Biotech

Validation Report

USD3151a

Supor® EKV Membrane in Mini Kleenpak™ 20 Capsules

Part Numbers KM5EKVP2S and KM5EKVP2G

Contents

1 Scope

Supor EKV filter cartridges and capsules have been designed as 0.2 µm rated liquid sterilizing filters for use within the pharmaceutical industry. The Supor EKV filter media is comprised of two layers of polyethersulfone membrane. The coarser asymmetric upstream membrane layer provides built-in prefiltration for the finer downstream sterilizing membrane layer. The validation of larger size Supor filter cartridges and capsules is described in the Pall document USTR 2222a[(1)] "Validation Guide Pall Supor EKV Sterilizing Grade Filters" and substantiates the bacterial retention performance of Supor EKV filter media in a variety of filter styles.

Mini Kleenpak 20 capsules with an effective filter area of 20 cm^2 have been specially designed for the filtration of small volumes. Their small hold-up volume guarantees minimal product loss. Mini Kleenpak 20 capsules with Supor EKV media, part numbers (p/n) KM5EKVP2S and KM5EKVP2G use the same membrane combination as the larger size Supor EKV cartridges and capsules, allowing for direct and fast scale-up.

This report summarizes the validation tests performed on Mini Kleenpak 20 capsule filters with Supor EKV media. The tests aimed to validate the integral sealing of Supor EKV media in Mini Kleenpak 20 capsules, their ability to meet the lot release specifications for fabrication integrity, bacteria retention for *Brevundimonas diminuta (B. diminuta)* (American Type Culture Collection (ATCC♦) 19146) at a minimum challenge level of 10^7 colony forming units (CFU) per cm^2 of effective filtration area, endotoxins and cleanliness, and confirming the suitable strength of the filter capsule by burst testing. Table 1 summarizes the physical dimension and specification for use, while Table 2. summarizes the lot release testing for Mini Kleenpak 20 capsule with Supor EKV media.

Table 1.

Physical dimensions and user specifications for Mini Kleenpak 20 capsules with Supor EKV media

Materials of Construction	Filter media: Hydrophilic polyethersulfone Housing components: Polypropylene
Inlet/Outlet Connectors	¼ in. – ½ in. hose barb
Dimensions	Diameter: 68 mm (2.7 in.) Length: 83 mm (3.3 in.)
Typical Effective Filtration Area	20 cm^2
Sterilization	KM5EKVP2S: Sterilized by gamma irradiation (25-40 kGy) KM5EKVP2G: Provided non-sterile; suitable for irradiation at up to 50 kGy and by autoclave using 1 x 60 min at 125 °C (257 F)
Maximum Operating Pressure and Temperature	1.4 bard at 22 °C (20.0 psid at 71 ºF)
Minimum User Bubble Point	3320 mbar (48.0 psi) when wetted with water

Table 2.

Lot release testing for Mini Kleenpak 20 capsules with Supor EKV media

Fabrication Integrity	Filter samples from this lot successfully passed a bubble point test integrity test as per internal manufacturing specifications.
Bacterial Retention	100% retention of > 10^7 colony forming units/cm^2 *B. diminuta* (ATCC 19146), using procedures described in Pall validation guides and ASTM Standard Test Method F838-15, in conformance with the applicable requirements of the FDA Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (September 2004).
Endotoxins	Meets the current requirement under USP Water for Injection, 0.25 EU/mL, when an aliquot from a soak solution is tested using Limulus Amoebocyte Lysate (LAL) reagent in accordance with USP <85> Bacterial Endotoxins Test.
Cleanliness	Meets with adequate safety margin after flushing, current limits under USP <788> Particulate Matter in Injections, with effluent counts determined microscopically. Counts serve to document conformance with the requirements for a non-fiber-releasing filter per Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 211.72 and 210.3 (b)(6).

Note:

The units of pressure quoted in this document are pounds force per square inch (psi) and bar.

