessing equipment cleanliness are based on TOC, but they can be adapted to product specific methods if required.

Setting the Acceptance Limits
Four different approaches for setting cleaning acceptance limits will be discussed. Each limit setting approach (Cleaning Process Capability, Safety Factor, Toxicology Threshold, and Performance Control) ensures patient safety and no impact to subsequent product quality. The assumption inherent in each approach is that product inactivation from the cleaning process conditions has been demonstrated, which provides the scientific rationale and assurance of no active product carryover. Every facility has unique characteristics, products, and operational variables to consider. The following approaches are not intended to be inclusive of all acceptable approaches to determine cleaning limits. The following approaches may be considered as an alternative to the MAC approach, which may have limited applicability for biopharmaceutical products.

Cleaning Process Capability Approach
The cleaning process capability approach sets the acceptance limits for equipment cleaning based on demonstration that all carbon containing process materials have been removed to the level that the cleaning process is capable. The basis for the cleaning process

capability limit is that equipment surfaces cannot be cleaner than the potential residual contribution from the last solution of the cleaning process to contact equipment surfaces. If TOC is used as the most suitable measure to demonstrate removal of process material, the limit of process capability of the cleaning process to measure cleanliness would be based on the potential TOC contribution of the final WFI rinse.

TOC results from surfaces that are below the cleaning process capability limit that cannot be differentiated from TOC intrinsic to the final WFI rinse or from potentially low levels of residual cleaning agent or process material. TOC results that are above the cleaning process capability limit would be as a result of residual cleaning agent or process material and not from the final water rinse. It should be noted that this is a conservative approach to setting limits for equipment cleaning verification and calculated limits are relatively low.

To calculate the TOC surface limit, the following variables are required: equipment surface area, smallest volume that the equipment could process (e.g., working volume), final rinse (WFI) TOC limit (source of potential TOC contribution), and swab surface area. It should be noted that the surface area and volume are specific to the equipment to be cleaned and not to the entire production train. When the MAC approach is used, there is a concern of a cumulative carryover of active product; which would not be removed through common purification steps of subsequent product production, which is the reason total surface area of all equipment in the production train is used (1). However, active product is not a concern once the product inactivation and rinsibility are completed because active, intact product would not be present after cleaning; product fragments, just as other non-product proteins (e.g., HCPs), would be removed during purification, and product fragments created after cleaning are "free rinsing" and easily removed from equipment surfaces. The cleaning process capability limit may be determined for each piece of equipment, or the "worst-case" piece of equipment in each production suite may be used to set a limit to be used for all equipment in the suite. The "worst-case" equipment will be the unit with the largest surface area to volume ratio.

The following equation is used to calculate the limit of TOC contribution on production equipment surfaces that could be from the final WFI rinse. This limit is determined by calculating the amount of TOC on the equipment surface that would not result in TOC concentration in minimum working volume allowed in the equipment that would be greater than the acceptable TOC limit of WFI (the final rinse source water):

$$\text{Maximum Surface Residual TOC (ng TOC/cm}^2\text{)} = \frac{\text{Minimum Equipment Volume (mL) x WFI TOC limit (ng TOC/mL)}}{\text{Equipment Surface Area (cm}^2\text{)}}$$

To convert the Maximum Surface Residual TOC limit into the limit for a swab sample, the following equation is applied:

Residual TOC Swab Limit = Maximum Surface Residual TOC (ng TOC/cm2) x SSA (cm2/swab) x 1 μg /1000 ng

Where:

Maximum Surface Residual TOC (ng TOC/cm2): The maximum amount of residual material that is allowed per square centimeter of production equipment.

SSA (cm2): Swabbed Surface Area, the area which his swabbed for sample analysis. For exam-

ple, 5 cm x 5 cm (2 inches x 2 inches) equals 25 cm2.

Unit Conversion (ng to μg): converts units of ng to μg where 1 μg equals 1000 ng.

Figure 1 illustrates the approach described above to calculate the residual TOC limit as measured by a swab sample.

An actual example of cleaning limit calculations using the approach describe above is presented below (Note: worst-case [tightest] limits will be calculated where the production equipment surface area relative to working volume is large as is typically observed in smaller equipment).

