Using A New, Fast Flow, Low Protein Binding Membrane for Sterile Filtration

By Joseph E. Gabriels, Applications Scientist
Millipore Corporation, Bedford, Massachusetts, USA

DOMINIQUE DUTSCHER SAS

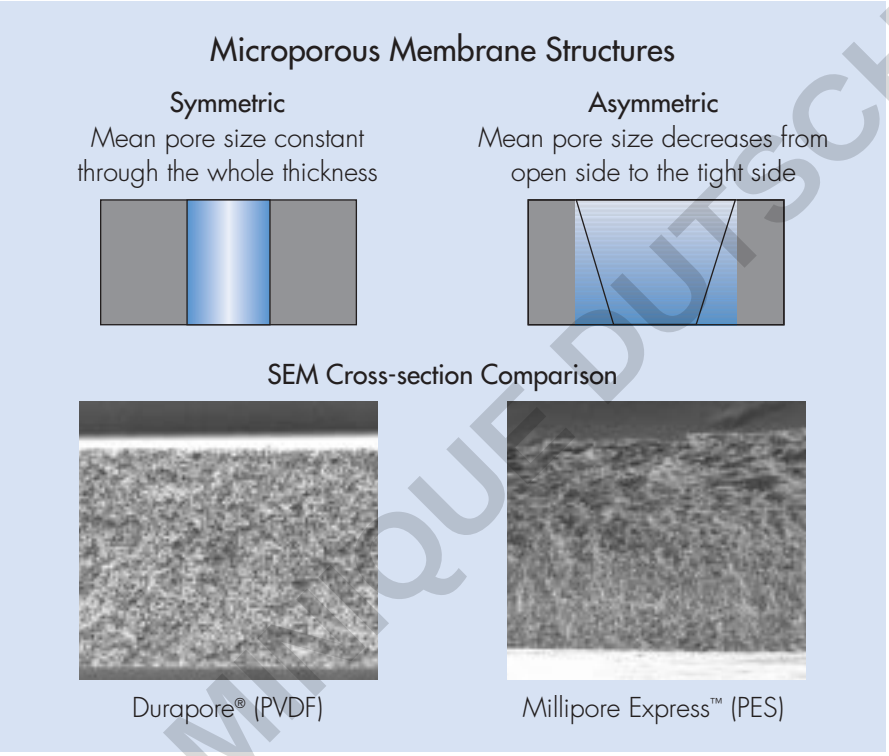


Figure 1. An SEM cross-sectional comparison of a traditional microporous filter (left), with a uniform pore structure from top to bottom, to a new fast-flow microporous membrane (right) capable of higher throughput because of the asymmetric pores. The new PES membrane is more open at the top, where filtration starts.

A variety of sterilization methods exist within the scientific laboratory. These methods include autoclaving, chemical sterilization and membrane filtration. If working within a biological system however, it is important to remember that many cell culture media components are heat sensitive. It is also important to remember that in addition to concerns about reactivity, chemical methods may leave unwanted by-products. Filtration is the method of choice for the sterilization of heat-sensitive tissue culture media and expensive protein additives. To improve on existing filter types, a new membrane was developed for speed, filtration capacity (throughput) and protein recovery.

Fast and Efficient Filtration

The key to this innovative membrane is in the asymmetry of its microporous structure. The Millipore Express polyethersulfone (PES) membrane used in this study is rated as a 0.22 µm filter. The SEM cross-section (Figure 1, right panel) of this membrane shows that it is more open at the top, where filtration starts, than at the bottom. The mean pore size decreases from the open side to the tight side. This membrane's unique architecture extends the useful life of the filter and promotes greater throughput while the 0.22 µm pores maintain bacterial retention. The open side acts as a built-in pre-filter catching large particles before they have a chance to clog the bacterially retentive tight side. In contrast, traditional membranes have a constant pore size throughout their whole thickness. The benefits of this new membrane design are also offered in a 0.1 µm Express (PES) filter.

