# Impact of sterile media filtration on the performance of cells cultured in WAVE Bioreactors

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# Impact of Sterile Media Filtration on the Performance of Cells Cultured in WAVE Bioreactors

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## Introduction

Disposable bioreactors have gained widespread acceptance in cell culture applications because they provide a flexible resource for multi-product facilities and speed the production of bio-molecules to the market. Disposable bioreactors offer a number of advantages for researchers including reduction of preparation time, elimination of cleaning and sterilization time, and ease of use. (Fries et al.) Of the many models of disposable bioreactors, WAVE bioreactors are the most popular—with over 2000 units in operation worldwide.

The operation of WAVE bioreactors is very straightforward. However, the feeding of cell culture media to the bioreactor continues to be a manual process which is associated with a heightened risk of contamination. Usually, the use of tube-fusers or welders ensures a sterile environment inside the reactors, inoculation bottles and tubes, but misuse or lack of this equipment may result in contamination without any simple method to test for sterility.

To minimize the risk of contamination, we propose that an ULTA™ Pure HC capsule filter be attached to the disposable Cellbag™ so that media can be fed directly through the filter after the initial inoculation. If successful, the pre-installation of the filter would eliminate the possibility of contamination during media feeding and the time spent performing a separate media sterilization step. The following experiments test the comparability of mammalian cell growth and viability in ULTA Pure HC filtered media versus media sterilized by other means.

## Materials

The following equipment and materials were used in these experiments.

*Consumables*

| | | |
|---|---|---|
| Standard Cellbag 10 | GE Healthcare | CB0010L10-02 |
| Custom Cellbag 10 w/ULTA | GE Healthcare | CB0010L10GE01-1 |
| ULTA Pure HC 2-inch capsule | GE Healthcare | KMPGHC9202HH |

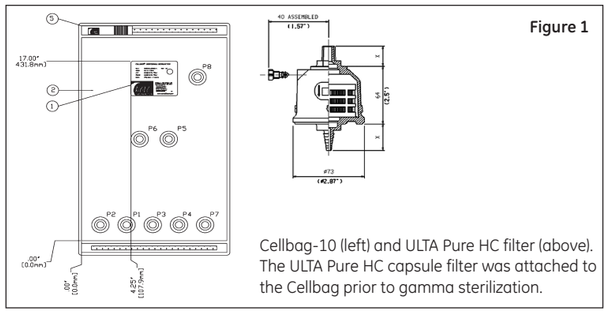


**Figure 1**

Cellbag-10 (left) and ULTA Pure HC filter (above). The ULTA Pure HC capsule filter was attached to the Cellbag prior to gamma sterilization.

*Equipment*

| | | |
|---|---|---|
| WAVE EH20/50 | GE Healthcare | 28-4115-03 |
| Biomedical Analyzer | Nova Biomedical | Bioprofile 400 |

*Cell Culture Media & Supplements*

| | | |
|---|---|---|
| SAFC EX-CELL | SAFC biosciences | C4567 |
| DMEM (Meth-A) | Cellgro | 50-014-PB |
| DMEM (Hybridoma) | Gibco | 10313 |
| FBS (Meth-A) | Gibco | 103B |
| FBS (Hybridoma) | Atlanta Biologicals | S11550 |
| Penicilin/streptomycin | Cellgro | 30-002-CI |
| Sodium pyruvate | Cellgro | 25-000-CI |
| L-glutamine | MP Biomedicals | 101806 |
| GlutaMax | Gibco | 35050 |
| Glucose | EMD | DX0145-3 |
| Sodium bicarbonate | Mallinckrodt | 7412-12 |

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**Table 1 – Filter Description**

| | |
|---|---|
| Type: | GE Healthcare ULTA Pure HC |
| Format: | 2-inch capsule w/pleated membrane |
| Prefilter membrane: | 0.6 µm polyethersulfone prefilter |
| Final membrane: | 0.2 µm polyethersulfone sterilizing-grade |
| Surface area: | 0.047 $m^2$ |

## Methods

### Media analysis

Three common cell culture media were prepared:

| Base Media | Supplements (per liter) |
|---|---|
| Ex-cell ACF CHO medium | 100mL FBS<br>20mL L-glutamine<br>10mL glucose<br>10mL penicillin/streptomycin<br>10mL sodium pyruvate |
| DMEM medium for hybridoma WN cells | 100 mL FBS<br>10 mL GlutaMAX<br>10 mL penicillin/streptomycin |
| DMEM medium for Meth-A cells | 100 mL FBS<br>10 mL penicillin/streptomycin<br>10 mL sodium pyruvate<br>0.87 g L-glutamine<br>2.0 g sodium bicarbonate<br>2.5 g glucose |

5L of each medium was warmed to 37°C and then filtered using an ULTA Pure HC filter. Filtered medium was collected to one 5L bottle and four 2mL samples were taken before filtration and after filtering every 0.5L of medium. Metabolite concentrations in the samples were analyzed using a NOVA Biomedical analyzer (Bioprofile 400).

