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White Paper

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Keywords or phrases:
Optimization of the antioxidant packages for Flexboy® and Flexsafe® single-use bags enables excellent cell growth.

Lot-to-lot consistency of cell growth performance is achieved with specifications and controls on the resins and films.

Cell Growth Performance in Single-use Bags

The uptake of single-use bags in upstream processing from R&D to cGMP clinical and commercial production requires superior and consistent cell growth performance.

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Abstract

Achieving consistent cell growth is critically important to the biotech industry. To optimize final drug product output, industry trends go toward higher cell densities and protein titers. Some manufacturers have reported inconsistent cell growth with the uptake of single-use bags in cell culture processes. Hammond & al – Amgen[1] identified a degradation product, the bis (2,4-di- tert-butylphenyl) phosphate (bDtBPP), derived from a commonly used antioxidant in polyethylene films. However, such antioxidants are necessary to keep films stable. They protect the polymer from oxidation during film extrusion and gamma irradiation. To ensure proper cell growth, it is extremely important to optimize and closely control the concentration of these additives. Sartorius has addressed the challenge of cell growth performance in single-use bags with our well-established Flexboy® bags and new Flexsafe® platform. The excellent cell growth performance in our bags is the result of optimized antioxidant packages. The Tris (2,4-di-tert-butylphenyl) phosphite is not present in the contact layer of the Flexboy® bags. For the new Flexsafe® bags, we have minimized and carefully control its concentration. Cell growth performance is established and demonstrated by both cell growth testing and extractable studies.

[1] Matthew Hammond, et al., PDA J Pharm Sci and Tech 2013, 67 123-134

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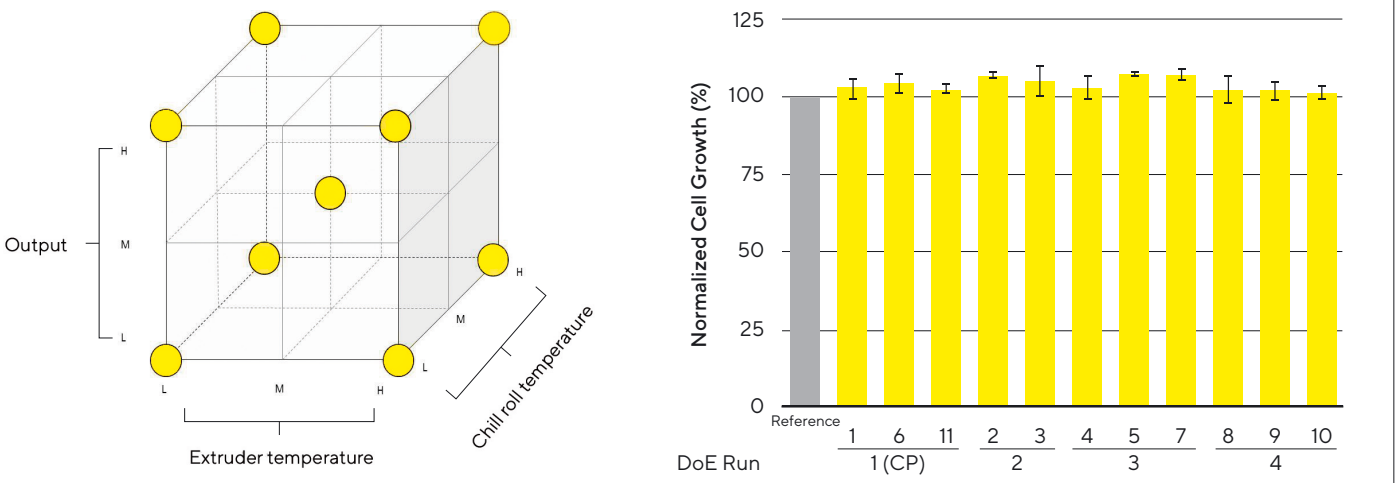
Flexsafe® PE Film Provides Excellent and Reproducible Cell Growth



Cell growth is demonstrated during process validation by running 33 cell growth tests in the entire extrusion design space.
No bDtBPP detected by LC-MS for each extrusion run after extraction in WFI.

