Clearance Services

A guide to planning your Cleaning Validation Study

BioReliance® by SAFC

0-0040508

Contents

Introduction

Why undertake a cleaning validation study?

Cleaning validation studies are performed to establish documented evidence which demonstrates—with a high degree of assurance—that an equipment-specific cleaning process will consistently yield results meeting specifications and quality attributes. GMP regulations explicitly state that manufacturers of finished pharmaceuticals must properly clean their facilities and equipment to ensure product safety:

> 21 CFR 211.67(a) Equipment and utensils shall be cleaned, maintained, and sanitized at appropriate intervals to prevent malfunctions or contamination that would alter the safety, identity, strength, quality, or purity of the drug product beyond the official or other established requirements.

The FDA has further clarified its definition of a properly validated cleaning method.[1] The expectations are very rigorous and include having written procedures for how cleaning processes are validated, who approves the validation studies, the acceptance criteria applied to these studies, and preparation of a final validation report indicating that residues have been reduced to an acceptable level. Analytical methods and sampling procedures need to be written into the validation protocols.

Cleaning validation studies are typically performed as a product goes through phase III clinical trials. Common situations that lead manufacturers to conduct cleaning validation studies include:

- When the same facility is used to manufacture multiple biologic products, especially when "change-over" validation is essential
- When both animal-derived and Animal Origin Free (AOF) supplies are used in the same facility
- When plant-derived materials are used, as they can be a source of mycoplasmas and a variety of adventitious agents
- When operating inside viral vaccine manufacturing environments (different viral constructs)

BioReliance has performed hundreds of cleaning validation studies in accordance to criteria set by various global regulatory bodies and we have processes in place to address differing requirements. Furthermore, we validate the cleaning procedures in our own manufacturing facilities using the same methodologies that we utilize for you. The information provided in this brochure outlines these procedures for everything from cleaning techniques to calculation methods used to validate cleaning study results, all of which are designed with your process in mind.

1. "Guide to Inspections Validation of Cleaning Process", http://www.fda.gov/ora/inspect_ref/igs/valid.html.

Process validation for removal and inactivation of bacteria, fungi, and viruses

BioReliance's cleaning validation studies are designed to quantify the elimination of bacteria, fungi, and viruses during the cleaning procedures used at your manufacturing facility. Our reports feature specific reduction factors for each cleaning measure studied, a method of reporting that is preferred by regulators, compared to a simpler plus or minus (+/–) result for the presence or absence of bacteria after a cleaning step. BioReliance's studies incorporate methodology and design analogous to the rigorous requirements for viral clearance studies, and are designed to withstand scrutiny by worldwide regulatory agencies. All work is performed under U.S. Food and Drug Administration Good Laboratory Practices per 21 CFR, part 58, and the U.K. GLP Regulations, the Japanese GLP Standard, and the OECD Principles of Good Laboratory Practice.

Selection criteria for potential contaminants

The selection of model microorganisms for validation studies is a critical part of developing a removal/inactivation protocol. The selection should take into account the nature and origin of equipment and raw materials used in production processes, and the model microorganisms should be known contaminants or appropriate related models. For example, bacterial and fungal species selected should be representative of environmental, human, and material source-derived microbial flora, and should include species of known antimicrobial resistance. An additional factor to consider for a model microorganism selection is its ability to grow as a high-titer stock in both standard microbiological and cell culture media, and its ease of detection in a sensitive and reliable assay. A combination of United States Pharmacopoeia (USP) strains and environmental isolates obtained from your site is recommended. Typical residual contaminants that can be important for cleaning validation studies include:

- Host-cell proteins
- Lipids
- DNA/host-cell nucleic acid
- Endotoxins
- Carbohydrates
- Membrane/chromatography matrix leachables
- Detergents
- Viruses
- TSEs
- Mycoplasmas, bacteria, fungi

Tables 1–3 list some of the most common agents BioReliance uses in these studies, but note that the final study design is developed with your specific process in mind. Studies typically involve 3–6 agents.

Table 1—Common bacteria and fungi used in cleaning validation studies.

