Designation: E3259 – 22

Standard Practice for Process to Remove Retroviruses by Small Virus Retentive Filters[1]

This standard is issued under the fixed designation E3259; 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 6.0 $\log_{10}$ removal of retrovirus (for example, MuLV).

1.2 This practice is applicable to monoclonal antibody (mAb), immunoglobulin G (IgG) fusion proteins, recombinant proteins, or other proteins produced using mammalian cell lines (for example, Chinese hamster ovary (CHO), murine hybridomas, murine myelomas, or human embryonic kidney (HEK) 293).

1.3 The step is performed on cell-free intermediates.

1.4 The log removal claim for retrovirus by small virus retentive filters can be used in conjunction with other clearance unit operations (for example, low pH inactivation, or inactivation of virus by surfactant) to assure sufficient total process clearance of potential virus contaminants, which would be supportive of early phase (clinical phase 1 or phase 2a trials) regulatory filings.

1.5 Retrovirus removal claim by filtration is limited to small virus retentive filters, as defined in the PDA Technical Report Virus Filtration (1)[2] in the context of this standard.

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

1.7 *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.*

1.8 *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 *bacteriophage PP7, n*—RNA bacteriophage that infects *Pseudomonas aeruginosa* bacteria that has a size of approximately 30 nm to 33 nm, often used as a surrogate for parvovirus in virus retentive filter studies, but not for regulatory clearance claims.

2.1.2 *bacteriophage PR772, n*—double-stranded DNA bacteriophage that infects *Escerichia coli* bacteria that has a size of approximately 80 nm, often used as a surrogate for retrovirus in virus retentive filter studies, but not for regulatory clearance claims.

2.1.3 *$\log_{10}$ reduction value (LRV) or log reduction factor (LRF), n*—used to describe the degree of reduction of a population; for virus filtration, LRV/LRF is calculated by comparing the virus quantity before and after filtration.

2.1.3.1 *Discussion*—Each log reduction (10^{-1}) represents a 90 % reduction in the population. For example, a process shown to achieve a 6-log reduction (10^{-6}) will reduce a population from a million (10^{6}) to 1. The calculation for log reduction is [$\log_{10}$ of total virus quantity in feed material or load] minus [$\log_{10}$ total virus quantity in filtrate or filtered product].

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

2.1.4.1 *Discussion*—Each module, or unit operation, in the purification scheme may be studied independently of the other modules. Different model proteins may be used to demonstrate viral clearance in different modules, if necessary. If the purification process of a specific product (protein) differs at any

[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.

Current edition approved Nov. 1, 2022. Published November 2022. DOI: 10.1520/E3259-22.

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

of the virus removal or inactivation modules from the model protein, this module must be studied independently from the model. (1)[3]

2.1.5 *monoclonal antibody, (mAb), n*—antibodies that have affinity for specific antigen(s) and are made from a master cell bank or working cell bank from a cloned from a parent cell.

2.1.6 *parvovirus, n*—small non-enveloped, single-stranded DNA virus, approximately 18 nm to 26 nm in diameter (2).

2.1.6.1 *Discussion*—Minute virus of mice (MMV) and porcine parvovirus (PPV) are small non-enveloped parvoviruses (approximately 18 nm to 26 nm) (3, 4). MMV and PPV are used in viral clearance studies as a model virus for small parvoviruses. Due to the mechanism of virus retention on virus retentive filters, the size of parvoviruses is regarded as a worst-case challenge for small virus retention filters (4).

2.1.7 *recombinant protein, n*—protein produced using recombinant DNA technology, expressed in living cells.

2.1.8 *retrovirus, n*—enveloped, single-stranded RNA virus that is propagated in host cell using a reverse transcriptase enzyme to produce viral cDNA from its RNA genome. Viral cDNA is incorporated into the host genome by viral integrase enzyme. Once the viral genome is integrated, virus can thereafter be replicated as part of the host cell DNA (2).

2.1.8.1 *Discussion*—Murine leukemia virus (MuLV) is a large, enveloped virus (approximately 80 nm to 110 nm) belonging to the retroviridae family (3, 4). MuLV, and various subtypes of MuLV, including xenotropic (xMuLV), ecotropic (eMuLV), and amphotropic (aMuLV), are used in viral clearance studies to model endogenous retrovirus or retrovirus-like particles.

2.1.9 *small virus retentive filter, n*—also known as parvovirus retentive filters and PP7-LRF4 filters, filters that are designed and claimed by the manufacturer or validated substitute vendor through filter lot release testing to retain parvovirus (18 nm to 26 nm) and be capable of retention of >4 $\log_{10}$ of bacteriophage PP7 (30 nm to 33 nm) across three lots of virus filters at load ratio of at least 50 L/m^2 and meet all of the stipulations defined in the PDA Technical Report Virus Filtration (1).

3. Significance and Use

3.1 Mammalian cell lines are widely used in the production of biological therapeutics, such as monoclonal antibodies and other recombinant proteins. Some of these cell lines, like rodent cell lines, are known to contain genes encoding endogenous retroviral-like particles or produce endogenous retrovirus, but there is no evidence of an association between rodent retrovirus and disease in humans. Adventitious viruses can be introduced into a drug substance manufacturing process from other sources, and contamination of human therapeutics is a safety concern (3).