MILLIPORE

Protein Binding

Since many media additives are proteins, researchers must be confident that when these solutions are filtered, either to ensure sterility or to remove particulates, the proteins are not lost. For example, concentrations of growth factors needed to maintain special cell types must remain constant before and after filtration. The Express (PES) membrane and the Durapore (PVDF) membrane meet this rigorous need. Figure 2 compares the binding of ^{125}I-labeled IgG to a variety of polymeric membranes. A 13 mm membrane disk was incubated in a 1 mg/ml solution of ^{125}I-IgG, washed and counted in a gamma counter to determine the amount of protein nonspecifically bound to the filter. The graph (Figure 2) shows that not only does protein binding vary considerably among polymer types but that even membranes made from the same polymer, polyethersulfone (PES), have different protein binding characteristics. Other PES membranes nonspecifically bound over 6 times more protein than the Express (PES) membrane. However, the Durapore (PVDF) membrane bound the least protein among the samples tested. It is important to note that both the Express (PES) and the Durapore (PVDF) membranes have been surface-modified to render them both hydrophilic and low protein binding.

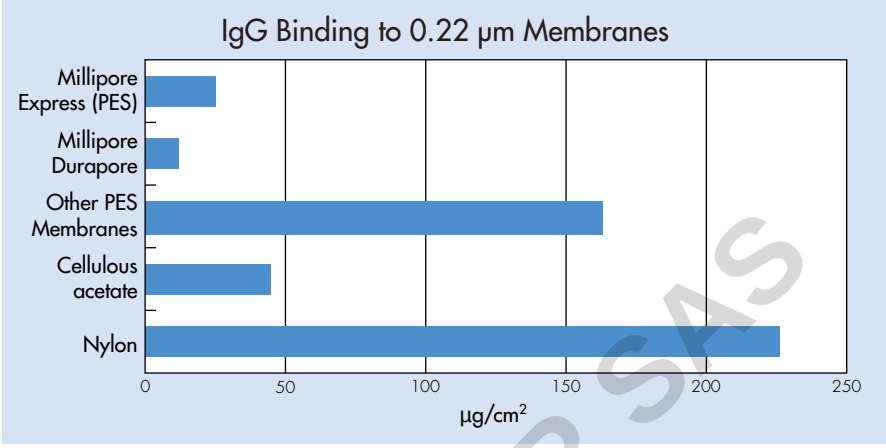


Figure 2. A comparative study was performed to assess nonspecific protein binding to 0.22 µm membranes. A 1 mg/mL solution of ^{125}I labeled IgG was offered to 13 mm membrane disks. After incubation, the amount of protein bound was determined. The other PES membranes nonspecifically bound over 6 times more protein than the Express (PES) membrane. Among all membranes tested, Durapore remains the lowest protein binding membrane available.

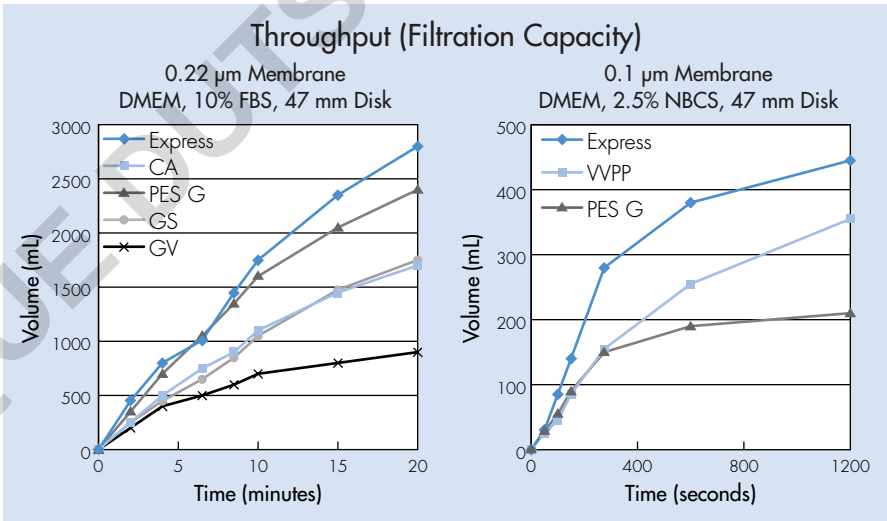


Figure 3. Several membrane types were compared for throughput or filtration capacity. 47 mm disks were challenged with either DMEM, 10% FBS (0.22 µm filters, left), or with DMEM, 2.5% NBCS (0.1 µm filters, right). In both cases, the Express (PES) filters exhibited the highest filtration capacity.

