Limitations:

— longer sample turn-around time
— relatively expensive

Special Validation Concerns:

— recovery, specificity, detection limit

7.5.10 Thin Layer Chromatography (TLC): Thin Layer Chromatography is a sensitive technique which uses very simple equipment and principles. TLC has been used for the analysis of residues of actives and cleaning agents.
Advantages:

— highly specific
— moderate-to-high sensitivity
— fairly inexpensive

Limitations:

— moderate-to-high sensitivity
— visual endpoint detection is not quantitative
— automatic readers are semi-quantitative
— lengthy process to perform sample preparation

Special Validation Considerations:

— recovery, specificity, detection limit

7.5.11 Capillary Zone Electrophoresis (CZE): Also known as capillary electrophoresis (CE), this technique has been applied mostly to the biotechnology industry and is effective for evaluating residues of proteins, amino acids, and certain cleaning agents. The technique is highly specific and quite sensitive. It's disadvantages are that only a single sample can be run at a time, thus a series of samples would require lengthy time periods and that most companies do not have this equipment in their labs requiring additional expenditures. CEZ works best for large bipolar molecules.
Advantages:

— highly specific
— highly quantitative
— sensitive

Limitations:

— expensive

Special Validation Concerns:

— recovery
— specificity
— detection limits

7.5.12 Fourier Transform Infrared (FTIR): FTIR involves the application of advanced mathematical concepts to multiple infrared scans of a sample. The technique is both qualitative and quantitative. FTIR is suitable for residues of actives as well as cleaning agents and has good sensitivity. It's major drawback is that the equipment is quite expensive and a library of spectra must be developed for comparison purposes.
Advantages:

— specific
— qualitative
— can be quantitative

Limitations:

— expensive
— requires extensive library of spectra

7.5.13 Enzyme Linked Immunosorbant Assay (ELISA): An ELISA assay is an antigen-antibody type reaction involving the use of specific chemicals developed especially for the residue involved. It is very specific. ELISA assays typically are used for analysis of protein residues resulting from manufacturing of biotechnology type products. While these assays are very sensitive, they are also costly to develop and validate. It should also be noted that an ELISA method developed for a protein will not detect the same protein once it is denatured. Many proteins are easily denatured by the cleaning process, and the method would fail to detect significant amounts of protein in the denatured form.
Advantages:

— ultimately specific
— very sensitive

Limitations:

— very expensive
— difficult to develop and validate
— labor intensive
— may not provide accurate results if proteins are denatured

7.5.14 Atomic Absorption/Ion Chromatography (AA/IC): AA/IC is another fairly complex technique which has been applied, although rarely, to analysis of cleaning samples. It has the advantage of being specific and very sensitive, but suffers the disadvantage of involving expensive equipment. AA/IC has a fairly narrow potential area of application in cleaning analysis; however, for the company manufacturing potent ionic or inorganic products it is a potentially useful application. For a company already having this equipment, it could also be readily applied to the analysis of residues of cleaning agents.
Advantages:

— very specific
— sensitive

Limitations:

— generally only useful for metals, salts and metal complexes
— expensive

7.5.15 Ultraviolet (UV) Spectrophotometry: Although UV has been applied to analysis of many products and raw materials, it often does not have the required sensitivity for pharmaceutical products. It is useful for those cases where the residue limits are high enough that an analytical technique of moderate sensitivity will suffice.
Advantages:

— moderately to highly specific
— high sensitivity
— may be used as a screening method or for confirmatory ID

Limitations:

— requires more technical expertise and more expensive equipment than some of the other methods.

Special Validation Concerns:

— recovery, specificity, linearity, detection limit, precision

7.6 Pass-Fail Testing Methods

Pass-fail type testing, also known as "go-no go" testing is used in many analytical situations and has been widely used over the years for detection of impurities in raw materials and products. In actual application to testing, the analyst is looking for a physical change such as a color change or development of a cloudy solution. The difficulty with the development of such tests for cleaning testing is in knowing the actual quantitative level of the transition, i.e., the break point between success and failure. Often the transition point is a range. If this is the case, the range must be known and it's relationship to the limits must be established in the validation process. Another difficulty is in not knowing how close to the transition point your actual sample may be. The actual result, although passing, could have been very close to failure and with normal plus/minus variation it could fail on the next sample.