Figure 1: Excellent and reproducible cell growth performance demonstrated in the entire extrusion design space

The resins of the S80 film of Flexsafe® bags are optimized, and the extrusion design space was established to ensure excellent and consistent cell growth.

Excellent Cell Growth

The antioxidant package used for the formulation of the S80 PE film of Flexsafe® bags was optimized to limit the concentration of the Tris (2,4-di-tert-butylphenyl) phosphite antioxidant. This limits the generation of the breakdown product, bis (2,4-di-tert-butylphenyl) phosphate (bDtBPP), detrimental to cell growth (1).

Reproducible Cell Growth

Resin specifications, controls of antioxidants levels and control of the extrusion process were established to ensure reproducible cell growth performance. Cell growth is demonstrated through cell growth testing. Verification of the absence of potential inhibiting extractable is then performed.

Flexboy® Eva Film Formulation Provides Excellent Cell Growth

The multi-layer EVA structure of the S71 film of Flexboy® does not contain the Tris (2,4-di-tert-butylphenyl) phosphite anti-oxidant in the contact layer.

Control of our resin and additive formulations, as well as the extrusion process, ensures consistent cell growth performance.

Extractable Testing Demonstrates the Absence of bDtBPP in Water and Ethanol Extracts

Extractable data for Flexsafe® and Flexboy® demonstrate the absence of bDtBPP after worst-case extraction in water for injectable and in ethanol.

We developed a targeted extractable method to detect trace amounts of the bDtBPP compound in films. Extraction was performed in water and ethanol, and extracts were analyzed by means of the most sensitive HPLC-UV.

Our analytical methods for detecting bDtBPP with HPLC-UV uses the bDtBPP standard developed by Amgen (for comparison).

This very sensitive HPLC-UV analytical method helps demonstrate the absence of bDtBPP in the WFI extracts for all Flexsafe® film extrusion runs of the entire design space.

Worst-case extraction in 100-percent Ethanol at 40°C for the central point of Flexsafe® film design space confirms the absence bDtBPP, as the result of the limited quantity and control of the Tris (2,4-di-tert-butylphenyl) phosphite anti-oxidant used for the S80 film formulation.

Additional testing also reveals that no Tris (2,4-di-tert-butyl-phenyl) phosphite could be detected in the WFI extract of Flexboy® bags.

