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White Paper

Single-Use Upstream Processing: Ultimus® Film Delivers Comparable Cell Growth Performance To Glass

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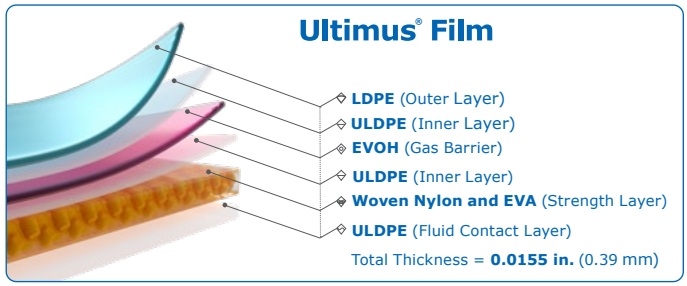
Introduction



Single-use technologies are increasingly being incorporated into the biomanufacturing workflow to achieve greater efficiency and productivity, reduce capital investment in facilities and equipment, and minimize the risk of cross-contamination. Along with the many benefits, however, single-use technologies can also pose some risks.

Irgafos® 168 (tris (2,4-di-tert-butylphenyl) phosphite), an anti-oxidant commonly used in single-use films, produces the by-product bis (2,4-di-tert-butylphenyl) phosphate (bDtBPP) following exposure to gamma irradiation. Studies have shown that bDtBPP, even at low concentrations, inhibits growth of Chinese Hamster Ovary (CHO) cells commonly used for mAb and recombinant protein cell culture processes[1].

This white paper describes studies that explore upstream bioprocessing performance with Ultimus® film, a single-use, multi-layer film with a fluid contact layer free of Irgafos® 168. Results confirm that cell growth, protein production, and protein quality were equivalent in cultures using media stored in single-use bags made with Ultimus® film and the more traditional glass bottles.

Methods and Materials

Cell culture studies

Bags (9.5 in. x 6 in.) were made with Ultimus® film and another commercially available film, Film A, that contained Irgafos® 168. All bags were sterilized with gamma irradiation at 25-40 kGy then filled with the various cell culture media (2 mL/cm^2) within 24 hours of sterilization. As a control, glass bottles were sterilized by autoclaving, then filled with cell culture media. Three replicates (bags or bottles) were prepared for each test condition.

Media in both the bottles and bags were incubated for three days at 37 °C, then transferred to spin tubes and inoculated with various CHO cell lines at a density of 3-5 x 10^5 cells /mL **(Table 1)**. In these batch cultures, growth and viability of the cell lines were recorded for 5 days.

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Table 1. Cell culture media and cell lines used to evaluate performance of Ultimus® film.

Cell Line	Host	Recombinant Protein	Media	Feed for Fed-batch
1	CHO-S	N/A	OpticCHO™	N/A
2	CHO-S	Antibody	Cellvento® 4CHO COMP	Cellvento® 4Feed COMP
3	CHOZN® GS -/-	Antibody	EX-CELL® Advanced CHO Fed-Batch medium	Cellvento® 4Feed COMP
4	CHOZN® GS -/-	Antibody	EX-CELL® Advanced CHO Fed-Batch medium	N/A
5	CHOZN® GS -/-	Fusion Protein	EX-CELL® Advanced CHO Fed-Batch medium	EX-CELL® Advanced CHO Feed 1
6	CHOZN® CHO-K1	Antibody	EX-CELL® Advanced CHO Fed-Batch medium	EX-CELL® Advanced CHO Feed 1 & Cellvento® 4Feed COMP
7	CHO K1	Antibody	Cellvento® CHO-210	N/A

The effect of Ultimus® film on cell culture performance was next assessed with fed-batch cultures of four cell lines. Cultures were initiated at 3-5 $\times 10^5$ cells/mL, fed every two to three days and grown until viabilities had declined to < 80%. Growth, viability and recombinant protein titer were monitored for the duration of the study. As a final check on performance, growth of CHOZN® GS -/- (cell line #3) was evaluated using bags made from two different lots of Ultimus® film. The quality attributes of the secreted antibody produced by this cell line were characterized, **Figure 1**.

mAb Characterization

Titer: recombinant IgG antibody titer was determined using a Poros® A/20 Protein A column, (2.1 x 30 mm) with 0.1 M NaPi, 0.5 M NaCl, pH 6.9 equilibration and pH 2.5 elution buffer system.