7.7 Analytical Methods Validation

The analytical methods used for testing cleaning samples must be validated for accuracy, precision, linearity, ruggedness, robustness, sensitivity and recovery. The reader is encouraged to refer to appropriate sources on analytical method validation (e.g., ICH guidelines, etc.).

7.8 Microbial and Endotoxin Detection and Testing

Testing methods used to isolate, quantitate, and speciate bacteria and associated endotoxins for cleaning studies are the same as those used routinely in the microbiology laboratory. Sterile swabs and samples from rinse solutions can be used as vehicles to generate samples for microbial testing. Alert levels and/or action levels should be established. In addition, cleaning agents should be checked to identify their level of bioburden, if any. Refer to the literature for more detail on endotoxin detection methods including gel clot, chromogenic and turbidometric LAL methods or rabbit pyrogen.

Isolated microorganisms should be identified to an appropriate level to determine whether they are of particular concern (pathogens, gram negative, etc.). Special cleaning and depyrogenation methods may be necessary depending on the nature of the bioburden.

8. Limits Determination

The determination of cleaning limits and acceptance criteria is a crucial element of a good cleaning validation program. A limit is an actual numerical value and is one of the requirements of the acceptance criteria of a cleaning validation protocol. Limits and acceptance criteria should be:

— practical
— verifiable
— achievable
— scientifically sound

The limits should be practical in the sense that the limit chosen should be appropriate for the actual cleaning situation to be validated. Also, the limits must be verifiable by some means of detection. In addition, the limits must be achievable by the analytical methodology available for the specific product. Most importantly, the company should develop a scientifically sound rationale for the limits chosen.

8.1 The Scientific Rationale for Cleaning

It is very important that cleaning limits not be selected arbitrarily but, rather, that there be a logical and scientific basis for the numerical limit selected. The scientific rationale is normally included in the limits section of the protocol for the cleaning validation. The scientific rationale which supports the actual limit should be logical, comprehensive, and easily understood.

8.2 Contamination of the Next Product

Product residue remaining on equipment contaminates a subsequently manufactured product. Thus, it is important to have information about the potential contaminant as well as the product which could become contaminated.

8.3 Considerations for Developing Limits

There are many areas that should be considered prior to establishing cleaning validation limits (see Table 1). Once these areas have been considered, one can establish a risk assessment factor appropriate for use in determining limits.

8.4 Limits Based on Medical or Pharmacological Potency of the Product

One basis of establishing limits is a mathematical calculation which allows a certain fraction of the therapeutic dose to carry over into each dosage unit of the following product.

Possible approaches to safety factor determination are discussed in Sections 8.5 and 8.6, below. The fraction of dose reduction is a measure of the risk involved and should be assessed by the company depending on the actual manufacturing situation.

8.5 The Basis for Quantitative Limits

Actual numerical limits are usually based on one of the following:

— the medical or pharmacological potency of the product

TABLE III
Safety Factor

Approach	Approach Typically Applicable To
1/10th to 1/100th of a normal daily dose	topical products
1/100th to 1/1000th of a normal daily dose	oral products
1/1000th to 1/10,000th of a normal daily dose	injections, ophthalmic products
1/10,000th to 1/100,000th of a normal daily dose	research, investigational products

— the toxicity of the residue
— the analytical limit of detection

Different manufacturing and cleaning situations may require different approaches and each approach will be discussed individually. It is also important to factor the following product to be manufactured in the same equipment into the limit calculation. Factors such as the batch size of the following product, the route of administration, and the largest daily dose of subsequent product which might be administered are important in the calculation.