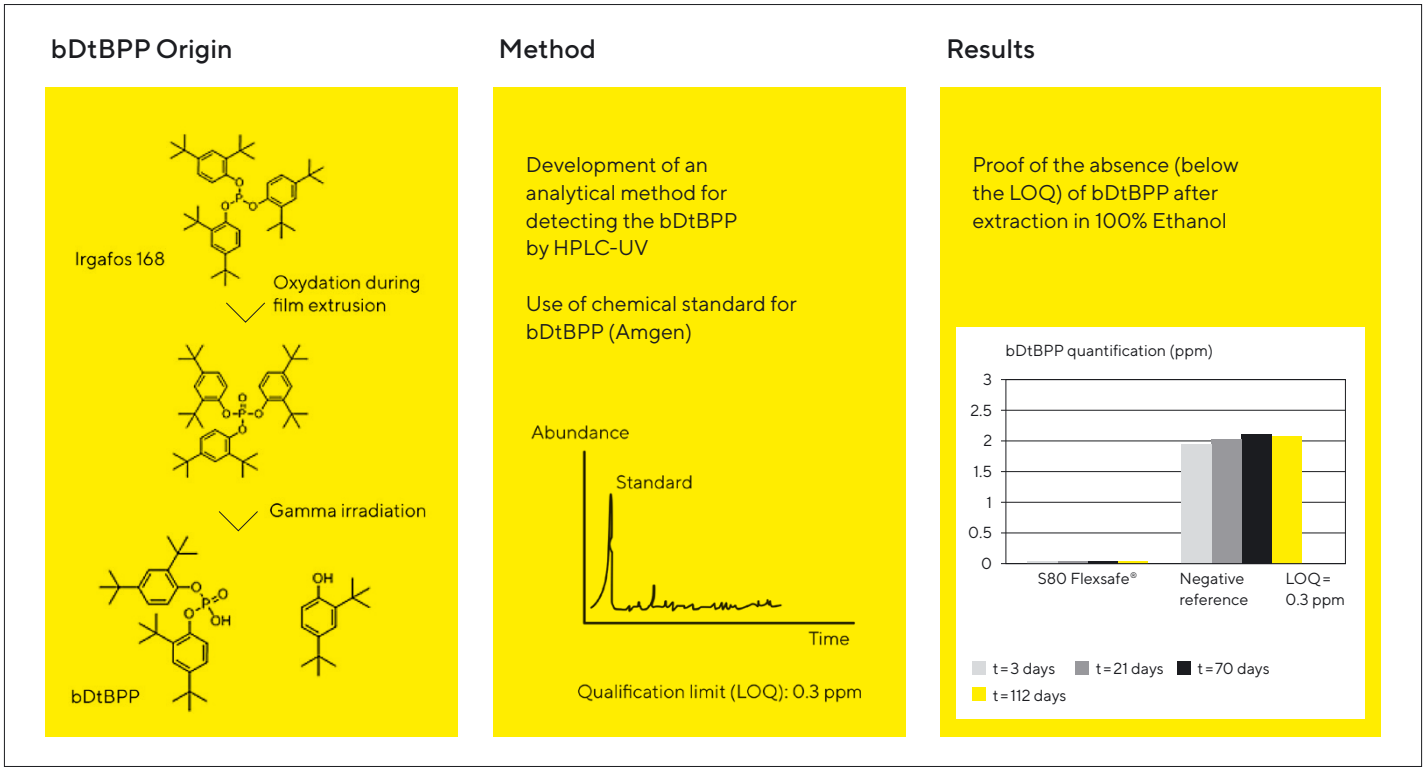


Figure 2: HPLC-UV analytical method shows the absence of bDtBPP in the ethanol extract of Flexsafe®

Extractable Profile for Different PE Films

We used the analytical method for quantifying the bDtBPP in worst-case conditions for several PE films marketed from other suppliers (A to G). This study demonstrates that:

- Sartorius PE films (S80 and S40) showed no detectable bDtBPP levels;
- The negative control sample expressed a level below 2 ppm;
- Other films and bags showed variable levels of bDtBPP suspected to be detrimental to cell growth.

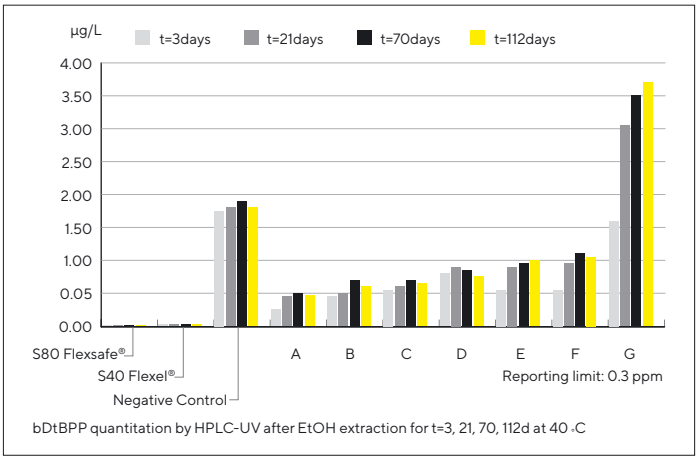


Figure 3: HPLC-UV analysis of 100% EtOH extract does not detect bDtBPP in S80 and S40 PE films.

Cell Growth Performance Demonstrated by Cell Growth Testing

Materials and Methods

We developed a standardized media extraction and CHO cell growth assay applied to both Flexsafe® and Flexboy® bags to demonstrate reproducible cell growth performances across their lifecycles.

