This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

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Standard Practice for Process Step to Inactivate Rodent Retrovirus with Triton X-100 Treatment[1,2]

This standard is issued under the fixed designation E3042; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice assures effective inactivation of ≥4 $\log_{10}$ of infectious rodent retrovirus (that is, reduction from 10 000 to 1 infectious rodent retrovirus or removal of 99.99 % of infectious rodent retroviruses) in the manufacturing processes of monoclonal antibodies or immunoglobulin G (IgG) Fc fusion proteins manufactured in rodent-derived cell lines that do not target retroviral antigens. Rodent retrovirus is used as a model for rodent cell substrate endogenous retrovirus-like particles potentially present in the production stream of these proteins.

1.2 The parameters specified for this practice are clarification, Triton X-100 detergent concentration, hold time, pH, and inactivation temperature.

1.3 This practice can be used in conjunction with other clearance or inactivation unit operations that are orthogonal to this inactivation mechanism to achieve sufficient total process clearance or inactivation of rodent retrovirus.

1.4 This detergent inactivation step is performed on a clarified, cell-free intermediate of the monoclonal antibody or IgG Fc fusion protein.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Terminology

2.1 *Definitions of Terms Specific to This Standard:*

2.1.1 *clarified, cell free intermediate, n*—in-process pool located downstream of the cell clarification unit operation(s), which should include a filtration step of ≤0.2 µm nominal pore size, and upstream of the initial purification step in the purification process of a monoclonal antibody or IgG Fc fusion protein.

2.1.1.1 *Discussion*—Cell clarification unit operations are performed on the cell culture supernatant. Cell clarification unit operations can be one or more of the following operation(s): microfiltration, centrifugation, depth filtration, or flocculation, or combination thereof. The primary purpose of cell clarification unit operation(s) is to remove cells used to generate monoclonal antibody or IgG Fc fusion protein and some proportion of cellular debris from the cell culture supernatant before the initial purification step. All clarification steps must include ≤0.2 µm nominal pore size filtration to minimize the presence of virus aggregates, prior to detergent inactivation. Freezing or prolonged storage between ≤0.2 µm filtration and detergent inactivation should be avoided.

2.1.2 *enveloped virus, n*—viruses in which the nucleic acid component of the virus is surrounded by a lipid containing envelope acquired from the host cell during virus assembly and budding.

2.1.2.1 *Discussion*—Some examples of enveloped viruses are from the families *orthomyxoviridae* (influenza), *paramyxoviridae* [mumps and measles], *retroviridae* [human immunodeficiency virus (HIV) and murine leukemia virus (MuLV)], and *herpesviridae* [human herpes virus (HHV), varicella-zoster virus (VZV), and pseudorabies virus (PRV)].

2.1.3 *hold time, n*—amount of time, after sufficient mixing takes place, that the biological drug intermediate and retrovirus interact with a specific chemical, in this case, the amount of time the biological drug intermediate and retrovirus interact with the Triton X-100.

[1] This practice is under the jurisdiction of ASTM Committee E55 on Manufacture of Pharmaceutical and Biopharmaceutical Products and is the direct responsibility of Subcommittee E55.12 on Process Applications.

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[2] Triton X-100 is a trademark of The Dow Chemical Company, Midlands, Michigan, http://www.dow.com. The sole source of manufacture of the material known to the committee at this time is The Dow Chemical Company. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

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2.1.3.1 *Discussion*—Demonstration of sufficient mixing is the responsibility of the manufacturer.

2.1.4 *immunoglobulin G, IgG, n*—antibody molecule composed of four peptide chains—two gamma heavy chains and two light chains.

2.1.4.1 *Discussion*—Each IgG has two antigen binding sites. IgG constitutes 75 % of serum immunoglobulins in humans. IgG molecules are synthesized and secreted by plasma B cells. There are four IgG subclasses (IgG1, 2, 3, and 4) in humans named in order of their abundance in serum (IgG1 being the most abundant).

2.1.5 *immunoglobulin G (IgG) fusion protein, n*—dimeric proteins comprised of two monomers, each monomer consisting of a peptide sequence (usually a human receptor-like protein or protein fragment) fused to a human IgG antibody Fc domain.

2.1.6 *effective viral clearance, n*—a viral clearance unit operation that removes or inactivates ≥4 $\log_{10}$ reduction value of virus.

2.1.6.1 *Discussion*—Inactivation requires a loss of infectivity.