Example: 200 L Reactor (150 L minimum working volume):

$$\text{Maximum Surface Residual TOC (ng TOC/cm2)} = \frac{150{,}000 \text{ mL x } 500 \text{ ng TOC/mL}}{28{,}573 \text{ cm}^2}$$

= 2625 ng TOC/cm2

Residual TOC Swab Limit = 2625 ng TOC/cm2 x 25 cm2/swab x 1 μg /1000 ng

= ≤66 μg TOC/swab

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Safety Factor Approach

This approach is to determine the safety factor involved; that is to calculate 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% (4), which is the equivalent to a Safety Factor of 1,000.

$$\text{Safety Factor} = \text{Concentration (mg/mL) x } \frac{1 \text{ ppm}}{1 \text{ μg/mL}} \text{ x } \frac{1000 \text{ μg}}{1 \text{ mg}} \text{ x } \frac{1}{\text{TOC Acceptance Limit (ppm)}} \text{ x } 50\%$$

Concentration is the amount of active ingredient in the drug substance/drug product.

Fifty-percent represents the approximate amount of carbon in protein (5). This may also be calculated based on the molecular makeup of the API if available.

The initial cleaning acceptance limits are typically in the range of 1-10 ppm TOC for swab and rinse samples. An example calculation is shown below; a 2 ppm acceptance limit with a product concentration of 100 mg/mL yields a Safety Factor of 25,000. Since this is greater than a 1,000 Safety Factor, the 2 ppm acceptance limit has been appropriately set to demonstrate adequate removal of residual active ingredient.

$$\text{Safety Factor} = \text{Concentration (mg/mL) x } \frac{1 \text{ ppm}}{1 \text{ μg/mL}} \text{ x } \frac{1000 \text{ μg}}{1 \text{ mg}} \text{ x } \frac{1}{\text{TOC Acceptance Limit (ppm)}} \text{ x } 50\%$$

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

$$= 25{,}000$$

Another example calculation is shown below, wherein a targeted Safety Factor of 10,000 (i.e., a 4-log reduction) is used to set the acceptance limit for a product with a concentration of 100 mg/mL and a molecular makeup of 53% carbon:

$$\text{TOC Acceptance Limit (ppm)} = 100 \text{ mg/mL} \times \frac{1 \text{ ppm}}{1 \text{ µg/mL}} \times \frac{1000 \text{ µg}}{1 \text{ mg}} \times \frac{1}{10{,}000} \times 53\%$$

$$= 5.3 \text{ ppm (or round down to 5 ppm)}$$

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

$$\text{Residual TOC Swab Limit} \leq 5 \text{ ppm} \times \frac{30 \text{ mL}}{25 \text{ cm}^2} \times \frac{1 \text{µg/mL}}{\text{ppm}}$$

$$\leq 150 \text{ µg TOC/25 cm}^2 \text{ (swab)}$$

Where:

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

Continuing from the example above, the calculation is shown below with a 5 ppm acceptance limit, 25 cm^2 swab surface area, and 30 mL desorption solution:

$$\text{Residual TOC Swab Limit} \leq 5 \text{ ppm} \times \frac{30 \text{ mL}}{25 \text{ cm}^2} \times \frac{1 \text{µg/mL}}{\text{ppm}}$$

$$\leq 150 \text{ µg TOC/25 cm}^2 \text{ (swab)}$$

Note: The Residual TOC Swab Limit is adjusted, as necessary, based on surface area sampled where it is not practical or possible to swab 25 cm^2.

Toxicology Threshold Approach

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 residues (degraded and inactivated fragments) affecting the next biopharmaceutical produced (6-9). Once an appropriate TTC has been determined based on structural class of process residuals, a calculation such as the one below can be applied.

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

Where:

ARL = Acceptable Residual Limit = µg/cm2
TTC = Toxilogical 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

For example, degraded biopharmaceutical product fragments may be considered to be Class I chemicals with a residual soil threshold of 100 µg/day. A 200 L Final Product Vessel may have a surface area of 28,573 cm2: minimum batch size is 400 g, and maximum daily dose is 50,000 µg/day.

$$\text{ARL (µg/cm}^2) = \frac{100 \text{ µg/day} \times 400{,}000{,}000 \text{ µg}}{50{,}000 \text{ µg/day} \times 28{,}573 \text{ cm}^2}$$

$$= 28 \text{ µg/cm}^2$$

To calculate the TOC limit of a swab sample using the ARL determined above, the following equation would be used:

Residual TOC Swab Limit = Acceptable Residual Limit (µg/cm2) x SSA (cm^2/swab) x 50%

Where:

Acceptable Residual Limit (µg TOC/cm^2): The maximum amount of residual material that is allowed per square centimeter of production equipment.
SSA (cm^2): Swabbed Surface Area, the area which his swabbed for sample analysis. For example, 5 cm x 5 cm (2 inches x 2 inches) equals 25 cm^2.
50%: Represents the approximate amount of carbon in protein/protein fragments.

