



Validation Guide

LeClafine[®] Depth Filters

A light gray silhouette of a city skyline, including the Oriental Pearl Tower, is positioned at the bottom of the page.

Shanghai LePure Biotech Co., Ltd.

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1. Effluent Quality

The performance of LeClafine® depth filters must meet the requirements of the U.S. Pharmacopeia. In this guide, we will conduct USP testing on the flush of the filtration media, as shown in Table 1.

Table 1 Test items and reference standards

Regulations and Standards	Test items
USP<643>	Total Organic Carbon (TOC)
USP<645>	Conductivity
USP<791>	pH
USP<232>/<233>/ICH* Q3D	Elemental Impurities
USP<788>	articulate Matter
USP<85>	Bacterial Endotoxins

The LeClafine® depth filters are produced in-house by LePure® in-house factories with traceable raw materials and processing. 5 randomly selected samples from the batches of filter media in this validation guide were tested, and all tests were conducted at ambient temperature and pressure, if not otherwise specified.

1.1 USP<643> Total Organic Carbon (TOC)

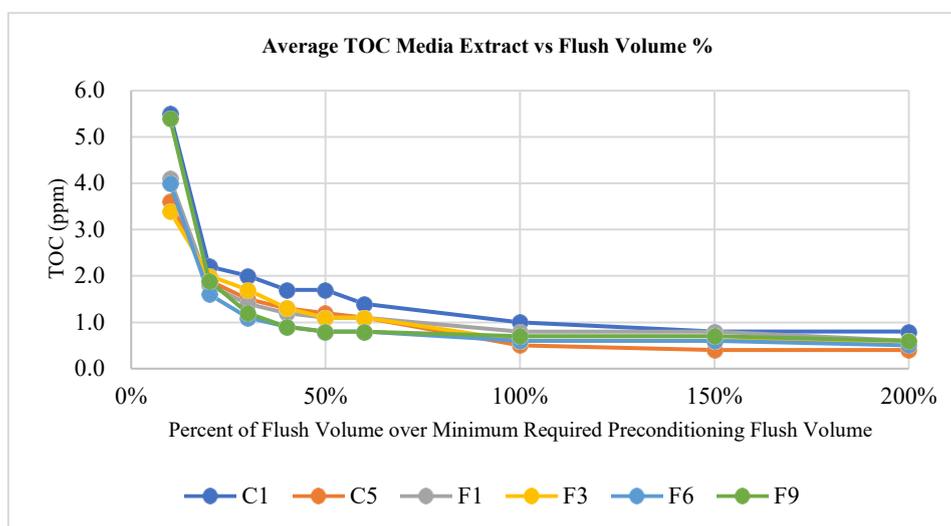
The 47-mm discs of LeClafine® media C1、C5、F1、F3、F6、F9 made at manufacturing facilities were flushed with 18 Megohm water (25°C) at a flux of 1200 LMH to a total volume of two times of minimum required preconditioning flush volume of 54 L/m2. Filtrate samples were collected at 10%, 20%, 30%, 40% et al at 10% increment to 200% of the preconditioning flush volume. The samples were then analyzed for total organic carbon (TOC).

The TOC data at selected preconditioning flush volume percentages are shown in Table 2, 3 and Figures 1, 2. After the minimum required preconditioning flush volume of 54 L/m2, the extractable TOC levels of all tested LeClafine® media were less than 3 ppm.

Table 2 TOC of LeClafine® Media Extract [ppm]