Conversion:

- 1 psi = 0.0689476 bar
- 1 bar = 14.5038 psi

For conversion to pascal (Pa) use:

- 1 bar = 1 x 10^5 Pa
- 1 psi = 6.89476 x 10^3 Pa

2 Summary of Results

The results presented in this validation report confirm that:

- The Mini Kleenpak 20 capsule design maintains the sterilizing grade bacteria performance characteristic of Supor EKV media.
- Mini Kleenpak 20 capsules with Supor EKV media can be integrity tested by means of the integrity test method bubble point wetted with water. Capsules meeting the minimum user bubble point of 3320 mbar (48 psi) provide a sterile filtrate when challenged with > 10^7 CFU of *B. diminuta* per cm^2 effective filtration area.
- Mini Kleenpak 20 capsules with Supor EKV media meet the lot release requirements for endotoxins and effluent cleanliness.
- Mini Kleenpak 20 capsules with Supor EKV media provide a high safety margin for the operating pressure claim of 1.4 barg (20 psig) as they have been found to display a minimum burst pressure of 120 psig (8.27 barg).

3 Fabrication Integrity

3.1 Methods

The correct sealing of the Supor EKV filter media into Mini Kleenpak 20 capsule housings was monitored during manufacturing on a statistical basis, using bubble point testing wetted with water to confirm membrane seal and integrity and ensuring with appropriate safety margin that the minimum user bubble point specification of 3320 mbar (48.0 psi) is met.

For the validation study one hundred-twenty (120) Mini Kleenpak 20 capsules with Supor EKV media from three (3) manufacturing lots were subjected to the manufacturing bubble point test as per the following workflow steps:

- 0.2 µm filtered water was introduced into the upstream side of the Mini Kleenpak 20 capsule using a 60 mL syringe to fully wet the filter media.
- Air present on the upstream side of the capsule was evacuated through the vent valve of the capsule.
- Water was flushed through the filter media for 10-20 seconds at a pressure not greater than 2.1 bar (30.0 psi).
- The capsule was connected to pressurized air via a multi-station manifold with a regulated air source and an automated controlled pressure ramping system with the outlet port facing upwards.
- The air pressure was progressively increased at a rate of 140 mbar (2.0 psi) per second, until bubbling was observed at the outlet port of the capsule. The pressure at which this bubbling appeared was recorded as the bubble point of the capsule.

*Notes of **CAUTION** for filter users when wetting Mini Kleenpak 20 capsules with Supor EKV media:*

- *Systems using automated valves should not be used due to potential for hydraulic shock that can damage the membrane.*
- *Take care to avoid excessive pressure that can be created during hand syringe flushing.*

3.2 Results

The bubble point test results of Mini Kleenpak 20 capsules with Supor EKV media are summarized in Table 3. All filters displayed bubble point test results above the specified minimum user bubble point 3320 mbar (48.0 psi) with appropriate safety margin.

Table 3.

Bubble point (BP) test results for Mini Kleenpak 20 capsules with Supor EKV media

Lot Number	21695103[a]	21695104[a]	21695105[a]	2764[b]	Overall
Number of tested capsules	32	56	32	6	126
Minimum BP value	59 psi (4.10 bar)	53 psi (3.65 bar)	54 psi (3.72 bar)	56 psi (3.86 bar)	53 psi (3.65 bar)
Maximum BP value	70 psi (4.83 bar)	66 psi (4.55 bar)	65 psi (4.48 bar)	61 psi (4.21 bar)	70 psi (4.83 bar)
Average BP value	64 psi (4.41 bar)	61 psi (4.21 bar)	61 psi (4.21 bar)	58 psi (4.00 bar)	62 psi (4.27 bar)
Standard deviation	3 psi (0.207 bar)	2 psi (0.138 bar)	3 psi (0.207 bar)	2 psi (0.138 bar)	3 psi (0.207 bar)
Within specification	Yes	Yes	Yes	Yes	

[a] *S option as supplied irradiated at 25-40 kGy*
[b] *G option after irradiation at 50 kGy*

3.3 Conclusion

The integrity of Mini Kleenpak 20 capsules with Supor EKV media can be assessed by bubble point testing wetted with water applying a minimum user bubble point of 3320 mbar (48.0 psi).

4 Bacterial Retention

4.1 Methods

Eighteen (18) Mini Kleenpak 20 capsules with Supor EKV media from four (4) manufacturing lots were evaluated using liquid bacterial challenge testing with *B. diminuta*. The tests served to confirm that Mini Kleenpak 20 capsules maintain the sterilizing grade filtration abilities of Supor EKV media, i.e., that the capsules will deliver a sterile filtrate when challenged with > 10^7 CFU/cm^2. The challenge tests were carried using Mini Kleenpak 20 capsules with Supor EKV media that had been sterilized by gamma irradiation as per the following workflow steps:

- Suspension of *B. diminuta* was prepared in saline so that a total challenge of more than 10^7 CFU/cm^2 of filter area would be met during the test. A sample of the challenge suspension is taken for challenge concentration verification and cell counts determined.
- The challenge suspension was aseptically poured into a pre-sterilized pressure vessel.
- The test capsule was aseptically connected to the sterile challenge rig.
- The downstream side of the test capsule was connected to a sterile MicroFunnel™ device fitted with a 0.2 µm Supor 200 recovery filter membrane.
- With all valves closed, the vessel was pressurized to 2.0 barg (29.0 psig).
- The challenge suspension was admitted onto the test capsule by partially opening the valve upstream of the test capsule. The test capsule was vented by opening the capsule vent valve under the protection of a disinfectant soaked paper.
- The valve upstream of the test capsule was fully opened and 100 mL of challenge suspension was transferred through the test capsule.

- After the challenge was completed, the test capsule was isolated.
- The MicroFunnel device was set on a vacuum manifold and a 10 inHg vacuum was applied to filter the test capsule effluent. The funnel was rinsed with at least 50 mL of saline.
- The Supor 200 recovery membrane was aseptically removed from the MicroFunnel and placed onto sterile 50 mm tryptic soy agar (TSA) plate.
- All plates, including a negative control plate were incubated at 35 °C ± 1 °C (95 °F ± 1.8 °F) for 48 hours.

4.2 Results

The results of the bacteria retentions testing are shown in Table 4. All tested filters yielded a sterile effluent, thus passing the acceptance criteria of the test.

Table 4.

Results of bacterial challenge test using B. diminuta on Mini Kleenpak 20 capsule with Supor EKV media

Lot Number	Number of Tested Capsules	Challenge Level (CFU/cm$^{2)}$	Downstream Counts	Sterile Effluent
21695103[a]	5	> 10^7	0	Yes
21695104[a]	5	> 10^7	0	Yes
21695105[a]	5	> 10^7	0	Yes
2764[b]	3	> 10^7	0	Yes

[a] *S option as supplied irradiated at 25-40 kGy*

[b] *G option after irradiation at 50 kGy*

4.3 Conclusion

Capsules meeting the minimum user bubble point of 3320 mbar (48 psi) provide a sterile filtrate when challenged with > 10^7 CFU of *B. diminuta* per cm^2 of effective filtration area. These results confirm that the Mini Kleenpak 20 capsule design maintains the sterilizing grade bacteria performance characteristic of Supor EKV media.

5 Endotoxins

5.1 Methods

Fifteen (15) Mini Kleenpak 20 capsules with Supor EKV media from three (3) manufacturing lots were subjected to endotoxin testing as per the following workflow steps:

- Each capsule was flushed with twenty (20) mL of pyrogen-free deionized (DI) water and the effluent collected.
- The effluents of the five (5) capsules from one (1) lot were pooled to get a total of 100 mL.
- An aliquot of the pool was tested using a kinetic turbidimetric Limulus Amebocyte Lysate (LAL) test with a test sensitivity of 0.1 endotoxin units (EU)/mL.

5.2 Results

Table 5 shows the results of the endotoxin testing.

Table 5.

Endotoxin test results of pooled effluent determined by LAL testing

Lot Number	Number of Tested Capsules	Endotoxin Units in Effluent Pool (EU/mL)	Endotoxin Levels Meeting Specification
21695103	5	< 0.1	Yes
21695104	5	< 0.1	Yes
21695105	5	< 0.1	Yes

5.3 Conclusion

These results confirm that Mini Kleenpak 20 capsules with EKV media meet the lot release requirements for endotoxins.

6 Cleanliness

6.1 Methods

Nine (9) Mini Kleenpak 20 capsules with Supor EKV membrane from three (3) manufacturing lots were subjected to effluent cleanliness testing as per the following workflow steps:

Each capsule was flushed with one (1) liter of sterile DI water at a flow rate of approximately 200 mL/min and the effluent passed through a 47 mm diameter Metricel® black analytical membrane, installed in a disc holder to collect particles and fibers that could have been released from the tested capsule.

- The analytical membrane was removed from the disc holder and dried.
- The analytical membrane was examined under incident light using a microscope with a 10-fold magnification, counting all particles > 5 µm and fibers over the entire effective filter area using counting techniques as per applicable Pall procedure.

6.2 Results

The results of cleanliness testing are shown in Table 6.

Table 6.

