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Standard Practice for Process for Inactivation of Rodent Retrovirus by pH[1]

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1. Scope

1.1 This practice assures 5 log10 inactivation of non-defective C-type retroviruses, which are endogenous to murine hybridoma and CHO cells and are potentially present in the production stream of biopharmaceutical processes that use rodent derived cell culture.

1.2 The process parameters specified in this practice consistently assure 5 log10 inactivation of murine retrovirus by adjusting the pH of a process solution after initial affinity capture chromatography purification.

1.3 This practice is applicable to mAb, IgG fusion, or other recombinant proteins produced from rodent cell lines (for example, CHO or murine hybridoma), which do not target retroviral proteins. Additionally, the low pH step is performed on a cell-free intermediate, post initial capture using protein A chromatography.

1.4 The 5 log10 inactivation of murine retrovirus claimed by using this practice will be utilized in conjunction with other clearance unit operations (for example, chromatography and virus retentive filtration) to assure sufficient total process clearance of murine retroviruses, which will be supportive of early phase regulatory filings.

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

2. Terminology

2.1 *Definitions of Terms Specific to This Standard:*

2.1.1 *IgG fusion protein*—a dimeric protein comprised of two monomers, each monomer consisting of a peptide sequence (usually a human receptor-like protein or protein fragment) fused to the carboxyl-terminal of the Fc-domain of a human IgG antibody.

2.1.1.1 *Discussion*—Dimerization occurs by way of the Fc domain.

2.1.2 *immunoglobulin G (IgG)*—an antibody molecule composed of four peptide chains — two γ heavy chains and two light chains.

2.1.2.1 *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). Only human IgG1, IgG2, and IgG4 show significant affinity to protein A.

2.1.3 *log10 reduction value (LRV)*—typically used to describe the degree of reduction of a population, in this case rodent retrovirus, by the treatment process.

2.1.3.1 *Discussion*—Each log reduction (10^{-1}) represents a 90 % reduction in the population. So a process shown to achieve a 6-log reduction (10^{-6}) will reduce a population from a million (10^{6}) to 1.

2.1.4 *monoclonal antibody (mAb)*—monospecific antibodies which have affinity for the same antigen and are made from a master cell bank, cloned from a parent cell.

2.1.5 *murine leukemia virus (MuLV)*—retroviruses named for their ability to cause cancer in murine (mouse) hosts.

2.1.5.1 *Discussion*—MuLV is a member of the genus *Gammaretrovirus*. MuLV is an enveloped spherical RNA virus which has a diameter of 80–110 nm and has low chemical resistance. MuLV is used as a model for non-defective C-type endogenous retrovirus or retrovirus like particles produced by murine hybridoma and CHO cell lines. MuLV is used to assess rodent retrovirus clearance of protein purification processes that use rodent cells for production.

2.1.6 *recombinant protein*—produced from the expression of recombinant DNA within living cells.

2.1.6.1 *Discussion*—Recombinant DNA is genetically engineered by inserting foreign DNA into the DNA of an appropriate host so that the foreign DNA is replicated along with the host DNA.

2.1.7 *retrovirus*—an RNA virus that is propagated in a host cell using the reverse transcriptase enzyme to produce DNA from its RNA genome.

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

[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 E55.04 on General Biopharmaceutical Standards.

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2.1.8 *modular validation*—a modular clearance study is one that demonstrates virus removal or inactivation at individual steps during the purification process (column chromatography, filtration, heat treatment, solvent/detergent treatment, low pH treatment, etc.).

2.1.8.1 *Discussion*—Each module, or unit operation, in the purification scheme may be studied independently of the other modules. Different model mAb may be used to demonstrate viral clearance in different modules, if necessary. If the purification process of a product mAb differs at any of the virus removal or inactivation modules from the model mAb, this module must be studied independently from the model. The other, identical modules in the procedure may be extrapolated to the product mAb. The total LRV of a purification process can be obtained by adding the LRVs of the individual modules.[2]

3. Significance and Use

3.1 Rodent cell lines are widely used in the production of biological therapeutics such as monoclonal antibodies and recombinant proteins. These cell lines are known to contain genes encoding endogenous retroviral-like particles or to produce infectious endogenous retrovirus. Despite the lack of evidence for an association between such rodent retroviruses and disease in man, the potential contamination of human therapeutics poses patient safety concerns. Additionally, adventitious agents such as viruses can be introduced into a drug substance manufacturing process from other sources such as raw materials. Potential safety issues can be attributed to these biosafety testing for products made using rodent cell lines.[3]

3.2 Low pH inactivates retroviruses by denaturing the viral envelope proteins. Similar to all chemical reactions, this depends on reactant concentration (that is, H+ ion concentration as measured by pH), time of reaction and temperature of reaction. Implementing the parameters that give robust and effective rodent retrovirus inactivation established by this pratice, in conjunction with other clearance unit operations (for example, chromatography, virus retentive filtration) can assure sufficient purification process clearance of rodent retroviruses.[4]

3.3 This practice incorporates parameters that give robust and effective rodent retrovirus inactivation, which can be used as modular validation for the low pH viral clearance module using MuLV, the model non-defective C-type retrovirus endogenous to murine hybridoma and CHO cells.

4. Procedure

4.1 For this practice, the primary variables specified are the concentration range of buffer composition elements, pH, temperature, protein concentration, and time during hold conditions.

4.2 This practice will be applicable to mAb, IgG fusion, or other recombinant proteins, produced from rodent (for example, CHO or murine hybridoma) cell lines, which do not target retroviral proteins.

4.3 The low pH viral inactivation step must be performed on a cell-free intermediate, post initial capture using protein A chromatography.

4.4 The inactivation process and the corresponding log10 reduction of 5.0 are as follows:[5]

4.4.1 The hold temperature is ≥15°C,

4.4.2 The hold time is ≥30 minutes,

4.4.3 The hold pH is ≤3.6 throughout the course of the hold time, and

4.4.4 The buffer matrix will be glycine, citrate, or acetate based, and the concentration of components will be in the following ranges:

4.4.4.1 If utilized, sodium chloride will be ≤500 mM in concentration, and

4.4.4.2 The protein concentration is ≤25 g/L.

5. Keywords

5.1 inactivation; retrovirus; rodent

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[2] U.S. Food and Drug Administration (FDA), *Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use*, 1997, Department of Health and Human Services, Food and Drug Administration, Rockville, MD.

[3] The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), *Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, Q5A*, 1999, Geneva, Switzerland.

[4] Brorson, K., Krejci, S., Lee, K., Hamilton, E., Stein, K., et al., "Bracketed Generic Inactivation of Rodent Retroviruses by Low pH Treatment for Monoclonal Antibodies and Recombinant Proteins," *Biotechnology and Bioengineering* , Vol 82, No. 3, 2003, pp. 321–329.

[5] Miesegaes, G., Bailey, M., Wilkommen, H., Chen, Q., Roush, D., et al., "Proceedings of the 2009 Viral Clearance Symposium," Journal of Developmental Biology (Basel), Basel, Karger, Vol 133, 2010, pp. 25–42.