The cell growth test involves a media incubation in 0.8 L γ-irradiation bags (~50 kGy).

Bags were filled with 200 mL of 4Cell® XtraCHO Stock & Adaptation Medium and incubated for three days at 37°C. The control medium was incubated in a glass bottle to serve as a reference.

The extraction media were transferred to six-well plates for cell growth tests in triplicate using a CHO DG44 cell line (Cellca) at 2 × 10^5 cells/mL. A CERTOMAT® CTplus shaking incubator was used for agitation at 160 rpm @ 37°C. Cultivation was performed with a 10 mL/well for three days. VCD (Viable Cell Density) and CV (Cell Viability) cell count was performed with a Nucleocounter (Chemometec).

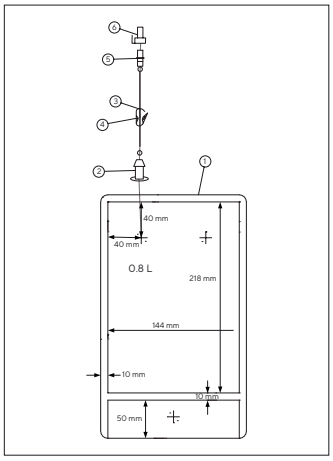


Figure 4: 0.8L bags are gamma irradiated and extracted with media for the cell growth test.

Bag sterilization	Filling with media	Media extraction	Cell growth assay	Qualitative cell culture analysis
0.8 L bags from 3 lots Gamma irradiation at 50kGy	Aseptic filling of 200 mL of 4Cell® XtraCHO Stock & Adaptation Medium into bags S/V = 3 cm^2 | mL	Test media incubation in bags: ▪ 37 °C, 3 days, static ▪ protected from light ▪ Media control incubated in glass bottle	10 mL of media transferred to 6-well-plates Cell growth tests with CHO DG44 cell line (Cellca) at 2 × 10^5 cells/mL for 3 days at 37°C Agitation with CERTOMAT® CTplus at 160 rpm	VCD (Viable Cell Density) and CV (Cell Viability) cell count with a Nucleocounter (Chemometec).

Results and Discussion

Cell growth performance of Flexsafe® and Flexboy® Bags after three years of shelf life

We examined the impact of polymer aging on the biological performance of the S80 & S71 films using an accelerated aging study. Bags were stored up to an equivalent of 36 months according to ASTM F1980 before being filled with media. We conducted the test according to the standardized cell growth method described previously at each due date. A glass bottle container was used as a reference.

Figure 5: Cell growth testing of Flexsafe® against glass bottle reference during the three-year shelf life.

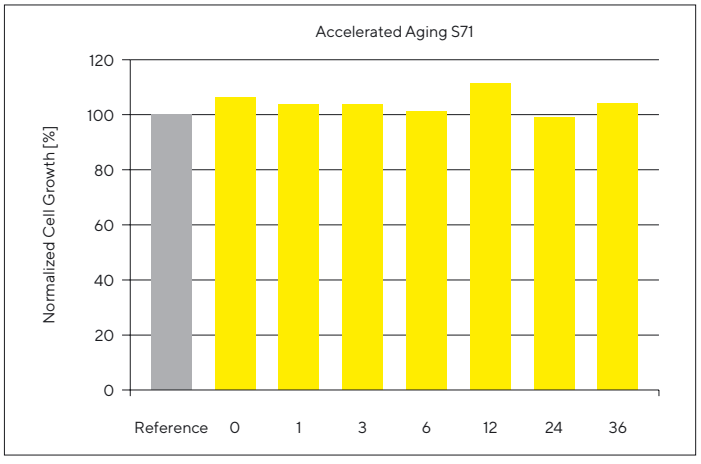


Figure 6: Cell growth testing of Flexboy® against glass bottle reference during the three-year shelf life.

Cell growth performance is comparable between the reference glass bottle) and the results obtained at each due date of aged Flexsafe® and Flexboy® bags under ASTM F 1980 (40°C, 75% humidity). We conclude that the cell growth performance is consistent from freshly irradiated bags up to 36 months of empty bag storage.