Media Grade		Flush Vol %								
		10%	20%	30%	40%	50%	60%	100%	150%	200%
C1	Avg	5.5	2.2	2.0	1.7	1.7	1.4	1.0	0.8	0.8
	Max	9.7	5.2	4.9	4.3	3.9	3.4	2.2	1.8	1.5
	Min	4.5	1.4	1.2	1.0	1.2	0.8	0.7	0.6	0.7
C5	Avg	3.6	1.9	1.5	1.3	1.2	1.1	0.5	0.4	0.4
	Max	4.7	2.5	2.1	1.8	1.5	1.5	1.0	0.9	0.9
	Min	3.3	1.7	1.4	1.1	1.1	1.0	0.4	0.3	0.2
F1	Avg	4.1	1.8	1.4	1.2	1.1	1.1	0.8	0.8	0.6
	Max	7.3	3.5	3.1	2.7	2.5	2.3	2.1	2.1	1.8
	Min	3.3	1.3	1.0	0.8	0.7	0.8	0.4	0.4	0.2
F3	Avg	3.4	2.0	1.7	1.3	1.1	1.1	0.6	0.6	0.6
	Max	7.8	6.1	5.3	3.9	3.3	3.5	1.8	1.9	1.5
	Min	2.2	0.9	0.8	0.6	0.5	0.4	0.3	0.2	0.4
F6	Avg	4.0	1.6	1.1	0.9	0.8	0.8	0.6	0.6	0.5
	Max	5.9	2.9	2.0	1.5	1.2	1.4	0.9	1.0	0.5
	Min	3.4	1.2	0.8	0.8	0.6	0.6	0.5	0.5	0.5
F9	Avg	5.4	1.9	1.2	0.9	0.8	0.8	0.7	0.7	0.6
	Max	6.4	2.6	1.6	1.1	1.0	0.8	0.9	0.8	0.6
	Min	5.1	1.8	1.1	0.9	0.5	0.7	0.6	0.6	0.5

Fig. 1 Average TOC of Media Extract vs Flush Volume %

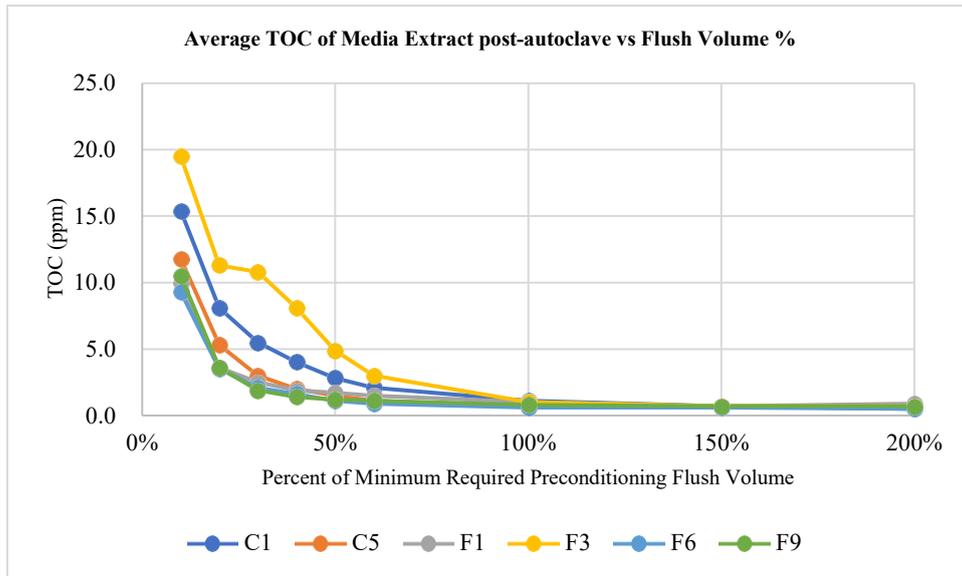


Filters were autoclaved for 60 minutes at 126 °C and then flushed and sampled by the same procedure for TOC test mentioned above. The samples were then analyzed for total organic carbon (TOC). The extractable TOC levels of all tested LeClafine® media were less than 3 ppm.

Table 3 Average TOC of LeClafine® Media Extract post-autoclave [ppm]

Media Grade	Flush Vol %								
	10%	20%	30%	40%	50%	60%	100%	150%	200%
C1	15.4	8.1	5.5	4.0	2.8	2.1	1.1	0.7	0.5
C5	11.8	5.3	3.0	2.0	1.5	1.1	0.7	0.7	0.6
F1	10.0	3.6	2.5	1.9	1.7	1.5	1.0	0.7	0.9
F3	19.5	11.3	10.8	8.1	4.9	3.0	1.0	0.7	0.7
F6	9.3	3.5	2.1	1.6	1.1	0.9	0.6	0.6	0.5
F9	10.5	3.6	1.9	1.4	1.2	1.1	0.8	0.7	0.7