Effluent cleanliness test results

Lot Number	Number of Tested Capsules	Counts for > 5 µm Particles and Fibers Meeting Specifications
21695103	3	Yes
21695104	3	Yes
21695105	3	Yes

6.3 Conclusion

These results confirm that Mini Kleenpak 20 capsules with Supor EKV media meet the lot release requirements for effluent cleanliness.

7 Burst Strength

7.1 Methods

This test assessed the structural strength of Mini Kleenpak 20 capsules with Supor EKV media when exposed to high pressure and thus support their operating pressure claim of 1.4 barg (20 psig) at 22 °C (71 °F). Gamma irradiation may weaken a polymeric material, such as polypropylene that is used for the capsule housing. Hence burst pressure testing was executed on capsules exposed to the maximum specified dose (50 kGy) for p/n KM5EKVP2G, presenting worst case conditions. Six (6) Mini Kleenpak 20 capsules with Supor EKV media from one lot were submitted to burst testing after exposure to gamma irradiation at a dose of 50 kGy as per the following workflow steps:

- The capsule was connected to a high gas pressure source and all ports closed.
- The inner volume of the capsule was exposed to increasing gas pressure until pressure loss occurs.
- The point of pressure loss was recorded as burst pressure.

7.2 Results

Table 7 shows the results of burst testing. All capsules displayed a minimum burst pressure of 120 psig (8.27 barg).

Table 7.

Burst testing results pressure

Capsule Lot Number	**2764**
Number of tested capsules	6
Minimum burst pressure	120 psig (8.27 barg)
Maximum burst pressure	140 psig (9.65 barg)
Average burst pressure	128 psig (8.83 barg)
Standard deviation	10 psi (0.689 bar)
Within specification?	Yes

7.3 Conclusion

All capsules displayed a minimum burst pressure of 120 psig (8.27 barg). These results confirm that the burst strength of Mini Kleenpak 20 capsules with EKV media provide a high safety margin for the operating pressure claim of 1.4 barg (20 psig).

8 Transmissible Spongiform Encephalopathy (TSE) / Bovine Spongiform Encephalopathy (BSE) Statement

Mini Kleenpak 20 capsules with Supor EKV media do not contain materials of construction that are considered TSE risk materials according to current legislation and guidance in both Europe and the United States:

1. The European CPMP Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathies via human and veterinary medicinal products. (EMA/410/01 Rev.3, applicable from 1st July 2011).

2. The U.S. Code of Federal Regulations, Title 9 of part 94.18, which sets forth restrictions on the geographical sourcing of products obtained from ruminants.
3. The U.S. Code of Federal Regulations, Title 21 of part 189.5, which defines specified risk materials obtained from cattle.

Pall has an established program with our raw material suppliers to assess whether animal derived products (e.g., bovine/ovine/caprine) are present in the materials employed for our pharmaceutical grade products. We have identified that polypropylene resins, used to manufacture plastic components of the referenced products, contain trace levels of additives, which may be derived from bovine tallow. Tallow derivatives are not considered specified BSE risk materials according to the current revision of Title 21, of the U.S. Code of Federal Regulations, part 189.5. Furthermore, the CPMP's Note for guidance (EMA 410/01) gives specific consideration to tallow derivatives and states they are unlikely to be infectious due to the rigorous processing steps used (an example of which is trans-esterification, or hydrolysis, at not less than 200 °C (392 °F) under pressure for not less than 20 minutes). The raw materials we purchase have been processed with these steps. Additionally, during the conversion of polypropylene resin into plastic components further high temperature steps are performed.

9 Sample Pharmaceutical Certificate of Test

Each Mini Kleenpak 20 capsule with Supor EKV media is supplied with a Certificate of Test confirming pharmaceutical industry requirements for biological safety testing, fabrication integrity, and effluent quality. The content of the Certificates of Tests for Mini Kleenpak 20 capsules with Supor EKV media p/n KM5EKVP2G and KM5EKVP2S are shown below.

9.1 Sample Certificate of Test for P/N KM5EKVP2G

We hereby certify that

Pall® **SUPOR® EKV MEMBRANE IN MINI KLEENPAK™ 20 FILTER CAPSULE**

Rated: **0.2 µm**

Part Number: **KM5EKVP2G**

Lot Number: (…)

was manufactured in a controlled environment. **These filters are not supplied sterile.**

Fabrication Integrity
Filter samples from this lot successfully passed a bubble point integrity test as per internal manufacturing specifications. The bubble point test parameters have been validated for bacterial removal by correlation with a microbiological challenge test. Recommended test values for integrity testing of Pall filters as installed must be obtained from Pall.

