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Application Note

Bioburden testing of a difficult-to-filter matrix using EZ-Fit® filtration units and Fluid K as the rinsing solution

Examining pharmaceutical products for microbial contamination is a critical task that comes with pharmaceutical manufacturing. All raw materials and additives must be tested to specifications to ensure product and patient safety.

Membrane filtration is the method of choice for bioburden testing, and the EZ-Fit® filtration units offer a convenient solution. They are disposable filtration devices suitable for testing liquid samples such as water, process samples or final products.

In this study, a difficult-to-filter pharmaceutical product, a nasal spray, was selected for bioburden testing with the EZ-Fit® filtration units after being spiked with contaminant microorganisms. The nasal spray was spiked with known organisms shown in the table to the right. This product contains an antibiotic agent (oxymetazoline hydrochloride) and a preservative, benzalkonium chloride. These compounds can accumulate on the membrane during filtration and possibly inhibit the growth of microorganisms. Several rinsing steps may be needed to remove these compounds.

The aim of the study was to evaluate the efficiency of ready-to-use Fluid K solution versus self-prepared Fluid K solution, when testing a difficult-to-filter matrix using EZ-Fit® filtration units and a panel of different microorganisms (table 3).

Table 1: Filtration device and equipment

Cat.No.	Filtration Unit
EFHAW10MS	EZ-Fit® filtration unit pink (without pad)
EZFITBASE3	EZ-Fit® manifold, 3-place
EZFITMVHE3	EZ-Fit® filtration head for EZ-Fit® filtration units
EZSTREAM1	EZ-Stream® pump

Table 2: Culture media and rinsing fluids

Cat.No.	Culture Media
146431	ReadyPlate™ TSA ISO, FDA-BAM, EP+USP
146075	R2A Agar
146237	Sabouraud Dextrose (4%) Agar – LI acc. EP/USP
STBMRFK34	Fluid K
1.10582	Sodium chloride peptone broth (buffered)

Table 3: Test strains, media, incubation temperature and duration

Strain	Media		Incubation
Staphylococcus aureus ATCC® 6538 (WDCM00193)	TSA	R2A	32.5 °C < 3 d
Bacillus subtilis ATCC® 6633 (WDCM00003)	TSA	R2A	
Pseudomonas aeruginosa ATCC® 9027 (WDCM00026)	TSA	R2A	
Serratia marcescens In-house Strain	TSA	R2A	
Candida albicans ATCC® 10231 (WDCM00054)	TSA	SDA 4%	22.5 °C < 7 d
Aspergillus brasiliensis ATCC® 16404 (WDCM00053)	TSA	SDA 4%	

MERCK

Matrix Nasal spray

The active ingredient, oxymetazoline, and a preservative, benzakonkum chloride, make this a challenging product to filter. These compounds can accumulate on the membrane during filtration, resulting in microbial inhibition or death. This can impact the result of bioburden testing.

Composition: water for injection, oxymetazoline hydrochloride (active agent), citric acid monohydrate, sodium citrate, glycerol 85%, benzalkonium chloride (preservative).

Rinsing solution: Self-prepared NaCl-Peptone with 3% Tween® 80

16.1 g sodium chloride peptone broth (buffered) was dissolved in 800 mL demineralized water (solution 1). 30 mL Tween® 80 was solved in 200 mL dem. water (solution 2). Both solutions were mixed and sterilized. Completely dissolving of Tween® 80 can take up to 5 days.

Rinsing solution: Fluid K

Fluid K is a rinsing solution recommended by the European Pharmacopeia (EP), United States Pharmacopeia (USP) and the Japanese Pharmacopeia (JP) for microbiological examinations by membrane filtration.[1,2,3] Fluid K contains a suitable emulsifying agent and is recommend for rinsing medical devices and samples that are "difficult" to filter or dissolve.

Composition of Fluid K (per liter of purified water): meat peptone (5.0 g), beef extract (3.0 g), Polysorbate 80 (10.0 g).

Method

Filtration was performed according to pharmacopoeia and the microbiological recovery rate determined using the microorganisms specified in the standards. *Serratia marcescens* was used to check the pore size of membrane filters.

The membrane filter was pre-wetted with 50 mL NaCl-Peptone, followed by sample filtration. Samples were prepared under sterile conditions by adding 10 mL nasal spray to each funnel, followed by 90 mL NaCl-Peptone Buffer.

After filtration, the membrane was rinsed with 100 mL of either ready-to-use Fluid K or self-prepared Fluid K. Two rinsing steps with NaCl-Peptone Buffer followed, with the inoculum containing 10 to 100 CFU in 100 µL being added in the last rinsing step.

The membranes were immediately transferred to either TSA, R2A or 4% SDA agar plates that were subsequently incubated (table 3).

Membrane filtration was conducted in triplicate, and the mean CFU was calculated for each filtration unit. Typical colony forming units (CFU) were counted when clearly visible and easy to identify with the naked eye.

Results

The recovery rates were calculated as the mean number of CFUs determined by membrane filtration as a percentage of the mean number of CFUs on the spread plate controls. The product recovery was determined using the method specified in ISO 7704:1985.[4]

PART 1 Using self-prepared Fluid K

Figure 1 shows the recovery rates on the selected agar media after EZ-Fit® membrane filtration of the nasal spray using conventional, self-prepared rinsing solution for rinsing (NaCl-Peptone with 3% Tween® 80). The three strains were selected to give a general impression of the growth conditions for microorganisms.