Species*	Gram reaction/ cell morphology	0_2 requirement	Bacteria or fungi	Lab assay time	Resistance to physical/chemical inactivation
Pseudomonas aeruginosa	Gram negative/rod	Obligate aerobe	Bacteria	3–14 days	Moderate
Candida albicans	Yeast	Facultative anaerobe	Fungi	5–14 days	Low
Aspergillus niger	Mold	Aerobe	Fungi	5–14 days	High
Clostridium sporogenes	Gram positive/ spore forming rod	Obligate anaerobe	Bacteria	3–14 days	Moderate
Staphylococcus epidermidis	Gram positive/cocci	Facultative anaerobe	Bacteria	3–14 days	Low
Bacillus isolates	Gram positive/rod	Aerobe	Bacteria	3–14 days	High
*We also recommend using client-specific environmental isolates.					

Table 2—Common viruses used in cleaning validation studies.

Virus*	Genome	Envelope	Family	Size (nm)	Lab assay time	Resistance to physical/ chemical inactivation
Xenotropic murine leukemia virus (XMuLV)	RNA	Yes	Retro	80–110	7–9 days	Low
Murine minute virus (MMV)	DNA	No	Parvo	20–25	10–13 days	High
Porcine parvovirus (PPV)	DNA	No	Parvo	20–25	7–9 days	High
Pseudorabies virus (PRV)	DNA	Yes	Herpes	150–250	4–6 days	Medium
Bovine viral diarrhea virus (BVDV)	RNA	Yes	Flavi	40–70	7–9 days	Medium
Adenovirus (Adeno)	DNA	No	Adeno	70–90	12–14 days	High
Reovirus (Reo)	RNA	No	Reo	60–80	7–9 days	High
Hepatitis A virus (HAV)	RNA	No	Picorna	~30	18–21 days	High
*We also recommend using client-specific constructs/isolates.						

Table 3—Common species of mycoplasmas used in cleaning validation studies.

A. laidlawii	*M. orale*
M. gallisepticum	*M. pneumoniae*
M. hyorhinis	*M. synoviae*

Study process

Pre-study protocol

The first step in the process is a pre-study to assess the potential for bacteriostasis and/or fungistasis in the test system and to verify the suitability of the recovery methods and media selected for the test articles to be evaluated. This will establish the degree to which the process intermediates interfere (i.e., cause inhibition) with the accurate recovery of microbial contaminants.

Although specific protocols can vary, the general approach is that individual samples of each process intermediate are spiked separately with less than 100 CFU of each challenge organism. After a four hour hold at 2–8°C, the samples are filtered through a sterile microbiological membrane filter, rinsed three times with an appropriate sterile diluent, and plated on an appropriate growth medium. Upon the satisfactory completion of the interference study, an appropriate microbial recovery procedure will have been established.

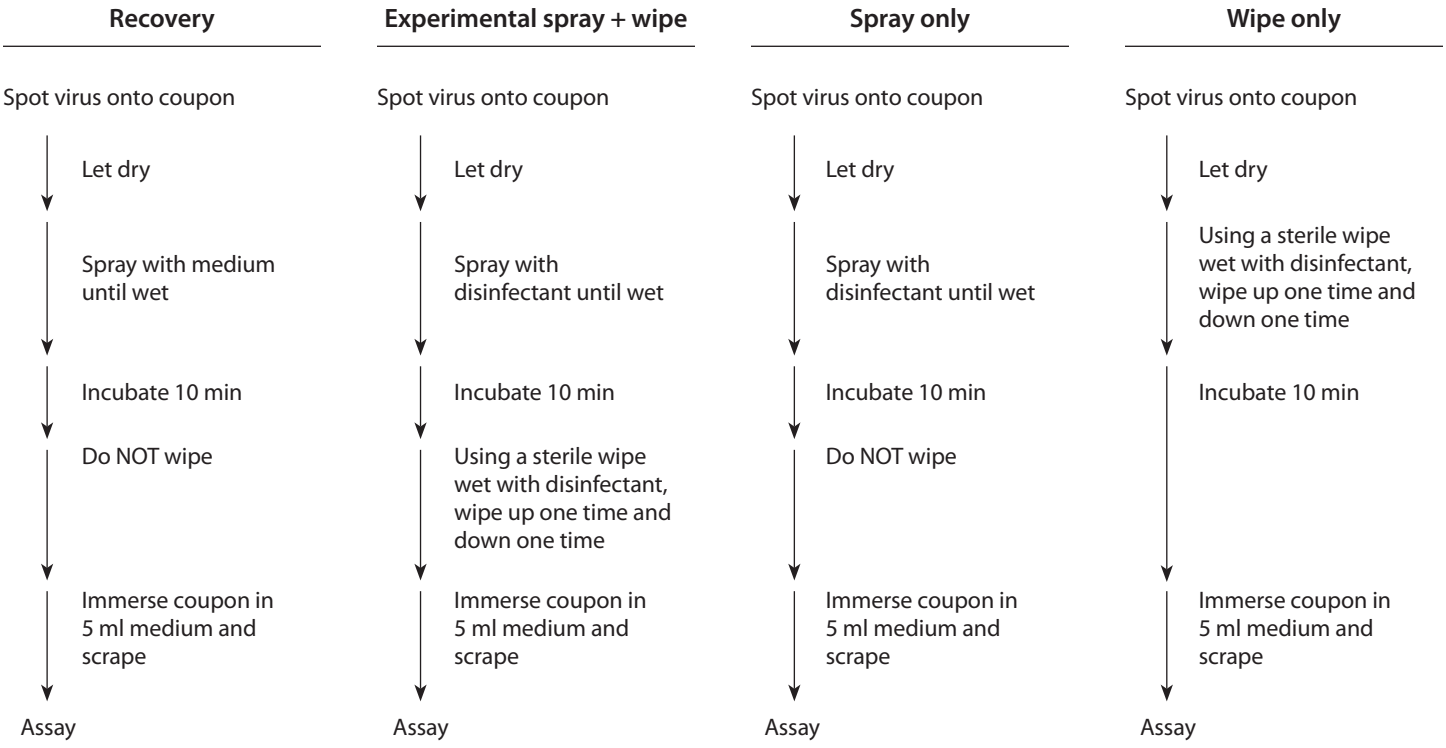
Figure 1—Example study design #1.