We tested other readily available bags from different suppliers and demonstrated that cell growth performance can vary with storage time. The results in Figure 7 show that bags with no control of the additive packages can significantly impact cell growth and more significantly during the early stage of shelf life.

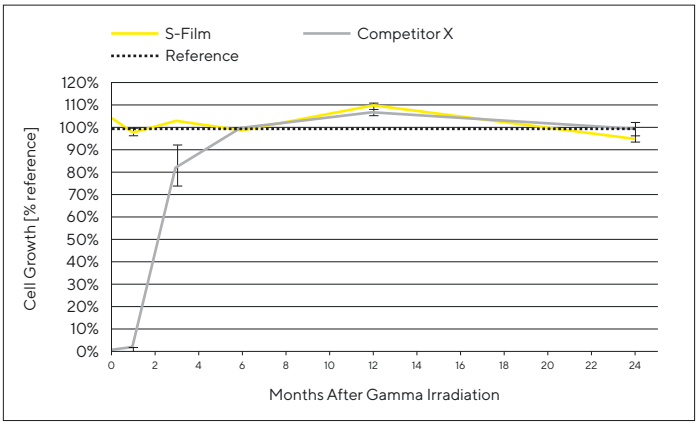


Figure 7: The age of bags can impact cell growth if the additive package is not under control.

Cell growth performance of Flexsafe® Bags after nine months of' media storage

We conducted a prolonged media extraction trial to evaluate the potential migration of toxic leachables from Flexsafe® Bags into cell culture media during normal media storage conditions. The test was conducted according to the standardized cell growth method described previously at each due date up to nine months in storage at 4 – 6°C.

Cell growth performance was comparable between the reference (glass bottle) and the results obtained at each due date of media storage. We concluded that cell growth performance is consistent for media storage from time = 0 up to nine months.

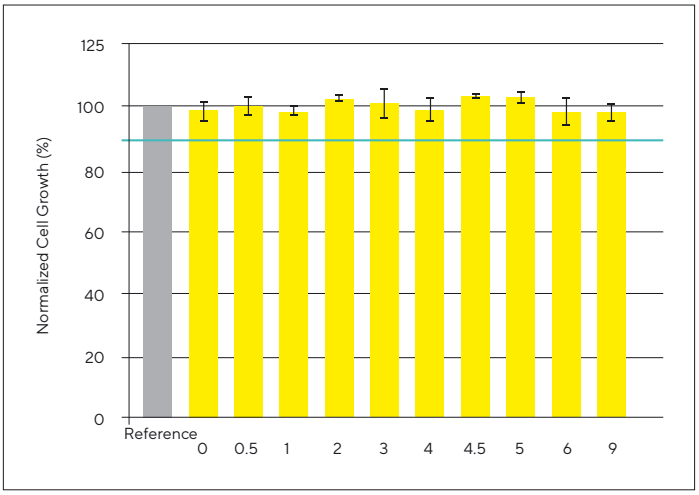


Figure 8: Cell growth testing of Flexsafe® against glass bottle reference shows excellent cell growth with media stored in bags at 4-6°C up to nine months.

Reproducible cell growth performance of Flexsafe® bags established during qualification and routine production

Lot-to-lot reproducible cell growth for Flexsafe® was established during process qualification and product validation and was further controlled during production using our internal cell growth test.

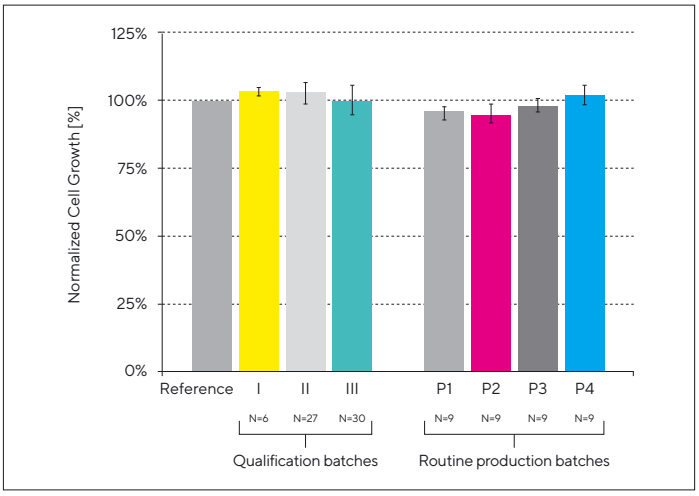


Figure 9: Reproducible cell growth established during validation and controlled during production