Continuing with example above to calculate the ARL, the following is an example limit for Residual TOC on a swab from production equipment:

Residual TOC Swab Limit (µg TOC/swab) = 28 µg/cm^2 x 25 cm^2 x 50% TOC
= 350 µg TOC/swab

Performance Control Limit Approach

Performance Control Limits may be considered once the cleaning validation studies have been completed and routine cleaning consistently demonstrates the equipment cleaning process removes process residue below the acceptance limits, especially if the data is considerably lower than the acceptance limit. The Performance Control Limit approach does not change the level of carryover that has previously been determined to be acceptable, but it will establish a limit that is more reflective of the performance of the cleaning process. The Performance Control Limit, sometimes referred to as an Alert Limit, enables detection of a change in the performance of the cleaning process and allows for a proactive investigation into a potential cleaning process issue.

The Performance Control Limit approach discussed below is based on the TOC data collected during on-going cleaning studies. The evaluation of data should be statistically based and strike an appropriate balance between sensitivity to data shifts and excessive false signals. 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

Figure 2: Example Histogram of Cleaning Verification Data.
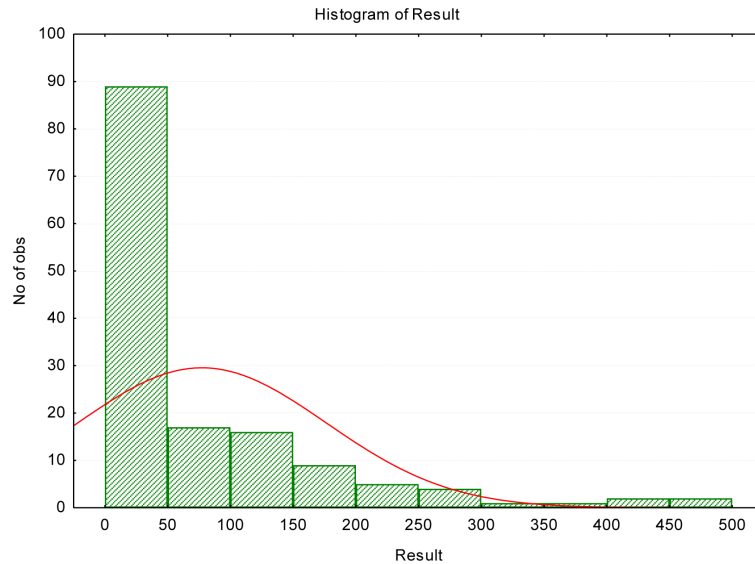
approach for setting a control (or performance) limit, but it assumes a normally distributed dataset. A control limit at three standard deviations from the mean ensures a false out-of-tolerance (OOT) rate of 0.27%. This 0.27% value is referred to as the alpha rate. The problem with the data typically generated from effective cleaning processes is that the data are not normally distributed, as shown in the following example in Figure 2.

Because the data are not normally distributed, data transformation techniques such as Box-Cox, mean scores, reciprocal, negative binomial, etc. are to be used to normalize data to apply appropriate statistical tools to establish an appropriate Performance limit (10). The Box-Cox method is a log transformation that optimizes the normality of the data set and was used to transform the dataset presented above.

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 TOC value, which must be > 0.

If the dataset contains an excessive number of zero values, 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. In the example dataset, 428 of 1034 results are "0." The alpha rate (0.027) is therefore adjusted according to the number of "0" results relative to the total number of results as described in the equation below:

$$\frac{0.0027}{1 - (428/1034)} = 0.0046$$

After the review and adjustment for the "0" data results, the Box-Cox transformation is performed using the adjusted alpha rate (in the example dataset, the adjusted alpha rate is 0.0046). Figures 3 and 4, below, depict the example TOC data that have been transformed using the Box-Cox method.