Fig. 2 Average TOC of Media Extract post-autoclave vs Flush Volume %



1.2 USP<645> Conductivity

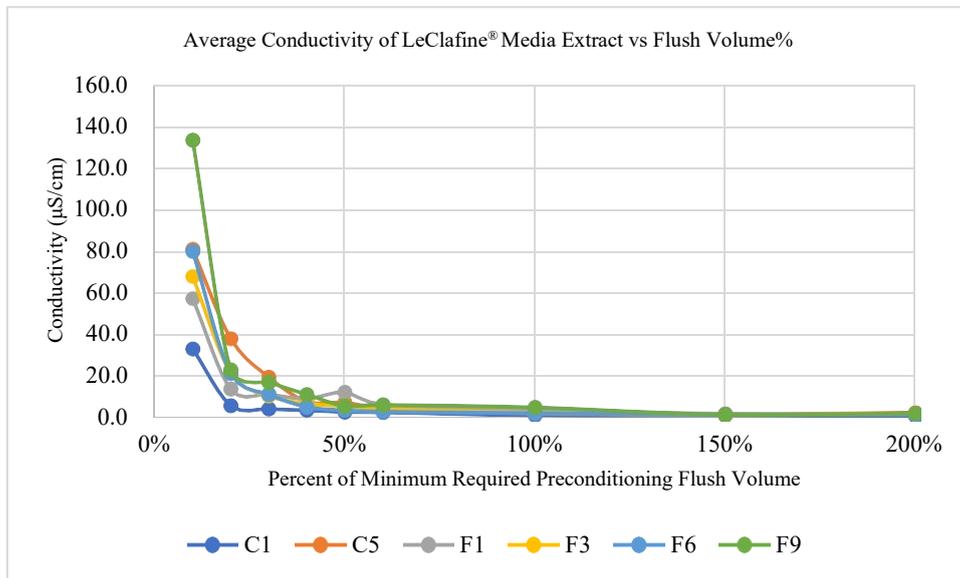
The 47-mm discs of LeClafine® media C1, C5, F1, F3, F6, F9 made at manufacturing facilities were flushed with 18 Megohm water (25°C) at a flux of 1200 LMH to a total volume of two times of minimum required preconditioning flush volume of 54 L/m². Filtrate samples were collected at 10%, 20%, 30%, 40% et al at 10% increment to 200% of the preconditioning flush volume. The samples were then analyzed for conductivity.

The conductivity data at selected preconditioning flush volume percentages are shown in Table 4 and Figures 3. After the minimum required preconditioning flush volume of 54 L/m², the conductivity of all tested LeClafine® media were less than 6 µs/cm and 3 µs/cm in 200% of the preconditioning flush volume.

Table 4 Conductivity of LeClafine® Media Extract [$\mu\text{S}/\text{cm}$]

Media Grade		Flush Vol %								
		10%	20%	30%	40%	50%	60%	100%	150%	200%
C1	Avg	33.5	6.3	4.7	4.0	3.2	3.0	1.6	1.5	1.3
	Max	33.8	6.5	4.7	4.0	3.2	3.0	1.6	1.5	1.3
	Min	33.2	6.2	4.7	4.0	3.2	3.0	1.6	1.5	1.3
C5	Avg	81.2	38.4	19.9	8.2	7.8	5.2	2.5	2.1	2.9
	Max	81.5	38.5	19.9	8.3	8.0	5.3	2.5	2.1	3.0
	Min	81.0	38.4	19.8	8.1	7.7	5.0	2.4	2.1	2.8
F1	Avg	57.6	14.2	11.7	9.6	12.7	6.7	4.2	2.4	2.1
	Max	57.9	14.2	12.5	9.6	13.2	6.7	4.3	2.4	2.2
	Min	57.2	14.2	11.1	9.6	12.4	6.7	4.2	2.4	2.1
F3	Avg	68.1	21.8	11.0	7.2	6.0	4.6	2.8	1.8	2.4
	Max	68.2	21.9	11.0	7.2	6.0	4.6	2.8	1.8	2.4
	Min	68.0	21.7	11.0	7.1	6.0	4.6	2.7	1.7	2.3
F6	Avg	80.2	21.7	11.6	5.6	4.0	3.3	2.6	1.9	1.9
	Max	80.4	21.8	11.6	5.7	4.0	3.4	3.0	2.0	2.3
	Min	80.1	21.7	11.5	5.6	3.9	3.3	2.4	1.9	1.3
F9	Avg	133.5	23.5	17.4	11.6	6.1	6.6	5.4	1.9	2.5
	Max	133.7	23.5	17.9	12.1	6.6	6.8	5.4	2.0	2.7
	Min	133.4	23.3	16.5	10.5	5.8	6.2	5.3	1.9	2.1

