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Technical Regulatory Topic

Evaluation of Monodispersion in *Brevundimonas Diminuta* Suspensions

Sterile Filter Validation

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Background

For many years, Pall has confirmed that *Brevundimonas diminuta* (*B. diminuta*) used in process-specific bacterial retention testing is in a monodispersed state using two different methods. However, as part of a global harmonization of procedures, Pall has decided to use only one test for all standard filter validation tests. The technical justification for this change is documented in this report.

Technical Rationale

For most sterilizing grade filters, one of the primary mechanisms for bacterial removal is size exclusion, which incorporates surface screening and/or entrapment within the depth of the filter membrane.

To perform process-specific filter validation studies, the industry standard for testing bacterial retention in any sterilizing grade filter is to use *B. diminuta* ATCC♦ 19146. This bacterium was selected due to the observation by Dr. Frances Bowman that it could repeatedly penetrate a 0.45 µm rated filter but was fully retained by a 0.2 µm filter [1]. Consequently, it was accepted by the regulatory authorities as a suitable bacterium for testing the ability of a filter to provide a sterile product.

Growth of the *B. diminuta* under defined conditions yields cells of approximate size 0.3 – 0.4 µm x 0.6 – 1.0 µm [2]. However, in addition to maintaining a minimal cell size, it is critical that any sterilizing grade filter is challenged with *B. diminuta* cells that are in a non-aggregated, monodispersed state. While this can be determined microscopically, demonstrated penetration through a 0.45 µm filter is considered a more sensitive test. Consequently, for process-specific bacterial retention studies, a 0.45 µm rated control filter is challenged in parallel with the test 0.2 µm sterilizing grade filters and confirmed penetration of *B. diminuta* through a 0.45 µm rated control filter is required to consider the bacterial challenge test valid. Pall's microscopic test to evaluate monodispersion involves assessing a minimum of 20 cells of *B. diminuta* and calculating the percent of monodispersed (single) cells from those evaluated. However, it is important to note that this test is typically performed post-bacterial retention testing on the post-input bacterial sample. As such, because this is not a real-time test, it could be argued that the test is not an accurate reflection of the extent of aggregation during the actual bacterial challenge test. Conversely, by testing the 0.45 µm rated control filter in parallel with the 0.2 µm sterilizing grade filters, this issue is avoided.

It should also be noted that in Pall's experience, microscopic evaluation of *B. diminuta* cells does not necessarily predict the ability of the *B. diminuta* challenge suspension to penetrate a 0.45 µm filter. This is partly due to the very small sample size used for the microscopic evaluation, compared to the actual *B. diminuta* concentrations used during bacterial retention testing (n = 20 cells vs 10^7 cells, respectively). Further, with no industry recognized acceptance criteria for the minimal percent of monodispersed cells via microscopic evaluation, this test becomes very user subjective.

Consequently, for all bacterial retention testing performed as part of Pall's standard filter validation package, proof of a monodispersed, non-aggregated *B. diminuta* suspension will be shown by penetration through the 0.45 µm filter only.

References

[1] Bowman FW, Calhoun, MP &. White, M. J. Pharm. Sci., 56(2), 453–459 (1967).

[2] American Society for Testing and Materials (ASTM) Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration F838, pp 1-6, ASTM, West Conshohocken, PA, 2005.

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