The top-left histogram describes the distribution of the original TOC dataset. This dataset is non-normal, being truncated at zero. 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

Figure 3: Box-Cox Transformation.
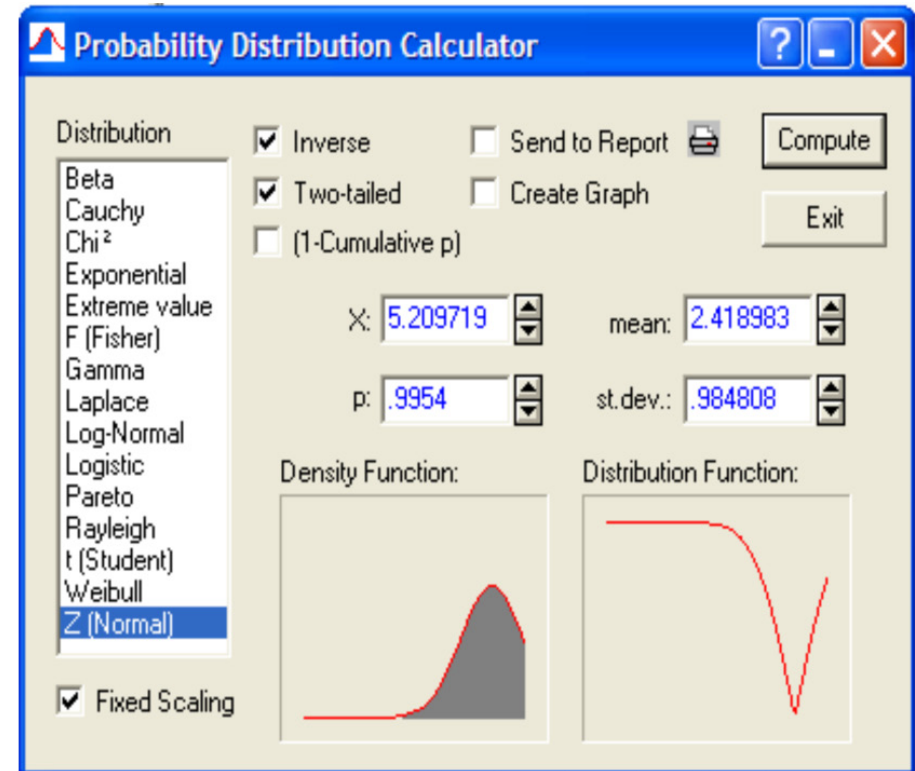
	Data statistics (Swab Data) Include condition: v7 <> 0			
Transformed variable(s)	Lambda	Shift	Mean	Standard deviation
Result	-0.126233	0.00	2.418983	0.984808

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Figure 4: Effect of Using the Box-Cox Transformation.

Histogram & normal probability plots (Result)
lambda = -0.126233 shift = 0.000000
Non-Zero Swab Data
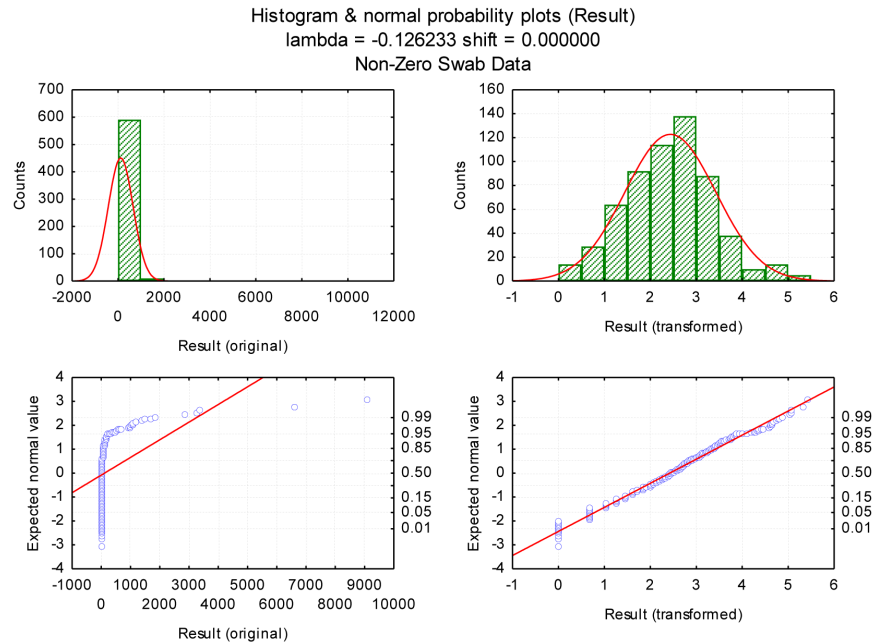
Figure 5: Performance Control Limit from Example Dataset.