Title: Validation of Agent Elimination in Cleaning Procedures

Process step: surface inactivation/removal studies (triplicate runs)

Spiking agents: bulk virus, purified virus, virus and stabilizer

Temperature: ambient



Note: Samples will be treated immediately upon collection to quench the inactivation reaction. Samples will then be tested immediately.

Study protocol including microbial spiking

Proper study design must take into account multiple variables, including the type, concentration, and preparation of the cleaning agents, as well as the contact time and temperature. Microbial spiking studies are performed in either duplicate or triplicate on coupons representative of surfaces found in your manufacturing facility. BioReliance produces titered stocks of microbial strains needed, operates the coupon cleaning studies, and collects and neutralizes the samples as needed. Note that all challenge organisms will be characterized and identified prior to the start of the study to ensure that the organisms are verified to be the species intended. Several examples of different study designs are shown in Figures 1–4.

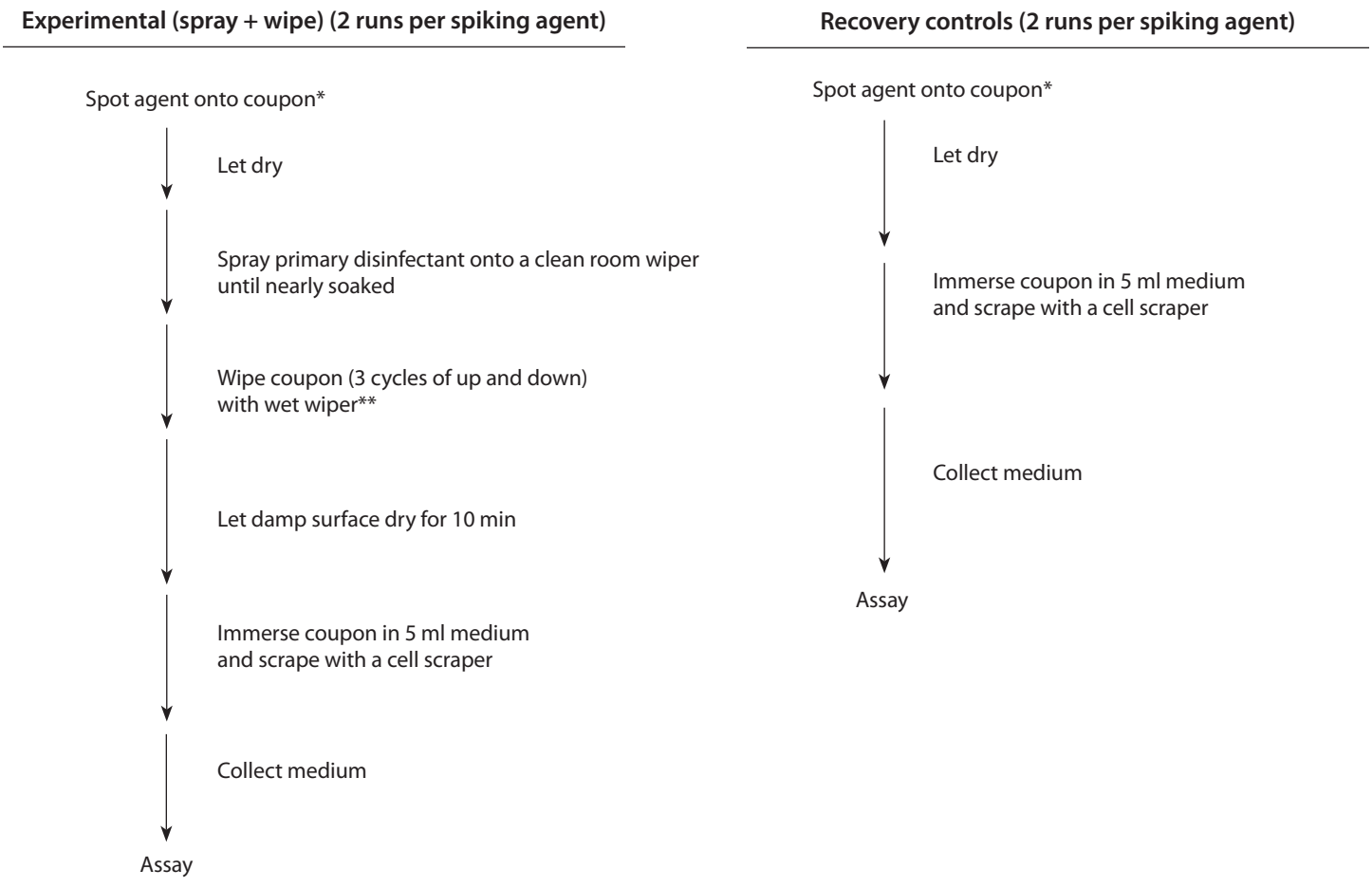
Figure 2—Example study design #2.

Title: Validation of Spiking Agent Elimination by Surface Cleaning Procedures

Process step: primary disinfectant efficacy testing

Spiking agent: viruses and microbial agents

Temperature: ambient



* 2 inch square piece of 304 stainless steel. **Use long, straight, overlapping strokes and moderate pressure. For each stroke (up and down), fold the wiper so that it presents a clean face.

Note: Samples will be treated immediately upon collection to quench the inactivation reaction. Samples will then be tested immediately.

