Millipore®
Preparation, Separation,
Filtration & Monitoring Products

Application Note

Comparison of Dehydrated Culture Media for Efficient Media Fill Testing by Evaluating Cold Filterability

The standard medium for Media Fill Tests under aerobic conditions is Soybean Casein Digest Medium (SCDM), also referred to as Tryptic Soy Broth (TSB). In order to perfectly match with EP recommendations, there is a market tendency to replace the animal-derived peptones by vegetable sources in order to reduce the risk of contaminating controlled production areas with prions.

The European Pharmacopoeia Chapter 5.2.8 recommends in the scientific principles for minimizing contamination risk (3 – 1.), "… the use of materials from 'non TSE-relevant animal species' or non-animal origin …". Furthermore, for peptone there "… is considerable interest in the use of vegetable protein as an alternative to animal-sourced protein …" (6 – 9).

Further to the practical aspects, avoiding animal-derived materials also obsoletes animal origin documentation requirements. Vegetable Peptones Broth has become a well-accepted alternative to Tryptic Soy Broth, and is commercially available from a variety of manufacturers. Both media types are therefore included in this study.

As the majority of aseptic filling processes does not permit autoclaving prior to filling, process simulations should also avoid this step. This presents a contamination risk to the aseptic filling lines. Sterile filtration is conducted in most cases but is insufficient to eliminate this contamination risk, as a number of organisms such as Mycoplasma and Acholeplasma are small enough to pass through the 0.2 µm filters. Gamma-irradiation is therefore an absolute necessity to counter this risk, and is the reason for selecting gamma-irradiated products for this study, which are deemed suitable for safe introduction into controlled areas.

During the sterile filtration step of the aseptic process simulation, a high-throughput rate of the media through the 0.2 µm membrane is essential, and is the main focus of this study.

MERCK

Materials

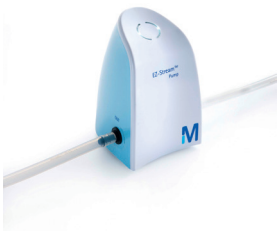
Table 1. Dehydrated Culture Media

Culture Media Type	Brand	Gamma-irradiated	Packaging	Format
Tryptic Soy Broth (**Cat. No. 1.00800.0500**)	Millipore®	yes	triple-wrapped	granulated
Tryptic Soy Broth	Brand A	yes	triple-wrapped	powder
Tryptic Soy Broth	Brand B	yes	triple-wrapped	powder
Tryptic Soy Broth	Brand C	yes	double-wrapped	powder
Tryptic Soy Broth	Brand D	no	single-wrapped	powder
Vegetable Peptone Broth (**Cat. No. 1.00550.0500**)	Millipore®	yes	triple-wrapped	granulated
Vegetable Peptone Broth	Brand A	yes	triple-wrapped	powder
Vegetable Peptone Broth	Brand B	yes	triple-wrapped	powder
Vegetable Peptone Broth	Brand C	yes	double-wrapped	powder

Table 2. Filtration Materials

Product Name	Filter Type	Pore Size	Diameter	Cat. No.
Millipore Express® PLUS Membrane Filter	PES (Polyethersulfone)	0.2 µm	47 mm	**GPWP04700**
Durapore® Membrane Filter	PVDF (Polyvinyliden-fluoride)	0.22 µm	47 mm	**GVWP04700**
Nylon Membrane Filter	Nylon	0.2 µm	47 mm	**GNWP04700**
MF-Millipore™ Membrane Filter	Mixed Cellulose Esters	0.22 µm	47 mm	**GSWP04700**
Microfil® Funnel, 250 mL	N/A	N/A	N/A	**MIHAWG250**

Table 3. Equipment

EZ-Stream® Vacuum pump	EZ-Fit® Manifold (1-place for Microfil® funnels and membranes)	Silicon hose—autoclavable
EZSTREAM1	**EZFITMIC0**	**STREAMTUB**
		

Method

For the comparison study, the dehydrated culture media was dissolved in two liters of purified water and only used when the medium had completely dissolved (see Note, page 4). Three runs of every combination of media and membrane filters were performed. Two batches per type of our filter membranes were evaluated.

Filterability of culture media is often measured in liters per square meter of membrane, and does not take into account the time elapsed until clogging occurs. As filtration time is an important factor when assessing the practical usability of culture media for media fill applications, an alternative approach was selected for measuring the time required to filtrate a given volume regarded as a minimum requirement. In laboratory scale, this volume is set to two liters on a 47 mm diameter membrane, with a maximum acceptable filtration time of 30 minutes. The specifications applied in this study were defined in line with the method for filtration performance testing applied at Merck's DCM manufacturing department. In the scope of this study, it was decided to filter until completion or clogging, irrespective of the 30-minute threshold.

Two liters on a 47 mm diameter membrane (with an actual filtration area of 40 mm diameter) are equivalent to about 1,592 liters per square meter.

Figure 1. EZ-Stream® pump and 1-place EZ-Fit® manifold. Merck Vegetable Peptone Broth poured in a 250 mL Microfil® funnel.

Controls: To confirm vacuum generation, the EZ-Stream® pump was checked once by our calibration service.

Filtration time measurement was initiated daily with a system check referred to as the "blank reference test", which comprises three runs of time measurement with two liters of purified water and the defined filter membrane. The reference values for water shown in the following data tables are average values of all these blank reference tests.

Note: Solubility of the Media Fill culture media

Selection: A contributing factor for an effective Media Fill process simulation is the complete solubility of the DCM, preventing particles from clogging the membrane. To change the filter during the process simulation is time-consuming, expensive and a contamination risk. Therefore, a good solubility and a high liquid-throughput rate of the membrane filter are high-priority criteria for selecting the most suitable media for process simulations.

Solubility of unheated media: The solubility of the Millipore® non-animal and animal-origin DCM was very good, dissolving completely. Competitor Brand A TSB and VPB as well as Brand B TSB powder also dissolved completely.

However, the color indicator of the non-animal VPB of Brand B did not dissolve. Solubility could not be improved by replacing purified water with Milli-Q® water or by leaving the broth overnight to dissolve in purified water. Therefore, the dissolving time for this medium was set to four hours under agitation for this comparison study.

Solubility of heated media: Brand C and Brand D were heated according to manufacturer's instructions, to ensure the broth was dissolved, and allowed to cool to room temperature.

Please note that growth promotion tests were not performed after the defined heating time.

Figure 2. EZ-Fit® 1-place manifold with PVDF membrane filter and Vegetable Peptone Broth.

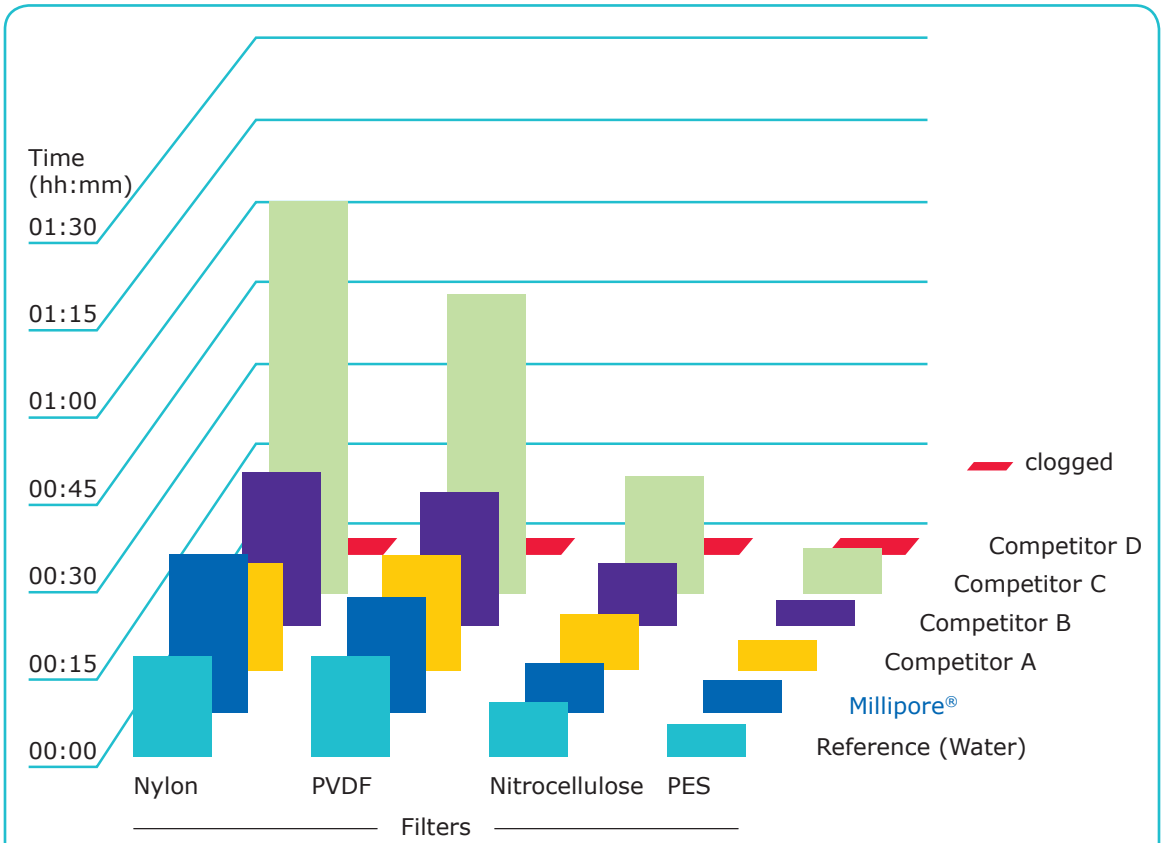
Results

The displayed results illustrate the average values from three filtration runs of each media and membrane batch combination.

With some combinations of membrane filter and broth, the membrane clogged and it was not possible to filtrate two liters through the membrane.

Tryptic Soy Broth

TSB from Brands C and D were dissolved by heating, as recommended by the manufacturers. Millipore® TSB, and Competitor Brands A and B can be dissolved in cold, purified water. A heating step is unnecessary.



	Nylon	PVDF	Nitrocellulose	PES
Reference (Water)	00:17:38	00:17:40	00:08:07	00:05:29
Millipore®	00:27:06	00:20:14	00:09:23	00:06:09
Competitor A	00:21:49	00:21:54	00:09:58	00:06:30
Competitor B	01:28:44	01:25:52	01:10:51	01:06:26
Competitor C	00:14:14	00:58:33	00:16:27	00:08:00
Competitor D	clogged	clogged	clogged	clogged

Figure 3. Filterability comparison of Tryptic Soy Broth.

Figure 3 illustrates the average time to filtrate two liters of Tryptic Soy Broth through a 47 mm membrane filter.

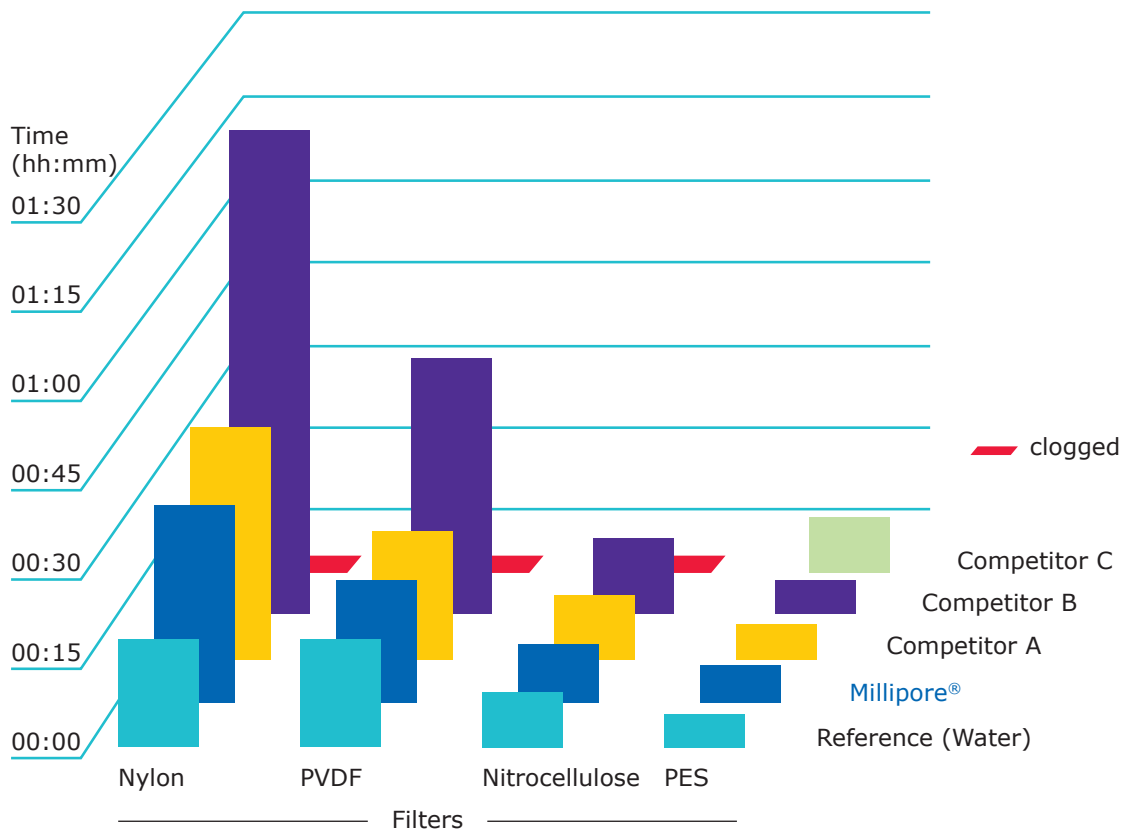
The figure demonstrates the excellent filtration properties of the Millipore® TSB with four types of membrane filters. With the most commonly used PVDF and PES, as well as the Nitrocellulose filters, Millipore® TSB was the fastest in comparison to the competitor products.

Vegetable Peptone Broth

Similar to Figure 3, Figure 4 shows all average filtration times from different Vegetable Peptone broth and membrane filter materials summarized in one graph.

Millipore® VPB, Brand A and Brand B can all be dissolved in cold water. The VPB from Brand C must be heated according to manufacturer's instructions. The VPB from Brand B includes a color indicator, which did not dissolve completely. The defined dissolving time for this media was 4 hours.

The figure demonstrates the excellent filtration properties of the VPB from Millipore® with all membrane filters, including the Nylon and PVDF membranes, which were found to be more difficult for filtering. In all cases, the Millipore® VPB membrane combinations produced the fastest filtration times.



	Nylon	PVDF	Nitrocellulose	PES
Reference (Water)	00:17:38	00:17:40	00:08:07	00:05:29
Millipore®	00:33:54	00:21:17	00:10:13	00:06:09
Competitor A	00:39:56	00:22:52	00:10:26	00:06:32
Competitor B	01:23:32	01:44:51	01:13:27	01:06:40
Competitor C	clogged	clogged	clogged	00:06:40

Figure 4. Filterability comparison of Vegetable Peptone Broth.

Interpretation

Before dehydrated culture media undergo sterile filtration, they need to be brought into solution. In many cases, media preparation takes place close to the aseptic filling area, typically a cleanroom D area. In order to not contaminate this area, it is preferable to use a product that is suitable for safe transfer into cleanrooms. Surprisingly, not all gamma-irradiated products on the market are triple-wrapped, and the overall packaging quality is heterogeneous (see Table 1 for details).

Even more important is the aspect of solubility itself. The volume of culture medium required for a single media fill run can be substantial, and dissolving powder in several hundred liters of water can be a time- and labor-consuming process. Even in the laboratory scale of this study, it was observed that granulated products, such as those provided by us, dissolve much easier and faster than any powder products. The different powdered products also showed important differences, such as the color indicator of one product failing to dissolve completely.

It should also be noted that some manufacturers recommend to heat their media to support dissolution. Besides being an additional process step, it is often not an option in the facilities where the media are prepared. Users who cannot heat the media should be aware of potential issues with some brands. This study also demonstrates that heating does not guarantee acceptable filtration times.

This comparison study includes four types of membrane filters for sterile filtration. The filter types applied largely depend on the substances produced, and culture media should provide sufficient filtration performance with all filter types. While PVDF and PES membranes are nowadays the most commonly used in the market, it is noticeable that there are considerable differences between the two with regard to how easily culture media and even water can be filtered.

PES membranes are by far the least demanding filters for both TSB and VPB. While Nitrocellulose membranes require only slightly more time, PVDF and Nylon are definitely much more challenging. With these filters, the differences between TSB and VPB become more obvious. It has to be noted that vegetable-source peptones are overall more difficult to filtrate than the animal-derived peptones in standard TSB. This is true for all tested brands and on all filter types.

Summarizing the test data, it can be stated that Millipore® Tryptic Soy Broth was the best performing with PVDF, Mixed Nitrocellulose Ester and PES membrane filters. Millipore® Vegetable Peptone Broth delivered the fastest results with all membrane filters.

Millipore® Tryptic Soy Broth and Vegetable Peptone Broth are overall the best choice for the most critical Media Fill setups. Packaging concept and cold-solubility performance allow for efficient media preparation at the same location as the actual product is prepared, with the least possible contamination risk, and the filtration performance of both products was best in the field of brands tested.

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