Fig. 3 Average conductivity of Media Extract vs Flush Volume %



1.3 USP<791> pH

The 47-mm discs of LeClafine® media C1、C5、F1、F3、F6、F9 made at manufacturing facilities were flushed with 18 Megohm water (25°C) at a flux of 1200 LMH to a total volume of

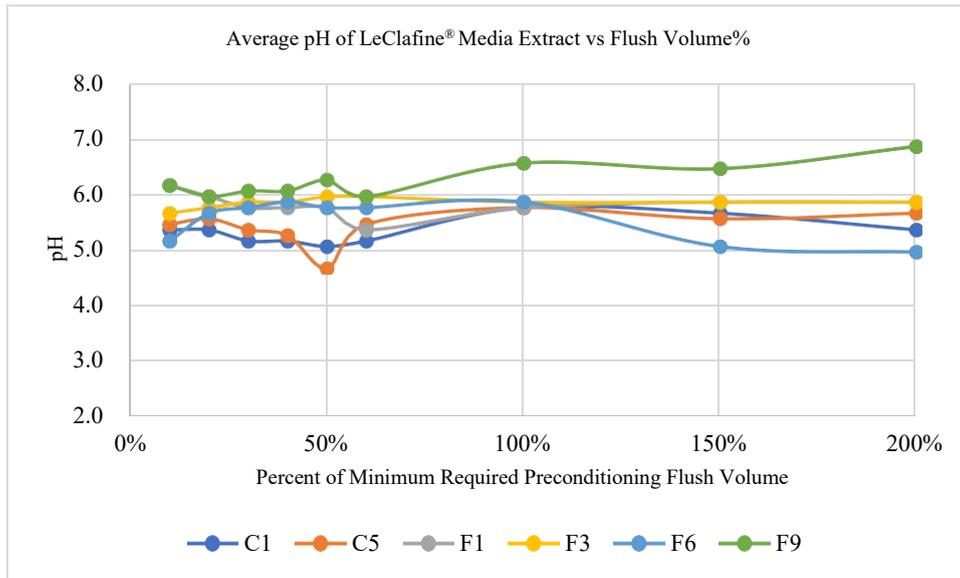
two times of minimum required preconditioning flush volume of 54 L/m². Filtrate samples were collected at 10%, 20%, 30%, 40% et al at 10% increment to 200% of the preconditioning flush volume. The samples were then measured for pH.

The pH data at selected preconditioning flush volume percentages are shown in Table 5 and Figures 4. After the minimum required preconditioning flush volume of 54 L/m², the extractable pH levels of all tested LeClafine[®] media were in the range of 5.8 to 6.6.