Bacterial Retention
Finished product has been sampled and successfully tested for retention of *Brevundimonas diminuta* (ATCC 19146), using procedures described in Pall Validation Guides and ASTM Standard Test Method F838-15, in conformance with the applicable requirements of the FDA **Guidance for Industry**: **Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice** (September 2004).

Materials of Construction
Representative filter components have met the requirements for biological reactivity, *in vivo*, under USP <88> for Class VI - 121°C plastics and *in vitro*, under USP <87> (Elution Cytotoxicity).

This product does not contain materials of construction that are considered specified TSE or BSE risk materials according to current legislation and guidelines (reference European CPMP EMA/410/01 and Title 21 of the U.S. Code of Federal Regulations (CFR), part 189.5).

Effluent Quality
Filter samples from this lot underwent the following tests and the lot was released by Quality Control when it was verified that their respective criteria were met:

Cleanliness
Meets with adequate safety margin after flushing, current limits under USP <788> Particulate Matter in Injections, with effluent counts determined microscopically. Counts serve to document conformance with the requirements for a non-fiber-releasing filter per Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 211.72 and 210.3 (b)(6).

Endotoxins
Meets the current requirement under USP Water for Injection, 0.25 EU/ml, when an aliquot from a soak solution is tested using Limulus Amoebocyte Lysate (LAL) reagent in accordance with USP <85> Bacterial Endotoxins Test.

In addition to the above tests, this product met manufacturing inspection standards and requirements for full traceability. This product is manufactured under a Quality System certified to ISO 9001. Consider only unopened, undamaged packages for use. Further information is available by contacting Pall.

9.2 Sample Certificate of Test for P/N KM5EKVP2S

We hereby certify that

Pall® **SUPOR® EKV MEMBRANE IN MINI KLEENPAK™ 20 FILTER CAPSULE**

Rated: **0.2 µm**

Part Number: **KM5EKVP2S**

Lot Number: (…)

was manufactured in a controlled environment. **These filters are supplied sterile after gamma irradiation.**

Fabrication Integrity

Filter samples from this lot successfully passed a bubble point test integrity test as per internal manufacturing specifications. The bubble point test parameters have been validated for bacterial removal by correlation with a microbiological challenge test. Recommended test values for integrity testing of Pall filters as installed must be obtained from Pall.

Bacterial Retention

Finished product has been sampled and successfully tested for retention of *Brevundimonas diminuta* (ATCC 19146), using procedures described in Pall Validation Guides and ASTM Standard Test Method F838-15, in conformance with the applicable requirements of the FDA **Guidance for Industry**: **Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice** (September 2004).

Materials of Construction

Representative filter components have met the requirements for biological reactivity, *in vivo*, under USP <88> (for Class VI - 121°C plastics) and *in vitro*, under USP <87> (Elution Test).

This product does not contain materials of construction that are considered specified TSE or BSE risk materials according to current legislation and guidelines (reference European CPMP EMA/410/01 and Title 21 of the U.S. Code of Federal Regulations (CFR), part 189.5).

Effluent Quality

Filter samples from this lot underwent the following tests and the lot was released by Quality Control when it was verified that their respective criteria were met:

Cleanliness
Meets with adequate safety margin after flushing, current limits under USP <788> Particulate Matter in Injections, with effluent counts determined microscopically. Counts serve to document conformance with the requirements for a non-fiber-releasing filter per Title 21 of the U.S. **Code of Federal Regulations** (CFR) parts 211.72 and 210.3 (b)(6).

Endotoxins
Meets the current requirement under USP Water for Injection, 0.25 EU/ml, when an aliquot from a soak solution is tested using Limulus Amoebocyte Lysate (LAL) reagent in accordance with USP <85> Bacterial Endotoxins Test.

In addition to the above tests, this product met manufacturing inspection standards and requirements for full traceability. This product is manufactured under a Quality System certified to ISO 9001. Consider only unopened, undamaged packages for use. Further information is available by contacting Pall.

Corporate Headquarters
Port Washington, NY, USA
+1.800.717.7255 toll free (USA)
+1.516.484.5400 phone

European Headquarters
Fribourg, Switzerland
+41 (0)26 350 53 00 phone

Asia-Pacific Headquarters
Singapore
+65 6389 6500 phone

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