Membrane Throughput or Filtration Capacity

Several membrane types were compared for their ability to filter a common tissue culture medium. The tissue culture medium used was Dulbecco's Modified Eagle's Medium (DMEM) with either 10% fetal bovine serum (FBS) or 2.5% newborn calf serum (NBCS). The Express (PES) 0.22 µm membrane filtered almost 3 liters of tissue culture medium with minimal decay in filtration rate (Figure 3, left). This is a direct benefit of the asymmetric architecture of this membrane. The increase in throughput is even more distinct using the 0.1 µm Express (PES) membrane. The asymmetric structure of the 0.1 µm Express (PES) membrane offered greater filtration capacity than both traditional symmetric membrane types tested (Figure 3, right).

DOMINIQUE DUTSCHER SAS

Bacterial and Mycoplasmal Retention

Consistent, quantitative removal of bacteria is the most important requirement of a sterilizing grade microporous membrane filter. Challenging a membrane with *Brevundimonas diminuta* (ATCC 19146) according to HIMA (Health Industry Manufacturers Association) guidelines assesses bacterial retention. The *B. diminuta* challenge concentration is maintained at equal to or greater than 1×10^7 cells per cm^2 of membrane filtration area. Assessment of the retentive nature of a sterilizing membrane is expressed as the log reduction value (LRV). The LRV is a mathematical expression of the microbial retention efficiency of a sterilizing membrane filter. It is defined as the logarithm to the base ten of the ratio of microorganisms in the challenge to the number of organisms in the filtrate. In the case where the filtrate is sterile, the LRV is expressed as greater than the log10 concentration of the total microbial challenge. When tested according to HIMA guidelines, the Express (PES), 0.22 µm membrane and devices were 100% retentive (Figure 4, top panel) for *Brevundimonas diminuta*. In addition, the new Express (PES) 0.1 µm membrane, when tested for the retention of the *Mycoplasma*, *Acholeplasma laidlawii* gave a *Mycoplasma* LRV of 7 (Figure 4).

IgG Binding to 0.22 µm Membranes

Pseudomonas dimunita

Manufacturer	Device Type	% Retention Failure	Quantity Tested	Membrane Type
Millipore	Millex GP25	0%	25	PES
Brand G	25 mm syringe filter	0%	25	PES
Brand W	25 mm syringe filter	4%	25	PES
Brand S	32 mm syringe filter	0%	25	cellulose acetate
Brand S	32 mm syringe filter	0%	25	glass fiber cellulose acetate

Manufacturer	Membrane Type	# of Membrane Lots Tested	% Retention Failure	Quantity Tested
Millipore	PES	5	0%	9
Brand G	PES	1	0%	2

Mycoplasma Acholeplasma laidlawii

Manufacturer	Membrane Type	Sample Tested	Quantity Tested	% Retention Failure	LRV
Millipore	0.1 µm PES	Sterivac VP20	6	0%	7
Millipore	0.1 µm PES	47 mm membrane disk	9	0%	7

Figure 4. Both 0.22 µm and 0.1 µm Express™ (PES) membranes and devices were 100% bacterially retentive when tested according to HIMA guidelines. In addition, the 0.1 µm Express™ (PES) membrane and devices, when tested for Mycoplasma retention, had a log reduction value (LRV) of 7.

Conclusion

It is important to consider all important performance properties when choosing a microporous membrane for filtration of tissue culture media and media additives. These performance characteristics include flow time, filter capacity, protein binding, and bacterial retention. The Millipore Express (PES) family of membranes offers fast flow, high filter capacity and low protein binding while remaining bacterially retentive. The unique asymmetric structure of these membranes extends their filtration capacity and useful lifetime by allowing them to tolerate higher particle loads and protein concentrations. This structure also yields a filter with a faster flow rate. The process by which the surface of this PES membrane is rendered hydrophilic is responsible for its low protein binding character. The fast flow, low binding, high throughput and bacterial retentive properties of the Millipore Express (PES) Membrane family provides scientists with a reliable, cost effective and time saving membrane for sterile filtration.

DOMINIQUE DUTSCHER SAS

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office.
In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**
In the U.S., Canada and Puerto Rico, fax orders to **1-800-MILLIFX (1-800-645-5439)**
On the Internet: **www.millipore.com**
Tech Service: **www.millipore.com/techservice**

Millipore Worldwide

AUSTRALIA Millipore Australia Pty. Ltd./9A Byfield Street/North Ryde/NSW 2113/Tel. 1 800 222 111 (toll free) or (02) 9888 8999/Fax (02) 9878 0788

AUSTRIA Millipore Ges.m.b.H./Hietzinger Hauptstrasse 145/A-1130 Wien/Tel. (01) 877 89 26/Fax (01) 877 16 54

BALTIC COUNTRIES Millipore Oy/Ruukinkuja 4/FIN-02330 Espoo, Finland/Tel. +358 9 804 5110/Fax +358 9 256 5660

BELGIUM AND LUXEMBOURG Millipore S.A.-N.V./Rue de la Fusée 60/Raketstraat 60/B-1130 Brussels/Tel. +32 2 726 88 40/Fax +32 2 726 98 84

BRAZIL Millipore Indústria e Comércio Ltda./Rua Prof. Campos de Oliveira, 430/CEP 04675-100/Saõ Paulo-SP/Tel. (011) 5548-7011/Fax (011) 5548-7923

CANADA **Life Sciences Division:** Millipore (Canada) Ltd./36 Antares Drive, Suite 280/Nepean, Ontario K2E 7W5/Tel. 1-800-645-5476/Fax 1-800-645-5439
BioPharmaceutical Division: Millipore (Canada) Ltd./109 Woodbine Downs Blvd, Unit 5/Etobicoke, Ontario M9W 6YI/Tel. 1-800-645-5476/Fax 1-800-645-5439
Laboratory Water Division: Millipore (Canada) Ltd./19 Thorne St. Suite 302/Cambridge, Ontario/N1R 1S3/Tel. 1-800-645-5476/Fax 1-800-645-5439

CHINA, PEOPLE'S REPUBLIC OF
Beijing: Millipore China Ltd./Suite 1209, 12/F, China Resources Building/No. 8 Jianguomenbei Avenue/Beijing 100005/ Tel. (86-10) 8519 1250, (86-10) 6518 1058/Fax (86-10) 8519 1255
Guangzhou: Millipore China Ltd./Room 1303, Office Tower, Citic Plaza/233 Tian He Bei Road/Guangzhou 510620/ Tel. (86-20) 8752 0187, (86-20) 8752 0173/Fax (86-20) 8752 0172
Hong Kong: Millipore China Ltd./Room 1701, 17/F, Harcourt House/39 Gloucester Road, Wanchai, Hong Kong/Tel. (852) 2803 9111/Fax (852) 2513 0313
Shanghai: Millipore China Ltd./Suite 901 Hong Kong Plaza (S)/No. 283 Huai Hai Road (M)/Shanghai 200021/Tel. (86-21) 5306 9100/Fax (86-21) 5306 0838

CZECH REPUBLIC Millipore spol. s.r.o./Ricanova 21/16900 Praha 6/Tel. 02-2051 3841/Fax 02-2051 4298

DENMARK Millipore A/S/Odinsvej 9-19, st./2600 Glostrup/Tel. 70 10 00 23/Fax 70 10 13 14

EASTERN EUROPE, C.I.S., AFRICA, MIDDLE EAST AND GULF
Life Sciences Division: Millipore S.A./BP 116/67124 Molsheim Cedex, France/Tel. +33 3 88 38 9536/Fax +33 3 88 38 9539
BioPharmaceutical Division: Millipore Ges.m.b.H./Hietzinger Hauptstrasse 145/A-1130 Wien, Austria/Tel. +43 1 877-8926/Fax +43 1 877-1654
Laboratory Water Division: Millipore S.A./BP 307/78054 Saint-Quentin/Yvelines Cedex, France/Tel. +33 1 30 12 7000/Fax +33 1 30 12 7180

FINLAND Millipore Oy/Ruukinkuja 4/02330 Espoo/Tel. (09) 804 5110/Fax (09) 256 5660

FRANCE Millipore S.A./BP 307/78054 Saint-Quentin/Yvelines Cedex/Tel. 01 30 12 7000/Fax 01 30 12 7180

GERMANY Millipore GmbH/Hauptstraße 87/65760 Eschborn/Tel. (06196) 494-0/Fax (06196) 43901

HUNGARY Millipore Kft./Budapest-1118/Ménesi út 23/a/Tel. 01-381-0433/01-381-0434/01-209-3232/Fax 01-209-0295

INDIA Millipore (India) Pvt. Ltd./50A 2nd Phase Ring Road/Peenya/560 058 Bangalore/Tel. (91) 80-839 46 57/Fax (91) 80-839 63 45

IRELAND **Life Sciences and Laboratory Water Divisions:** Millipore (U.K.) Ltd./Units 3 & 5 The Courtyards/Hatters Lane/Watford, Hertfordshire, U.K./WD18 8YH/ Tel +44 1923 816375/Fax +44 1923 818297
BioPharmaceutical Division: Millipore Ireland B.V./Carrigtwohill, County Cork/Tel. (021) 883 666/Fax (021) 883 048

ITALY **Milano:** Millipore S.p.A./Via XI Febbraio, 99/20090 Vimodrone/Tel. (02) 25.07.81/Fax (02) 26.50.324
Roma: Millipore S.p.A./Via D. Sansotta 100/00144 Roma/Tel. (06) 52.03.600/Fax (06) 52.95.735

JAPAN Nihon Millipore Ltd./Mita Kokusai Bldg./4-28, Mita 1-Chome/Minato-ku/Tokyo 108/Tel. (03) 5442-9711/ Fax (03) 5442-9736 (Life Sciences Division), (03) 5442-9737 (BioPharmaceutical Division), (03) 5442-9734 (Laboratory Water Division)

KOREA Millipore Korea Co. Ltd./Suite 711, Korea City Air Terminal Building/159-6, Samsung-Dong, Kangnam-Ku/Seoul 135-728/Tel. (822) 551-0230/ Fax (822) 551-0288

MALAYSIA Millipore Asia Ltd./Suite 3.03 Wisma KT/No. 14 Jalan 19/1/46300 Petaling Jaya/Selangor/Tel. 03-7957-1322/Fax 03-7957-1711

MEXICO Millipore S.A. de C.V./Ings. Militares 85-PB/Col. Argentina Poniente/11230 México, D.F./Tel. (55) 5576 9688/Fax (55) 5576 8706

THE NETHERLANDS Millipore B.V./Postbus 23249/1100 DS Amsterdam Zuidoost/Tel. 0900 7645645/Fax 0900 7645644

NORWAY Millipore AS/Postboks 214 – Manglerud/0612 Oslo/Tel. 22 67 82 53/Fax 22 66 04 60

POLAND Millipore Sp.z.o.o./ul. Jasnodworska 7/01745 Warszawa/Tel. 22-669 12 25/ 22-663 70 31/Fax 22-663 70 33

PUERTO RICO Millipore Cidra Inc./P.O. Box 11977/Cidra, P.R. 00739-1977/Tel. (787) 273-8495/Fax (787) 747-6553

SINGAPORE Millipore Singapore Pte Ltd./31 Kaki Bukit Road 3/#06-08/11 Techlink/Singapore 417818/Tel. 6842-1822/Fax 6842-4988

SPAIN AND PORTUGAL Millipore Iberica, S.A./Avda. Llano Castellano,13-3°/28034 Madrid, Spain/Tel. +34 917 283 960/Fax +34 917 292 909

SWEDEN Millipore AB/Box 7090/174 07 Sundbyberg/Tel. 08-628 6960/Fax 08-628 6457

SWITZERLAND Millipore AG/Chriesbaumstrasse 6/8604 Volketswil/Tel. (01) 908-30-60/Fax (01) 908-30-80

TAIWAN Millipore Biosciences Asia Ltd./13th Floor, 160, Sec. 6 Min Chuan East Road/Taipei, 114/Tel. 886-2-2792-9333/Fax 886-2-2792-6555

U.K. Millipore (U.K.) Ltd./Units 3 & 5 The Courtyards/Hatters Lane/Watford, Hertfordshire WD18 8YH/Tel. 01923 816375/Fax 01923 818297

U.S.A. Millipore Corporation/290 Concord Road/Billerica, MA 01821-7037/Tel. (781) 533-6000 or (800) 645-5476 toll-free/ Fax (781) 533-3110 or (800) 645-5439 toll-free

In All Other Countries

MILLIPORE INTERTECH P.O. Box 255 /Bedford, MA 01730 U.S.A./Tel. +1 (781) 533-8622/Fax +1 (781) 533-8630

Millipore is a registered trademark of Millipore Corporation.
Durapore and Millipore Express are trademarks of Millipore Corporation.
Lit. No. PF2001EN00
Printed in U.S.A. 01/03 03-037
©2003 Millipore Corporation, Billerica, MA. All rights reserved.

MILLIPORE

DOWNLOADED BY FISHER S&S