Excellent and Reproducible Cell Growth

Excellent cell growth obtained by optimization of the resin and& additive package formulation

- Limited concentration of antioxidant – Tris (2,4-di-tert-butylphenyl) phosphite – in the PE film contact layer
- Cell growth demonstrated by both cell growth testing and extractable study

Reproducible cell growth ensured by the establishment of the design space and & control of critical process parameters

- Cell growth reproducibility validated in entire extrusion design space
- Lot-to-lot reproducibility with complete control of our process from resin and additives to bags
- Routine cell growth testing in production

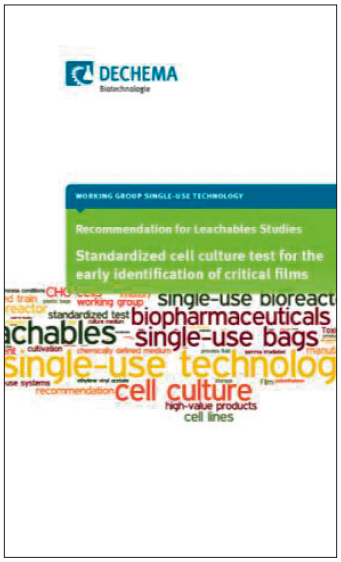
Cell Growth for Flexsafe® & Flexboy® Demonstrated on a Broad Panel of Cell Lines

In addition to the extensive internal testing program, we ordered an external study by an independent laboratory – Dechema[2]. The goal was to evaluate the cell growth performance of commonly used bags available on the general market with a broad panel of commonly used media and cell lines.

The external broad panel cell growth test study demonstrates the superior cell growth performance of Flexsafe® and Flexboy®.

Dechema Study

- Suppliers sent bags to the Zurich University of Applied Sciences (ZHAW)
- Bags were subjected to media extractions and& WFI extractions in blind tests.
- Positive control: borosilicate glass
- 11 films tested, including negative control
- Sartorius Stedim supplied S71 (EVA) and S80 (PE)



Dechema	WFI Extraction	Media Extraction
SSB	S80 passed S71 passed	S80 passed S71 passed
Other Suppliers	4 films passed 4 films failed	2 films passed 6 films failed

[2] Eibl et al: Standardized cell culture test for the early identification of critical films for CHO cell lines in chemically defined culture media Dechema: ISBN: 978-3-89746-149-9

Film	Maximum Cell Density									Viability									Cell Diameter			
	V1	V2	V3	V4	V5	V6	V7	V8	V9	V1	V2	V3	V4	V5	V6	V7	V8	V9	V1	V2	V3	V4
1	46%	+	20%	16%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	64%	30%	25%	21%	12%	25%	38%	+	61%	+	+	+	+	+	+	+	-	-	+	+	+	+
3	57%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4*	20%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
5	52%	21%	+	12%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6**	39%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	33%	+	25%	17%	+	+	+	+	38%	+	+	+	+	+	+	+	+	-	+	+	+	+
8 (NK)	68%	42%	22%	26%	50%	55%	95%	12%	80%	-	+	+	+	+	+	-	-	-	+	+	+	+
9	20%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10	50%	33%	21%	19%	+	+	+	+	57%	+	+	+	+	+	+	+	+	-	+	+	+	+
11	73%	+	23%	17%	13%	+	+	+	47%	+	+	+	+	+	+	+	+	-	+	+	+	+

* Film 4 - S71
**Film 6 - S80

(+) no deviation, (%) deviation from the control, (-) deviation occurred, (k) no or negligible deviation, (m) moderate deviation, (s) strong deviation NK negative control