Table 5 pH of LeClafine[®] Media Extract

Media Grade		Flush Vol %								
		10%	20%	30%	40%	50%	60%	100%	150%	200%
C1	Avg	5.4	5.4	5.2	5.2	5.1	5.2	5.8	5.7	5.4
	Max	5.4	5.5	5.3	5.2	5.3	5.3	5.9	5.7	5.4
	Min	5.4	5.4	5.2	5.2	5.1	5.2	5.7	5.7	5.3
C5	Avg	5.5	5.6	5.4	5.3	4.7	5.5	5.8	5.6	5.7
	Max	5.5	5.6	5.4	5.5	4.8	5.5	5.9	5.7	5.8
	Min	5.5	5.6	5.4	5.1	4.5	5.4	5.7	5.6	5.7
F1	Avg	6.2	6.0	5.8	5.8	5.8	5.4	5.8	5.9	5.9
	Max	6.3	6.1	5.9	5.9	6.0	5.5	5.8	5.9	6.0
	Min	6.2	6.0	5.8	5.7	5.7	5.4	5.8	5.8	5.8
F3	Avg	5.7	5.8	5.9	5.9	6.0	6.0	5.9	5.9	5.9
	Max	5.8	5.9	5.9	6.0	6.0	6.2	6.2	6.1	6.0
	Min	5.7	5.8	5.8	5.8	5.9	5.8	5.8	5.7	5.8
F6	Avg	5.2	5.7	5.8	5.9	5.8	5.8	5.9	5.1	5.0
	Max	5.4	5.7	5.9	6.0	5.9	6.0	6.0	5.1	5.1
	Min	5.2	5.7	5.7	5.8	5.8	5.7	5.8	5.1	4.9
F9	Avg	6.2	6.0	6.1	6.1	6.3	6.0	6.6	6.5	6.9
	Max	6.3	6.2	6.3	6.2	6.5	6.1	6.9	6.5	7.0
	Min	6.1	5.8	6.0	6.0	6.3	5.9	6.4	6.4	6.7

Fig. 4 Average pH of Media Extract vs Flush Volume %



1.4 USP<85> Bacterial endotoxin

The 47-mm discs of LeClafine® media C1、C5、F1、F3、F6、F9 made at manufacturing facilities were flushed with Sterile Water for Injection (SWFI) at a flux of 900 LMH to a total volume of 100% of the minimum required preconditioning volume. The filtrate samples collected at the end of flush were analyzed for the test of extractable endotoxin concentration by LAL reactivity method.

The LeClafine® filter media tested met the bacterial endotoxin limits for PFW/WFI of ≤ 0.25 EU/mL, as shown in Table 6.

Table 6 Extractable Endotoxin of LeClafine® Media [EU/mL]

Media Grade	Extractable Endotoxin [EU/mL]	Acceptance [EU/mL]
C1	<0.125	<0.25
C5	<0.125	<0.25
F1	<0.125	<0.25
F3	<0.125	<0.25
F6	<0.125	<0.25
F9	<0.125	<0.25

1.5 USP<788> Particulate matter

The 47-mm discs of LeClafine® media C1、C5、F1、F3、F6、F9 made at manufacturing facilities were flushed with 18 Megohm water (25°C) at a flux of 1200 LMH to a total volume of two times of minimum required preconditioning flush volume of 54 L/m². Filtrate samples were collected at 33%, 66%, 100%, 200% of the preconditioning flush volume. Media discs were then soaked in DI water for one hour. The samples were then analyzed for Particulate Matter.

The particle counts in the size ranges of 10-25 and ≥ 25 micron are shown in Table 7 and Figure 5, 6. The tests showed that minimum required preconditioning flush of the LeClafine® filter media reduced the particulate matter of effluent.

Table 7 Particulate Matter of LeClafine® Media Extract (Average Cumulative Counts per ml - Control Corrected) [#/mL]

Media Grade	Particle size		Flush Vol %				
			33%	66%	100%	200%	Soak
C1	>10 μ m	Avg	9	1.5	0.9	0.2	0.7
		Max	18.6	3	1.8	0.6	1.2
		Min	1.6	0.4	0	0	0
	>25 μ m	Avg	0.1	0	0	0	0
		Max	0.6	0	0	0	0
		Min	0	0	0	0	0
C5	>10 μ m	Avg	98.8	18.8	9.4	8.2	11.4
		Max	146.2	26.2	16.8	11.2	17.2
		Min	56.8	12.2	3.2	3.4	6.8
	>25 μ m	Avg	2.1	0.1	0	0	0.2
		Max	3.2	0.4	0.2	0.2	0.8
		Min	0.2	0	0	0	0
F1	>10 μ m	Avg	71.4	9.6	10.5	13.9	10.5
		Max	130	12	17.4	20.2	15.8
		Min	15.6	6.6	5.2	8.6	5.8
	>25 μ m	Avg	4.3	1.2	0.2	0.4	0.1
		Max	14	7.3	0.6	1.8	0.6
		Min	0.2	0	0	0	0
F3	>10 μ m	Avg	153.3	20.8	20.4	13.6	28
		Max	218.6	27.6	32.2	20.6	48
		Min	108	15	9.8	6	7.6
	>25 μ m	Avg	5.7	0.1	0.4	1	0.7
		Max	11	0.6	1.2	3.6	1.6
		Min	4	0	0	0	0
F6	>10 μ m	Avg	195.8	49.8	11.1	3.6	10.2
		Max	287.6	66	13	6	14.6
		Min	117.4	29.4	7.4	2	8
	>25 μ m	Avg	3.3	0.3	0	0	0
		Max	5.2	0.6	0.2	0	0.2
		Min	1.4	0	0	0	0
F9	>10 μ m	Avg	157.9	26	33.5	11	6.5
		Max	216.6	58.6	86.2	26	8.6
		Min	87	12	3	1.2	3.8
	>25 μ m	Avg	5.6	5	0.9	0.2	0
		Max	13.1	23.4	2	0.6	0.2
		Min	1.6	0	0	0	0