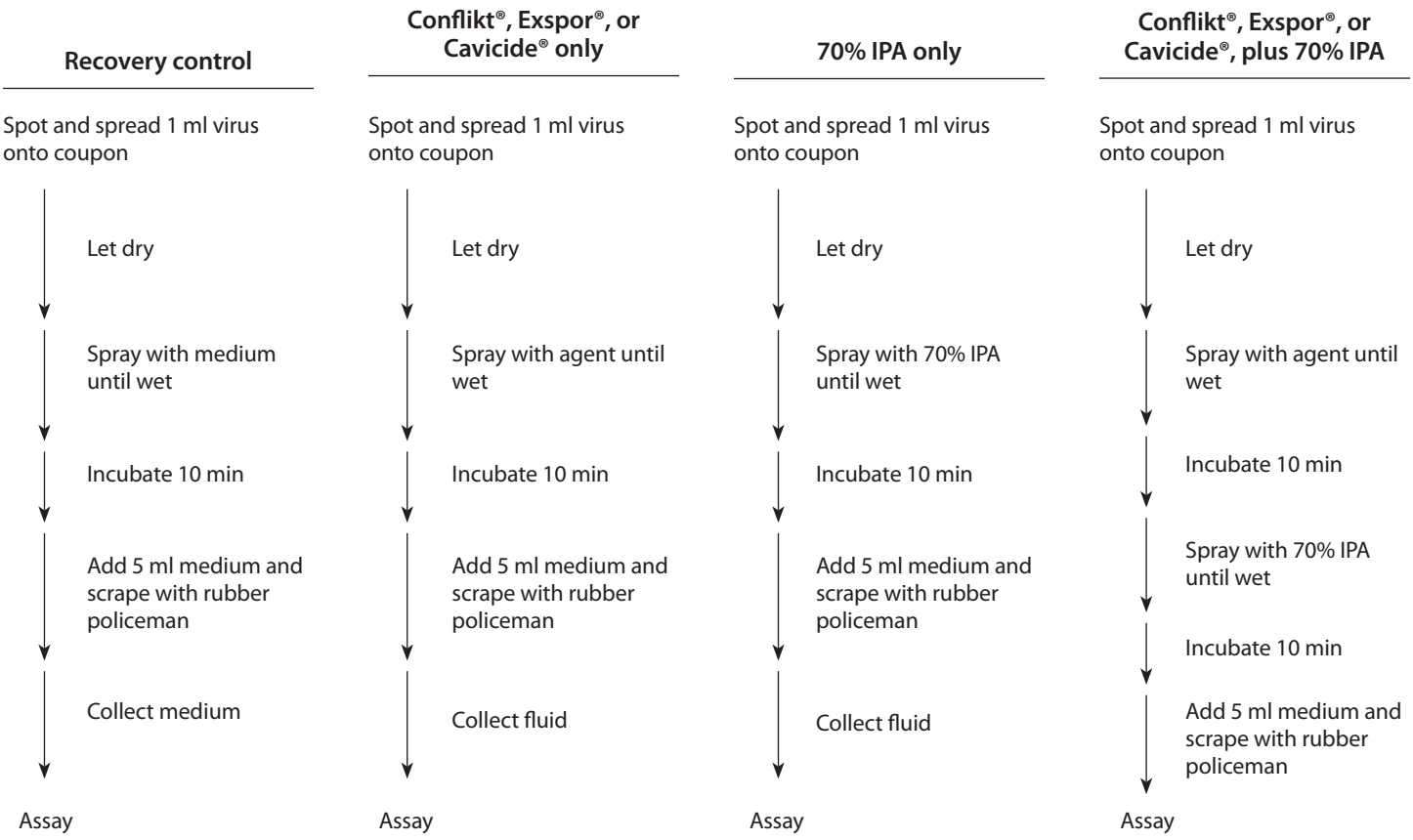
Figure 3—Example study design #3.

Title: Validation of Virus Elimination Using Solid Surface Cleaning Procedures

Process step: stainless steel cleaning

Viruses: XMuLV, Ad2, and PRV