Purification: used Eshmuno® A Protein A resin packed into a Pierce™ mini-column equilibrated with PBS. The clarified CHO feed (700 µL) was loaded in five sequential cycles to concentrate the antibody on the resin before elution with 600 µL of 50 mM NaOAc at pH 2.5. Elution fractions were neutralized to pH 5.5 with 2 M Tris-base.

Aggregation and/or fragmentation analysis: SEC analysis with a Tosoh TSKgel® G3000SWXL column, 0.05 M NaPi, 0.3 M NaCl pH 6.9 at 0.75 mL/minute. A total of 100 µg of antibody was loaded for analysis.

Charge heterogeneity: determined using WCX-10 analysis with YMC BioProSP-F column and CX-1 Buffer A (pH 5.6) and B (pH 10.2) at 0.8 mL/minute. Fluorescence detection was set at 280 nm/340 nm and 5 µg were injected for analysis.

Glycan analysis: 200 µg of the antibody was deglycosylated using PNGase Fast, labeled with 2-AB (ProZyme®), cleaned with GlykoPrep® Cleanup Module (ProZyme®), and analyzed by Agilent UPLC 1290 with Water's ACQUITY UPLC®BEH Glycan Column (1.7 µm, 2.1 x 150 mm) at 0.5 mL/minute. Buffer A was 100% of acetonitrile and buffer B was 100 mM ammonium formate at pH 4.0.

Figure 1. Methods for mAb Characterization

Statistical analysis

Minitab® software was used to identify differences in perfomance between cells grown with media stored in bags made from Ultimus® film and those with media stored in glass bottles. A two-sample t-test was used to analyze differences in integrated viable cell density (IVCD) ($\times 10^6$ cells*hour/mL), viability (%), and titer (g/L).

Statistical analysis of the mAb producing CHOZN® GS -/- (cell line #3) performance grown in bags made from two lots of Ultimus® film and bottles were compared with a one-way analysis of variance (ANOVA).

Results & Discussion

As biomanufacturers expand their use of single-use technologies in bioprocessing operations, suppliers are innovating to meet their needs. Single-use films for use in cell culture processes must be able to support healthy cell growth while providing strength and resistance to leaking, particularly when processing large volumes.

Ultimus® film was developed as a single-use solution to meet the challenges of large volume processing, and has superior abrasion and tear resistance, puncture strength and durability[2].

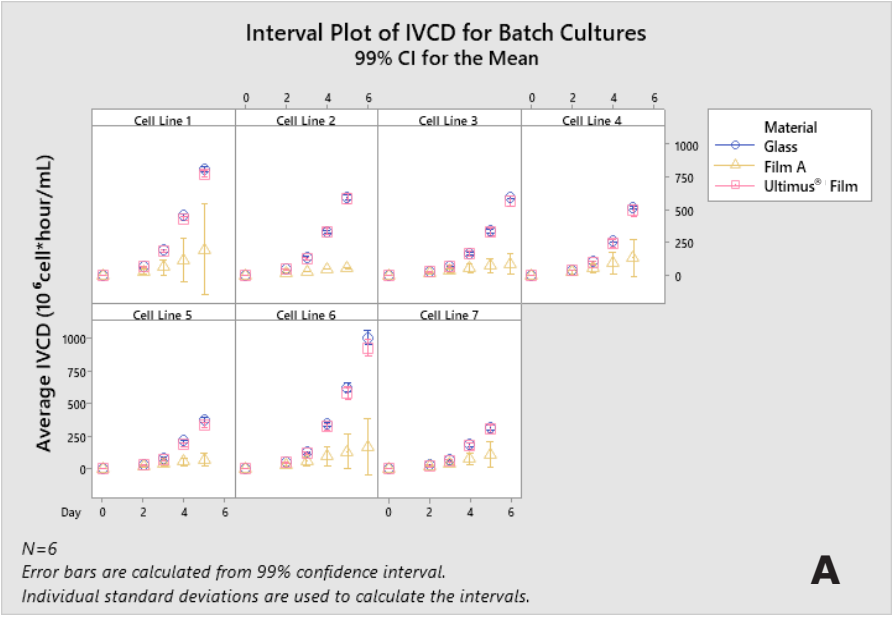
The purpose of these studies was to assess cell culture performance of a panel of CHO cell lines grown in spin tube cultures with cell culture media that had been stored in bags made with Ultimus® film and to benchmark performance with that of spin tube cultures grown in cell culture media stored in glass bottles.

