May 2005
Objectionable Microorganism Concept in Cleaning Validation
A special Cleaning Memo authored by Dr. Tony Cundell

A common question asked of pharmaceutical microbiologists, by their counterparts in validation, is what microorganisms should be absent from the surface of pharmaceutical manufacturing equipment when monitored for microorganisms during cleaning validation?

For equipment used for the manufacture of non-sterile pharmaceutical and over-the-counter drug products, we tend to relate the cleaning validation requirements back to the microbiological attributes of the pharmaceutical dosage form. The compendial requirements for Microbial Limits consist of limits for the Total Aerobic Microbial Count (TAMC) and Total Combined Yeast and Mold Count (TCYMC) and the absence of specified microorganisms. These requirements will vary with dosage form, with the risk to the user being related to the route of administration of the drug product. The proposed harmonized microbiological quality criteria of non-sterile dosage forms based on their route of administration are listed in Table 1.

Table 1: Criteria for the Microbiological Quality of Non-sterile Dosage Forms (After Pharmacopoeial Forum 29 (5) Sept-Oct. 2003)

Route of Administration	TAMC (cfu/g or mL)	TCYMC (cfu/g or mL)	Specified Microorganism(s) to be Absent (per 1 g or 1 mL)
Oral solids, i.e., compressed tablets, powder- and liquid-filled capsules	1000	100	*Escherichia coli*
Oral liquids	100	10	*Escherichia coli*
Rectal ointments, creams and suppositories	1000	100	
Topical lotions, ointments and creams; Nasal sprays	100	10	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Vaginal ointments, creams and suppositories	100	10	*Staphylococcus aureus* *Escherichia coli* *Candida albicans*
Transdermal patches (limit per patch)	100	10	*Staphylococcus aureus* *Pseudomonas aeruginosa*
Inhalation aerosols	100	10	*Staphylococcus aureus* *Pseudomonas aeruginosa* Bile-tolerant Gram-negative bacteria

Although pharmacopoeial articles are screened for indicator/specified microorganisms that vary for the respective pharmaceutical dosage forms, it is widely recognized that other microorganisms found in products may be objectionable. According to 21 CFR 211.113 "Control of microbiological contamination", pharmaceutical manufacturers need written procedures describing the systems designed to prevent objectionable microorganisms in both non-sterile and sterile drug products. An objectionable microorganism can be defined as either (1) an organism that can

proliferate in a product adversely affecting the physical and therapeutic attributes of that pharmaceutical product, or (2) an organism that due to its numbers in the product and its pathogenicity can cause infection in the patient when treated with that pharmaceutical product. The absence of the specified microorganisms, e.g., *E. coli, S. aureus, P. aeruginosa* and *Salmonella spp.* will indicate that the pharmaceutical ingredients, manufacturing environment and the actual products were not exposed to gross contamination with recent sewage, fecal material, and excessive human contact. With the high level of cGMP compliance within the pharmaceutical industry, this type of gross contamination is unlikely.

One obvious approach is to apply the compendial microbiological quality criteria for the absence of specified microorganism to cleaning validation. For example, for a mixing tank used to manufacture a topical cream, we could set the cleaning validation requirement of the absence of *S. aureus* and *E. coli* from the equipment surface after cleaning and storage prior to use. This could be done by swabbing a unit area of the mixing tank, conducting a general microbiological enrichment of the contents of the swab, and proceeding with the absence of specified microorganism screening as outlined in the compendial test. However, as the presence of a single cell of the specified microorganism could give rise to a positive result, this approach is probably too stringent. Remember considerable microbial counts are needed on the equipment to impact the product processed in the equipment resulting in the possibility of exceeding the microbial limit.

Since the presence of so-called objectionable microorganisms for the specific dosage form on the equipment may be a concern, I generally suggest that alert and action levels for microbial counts per unit area should be included in the cleaning validation acceptance criteria. When the counts from a swab or contact plate exceed the alert level, all the representative colonies should be purified by subculture, identified, and compared to list of specified/objectionable microorganism for that dosage form. Using this approach, the cleaning validation acceptance criteria would not be dependant on the presence of a single colony-forming unit with a monitoring sample. This approach would not give rise to *S. aureus* shed by an equipment operator or sampler resulting in a cleaning validation failure.

An understanding of objectionable microorganisms for a particular dosage form is required with this approach. Unlike some microbiologists I would not obtain a list of objectionable organisms by using the index of the ASM Manual of Clinical Microbiology. The objectionable organisms list would be limited to frank pathogens that would result in infection when entering the human body via the route of administration of the dosage form, microorganisms capable of being isolated on the compendial media, and microorganisms with a known history of proliferating within the dosage form and degrading the physical and therapeutic attributes of the product.

To illustrate the shortness of the list of objectionable microorganisms for a dosage form, the microorganisms objectionable in a topical cream are given in Table 2. Some microorganisms, e.g., *Clostridium perfingrens* and *Propionibacterium acnes*, associated with skin, wounds, and burns infection would not be isolated using soybean-casein digest and Sabouraud dextrose agar.

The approach given is one rational scheme with a sound scientific basis to address microbial issues in a cleaning validation program for process equipment.

Table 2: Example of Specified and Objectionable Microorganisms

Pharmaceutical Dosage Form	Absence of Specified Microorganism Requirement (per 1 g or 1 mL)	Objectionable Microorganisms due to their ability to overcome preservative system or cause infection
Topical Cream	*Staphylococcus aureus* *Pseudomonas aeruginosa*	*B. cepacia, S. marcescens* and *S. pyogenes*

Note: This Cleaning Memo was written by and is copyright by Dr. Tony Cundell. It is used with permission.