Temperature: ambient



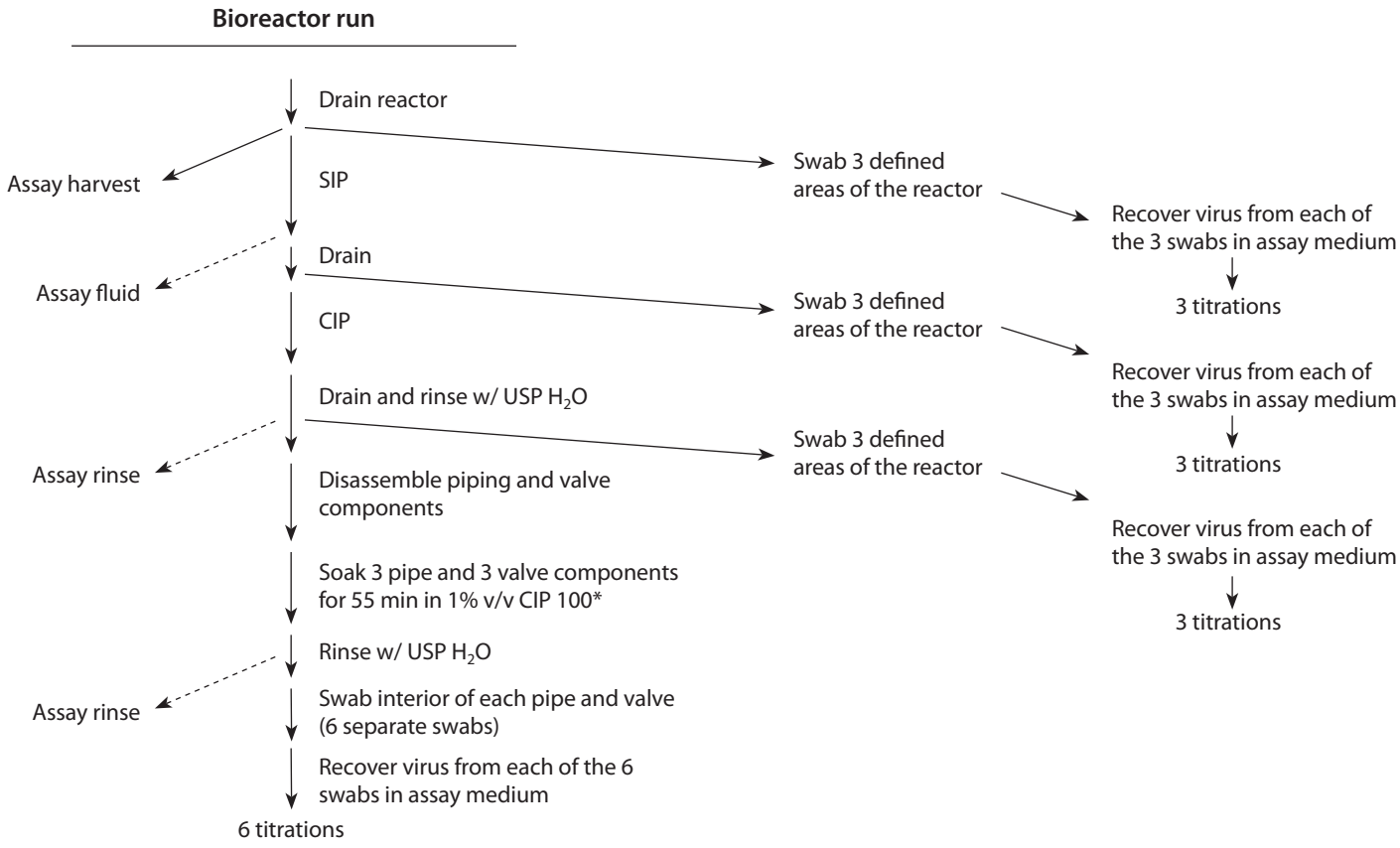
Note: Samples will be treated immediately upon collection to quench the inactivation reaction. Samples will then be tested immediately.