Batch cultures: Ultimus® film does not negatively impact cell line growth and viability

Figure 2 shows the integrated viable cell density (IVCD) and cell viability results from batch cultures of the seven CHO cell lines. No differences were identified in any of the cell lines between the cultures grown in media stored in bags made with Ultimus® film, and that stored in glass bottles. Statistical analysis with a two-sample t-test at a 99% confidence level revealed that the mean IVCD and viability of cells was not statistically significant or less than 10% different due to instrument variability. The exception was the CHOZN® GS -/- cell line producing a fusion protein (cell line #5), where IVCD was slightly different. However, further analysis of fed batch cultures of this cell line did not reveal any significant differences in growth.

By contrast, the growth of all seven CHO cell lines grown in media stored in bags made from another commercially available film that contained Irgafos® 168, designated Film A, was markedly different to growth using media stored in glass bottles or Ultimus® film: both IVCD and viability were notably lower.

These results illustrate the potential negative impact of single-use film that contains Irgafos® 168 on cell culture performance and highlights the benefits of a fluid contact layer free of Irgafos® 168 designed specifically for challenging bioprocessing applications.



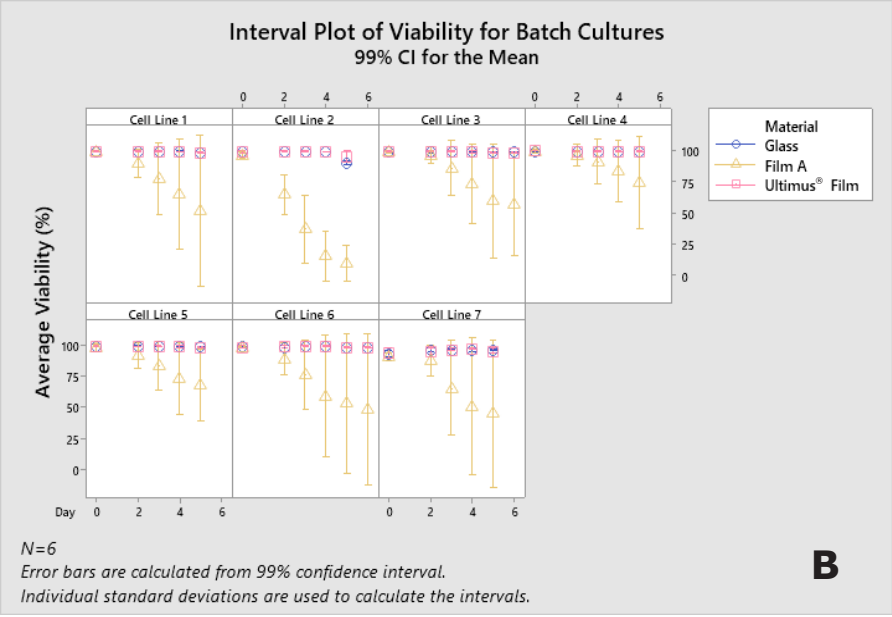


Figure 2. IVCD (A) and viability (B) of batch cultures of seven CHO cell lines grown in media stored in bags made from Ultimus® film, glass, or another commercially available film, Film A. Each data point represents the mean of 6 replicates, and error bars were calculated from the 99% confidence interval.