Fig. 5 Particulate Matter of Media Extract vs Flush Volume %

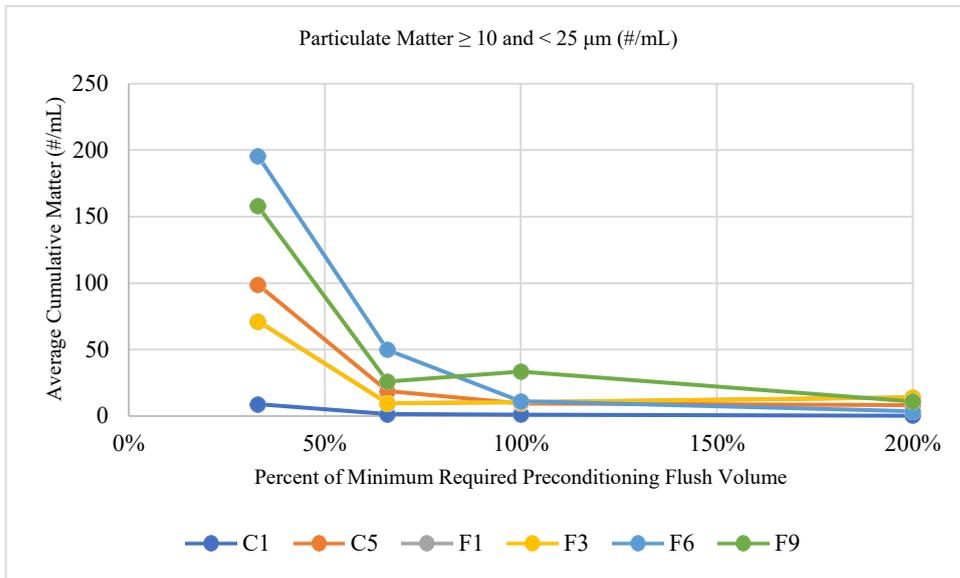
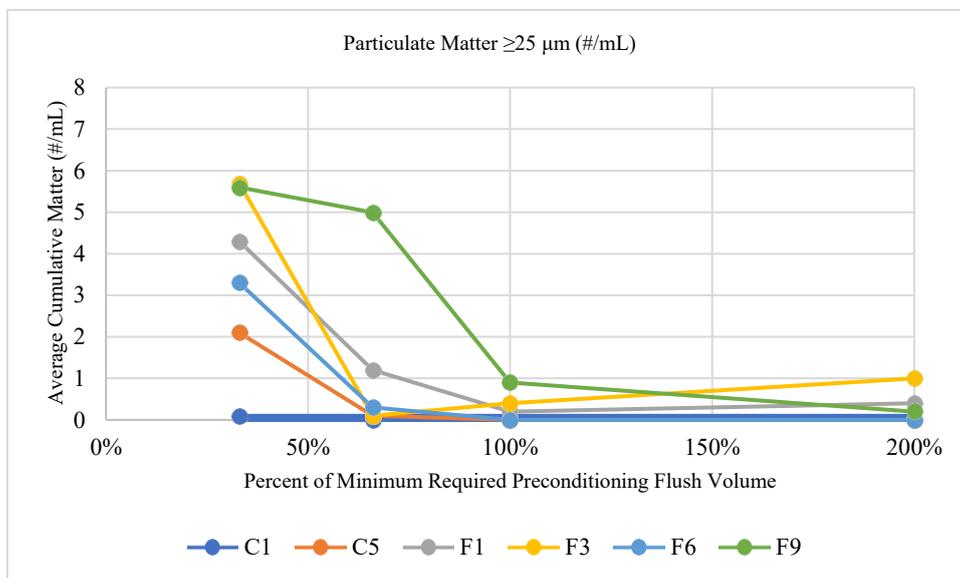