3.2 Virus filtration, an orthogonal technology in a virus clearance platform to such steps as low pH or surfactant inactivation, has traditionally been accepted as a robust method for virus clearance when well designed. Size exclusion has been shown to be the primary mechanism of virus removal by virus retentive filtration, that is, larger viruses are more easily retained than smaller viruses such as parvoviruses (4, 5). Large virus retention has also been shown to be insensitive to process fluid characteristics such as protein type, protein concentration, pH, and ionic strength (4, 6, 7, 8, 9, 10). In contrast, for small viruses, aspects like flow pausing and/or flux decay can impact clearance (4, 6, 11).

3.3 Large virus retentive filters, or retrovirus filters, are tested for removal of larger enveloped viruses like retrovirus or MuLV (80 nm to 100 nm) and have undetectable levels of the large bacteriophage PR772 (64 nm to 82 nm) (1). Small virus retentive filters, or parvovirus filters, are designed to remove parvovirus, like MMV (18 nm to 26 nm) (1). Since size exclusion has been demonstrated as the mechanism of virus retention, retroviruses, which are three to four times larger than parvoviruses, should be large enough to be completely retained, with undetectable levels of retrovirus in the filtrate, by all small virus retentive filters designed to remove parvovirus.

3.4 Numerous published studies and reviews encompassing data from the last 20 years have shown both large and small virus retentive filters are effective and consistent for removal of retrovirus. In published reviews of regulatory submissions from 1990 through 2010, rare occurrences of retrovirus breakthrough did occur across both large and small virus retentive filters. These anomalies, however, were not resolved and could be attributed to study design, experimental artifacts, or limitations of the meta-analyses performed on the regulatory submission (12). In a summary of 89 submissions to Paul Ehrlich Institute (PEI), processes using either large or small virus retentive filters showed no detection of any infectious particles from large viruses (12). A collection of viral filtration results across eight biopharmaceutical companies showed no large virus breakthrough across any small virus retentive filter for all 198 experiments reviewed (7). Additionally, a recent review of 20 plus years of small virus retentive filter experiments from two viral clearance testing houses showed only 0.61 % (14 out of 2311 experiments) viral filtration studies performed with larger viruses had detectable, replicated virus (10). This manuscript further suggests that all positive results were not due to viral breakthrough of integral small virus retentive filters, but rather to other causes that included virus detection assay, aerosolization of virus during filtration, splashes, spills, or potential use of non-integral laboratory scale filters.

3.5 The level of justifiable LRV for a modular claim from a censored dataset, that is, all observations are below a certain LRV level, can be difficult to estimate (13), and is almost always an underestimation of an actual value. LRV approximations from censored data are influenced by viral spike volume, viral spike titer, load volume, and assay sensitivities to product matrices, in addition to small virus retentive filter performance. In the Mattila review, where no large virus was detected in 198 experiments, small virus retentive filters showed an average clearance >5.86 LRV ±0.91 for Reo3, a medium-sized virus (60 nm to 80 nm), suggesting at least equal or better clearance for the larger retrovirus, MuLV (7). For creation of modular claim from censored values, Stuckey, et al.

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

proposed that the highest LRV in set of censored data could be used for a modular claim (**6**). In these experiments, load material spiked with both parvovirus and retrovirus was used to challenge small virus retentive filters. Parvovirus breakthrough was observed but no retrovirus breakthrough was detected, further supporting both a size exclusion-based mechanism of virus retention and the robustness of retrovirus retention using small virus retentive filters (**6**). Additionally, even large virus retentive filters, designed to retain larger viruses and having on average larger pore sizes than small virus retentive filters, are classified as having the ability to retain >6 logs of PR772, a 64 nm to 82 nm bacteriophage often used as a surrogate for retrovirus, in virus filtration studies (**1, 14**). These large virus filters have been shown to clear >8 logs of PR772 (**15**), and by definition clear more than 6 $\log_{10}$ of retrovirus (**12**). These published data, collectively, support a modular claim for small virus retentive filters of >6.0 LRV for retrovirus (MuLV).

3.6 Implementing parameters of small virus retentive filtration established by this practice can provide robust retrovirus removal and can be used as a modular retrovirus validation of the virus filtration step. In conjunction with other clearance unit operations (for example, chromatography and inactivation by pH or surfactants), sufficient overall retrovirus clearance can be achieved (**3**).

4. Procedure

4.1 Retrovirus removal claim by filtration is limited to small virus retentive filters, as defined in the PDA Technical Report Virus Filtration (**1, 16**) in the context of this practice.

4.2 To obtain an LRV of 6.0 for retrovirus, viral filtration must be performed within filter manufacturer recommended operating conditions for the following operating parameters defined for each unique small virus retentive filter.

4.2.1 Differential pressure maintained within the specified limits provided by specific small virus retentive filter manufacturer.

4.2.2 Passing of appropriate pre-use assurance filter integrity test, performed by either the viral filter manufacturer (Certificate of Analysis for each unique filter lot used) or biopharmaceutical manufacturer (pre-use integrity test result), or both.

4.2.3 Passing of appropriate post-use integrity test.

4.2.4 Any additional specifications for operation provided by the small virus retentive filter manufacturer must also be adhered to during filtration. The end user of the small virus retentive filter and this standard practice must show that these parameters were within the specified limits provided from each specific small virus retentive filter manufacturer during the filtration operation.

5. Keywords

5.1 modular claim; modular retrovirus claim; viral clearance; virus filtration; MuLV; virus filter; parvovirus; retrovirus; virus removal; small virus retentive filter

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