All of these factors mentioned previously are usually summarized in an equation which may take the following general form:

$$MAC = \frac{TD \times BS \times SF}{LDD}$$

where:

MAC = the maximum allowable carryover
TD = a single therapeutic dose
BS = the batch size of the next product to be manufactured in the same equipment
SF = the safety factor
LDD = the largest daily dose of the next product to be manufactured in the same equipment

This mathematical equation shows that the batch size of the next product as well as the largest daily dose of the next product are required for the calculation. If the next product to be manufactured is not known, then the smallest batch size of any product manufactured previously in the equipment can be used. When a new worst case is to be manufactured in the equipment, then this would be evaluated by the change control process and new limits could be imposed on the cleaning for the equipment.

As an example of the calculation of an overall limit, consider the case of a product A having a single therapeutic dose of 100 mg. Assume that the product is given by the oral route of administration. Let's also assume that the next product to be manufactured in the same equipment has a batch size of 10 Kg, and a largest daily dose of 800 mg. Use a safety factor (SF) of 1/1000. In this case, the calculation would be:

$$MAC = \frac{TD \times BS \times SF}{LDD}$$

$$= \frac{100\ mg \times 10{,}000{,}000\ mg \times 1/1000}{800\ mg} = 1250\ mg$$

This is the total limit for all residues on all equipment used to manufacture the product.

8.6 Limits Based on the Toxicity of the Residue

Using the therapeutic dose as the basis of limits calculations is appropriate for situations where the material is an active ingredient and therapeutic dosage levels are known. There are other situations, however, where the material is not medically used and there are no known therapeutic dose data available. Examples are precursors and intermediates used in chemical synthesis (i.e., manufacture of active pharmaceutical ingredient (APIs)), and cleaning agents. These materials have no quantitative therapeutic dosage levels. Yet, they may have a medical or toxic effect in the body. In these cases, it is necessary to base the limits calculations on the toxicity of the material.

This can be done by using the method described above for pharmacological activity with substitution of the toxic dose. Alternatively, where toxicity is expressed as LD_{50}, the following methodology can be used.

$$NOEL = LD_{50} \times \text{emperical factor}$$

$$ADI = NOEL \times AAW \times SF$$

where:

NOEL = no observed effect level
LD_{50} = lethal dose for 50% of animal population in study
empirical factor = derived from animal model developed by Layton, et. al.
ADI = acceptable daily intake
AAW = average adult weight
SF = a safety factor

This equation can be applied to a pharmaceutical cleaning validation study for the purpose of calculating a limit. The result would be as follows:

$$MAC = \frac{ADI \times B}{R}$$

where:

MAC = maximum allowable carryover
B = smallest batch size of any subsequent product
R = largest daily dose of any product made in the same equipment

The only changes made to the equation are those representing the batch size and the largest daily dose of the subsequent product. The basic value of this approach is, as indicated previously, that a limit can be calculated for cleaning validation purposes based solely on the toxicity of the material. It is important that the LD_{50} be from the same route of administration as the product for which the limit is calculated. For example, if the product is an oral product, then the LD_{50} should be from the oral route of administration. Likewise, if the product is an intravenous injection, then the LD_{50} should also be by the intravenous route of administration.

8.7 Limits Based on the Analytical Limitations

These approaches to establishing limits are based on the cleaning limit being the limit of analytical detectability. In some cases, where the danger of contamination and the consequences are of a critical nature, this may be a viable approach. However, in the great majority of cleaning situations, this extreme degree of cleaning is neither necessary nor justified. When carried to extremes the cost of cleaning can easily surpass the cost of the product. The key to selecting this approach is the nature of the product (i.e., its

toxicity and stability) and the nature and use of other products made in the same equipment. A further problem with using these approaches is that constant advances in analytical technology means that more sensitive analytical procedures are constantly being developed.

8.8 The Meaning of "None Detected"

The use of the term "none detected" is very common in the laboratory. It is important to correctly interpret the meaning of this term, especially where cleaning samples are involved. "None detected" does not equal zero, i.e., it does not mean there was no residue present. All that can be stated about such results is that the level of residue was below the detection capability of the analytical technique or instrument, often referred to as the sensitivity of the method. The sensitivity parameter is one of the most important parameters of an analytical method and sensitivity must be validated as a part of the analytical methods validation.