Figure 4—Example study design #4.

Title: Steam in Place and Clean in Place for Bioreactors

Process step: SIP/CIP bioreactor studies

Viruses: client-specific viral construct



*CIP 100 volume sufficient to cover pipe/valve components. Soak performed at RT, but solution preheated to 59°C.
Note: Samples will be neutralized to pH 6–8 and/or diluted as needed upon collection.

Cleaning technique

Coupons

While stainless steel coupons are commonly used for microbial spiking experiments, in some cases it is appropriate to evaluate a wider variety of surface materials. For example, in some manufacturing environments, wall, floor, curtain, glass, and linoleum surfaces are all tested. Furthermore, there can be important differences between new stainless steel and the older, pitted surface of well-used stainless steel. Coupon choice needs to reflect the conditions that are found in your actual manufacturing environment.

Swabbing validation

All steps in cleaning studies need to be carefully controlled—even the technique for swabbing a surface needs to be performed in a highly reproducible manner. For example, the pattern should be consistent (e.g., perpendicular, zigzag, or clockwise) if an operator is wiping a surface, and the cleaning validation studies need to reflect the procedures already established by your organization.

Cleaning agents

The cleaning agents to be used in the studies depend upon your manufacturing process. Table 4 lists common cleaning agents. BioReliance has experience with virtually all commonly used cleaning agents.

Table 4—Common cleaning agents tested.

Cavicide®	Hypochlorite
Conflikt®	IPA
Decon-Cycle®	LpH®se
Decon-Phene®	Septihol®
Decon-Spore 100®	Spor-Klenz®
Decon-Spore 200 Plus®	Vesphene® II
Ethanol	Vespore
Exspor®	

TSE agents

Due to the risk of Transmissible Spongiform Encephalopathy (TSE) originating from infected bovine products, these agents represent potential contaminants for biologic products that deserve special attention. Study design for cleaning validation studies involving TSEs can be similar to the examples shown above, but note that direct testing methodologies do not currently exist for the detection of the TSE infectious agents. BioReliance has more experience with TSE studies than any other testing lab in the world, and we have developed a significant amount of technical knowledge to apply to these studies. As is the case with cleaning validation studies for the previously described microbial contaminants, the selection of appropriate model agents is an important part of the study design (Table 5). It is important that model agents can be grown in high-titer stocks and that the agents utilized can be safely handled by laboratory staff.