Fed-batch cultures: Ultimus® film does not negatively impact cell line growth, titer, and viability

The impact of Ultimus® film was further evaluated in fed-batch cultures, 3 of the 4 cell lines are shown in **Figure 3** with the fourth cell line shown in **Figure 4**. As with the batch culture, no differences in growth were identified between the cultures grown in media stored in bags made with Ultimus® film and that stored in glass bottles. In addition, antibody titers were not impacted by cell culture media storage conditions. Statistical analysis confirmed differences were either not statistically significant or less than 10% different due to instrument variability.

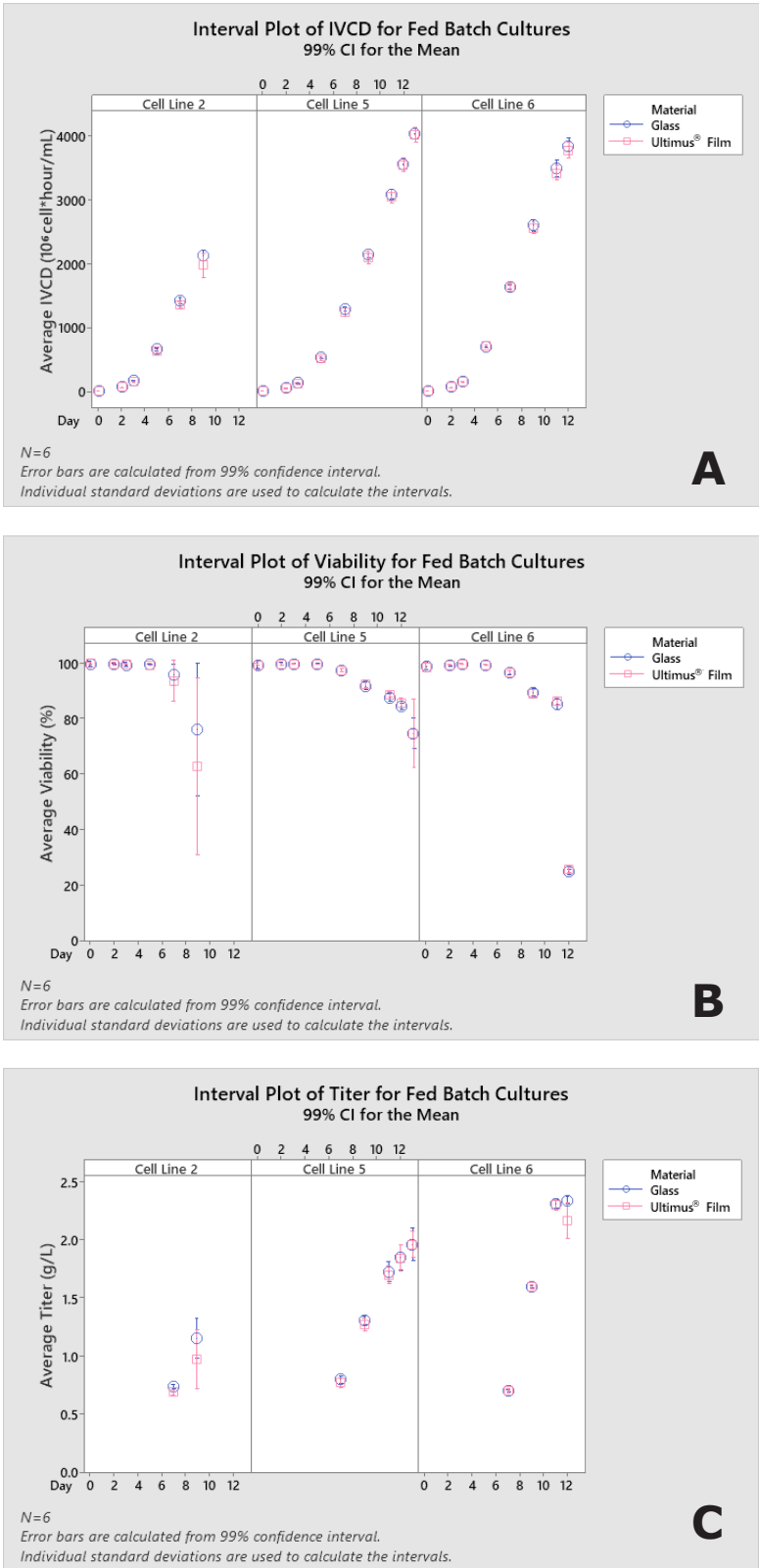
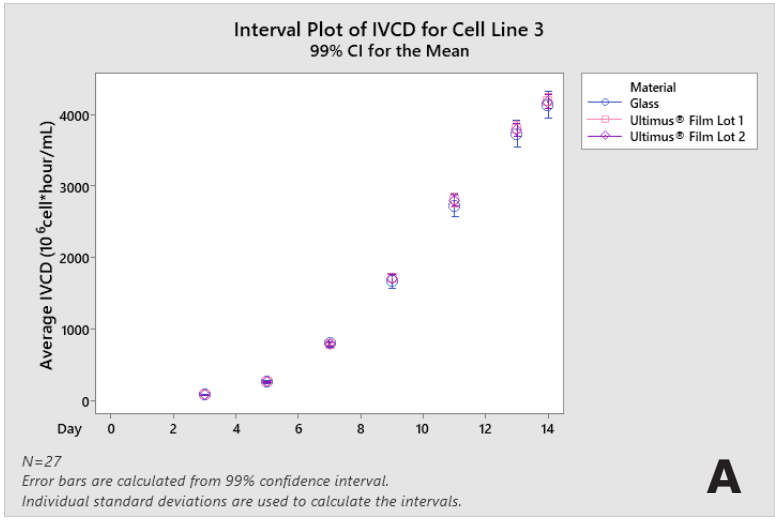
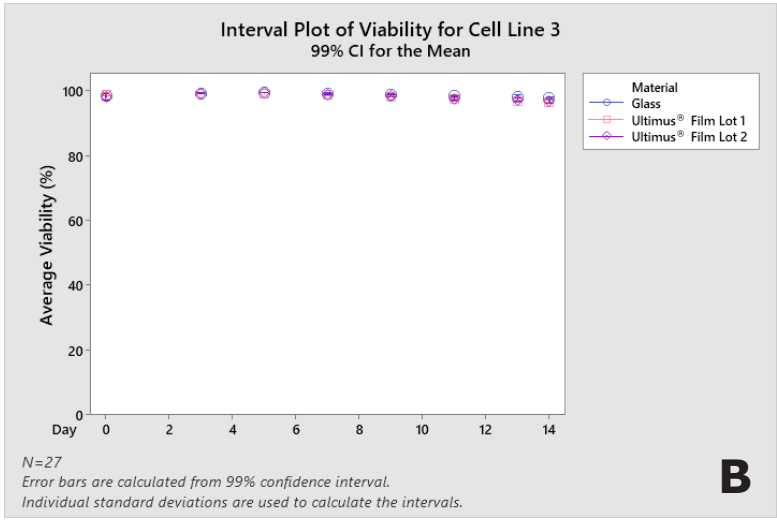


Figure 3. IVCD (A), viability (B) and titer (C) of fed-batch cultures of three of the four CHO cell lines grown in media stored in bags made from Ultimus® film or glass. Each data point represents the mean of 6 replicates and error bars were calculated from the 99% confidence interval.

A more comprehensive study of the fourth cell line, an antibody producing CHOZN® GS -/- (cell line #3), assessed growth of fed-batch cultures with media stored in bags made from two different lots of Ultimus® film. Statistical analysis (ANOVA, with 99% confidence) confirmed that differences in IVCD, viability, and titer between cells grown from media stored in different lots of Ultimus® film and glass, were either not statistically significant or less than 10% different, **Figure 4**.