Experiment	V1	V2	V3	V4	V5	V6	V7	V8	V9
Cell Type	CHO DG44	CHO DG44	CHO K1	CHO K1	CHO K1	CHO K1	CHO K1	CHO DG44	CHO K1

Figure 10: Inter-laboratory test for detection of leachables arising from single-use bags - DECHEMA

Quality by Design and Process Control Ensures Reproducible Cell Growth

Multiple factors such as the additive formulation, extrusion parameters and gamma sterilization can affect the release of bDtBPP. Reproducible cell growth can only be obtained with complete control of the entire process, from the resins and film extrusion to the final bag assembly. Quality by design principles, material science, film extrusion know-how and bag making expertise is needed to ensure consistency of cell growth and extractable profile.

A detailed knowledge of the formulation of our resins and additives also enables the accurate quantification and identification of the extractable profiles of Flexsafe® bags.

Control of the resins and all other critical process steps such as extrusion, welding and gamma radiation ensure lot-to-lot consistent extractable profiles. The extractable data contained in our extractable guides or the specific leachable studies performed by our Confidence® Service remain representative and valid for risk assessments throughout the product life cycle.

The excellent and reproducible cell growth of our Flexsafe® and Flexboy® bags is established with the control of raw materials and processes and demonstrated by both our internal and the external Dechema studies. All of our bags provide reliable, consistent and economic upstream processing, with the most sensitive commercial cell lines.

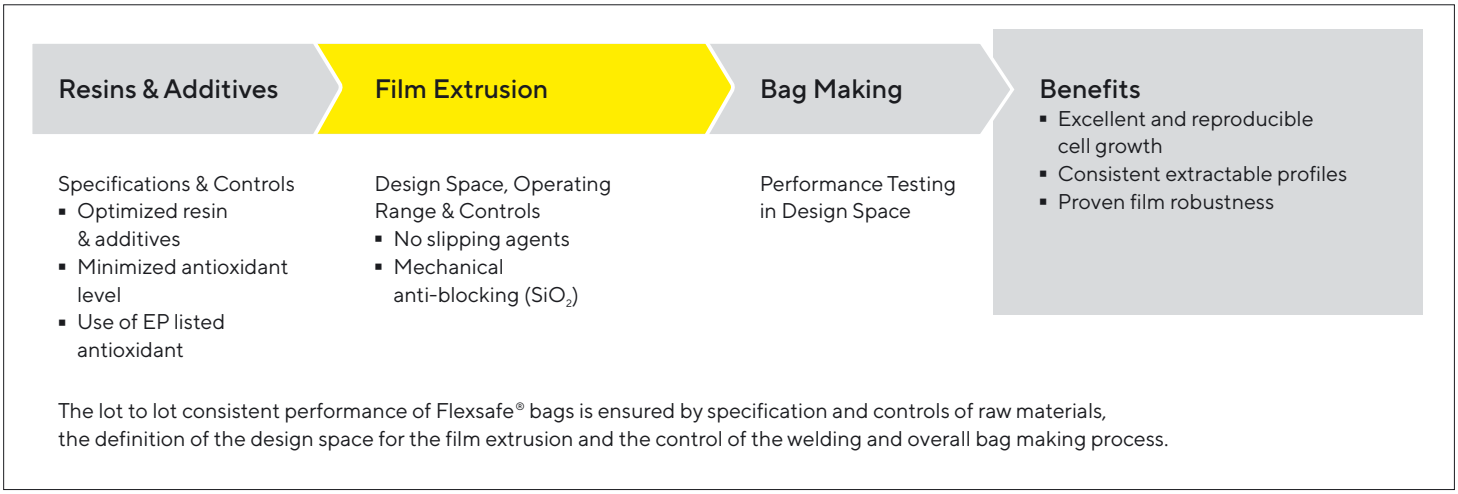


Figure 11: Quality by design principles for the development and validation of Flexsafe® bags.

Sales and Service Contacts

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