TOC Swab Results by Unit Operation
Proposed Alert Limit = 4876
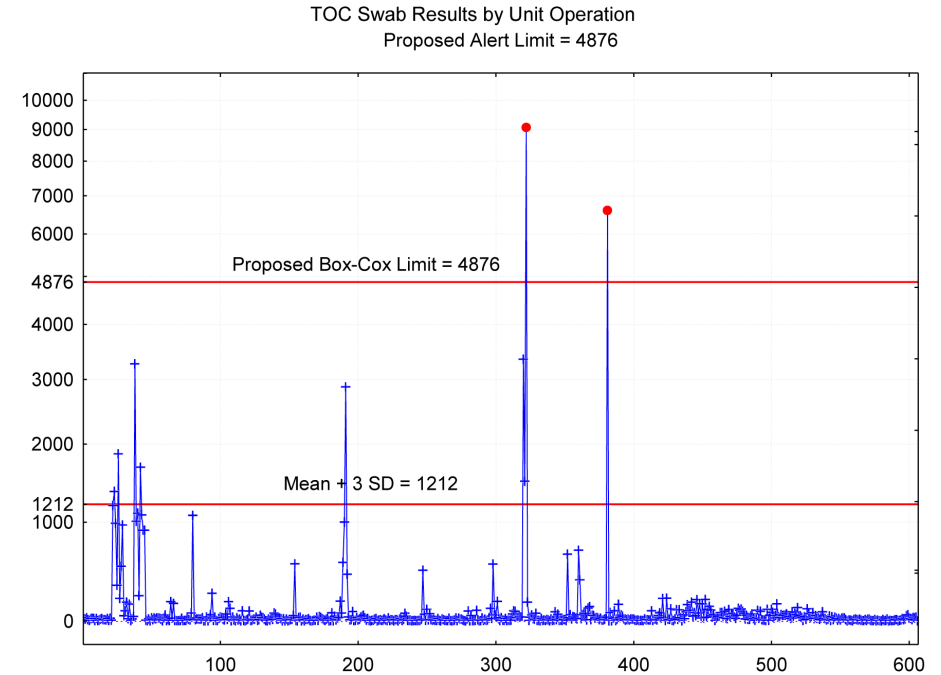
are normally distributed as evidenced with the normal probability plot in the lower-right.

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

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

The Box-Cox transformed Performance Limit from the example data is 4876 ppb TOC and is shown in Figure 5.

Finally, as further cleaning studies are conducted, additional TOC data will be collected. An appropriate review of the overall dataset should be conducted, and the performance limits adjusted if performance changes for reasons that should be well understood.

Conclusion

Setting acceptable limits for process residue following equipment cleaning in multiproduct 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.

Acronyms and Definitions

Action Limit — An empirical limit that the cleaning process cannot exceed without potential impact to product quality or patient safety.

PDE — Permissible Daily Exposure (also called ADE, Acceptable Daily Exposure) which represents a dose of a drug to which a human may be exposed per day or per dose (for biologics) without any anticipated pharmacologic or toxicological effects, so in the event of potential carry-over of one API to another, there would be no risk to the patient.

Alert Limit — An empirical limit, statistically established from study data, which is used to monitor the quality of the cleaning process.

API — Active Pharmaceutical Ingredient

Degrade — To cause the cleavage and hydrolysis of chemical bonds within peptides and amino acid strings, such that the biological activity is diminished or eliminated.

Denature — To cause the tertiary structure of a biological product to unfold, as with heat, alkali, or acid, so that some of its original properties, especially its biological activity, are diminished or eliminated.

MAC — Maximum Allowable Carryover

Peptides — A chemical compound containing two or more amino acids (amino acid polymers) that are coupled by a peptide bond. Peptides are often classified according to the number of amino acid residues. Oligopeptides have 10 or fewer amino acids. Molecules consisting from 10 to 50 amino acids are called peptides. The term protein describes molecules with more than 50 amino acids.

TOC — Total Organic Carbon

TTC — Toxicological Threshold of Concern

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