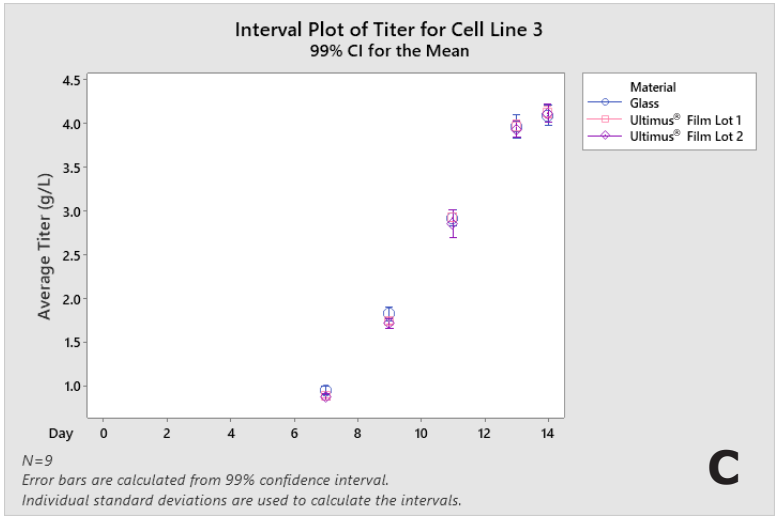


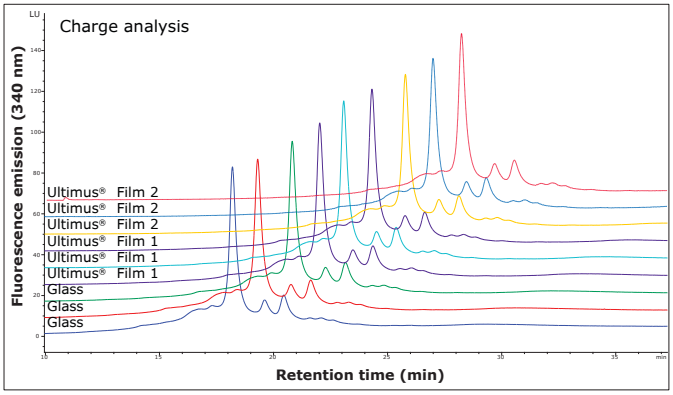
Figure 4. IVCD (A), viability (B) and titer (C) of fed-batch cultures of antibody producing CHOZN® GS -/- (cell line #3), grown in media stored in bags made from two lots of Ultimus® film or glass bottles. Each data point represents the mean of 27 replicates for IVCD and viability and 9 replicates for titer; error bars were calculated from the 99% confidence interval.

Ultimus® film does not negatively impact product quality

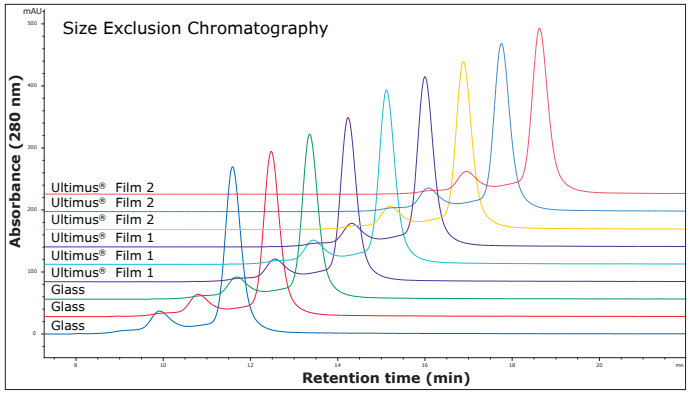
Although results with Ultimus® film had not indicated any issues in cell culture performance, the antibody produced by CHOZN® GS -/- (cell line #3) was evaluated to determine if the storage conditions of cell culture media in bags made from Ultimus® film changed product quality of the secreted antibody.