The recovery rates show that the inhibitory matrix nasal spray ingredients do not accumulate on the membrane if using EZ-Fit® filtration unit with the self-prepared rinsing solution. Both selected agar media deliver recovery rates of 70% or greater, which is according to regulations.

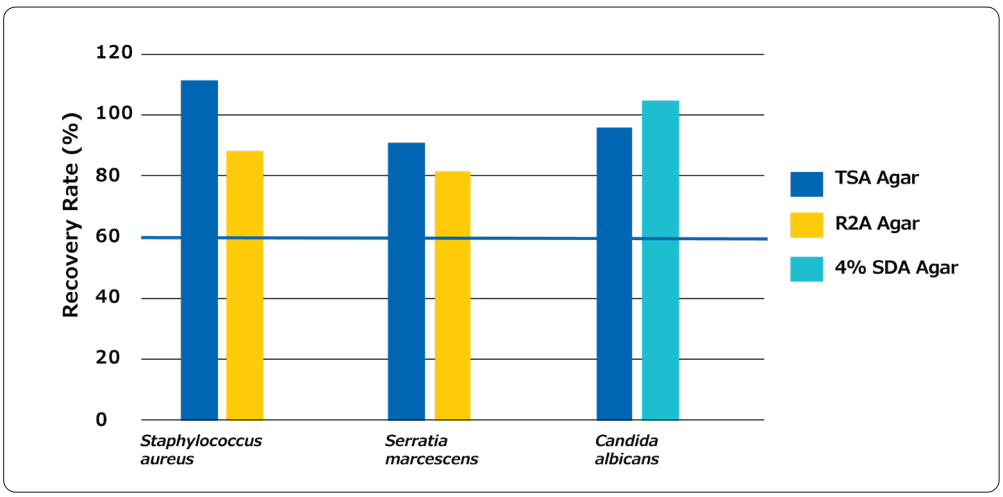

Figure 1. Recovery rate with self-prepared rinsing solution.

PART 2 Using ready-to-use Fluid K

Part 1 showed conforming recovery rates with EZ-Fit® filtration units when using the self-prepared Fluid K. However, dissolving Tween® 80 in NaCl-Peptone broth can take up to five days, causing QC labs to lose valuable time. Using sterile, prepared Fluid K saves the time and cost needed to manually prepare rinse fluids.

Strains of two Gram positive bacteria, two Gram negative ones, and one mold were used to determine recovery rates using Fluid K. *Aspergillus brasiliensis* was chosen as a standard mold species used in QC testing.

Figure 2 illustrates the calculated recovery rates on TSA, R2A and 4% SDA agar when using ready-to-use Fluid K for rinsing. The EZ-Fit® filtration units showed 70% or greater recovery for all organisms upon accurate counting. The morphology of the colonies was typical of the respective strains.

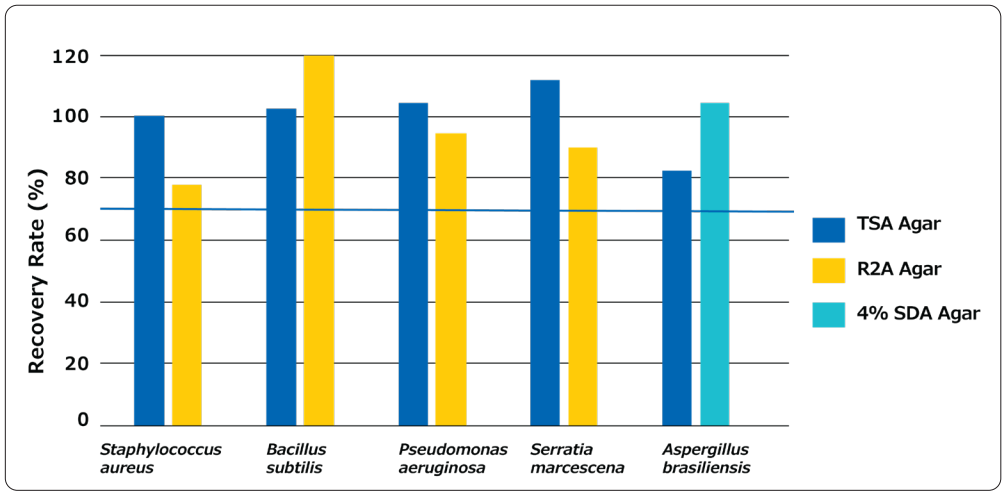

Figure 2. Recovery rate of EZ-Fit® filtration units with ready-to-use Fluid K.

Conclusion

This study demonstrates that EZ-Fit® filtration units achieve conforming recovery rates on TSA, SDA, and R2A agars, even when testing difficult-to-filter matrices such as nasal spray.

In all the filtration experiments (i.e. with the self-prepared Fluid K and with ready-to-use Fluid K) the inhibitory ingredients could be washed away from the membrane filter and false-negative results prevented.

The self-prepared Fluid K and ready-to-use Fluid K showed similar recovery rates.

Using ready-to-use Fluid K has clear advantages: consistent quality, no risk of contamination during preparation and storage, and no loss of time to re-dissolve Tween® 80, which can take up to 5 days. This leads to lower lab costs and time savings without compromising test performance.

Literature

1. European Pharmacopoeia Chapter 2.6.12: Microbiological examination of non-sterile products: Total viable aerobic count
2. U.S. Pharmacopeia <1231> Water for Pharmaceutical Purposes
3. Japanese Pharmacopeia 16th Edition JP Chapter 4.4.2
4. ISO International Standardization Organization: Water quality - Evaluation of membrane filters used for microbiological analyses ISO 7704:1985

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