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Protocol of microbial reduction by the (E.A.M Benelux Hand Shower Filter Complete, QF10202WH) as based upon the ASTM Standard F838-05 for determining the bacterial retention of membrane filters utilized for liquid filtration.

1. Introduction.

The purpose of this test method is to evaluate the purifier status of the E.A.M Benelux Hand Shower Filter for the Water Quality Association. The test organism used for this test is *Legionella pneumophila* serotype 9 instead of *Pseudomonas diminuta*. After the start of flushing the filter with sterile water the filter is flushed with tap water. In order to test the removal capacity of the filter a challenge test is carried out. Therefore a suspension with a high concentration of *Legionella pneumophila* serotype 9 is filtered through the membrane filter. Based upon the concentration of target constituent added and the number observed in the effluent, percentage removal or log reduction can be calculated.

2. Media/Reagents.

2.1 Buffered Charcoal Yeast Extract Agar)BCYE)
2.2 Sterile distilled water

3. Microorganisms.

3.1 Bacteria

3.1.1 *Legionella pneumophila* serotype 9

3.1.2 Culture Maintenance
3.1.2.1 Storage, growth and sample preparation of *L. pneumophila*.
L. pneumophila will be cultured first on BCYE agar at 37 °C, following by an additional culturing of a single colony on BCYE agar at 37 °C. Then a few colonies will be scratched from the BCYE and inoculated in sterile water. The suspension is mixed thoroughly and washed three times with sterile water by centrifugation at 3000 g for 10 minutes. After the washing procedure the pellet will be resuspended and the actual count will be determined by plate count on BCYE agar. The prepared suspension is stable for seven days when stored at 5 ± 3 °C. B

4. Test Water.

4.1 General Test Water #1 (GTW 1)
4.1.1 Chlorine Free Potable Water
4.1.2 pH 6.5 - 8.5
4.1.3 TOC, 0.1 to 5.0 mg/L
4.1.4 Turbidity, 0.1 to 5 NTU
4.1.5 Temperature, 20°C $\pm$ 5°C
4.1.6 TDS, 50 - 500 mg/L

5. Equipment.

5.1 Disposable pipettes
5.2 Pipetting devices
5.3 Stir Plate and Sterile Stir Bars
5.4 37°C Incubator
5.5 Flasks, beakers and graduated cylinders (various)
5.6 Centrifuge tubes, sterile (various)
5.7 Sample bottles, (various)
5.8 Water treatment devices (QF10202WH); supplied by E.A.M Benelux.

6. Procedure.

6.1 Preparation of Test Water.
6.1.1 Test water will be prepared as described in Test Water section above.

6.2 Preparation of the challenge suspension
6.2.1 *L. pneumophila* will be added to Test Water to a final concentration of around 10^8 CFU/L during challenge periods.

6.3 Assembly of Hand Shower Filter.
6.3.1 The Hand Shower Filter will be mounted on the test rig following manufacturer instructions.
6.3.2 Water will be applied at 60 psi dynamic pressure.
6.3.3 Flow rate will be determined per unit (approx. 4 Lpm).
6.3.4 There will be an initial flush of 1 L through each unit at 30 psi with test water.
6.3.5 Void volume of unit approx. 1 L. 10 void volumes = 10 L.
6.4 The entire test will be carried out with GTW 1 water.

6.5 Challenge with *L. pneumophila*.

6.5.1 The Hand Shower Filter will be mounted on a test-bed system to allow for the passage of a sufficient volume of GTW1. The units will be positioned in a configuration to allow the challenge water to travel through the devices in the proper orientation.

6.5.2 The test schedule is presented in Table 1. The Hand Shower Filter will be assembled in-line so that the units will be under pressure only when water is flowing through them. For the challenge 1000 ml of GTW1 containing *L. pneumophila* in a concentration of around 10^8 CFU/L is flushed through each filter. A blank sample, the influent and the effluent are sampled and analyzed for the concentration of *L. pneumophila.*

1. TABLE 1. CHALLENGE/SAMPLE SCHEDULE			
Test Point	Test Water Challenges	Influent *L. pneumophila*	Effluent *L. pneumophila*
Day 1 start Flush 10L GTW1 Challenge Flush 100L GTW1 Stagnation min 16 hr Day 2 Flush 400L GTW1 16 h stagnation Day 3 Flush 500L GTW1 16 h stagnation Day 4 – Day 9 Flush 500L GTW1 16 h stagnation	General (GTW 1)	X	X Blank
Day 10 Challenge Flush 500L GTW1 Stagnation min 16 hr Day 11 Flush 500L GTW1 Stagnation min 16 hr		X	X Blank
Day 12 Challenge Flush 500L GTW1 Stagnation min 16 hr		X	X Blank

6.5.3 All samples will be stored at 4°C. Aliquots of the sample will be portioned for analysis. Bacteriological samples will be processed within six hours of collection. Virus samples will be processed within 24 hours of collection, or, preserved and stored for up to three days at 5 ± 3°C.

6.6 If the flow rates decrease by 95% within the 3 day testing period, the last challenge will be performed at that time.B

7. Microbial Assay Techniques.

7.1 Bacteriological Analysis
7.1.1 *L. pneumophila* analysis is done according NEN 6265.

8. Quality Control.

8.1 Bacteriological controls will include:
8.1.1 Positive and negative cultures on all batches of growth media prepared.
8.1.2 Positive and negative culture controls during incubation of samples.

9. Calculations.

9.1 Bacteriological.
9.1.1 Plates will be evaluated for countable colonies (20 to 80 CFU/plate).
9.1.2 Counts will be made under magnification manually.
9.1.3 The number of colonies per plate will be recorded on a worksheet and the mean (average) value from the total of two plates will be determined.
9.1.4 The final number will be the mean value of the two plates multiplied by the appropriate dilution factor.
9.1.5 The final answer will be expressed in CFU/L.
9.1.6 Log reductions are calculated by determining the log10 of the average number in the influent and subtracting the log10 of the average number in the effluent.
9.1.7 "Less than" values are calculated as an average equal to "1" CFU, and log reductions expressed as "greater than" the highest dilution tested.

10 References

10.1 ASTM F838-05; Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration.