

Flow rate and throughput of Thermo Scientific Nalgene® 0.1 micron Rapid-Flow™ PES filter units with cell culture media

The first Nalgene® vacuum filter was launched in 1965. Since then, filter units and bottle top filters have become an indispensable method for sterile filtration of media, serum and buffers used in cell culture, providing a last line of defense against loss of cell cultures due to inadvertent contamination of fluids. Filter units with 0.2 micron membranes are typically used for this purpose. However, although 0.2 micron membranes are considered sterilizing in that they remove bacteria, the removal of smaller contaminants such as

Mycoplasma requires the use of 0.1 micron filter membranes. Thermo Scientific Nalgene 0.1 micron filters are available in four sizes; 150 mL, 250 mL, 500 mL and 1000 mL capacity. All sizes have the unique Nalgene Rapid-Flow™ membrane support system. The Rapid-Flow system consists of a multi-column array that provides more uniform membrane support and stability than does the radiating spoke design used by other filter manufacturers. This design feature allows for increased flow rates and greater fluid throughput.

The purpose of this paper is to compare the performance of Nalgene 0.1 micron filter units with similar devices made by Corning and Millipore.

Materials

Table 1:
Filters Tested

| Description | Catalog # | Membrane Pore Size and Material | Membrane Diameter (mm) | Effective Membrane Surface Area (cm ²) | Receiver Bottle Capacity (mL) |
|---|-----------|---------------------------------|------------------------|--|-------------------------------|
| Nalgene Rapid-Flow™ 1000 mL Filter Unit | 567-0010 | 0.1 µm PES | 90 | 57.3 | 1000 |
| Millipore Stericup® 1000 mL Filter Unit | SCVPU11RE | 0.1 µm PES | 70 | 36.6 | 1000 |
| Nalgene Rapid-Flow 500 mL Filter Unit | 566-0010 | 0.1 µm PES | 75 | 38.8 | 500 |
| Corning 500 mL Filter Unit | 431475 | 0.1 µm PES | 63 x 63 mm square | 33.2 | 500 |

Test fluids

Cell Media: DMEM + 10% FBS (fetal bovine serum) and RPMI + 10% BGS (bovine growth serum). Both are typical cell growth media.

Serum: 100% BGS (bovine growth serum) was used. 100% serum is a common component of cell growth media and also a severe filtration challenge.

Test method

Flow Rate and Clogging Volume

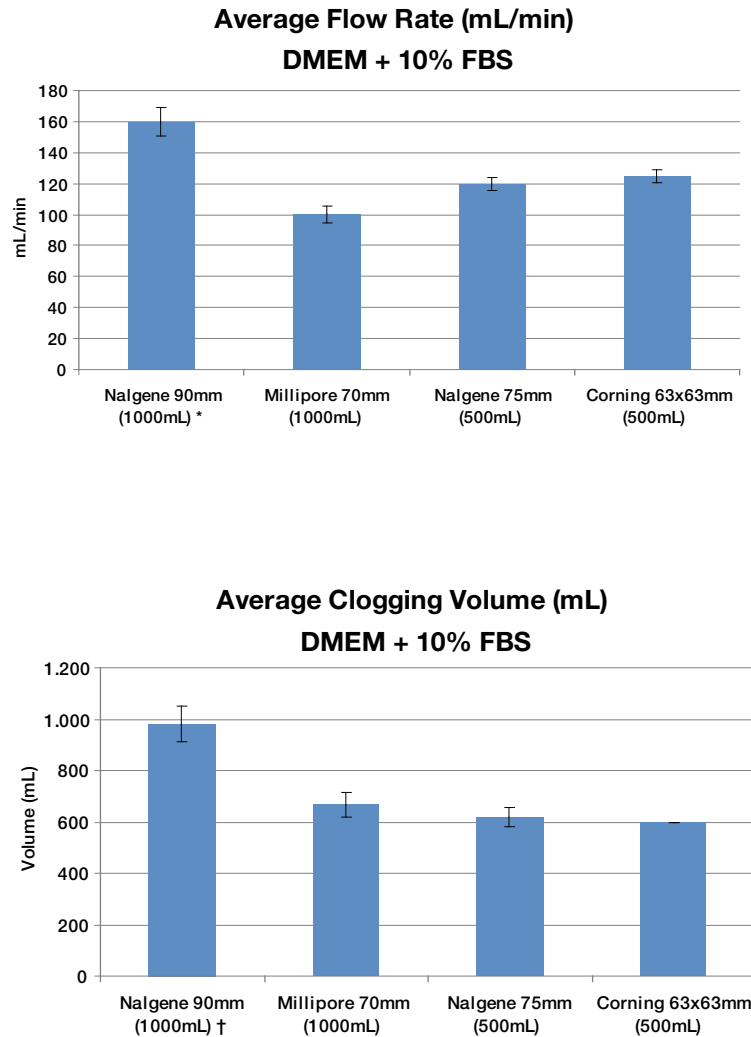
Filtration was conducted between 25 and 27" Hg vacuum. The plastic receiver bottle for each unit was replaced by a 2 L calibrated glass receiver that was used to measure flow volumes. Nalgene Rapid-Flow 1000 mL filter units, Millipore Stericup 1000 mL filter units, Nalgene Rapid-Flow 500 mL filter units and Corning 500 mL filter units were tested in random order. The filter units were filled to the top with test solution and the vacuum stopcock at the test stand was opened. Time points were taken every 100 mL for DMEM + 10% FBS, RPMI 1640 + 10% BGS and every 50 mL for 100% BGS until clogging was reached (defined as 1 drop of fluid per second or a flow rate such that the time to filter 100 mL exceeded two minutes) or 1 Liter of fluid had been filtered for DMEM + 10% FBS, RPMI 1640 + 10% BGS, and 500 mL for 100% BGS. Fluid was replenished as needed to keep approximately 200 mL of test solution in the filter unit at all times.

Data analysis

Two sample t-tests assuming unequal variances were used to determine the significance of filter unit performance (flow rate and clogging volume) for DMEM + 10% FBS and RPMI 1640 + 10% BGS between a) the Nalgene Rapid-Flow 1000 mL filter unit and the Millipore Stericup 1000 mL filter unit; b) the Nalgene Rapid-Flow 500 mL filter unit and the Corning 500 mL filter unit; and c) the Nalgene Rapid-Flow 500 mL filter unit and the Millipore Stericup 1000 mL filter unit; ($p \leq 0.01$).

Results

Table 2: Flow Rate (mL/min) and Clogging Volume (mL) for Filter Units with DMEM + 10% FBS



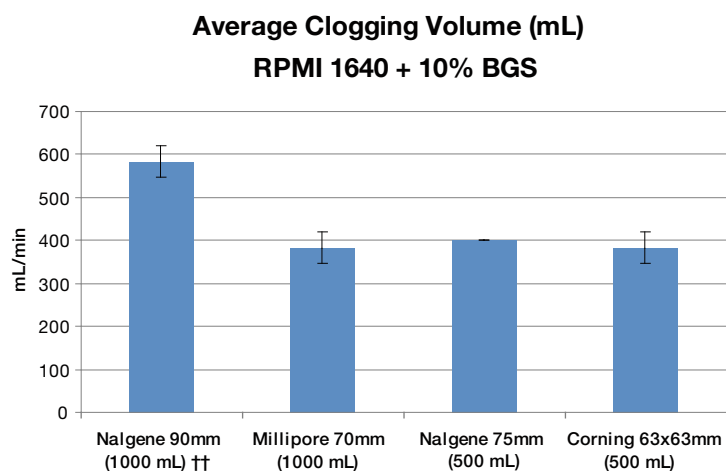
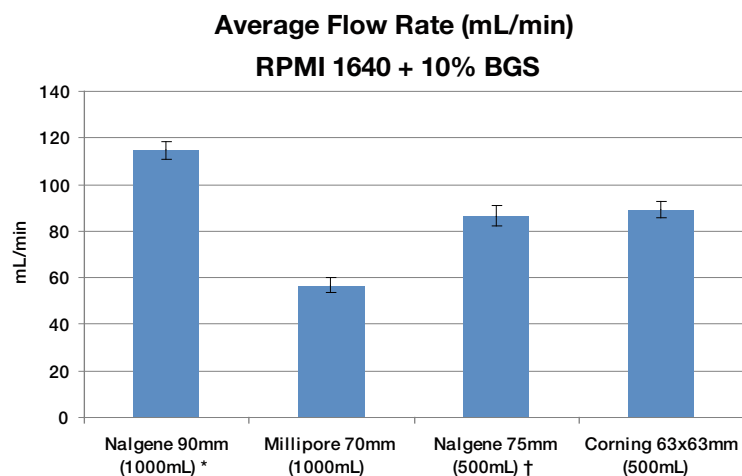
| 0.1 μ m PES Filter Unit | N | Flow Rate (mL/minute) | Clogging Volume (mL) |
|---|---|-----------------------|----------------------|
| | | Mean \pm Std. Dev. | Mean \pm Std. Dev. |
| Nalgene Rapid-Flow™ 1000 mL Filter Unit | 6 | 159.9 \pm 9.4 | 983.3 \pm 68.7 |
| Millipore Stericup 1000 mL Filter Unit | 6 | 100.2 \pm 5.3* | 666.7 \pm 47.1† |
| Nalgene Rapid-Flow 500 mL Filter Unit | 6 | 119.9 \pm 4.1 | 616.7 \pm 37.3 |
| Corning 500 mL Filter Unit | 6 | 124.8 \pm 3.9 | 600.0 \pm 0.0 |

* Nalgene Rapid-Flow 1000 mL filter is significantly faster than the Millipore Stericup 1000 mL filter at $p < 0.01$.

† Nalgene Rapid-Flow 1000 mL filter is able to flow significantly more volume than the Millipore Stericup 1000 mL filter at $p < 0.01$.

Table 3:

Flow Rate (mL/min) and Clogging Volume (mL) for Filter Units with RPMI 1640 + 10% BGS



| 0.1 µm PES Filter Unit | N | Flow Rate (mL/minute) | Clogging Volume (mL) |
|---|---|-----------------------|----------------------|
| | | Mean ± Std. Dev. | Mean ± Std. Dev. |
| Nalgene Rapid-Flow™ 1000 mL Filter Unit | 6 | 114.8 ± 3.6 | 583.3 ± 37.3 |
| Millipore Stericup 1000 mL Filter Unit | 6 | 56.8 ± 3.1* | 383.3 ± 37.3†† |
| Nalgene Rapid-Flow 500 mL Filter Unit | 6 | 86.5 ± 4.3† | 400.0 ± 0.0 |
| Corning 500 mL Filter Unit | 6 | 89.2 ± 3.5 | 383.3 ± 37.3 |

* Nalgene Rapid-Flow 1000 mL filter is significantly faster than the Millipore Stericup 1000 mL filter at $p < 0.01$.

† Nalgene Rapid-Flow 500 mL filter is significantly faster than the Millipore Stericup 1000 mL filter at $p < 0.01$.

†† Nalgene Rapid-Flow 1000 mL filter is able to flow significantly more volume than the Millipore Stericup 1000 mL filter at $p < 0.01$.

Flow Rate (mL/min)

When the 1000 mL filter units were compared, the Nalgene Rapid-Flow product performance advantage was apparent. The results showed that the 0.1 micron Nalgene Rapid-Flow 1000 mL filter units performed significantly better than the Millipore Stericup 1000 mL filter unit when filtering both DMEM + 10% FBS and RPMI + 10% BGS. The flow rate of the Nalgene Rapid-Flow 1000 mL was also significantly faster than the Nalgene Rapid-Flow 500 mL filter units with the smaller membrane diameter, as expected.

When the 500 mL filter units were compared, the Nalgene Rapid-Flow 500 mL had a comparable flow rate to the Corning 500 mL filter unit when filtering DMEM + 10% FBS and RPMI + 10% BGS. The Millipore Stericup 1000 mL showed performance comparable to the Nalgene Rapid-Flow 500 mL filter units when filtering DMEM + 10% FBS. However, the Nalgene Rapid-Flow 500 mL was significantly faster than the Millipore Stericup 1000 mL when filtering RPMI + 10% BGS.

With 100% BGS, all of the 0.1 micron PES filter units had slow flow rates (data not shown).

Clogging Volume (mL)

With all of the test solutions, none of the filters tested achieved an average cut-off throughput of 1 Liter of fluid for DMEM + 10% FBS, RPMI 1640 + 10% BGS, and 500 mL for 100% BGS due to the small 0.1 micron pore size. However, for both DMEM + 10% FBS and RPMI 1640 + 10% BGS, the Nalgene Rapid-Flow mL 0.1 micron filter units achieved a significantly higher volume filtered before clogging in comparison to the Millipore Stericup 1000 mL, the Nalgene Rapid-Flow 500 mL or the Corning 500 mL filter units. The Millipore Stericup 1000 mL showed performance comparable to the Nalgene Rapid-Flow 500 mL filter units.

With 100% BGS, all of the filter units tested became clogged at 100 mL of 100% BGS (data not shown).

Discussion

Mycoplasma contamination can destroy cell cultures. Close attention to sterile technique and routine testing helps reduce the chance of inadvertent Mycoplasma contamination but cannot provide complete protection. 0.1 micron final filtration of culture media and buffers provides a last line of defense against these stubborn and dangerous microorganisms.

The fluids tested here reflect routine sterilizing filtration applications. DMEM and RPMI tissue culture media with 10% serum are among the most commonly filtered solutions for this type of device. Whole (100%) serum is a tough-to-filter material that is often filtered at a colder temperature, which further increases the difficulty of filtration. None of the 0.1 micron filter units tested here performed well with 100% serum. 0.2 micron membranes should be used for sterile filtration of 100% serum.

With a reduction in membrane pore size to 0.1 microns, there is an expected trade-off in the form of slower flow rates and lower clogging volumes. Flow is also restricted by the membrane support plate design. These effects

can be minimized by using filters with larger surface areas and by improving the design of the underlying membrane support to maximize flow. In most filter units, the support plate that lies under the membrane is designed with ribs which extend from the center of the membrane like spokes on a wheel. When a vacuum is applied, the spacing of the ribs allows distortion of the membrane resulting in a reduction of fluid flow. To overcome this effect, Nalgene Rapid-Flow filter units employ a multi-column array of posts which provides more uniform and less restrictive support, thus allowing higher flow rates and throughput.

The Nalgene Rapid-Flow 1000 mL filter unit has a larger surface area than the other filters tested. However, surface area alone cannot explain the performance advantages seen here. Nalgene Rapid-Flow 1000 mL has a 56% larger surface area than Millipore Stericup 1000 mL but showed a 102% faster RPMI 1640 + 10% BGS flow rate; Nalgene Rapid-Flow 500 mL has a 6% larger surface area than Millipore Stericup 1000 mL but showed a 34% faster RPMI 1640 + 10% BGS flow rate. The performance advantages are more likely due to the combined effects of larger surface area and the more efficient Nalgene Rapid-Flow membrane support system.

Conclusions

Under the controlled conditions of this testing, Nalgene Rapid-Flow 1000 mL 0.1 micron filter units had superior performance compared to Millipore Stericup 1000 mL 0.1 micron filter units when filtering culture media (DMEM + 10% FBS, RPMI 1640 + 10% BGS). Nalgene 1000 mL 0.1 micron filter units also outperformed all of the 500 mL 0.1 micron filter units tested. In fact, for RPMI + 10% BGS, the Nalgene Rapid-Flow 500 mL filter unit's mean flow rate was 34% faster than the 1000 mL filter unit from Millipore. These Nalgene performance advantages come from a combination of larger diameter membranes that provide more surface area and Nalgene's unique Rapid-Flow design that provides more uniform membrane support and thus better membrane stability during filtration, resulting in faster filtration and increased volume filtered.

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