The hamster-adapted 263K strain appears to be an especially good model for BSE, CJD, and other TSEs due to its well-known incubation period and a well-characterized brain histopathology. Positive identification of TSE can be seen through vacuolized lesions from the brain tissue.

There are two approaches for detecting TSEs: bioassays or western blots. BioReliance has an exclusive license on a unique TSE western blot assay that is fully validated and GLP compliant. This sensitive, specific assay is semi-quantitative over a 5.0 $\log_{10}$ range and can be used to rapidly screen and identify process steps. Table 6 gives an overview of the advantages and disadvantages of the two assays.

Table 5—Model agents for TSE studies.

Model agent	Technique	Result	Titer
Mouse adapted scrapie strain ME7	Intracranial injection into C57 BL mice	Infected mice show symptoms from days 160 to 450	10^7–10^8 LD_{80} units/ml
Hamster adapted 263K strain	Intracranial injection into Syrian golden hamsters	Infected hamsters show symptoms from days 70 to 200	10^8–10^9 LD_{50} units/ml

Table 6—TSE assay systems.

	Bioassay	Western blot
Advantages	• Measures both inactivation and removal • Generally accepted by all regulatory agencies • Good sensitivity	• Does not require the use of experimental animals • Involves less time and expense
Disadvantages	• Requires experimental animal use • Involves greater time and expense	• Assay only measures removal • Less likely to gain acceptance by regulatory agencies • Lower sensitivity

Calculation methods: logarithmic reduction factor

The logarithmic reduction factor (RF) for each individual purification or inactivation step is calculated similar to the calculation methods employed in a viral inactivation study in accordance with statistical methods in the CPMP/BWP/268/95, Final, "Note for Guidance on Virus Validation Studies: the Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses."

$$RF = \log_{10}\left[\frac{\text{Input titer/Volume x Input volume}}{\text{Output titer/Volume x Output volume}}\right]$$

An example: RF = 5.7

$$RF = \log_{10}\left[\frac{10^8 \text{ CFU/ml x 10 ml}}{10^2 \text{ CFU/ml x 20 ml}}\right]$$

Cleaning validation study results

Data handling and record maintenance

In addition to high testing standards, BioReliance provides uncensored data reporting. The report will include raw data collation and calculation of the reduction factors for each cleaning agent. We have a dedicated scientific writing team with experience in cleaning validation studies. Unaudited results are faxed within 72 hours. The average turnaround time for a final, 100% audited report is 3–4 weeks. The final study report (one per test agent) will include results of the test for clearance of spiked microorganisms by the sponsor's purification process material and a summary evaluation of the results.

All important records are maintained including: microbial spiking records, sample records, media sterility and growth promotion records, process treatment records, batch records (where appropriate), microbial titration records, dilution records, inoculation records, and records of observations.

Delivering the study report

Customers have the services of a dedicated client liason/project manager who will send biweekly status reports. Study directors also work directly with clients to discuss results and consult on scientific issues.

BioReliance Corp.
14920 Broschart Road
Rockville, Maryland 20850
Tel: 800.553.5372
Fax: 301.610.2590
Email: info@bioreliance.com

BioReliance Ltd.
Todd Campus
West of Scotland Science Park
Glasgow, Scotland G20 0XA
Tel: 44 (0) 141 946.9999
Fax: 44 (0) 141 946.0000
Email: info@bioreliance.com

BioReliance Ltd.
Innovation Park
Hillfoots Road
Stirling, Scotland FK9 4NF
Tel: 44 (0) 141 946.9999
Fax: 44 (0) 141 946.0000
Email: info@bioreliance.com

BioReliance, K.K.
c/o Sigma-Aldrich Japan K.K.
Tennoz Central Tower 4F
2-2-24 Higashi-Shinagawa
Shinagawa-ku
Tokyo 140-0002, Japan
Tel: +81 (0)3 5796 7430
Fax: +81 (0)3 5796 7435
Email: jpinfo@bioreliance.com

BioReliance Ltd.
c/o Sigma-Aldrich Chemicals Pvt Ltd
102 Alpha Building
Hiranandani Gardens
Powai, Mumbai 400076
Tel: 91 22 40872364
Fax: +91 22 25797589
Email: indiainfo@bioreliance.com

www.bioreliance.com
North America Toll Free: 800 553 5372 Tel: 301 738 1000
Europe & International Tel: +44 (0)141 946 9999 • Japan Tel: +03 5425 8491
Email: info@bioreliance.com

BioReliance® by SAFC