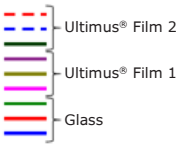
Figure 5 shows the results of charge (charge heterogeneity profile), aggregate profile and glycan structure (glycan profile) analysis of the secreted antibody. Overall profiles were similar between all samples, and confirmed that storing cell culture media in bags made with Ultimus® film did not negatively impact product quality attributes.

Charge Profile

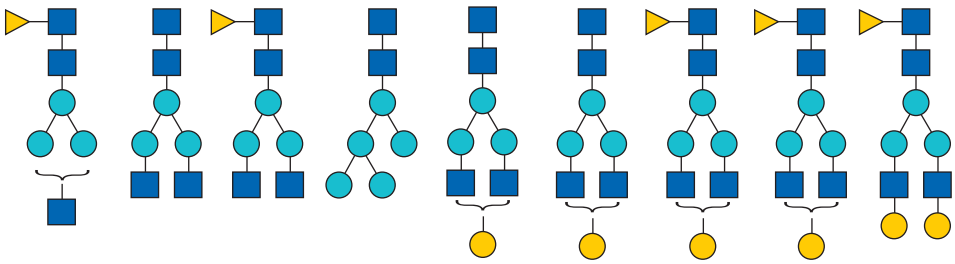


Aggregation Profile





Sample	Glycan Structure (Peak Area%)									
	G0F-N	G0	G0F	Man5	G1a	G1b	G1Fa	G1Fb	G2F	**
Glass	3.6	4.4	72.4	2.5	0.0	0.0	5.8	3.0	0.0	8.2
Glass	4.6	5.5	73.5	3.1	0.0	0.0	5.7	2.8	0.0	4.8
Glass	4.2	5.2	71.8	3.1	0.7	0.0	6.4	3.1	0.6	4.9
Ultimus® Film 1	4.5	5.6	74.1	3.0	0.0	0.0	5.1	2.5	0.0	5.3
Ultimus® Film 1	4.5	5.6	73.3	3.1	0.0	0.0	5.2	2.6	0.0	5.8
Ultimus® Film 1	4.3	5.3	73.3	3.0	0.5	0.0	5.4	2.7	0.0	5.5
Ultimus® Film 2	4.4	5.5	72.7	3.0	0.6	0.0	5.5	2.8	0.5	5.1
Ultimus® Film 2	4.3	5.2	72.6	3.1	0.6	0.0	5.6	2.9	0.0	5.7
Ultimus® Film 2	4.1	5.3	72.8	3.1	0.6	0.0	5.5	2.8	0.5	5.4



** = total of unidentified peaks

Figure 5. Comparison of charge profile, aggregate profile and glycan profile of secreted antibody grown in fed-batch cultures with media incubated in different lots of Ultimus® film or glass.

Conclusion

The results of this comprehensive study with seven CHO cell lines (six of which express either an antibody or recombinant protein), provide confidence in the use of bags made with Ultimus® film for single-use upstream cell culture processes.

Cell growth and productivity using media that was stored in bags made from Ultimus® film or glass containers showed equivalent performance within normal variations for growth, viability, recombinant protein production, and product quality. By contrast, when these same cell lines were cultured in media that had been stored in bags made from a film that contained Irgafos® 168, IVCD and viability was markedly reduced.

Bags made with Ultimus® film offer new opportunities for upstream cell culture processing: the fluid contact layer is free of animal origin components and Irgafos® 168 and offers low extractables and leachables while the innovative strength layer protects against leaks. Combined, Ultimus® film offers a smart design that minimizes risk to make your work more secure.

References

1. Hammond M, Nunn H, Rogers G, Lee H, et al. Identification of a leachable compound detrimental to cell growth in single-use bioprocess containers. PDA. J Pharm Sci Technol. 2013; 67:123-134.
2. Demonstrated Strength and Durability of Ultimus® Film, 2022, Lit. no. TB5661EN

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