The sensitivity of an analytical method is often expressed as either the limit of detection or the limit of quantitation. The sensitivity of the analytical method may be used to establish the actual cleaning limits. In cases where the cleaning validation study results in "none detected" the sensitivity of the analytical method could be used to calculate the maximum amount of residue which could be present.

8.9 Dividing a Limit Among Various Pieces of Equipment

In order to evaluate a processing operation composed of several unit operations, it is important to consider the accumulated residue from each piece of process equipment. This is the sum of all residues which were present on the various pieces of manufacturing and packaging equipment. The total residue is equal to the sum of all residues in the manufacturing "train" as represented in the following diagram:

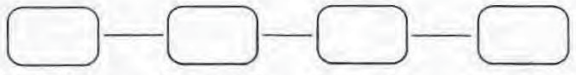

Figure 1

Equipment A could be, for example, a powder blender; equipment B could be a granulator; equipment C could be a compressing machine; and equipment D might represent a packaging filler for a solid dosage form such as a tablet. For a liquid product, equipment A could be a mixing tank; equipment B could be a holding tank; equipment C could be transfer piping to the packaging department; and equipment D might be the liquid filling equipment.

In many manufacturing operations each piece of equipment is a discrete unit. Since the equipment pieces are usually separate stand-alone units, it is necessary to determine limits for each individual equipment piece.

An equipment train should be delineated to separate those portions in which the residue would be evenly distributed (e.g., blender, granulator) from those in which the residue could be transferred to an individual dosage unit (e.g., tablet press, encapsulating machine, tablet filler).

Prior to the dose forming step the allowable residue may be distributed across the equipment. The dose forming step (compression, filling) must use a different, tighter limit to restrict potential carryover to a single product dose.

9. Ongoing Verification of Cleaning

9.1 Verification of Cleaning

Verification of cleaning involves the performance of testing which confirms that the cleaning method is adequately removing substances to established levels. The CGMP regulations require inspection of each piece of equipment immediately before use to ensure its cleanliness. However, additional verification may be necessary depending on the complexity of the equipment.

9.2 Monitoring of Automatic and Manual Cleaning

For automatic cleaning methods, ongoing verification may not be required. If the automated system is designed, installed and validated appropriately and the process reproducibility is confirmed, no further verification should be necessary. For semi-automated processes, a determination must be made about the predicted reproducibility of the process over time.

Manual cleaning generally requires periodic verification. Verification should confirm the ongoing appropriateness of the training program as well as the operator's ability to perform the cleaning process.

One way in which corporations provide for the ability to perform ongoing or occasional verification is to correlate rinse results to other residual data. Once this data is collected, it is possible to confirm that the residual levels meet the predetermined requirements. It may be used as an adjunct to cleaning validation or in a clinical supply setting where consistency of cleaning may not have been established.

10. Change Control

All aspects of cleaning should fall under the auspices of a change control policy. Cleaning standard operating procedures, assay methods, equipment, detergents, product formulations, batching methods and the like should all be documented at the time of the validation effort. Changes to these items will require formal documentation and approval. Typically, corporate change control policies are in existence which will govern the review and approval of these changes.

If a firm chooses not to pursue the verification of cleaning on a periodic or occasional basis, changes performed under the change control program will require reconfirmation of the cleaning validation results, or verification. If the change is deemed to be fundamental to either the grouping philosophy on which the validation was founded, or to the cleaning method, the change may require revalidation.

Revalidation, in contrast to verification, may require that portions of the initial cleaning validation program be repeated. Revalidation may differ from verification only by

the amount or type of sampling that is performed. Typically the sampling and testing that are performed during revalidation are more stringent than that performed during routine or occasional monitoring. Revalidation of cleaning may incorporate aspects of both validation and verification, but in accordance with a firm's internal policies may be restricted to one or the other.