Fig. 6 Particulate Matter of Media Extract vs Flush Volume %



1.6 Non-volatile residue (NVR)

The 60-mm discs of LeClafine[®] media C1, F3, F9 made at manufacturing facilities were flushed with 18 Megohm water (25°C) at a flux of 1200 LMH to a total volume of two times of minimum required preconditioning flush volume of 54 L/m². Filtrate samples were collected at 33%, 66%, 100%, 200% of the preconditioning flush volume. Media discs were then soaked in DI water for one hour. The samples were then analyzed for Particulate Matter.

Table 8 Non-Volatile Residues (NVR) of LeClafine® media C1, F3, F9

Extract [mg/L]

Flush volume [%]	C1			F3			F9		
	No. of Lots: 5			No. of Lots: 5			No. of Lots: 5		
	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min
33%	5	7	3	10	14	6	19	27	9
68%	4	6	2	8	12	5	9	19	3
100%	5	13	2	10	16	4	10	25	3
200%	9	15	3	5	9	1	16	27	9

1.7 USP<232>/<233> and ICH Q3D Elemental Impurity Levels

The 47-mm discs of LeClafine® media C1、F3、F9 made at manufacturing facilities were flushed with 18 Megohm water (25°C) at a flux of 1200 LMH to a total volume of two times of minimum required preconditioning flush volume of 54 L/m2. Filtrate samples were collected at 10%, 20%, 30%, 40% et al at 10% increment to 200% of the preconditioning flush volume. The samples of 10%, 100% and 200% extract samples were then analyzed.

Table 9 Extractable Elemental Impurities of LeClafine® media C1、F3、F9 Extract [ppb]

ICH Class	elemental	Method LOQ [ppb]	Instrument LOQ [ppb]	C1			F3			F9		
	冲洗量 Vol%	Flush Volume%		10%	100%	200%	10%	100%	200%	10%	100%	200%
1	As	10	0.006	ND	ND	ND	0.291	0.044	0.021	0.151	0.015	0.015
	Pb	10	0.006	0.071	0.673	0.023	0.039	0.024	0.078	0.036	0.035	0.045
	Cd	10	0.0007	0.005	0.002	ND	0.091	0.015	0.004	0.003	0.002	ND
	Hg	5	0.003	ND	ND	ND	ND	ND	ND	ND	ND	ND
2A	V	10	0.04	0.044	0.018	0.003	98.903	17.771	8.805	66.772	15.091	8.976
	Ni	10	0.008	22.552	2.147	1.041	0.010	ND	ND	ND	ND	ND
	Co	10	0.002	0.642	0.071	0.029	0.012	ND	ND	0.016	ND	ND
2B	Ag	10	0.009	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Au	10	0.008	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Tl	10	0.0003	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Pd	10	0.005	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Pt	10	0.0006	0.007	0.005	0.004	0.001	ND	ND	0.001	ND	ND
	Ir	10	0.0002	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Os	10	0.003	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Rh	10	0.00003	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Ru	10	0.002	0.001	ND	ND	ND	ND	ND	ND	ND	ND
	Se	10	0.118	ND	ND	ND	0.156	0.215	0.058	0.315	0.103	ND
3	Sb	10	0.005	ND	0.031	ND	0.104	0.021	0.023	0.166	0.080	0.052
	Ba	10	0.003	0.620	0.955	0.061	0.462	0.107	0.070	0.254	0.037	0.159
	Li	10	0.002	0.307	0.252	0.233	0.864	0.298	0.234	0.671	0.244	0.205
	Cr	10	0.01	0.046	0.153	0.004	0.147	0.041	0.011	0.040	0.027	0.002
	Cu	10	0.002	0.261	0.212	0.035	0.051	0.067	0.027	0.076	0.063	0.207
	Mo	10	0.001	0.061	0.024	0.022	12.844	2.050	1.065	0.866	0.110	0.049
Others	Sn	10	0.04	ND	ND	ND	ND	ND	ND	ND	ND	ND
	B	10	0.03	0.074	ND	ND	ND	ND	ND	1.071	ND	ND
	Fe	10	0.06	48.671	10.674	1.443	1.092	1.210	0.272	4.626	0.626	0.450
	Zn	10	0.03	4.339	5.411	2.123	3.045	2.418	2.177	2.868	2.584	2.790
	Mn	10	0.01	0.642	0.279	0.068	0.848	0.197	0.112	0.912	0.055	0.122
	Mg	500	0.04	25.858	9.933	3.147	22.452	6.244	4.584	64.768	7.513	15.012
	W	10	0.0009	ND	ND	ND	0.194	0.033	0.019	0.066	ND	ND
	Al	500	0.322	4.265	2.174	2.009	12.300	3.186	2.248	13.535	3.177	3.589
	Ti	10	0.04	0.079	ND	ND	ND	ND	ND	0.138	0.059	0.020
Sr	10	0.008	1.025	0.296	0.076	0.479	0.150	0.076	0.423	0.037	0.062	