2.1.7 *$\log_{10}$ reduction value, LRV, n*—$\log_{10}$ reduction is typically used to describe the degree of reduction of an organism population, in this case, rodent retrovirus, or other enveloped virus, by the treatment process.

2.1.7.1 *Discussion*—Each $\log_{10}$ reduction represents a 90 % reduction in the organism population so a process shown to achieve a "6 $\log_{10}$ reduction" will reduce a population from a million organisms to one.

2.1.8 *modular viral validation, n*—modular clearance study is one that demonstrates virus removal or inactivation by individual unit operations during the purification process (column chromatography, filtration, pasteurization, solvent/detergent, low pH, and so forth).

2.1.8.1 *Discussion*—Each unit operation, or module, in the purification scheme may be studied independently of the other modules. Different model monoclonal antibodies (mAbs) may be used to demonstrate viral clearance in different modules, if necessary. If the purification process parameters used in the manufacturing of a mAb product differs at any of the virus removal or inactivation modules from the model mAb, this module shall be studied independently from the model. The other, identical modules in the procedure may be extrapolated to the product mAb.

2.1.9 *monoclonal antibody, mAb, n*—monospecific, recombinant antibody manufactured using a production cell bank.

2.1.10 *murine leukemia virus, MuLV, n*—retrovirus named for its ability to cause cancer in murine (mouse) hosts.

2.1.10.1 *Discussion*—MuLV is a member of the genus Gammaretrovirus. MuLV is an enveloped spherical RNA virus of 80 nm to 110 nm and has low chemical resistance. MuLV is used as a model for C type endogenous retrovirus, retrovirus-like particles produced by rodent cell lines. MuLV, therefore, is used to assess retrovirus clearance of manufacturing processes that use rodent cells for production.

2.1.11 *inactivation temperature, n*—temperature (°C) of matrix in the container holding the Triton X-100 and the clarified, cell-free intermediate.

2.1.12 *retrovirus, n*—ribonucleic acid (RNA) virus that is propagated in a host cell using the reverse transcriptase enzyme to produce deoxyribonucleic acid (DNA) from its RNA genome.

2.1.12.1 *Discussion*—The DNA is then incorporated into the host's genome by an integrase enzyme. The virus thereafter replicates as part of the host cell's DNA. Retroviruses are enveloped viruses that belong to the viral family *Retroviridae*.

2.1.13 *Triton X-100 (polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether), n*—non-ionic surfactant; a liquid at room temperature.

2.1.13.1 *Discussion*—Triton X-100 is also known as polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether, octyl phenol ethoxylate, Octylphenol Ethoxylate (non-ionic), and Octoxynol-9. The CAS number for Triton X-100 is 9002-93-1. In this practice, the chemical polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether, CAS number 9002-93-1, will be referred to as Triton X-100.

2.1.14 *Triton X-100 concentration, n*—percentage of Triton X-100 (% weight : volume) in the Triton X-100 detergent solution.

3. Significance and Use

3.1 Rodent-derived cell lines are widely used in the production of biopharmaceutical drugs such as mAbs and Fc fusion proteins. These cell lines have been shown to contain genes encoding endogenous retroviral-like particles or endogenous retrovirus. Despite the lack of evidence for an association between such rodent retroviruses and disease in humans, the potential contamination of human therapeutics raises safety concerns for biopharmaceutical drugs. Additionally, adventitious agents such as viruses can be introduced into a biopharmaceutical drug substance manufacturing process from other sources, and potential safety issues can be attributed to these potential unknowns. For these reasons, effective viral clearance is an essential aspect of an integrated approach combining safety testing and process characterization which ensures virus safety for biopharmaceutical drug products made using rodent cell lines.

3.2 Solvent/detergent inactivation has been widely used for decades to inactivate enveloped viruses in blood plasma derived biopharmaceutical therapies (**1-3**).[3] Solvent/detergent systems using the detergents Triton X-100 or Polysorbate 80 along with the organic solvent tri(n-butyl)phosphate (TNBP) have been used to inactivate enveloped viruses by disrupting the viral envelope thereby reducing the ability of the enveloped virus to attach to and then infect the host cell (**4 and 5**).

3.3 Most manufacturers of mAbs, recombinant proteins, and Fc fusion proteins have focused on viral inactivation methods using the detergent Triton X-100 or Polysorbate 80 in the absence of TNBP (**6**), which can interfere with subsequent

[3] The boldface numbers in parentheses refer to a list of references at the end of this standard.