### Cell growth performance

| | WAVE 1A | WAVE 1B | WAVE 2A | WAVE 2B |
|---|---|---|---|---|
| Objective | Compare mammalian cell growth in filtered and unfiltered media. | | | |
| Cell Type | Meth-A | | | |
| Run Mode | Batch | | Fed-Batch | |
| Inoculation volume | 1.25L cells and media were inoculated , 3.75L of sterile medium was added for final volume of 5L. | 1.25L cells and media were inoculated, 3.75L of unsterile medium was added through ULTA Pure HC filter. | 1.25L cells and medium were inoculated sterilely. | 1.25L cells and medium were inoculated sterilely. |
| Media addition | No extra medium was added. | | 1.25L of medium was added sterilely when VCD reached 4e5 cells/mL. 2.5L of medium was added the next day for a final working volume of 5L. | 1.25L of unsterile medium was added through the ULTA Pure HC filter when VCD reached 4e5 cells/mL. 2.5L of unsterile medium was added the next day for a final working volume of 5 L. |
| Temperature | 37°C | | | |
| Rock angle/ speed | Initial rock angle was set at 6° and rock speed at 17 rpm. Rock speed was later increased to 21rpm to increase dissolved oxygen level. | | Initial rock angle was set at 5° and speed at 14rpm. Rock angle and speed were adjusted accordingly after each media addition. | |
| Sampling | Samples of 3-4mL were taken from each bag everyday for CEDEX and NOVA analysis | | | |

## Results

### Media Analysis

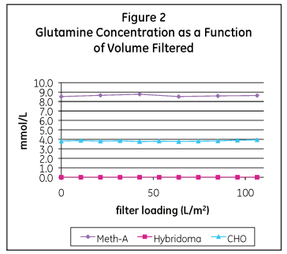


**Figure 2**
**Glutamine Concentration as a Function of Volume Filtered**

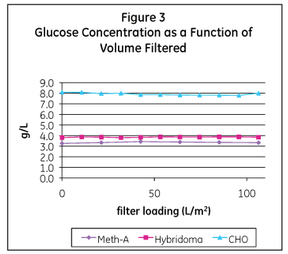


**Figure 3**
**Glucose Concentration as a Function of Volume Filtered**

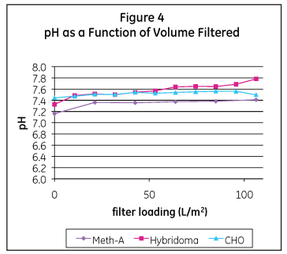


**Figure 4**
**pH as a Function of Volume Filtered**

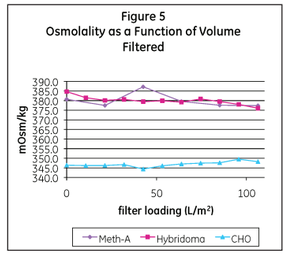


**Figure 5**
**Osmolality as a Function of Volume Filtered**

Media composition was not affected by filtration. The small changes in pH and osmolality observed are likely due to the media's carbonate buffering systems and the fact that the filtration was not performed in a carbon dioxide enriched environment. Note that this test result represents a worst-case scenario since the filter loading (~100 L/$m^2$) is far lower than typical filter capacities for cell culture media (>1000 L/$m^2$).

### Cell Growth Performance

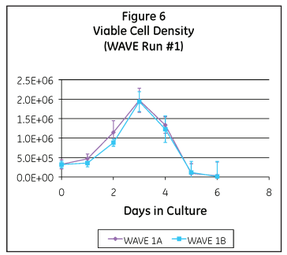


**Figure 6**
**Viable Cell Density (WAVE Run #1)**

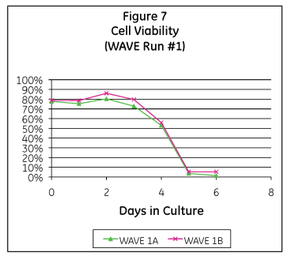


**Figure 7**
**Cell Viability (WAVE Run #1)**

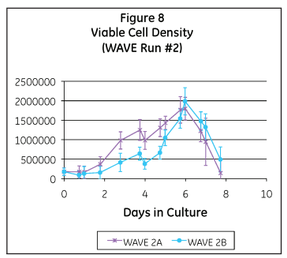


**Figure 8**
**Viable Cell Density (WAVE Run #2)**

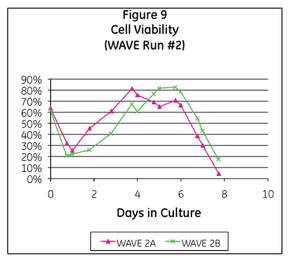


**Figure 9**
**Cell Viability (WAVE Run #2)**

No statistically significant differences in cell growth were observed. Maximum achieved viable cell density was within ±11% for both experiments. The eventual decline in viability observed in both experiments represents the natural life cycle of cells grown in batch or fed-batch reactors and is not attributable to the use of ULTA filters or Cellbag bioreactor chambers.

## Conclusions

Analysis of cell culture media composition before and after filtration indicates only minor changes in the media which are not associated with changes in cell growth performance.

The data presented here indicate that the integration of ULTA Pure HC capsule filters does not affect the growth of mammalian cells cultured in WAVE Bioreactors.

The use of ULTA Pure HC capsule filters improves the usability of the Cellbag by eliminating aseptic handling steps which are not only time consuming but also are associated with a heightened risk of contamination.

## Future Work

As a next step, we plan to extend this work to a cell line which produces an extracellularly expressed protein. By doing so, we can evaluate not only cell density but also protein production.

## Literature Cited

Fries, Serena et al, "Evaluation of Disposable Bioreactors – Rapid Production of Recombinant Proteins By Several Animal Cell Lines", BioProcess International, October 2005.

## Acknowledgments

We thank Carrie Miller (GE Healthcare) for her assistance in designing the custom Cellbag.
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## For Further Information

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