The careful planning and execution of the revalidation program will allow for compliance in the ongoing operations of the facility.

11. APPENDICES

11.1 Glossary of Terms

acceptable daily intake	an amount of a substance administered or consumed on a daily basis that will not produce a pharmacological or toxic response
analyte	substance for which an analysis is being performed
API	active pharmaceutical ingredient
automated cleaning	a cleaning procedure which relies on a sequence of programmed, reproducible steps (usually via mechanical and/or electronic devices)
batch production	a series of unit operations performed according to a single manufacturing order during the same cycle of manufacture to produce a specific quantity of a drug having uniform character and quality within specified limits
blank	analytical method control sample used to establish a baseline for the result, e.g., as in a titration where one or two drops of the titrant must be added to the blank to cause an indicator color change
bulk pharmaceutical	generally known as bulk pharmaceutical chemicals; also called primary pharmaceuticals or active pharmaceutical ingredients
campaign	processing of more than one product in the same facility and/or equipment in a sequential manner; only one product is present in any one manufacturing area of the facility at a time
CGMP	Current Good Manufacturing Practices
change control	a documented system for reviewing proposed or actual changes that might affect a validated system or process; change control includes the determination of any corrective action required to ensure that the system remains in a validated state
change-over	actions required for switching multi-product equipment and facilities from one product to another
clean (v.)	the implementation of procedures to render a piece of equipment, or a system, free of adulterants and contaminants
clean(liness) (adj.)	visually clean—absence of materials which would adulterate a product when inspected with the eyes detectbly clean—absence of materials which would adulterate a product down to the level of detection chemically clean—absence of all chemicals which would adulterate a product
clean-in-place (CIP)	cleaning without the need to disassemble equipment (may be either automatic or manual)
CIP system	a system, usually automatic, used to clean equipment in place
clean-out-of-place (COP)	the cleaning performed, usually manually, after disassembly of equipment or a system
COP system	a system which may be automatic, semi-automatic or manual, used to clean equipment out of place, e.g., a parts washer
cleaning agent	usually a detergent or surfactant that reduces the surface tension of a solvent to increase its effectiveness
cleaning validation	demonstrating that cleaning results are consistent and reproducible, usually by sampling critical and representative sites on the equipment after cleaning
contaminant	extraneous substance that exists in a product
continuous process	a series of operations performed according to a manufacturing order so as to provide a steady stream of a drug having uniform character and quality within specified limits
control parameters	those operating variables that can be assigned values that are used to regulate a process
coverage	the exposure of equipment surface area to the cleaning process
critical site	area of a piece of equipment on which residual materials are trapped or concentrated (e.g., because of location, surface or equipment design) and which is likely to contribute all of the contamination to a single dose (i.e., "hot spot")
dead leg	a pipe with restricted flow or agitation exceeding the length of six pipe diameters
dedicated equipment	equipment that is to be utilized for a single product or product family
degradation	breakdown of material during manufacture or after exposure to the cleaning process
depyrogenation	removal or destruction of pyrogens
detergent	a synthetic wetting agent and emulsifier that can be added to a solvent to improve its cleaning efficiency
disinfection	to adequately treat equipment, containers, or utensils by a process that is effective in destroying vegetative cells of microorganisms of public health significance, and in substantially reducing numbers of other undesirable microorganisms
endotoxin	lipopolysaccharide, usually from gram negative bacteria
equipment grouping	equipment closely related by design, as to be considered the same for the purposes of cleaning
equipment train	the sequence of equipment through which a product is produced or processed
final rinse	the last rinse of a piece of equipment during the cleaning procedure (see rinse)
hot spot	see critical site
impingement	to cause to strike
impurity	any extraneous substance or contaminant present in the drug substance or drug product
LD_{50}	the dose resulting in a fifty percent mortality of the test animal
largest daily dose	maximum daily dose of the next product to be produced in the equipment train
limit	a prescribed maximum and/or minimum tolerance

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