2. Chemical compatibility

To ensure that the filter can be used in different application scenarios, it is necessary to test the chemical compatibility of LeClafine® filter media. The testing conditions are: soaking the filter media in a chemical solution for 24 hours at 20°C, and analyzing the wet strength and water flow rate under constant pressure. As shown in Table 10, R represents tolerance, L represents partial tolerance, and N represents intolerance. In addition, the chemical compatibility is affected by various factors such as temperature, concentration, and pressure. If necessary, compatibility test can be conducted before actual use.

Table 10 Chemical compatibility table for filter media

	Fluids	Concentration	Temperature	Results
Acids	Hydrochloric Acid	5%	20°C	R
	Sulfuric Acid	10%		R
	Phosphoric Acid	10%		R
	Citric Acid	15%		R
	Acetic Acid	30%		R
	Iacial Acetic Acid	99%		R
Ikali	Potassium Hydroxide	2%		R
	Sodium Hydroxide	2%		R
Alcohol	Ethanol	95%		R
	N-Butanol	80%		R
	Isopropyl Alcohol	99%		R
Esters	Ethyl Acetate	99%		R
	Butyl Acetate	99%		R
Aromatic Hydrocarbons	Benzene	99%		R
	Toluene	99%		R
Others	Acetone	99%		R
	Dimethyl Sulfoxide	99%	R	

3. Compliance

3.1 USP<88> Class VI Biocompatibility

The USP <88> VI Biological Reactivity Tests In Vivo were performed on LeClafine® media and other components of LeClafine® Filter. The tests were performed by an accredited and independent laboratory following CNAS. The test is based on an extracting ratio of 0.2 gram of per ml and with leaching conditions of 72 hours at 50°C.

The LeClafine® depth Filter meets USP<88>Class VI biocompatibility requirements.

3.2 BSE/TSE

The LeClafine[®] depth Filter product from LePure Biologics does not pose a TSE/BSE risk based on the following two points:

- (1) The raw materials are chemically synthesized products, lignocellulose as well as inorganic minerals, and no animal or cell-derived materials are used in their manufacture.
- (2) Applicable procedures are in place to avoid cross-contamination of animal or cell-derived products into the production facility.

4. Shelf life

The aged LeClafine[®] depth Filters performance met the release specifications. Based on that, with a safety factor considered, a 3 year shelf life claim is made for LeClafine[®] depth Filters.

Temperature 5°C - 40°C;

Humidity 10%-75%;

Not directly exposed to sunlight, with complete outer package;

Avoid collision.



Shanghai LePure Biotech Co., Ltd.

Website: www.lepure-bio.com

Building 3, 410 Yunzhen Road, Songjiang, Shanghai, China 201600

E-mail: [marketing @ lepure-bio.com](mailto:marketing@lepure-bio.com)