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Bacterial Challenge Verification Test Report of Cobetter MCE Grid Membrane

1. Experimental Purpose

The bacterial challenge test is a destructive integrity test method that needs to be correlated with a non-destructive integrity test method. The FDA guidelines state that, after properly verifying the filtration operation of a given product, process, and filter,it is important to use the same filter (filter membrane or filter of the same material) in continuous production. One method is to correlate the filter's bacterial retention capacity with filter integrity data. In general, the integrity test is performed after the filter is installed and sterilized, and before it is officially used. And more importantly, the integrity test is also performed after the filter is used, to detect the filter penetration risk that may occur during the filtration process.

According to the sterilization grade filter bacterial challenge test method in ASTM F838, conduct＞10^7 cfu/cm² *Serratia Marcescens* (ATCC® 14756) challenge test to verify its bacterial retention ability.

2. Test standards

ASTM F838-15a；

3. Test samples

No.	Sample Name	Item No.	Qty.	Specification
1	MCE Grid Membrane	YQJ4	3pcs	Φ47mm 0.45μm
2		YQD5	3pcs	Φ47mm 0.45μm
3		YQS7	3pcs	Φ47mm 0.45μm

4. Test equipment

Integrity Tester Cobetter CF 5.2, Homemade membrane bacteria challenge test device, Vertical pressure steam sterilizer, Secondary biological safety cabinet, Biochemical incubator

5. Test method

5.1 Preparation of bacterial challenge solution

Take a slant test tube strain, pick an appropriate amount of Serratia marcescens and inoculate in tryptone soy broth (TSB), and cultivate at 30±2℃ for 24h. Take several TSB cultures in nutrient broth (NB), mix well, and shake and culture at 30±2℃ for 24h. Ultrasonic treatment of the culture at room temperature in a water bath for 10 minutes, helps the cells maintain monodisperse properties. Take the appropriate volume of NB culture and add it to sterile saline to prepare a challenge suspension, which meets the requirement of 10_7 cfu/cm_2 EFA (test filter effective filtration area). The bacterial solution is freshly prepared, and a microscopic examination is performed before use to ensure that the bacterial condition meets the requirements. Under sterile operating conditions, dilute the bacterial suspension with 0.1% peptone water to 10_{-6}, then take 0.1 ml of 10_{-6}, 10_{-5}, and 10_{-4} dilutions respectively and count by coating method. Place the coated tryptone soy agar (TSA) plate at 30±2℃ for 24h. The concentration of the bacterial suspension was counted by the coating plate method.

5.2 Integrity testing

After wetting the filter with a suitable method, ensure that the air in the filter is exhausted during the wetting process. Use a Cobetter CF5.2 integrity tester to test the integrity of the filter.

5.3 Sterilization

The test filter is installed in the membrane bacteria challenge test device. The 0.45 μm analysis membrane is loaded into the downstream flat filter. After ensuring that the components of the test device are connected correctly, perform offline steam sterilization at 121℃ for 30 minutes.

5.4 Negative control test

After the filter is sterilized, the filter integrity test is performed aseptically, ensure that the filter is not damaged during the sterilization process. Subsequently, the filter device was rinsed with sterilized purified water, the rinse solution passed through the 0.45 μm analysis membrane, the analysis membrane was taken aseptically, placed on a TSA plate, and cultured at 30±2℃ for 7 days.

5.5 Bacterial challenge test

Add a sufficient amount of Serratia marcescens challenge suspension to the storage tank to meet the 10_7 cfu/cm_2 EFA. Slowly pressurize so that the challenge suspension fills the filter, while adjusting the venting port properly to ensure that the air in the filter has been exhausted. Pressurized to 0.2 MPa for filtration, all filtrate was filtered through 0.45 μm analysis membrane. Subsequently, the analysis membrane was removed aseptically, placed on a TSA plate, and cultured at 30±2℃ for 7 days. After the bacterial challenge is finished, the filter integrity test is performed again.

5.6 Calculation of results

$LRV=\log_{10}$(Number of colonies in challenge fluid / Number of colonies in the filtrate fluid)

6. Test results

Item No.	No.	Bubble point(MPa)	Challenge level（cfu/cm²）	Downstream colonies (cfu)	LRV/cm²
YQJ4	1	1805	1.81×10^7	0	7.25
	2	2023	1.81×10^7	0	7.25
	3	2045	1.81×10^7	0	7.25
YQD5	1	2052	1.64×10^7	0	7.21
	2	2158	1.64×10^7	0	7.21
	3	2143	1.64×10^7	0	7.21
YQS7	1	1962	1.52×10^7	0	7.18
	2	2054	1.52×10^7	0	7.18
	3	2038	1.52×10^7	0	7.18

7. Test conclusion

The number of colonies in the downstream of the bacterial challenge experiment of Cobetter Gridded Membrane is 0 cfu, and the LRV/cm² is greater than 7, which proved that the bacteria retention ability meet the requirements of 0.45μm filter membrane.