



# The Strength and Flexibility of Flexsafe<sup>®</sup>, Flexboy<sup>®</sup> & Celsius<sup>®</sup> Bags Ensure their Robustness in all Applications

Flexsafe<sup>®</sup>, Flexboy<sup>®</sup> and Celsius<sup>®</sup> bags allow safe and convenient bioprocessing in clinical and commercial biomanufacturing.



## Key Points

- Selection of the best raw materials and optimization of the production process for consistent robustness
- Robustness is qualified across the design spaces of all bag product lines
- The robustness of Flexsafe<sup>®</sup> has been demonstrated using the most stringent ASTM D 4169 liquid shipping test

Biomanufacturers commonly use single-use bags during the production of biopharmaceuticals. Increasingly, they apply the technology in every step along the bioprocess. However, manufacturers incur significant financial costs in the event a bag leaks.

These costs are highest when the leakage occurs during steps that are critical to patient safety.



Bag failures cost ~\$100K to \$1M per bag

**(R. Wong, Bayer)**



Leaks have been reportedly responsible for up to \$20 m worth of products per year at some larger facilities.

**(Bioplan)**

The industry must ensure, therefore, that single-use bags are robust. To achieve this, suppliers must understand the requirements of their customers' processes and applications and use their knowledge of raw material science to translate these requirements into bag quality attributes. The robustness of Sartorius films makes them safe & easy to use for all process steps & applications. The strength brings safety & ease-of-use for large-scale cell culture, mixing and storage of high value drug substances and drug products. The flexibility of these films ensures safety and ease-of-use for rocking motion cell culture, freezing, shipping and storage of high value drug substances and drug products.

Bags often fail because their construction is not robust or because they are mishandled during shipment and use. Stiff bags are not resistant to fatigue and can be difficult to handle. Highly flexible bags may not withstand high pressures.

Sartorius Stedim Biotech manufactures Flexboy® and Celsius® bags from an EVA, S71 film and has, to date, produced over 20 million bags. The new Flexsafe® bags are manufactured from a PE, S80 film and are setting a new benchmark in robustness and ease-of-use for safe, convenient single-use processing.

These films provide superior thickness, strength and flexibility for outstanding robustness in all applications. The strength

of the films reduces the risk of damage to the bags while their flexibility allows the bags to self-deploy.

To provide consistent robustness and ease-of-use for all our bags we have applied our expertise in material science. Our film expertise has allowed us to select the most suitable raw materials and film layers for best thickness, strength, flexibility and weldability. Consistent robustness across the entire extrusion design space has been ensured by applying Quality by Design (QbD) principles and thorough product validation (Figure 1). The control of the extrusion and welding processes and the routine quality control of films & bags guarantees lot-to-lot consistent robustness.

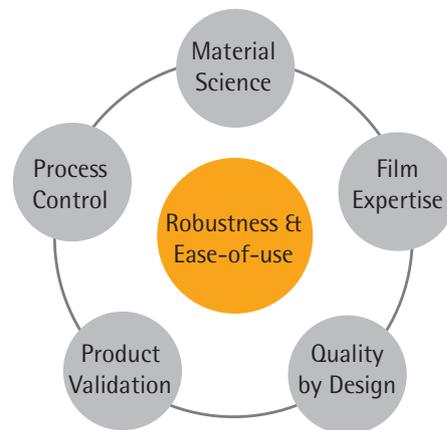


Figure 1: Robustness, Safety & Ease-of-use by Design

## Material Science Identified the Most Suitable Raw Materials

Sartorius has a thorough knowledge of all the resins and additive formulations used in the manufacture of our films. We have selected the basic type of polymer and molecular architecture to meet the requirements of all bioprocess applications. Our suppliers produce the resins we have selected in very large quantities. The use of these blockbuster resins provides assurance of supply.

Sartorius has optimized the use of antioxidant additives in the resin formulation to ensure low levels of extractable and leachable associated with the degradation of these compounds. This optimization

has not compromised the long-term performance and resistance to gamma radiation of the film.

The resin formulation was optimized by:

- Removing slipping agents
- Using mechanical rather than organic antiblocks
- Optimizing primary (long-term) and secondary (short-term) antioxidants packages
- Using additives specified in European Pharmacopeia to ease toxicological assessment

Our new S80 multilayer polyethylene film has been specified and designed in partnership with Südpack, the leading manufacturer of films. This partnership was enhanced by close collaborations with polymer and additive suppliers (Figure 2).

Sartorius performed extensive prototyping during which various polymers and film compositions were evaluated to achieve the best mechanical properties. This allowed the selection of the best resins in each layer of the S80 film and their associated extrusion parameters.

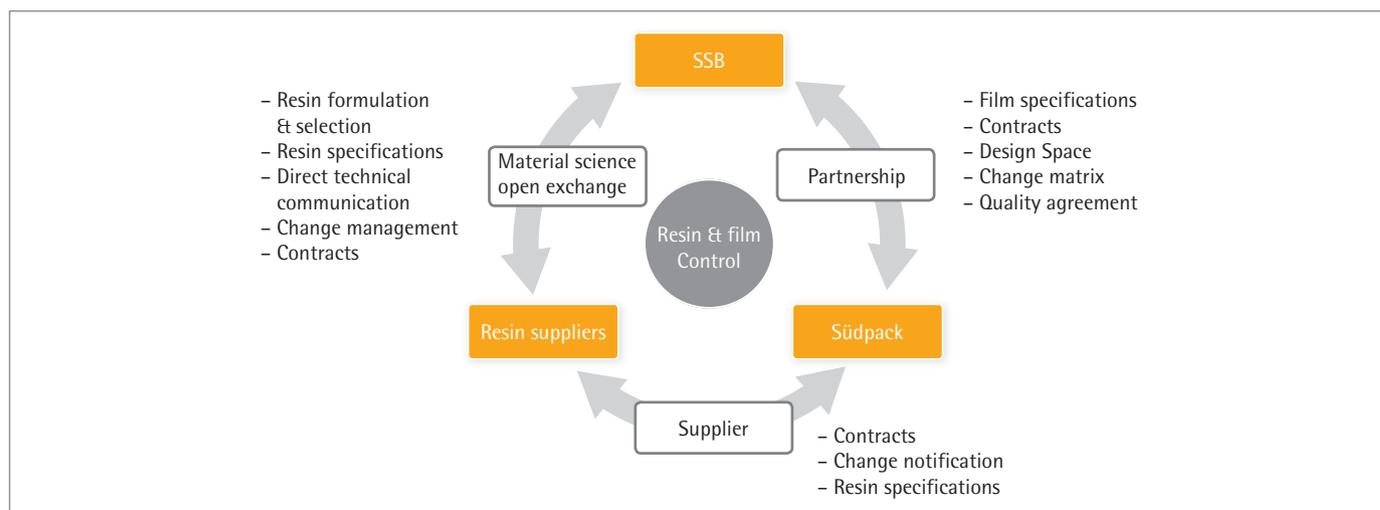


Figure 2: Partnerships & Supplier Agreements Help Achieve Raw Material – Resins Specifications and Control

# Film Expertise Helps Select the Best Layer Formulations and Combinations

Our films are highly resistant to impact and puncture, are easy to handle and resistant to fatigue. They are perfectly able to withstand the hydrostatic liquid pressure associated with large volume bags.

Our S71 and S80 film structures are described in Figure 3. Combining thickness, strength and flexibility for our PE & EVA films provides outstanding robustness and ease-of-use. The strength reduces the risk of accidental damage and the flexibility provides easy installation and self-deploying of 3D bags.

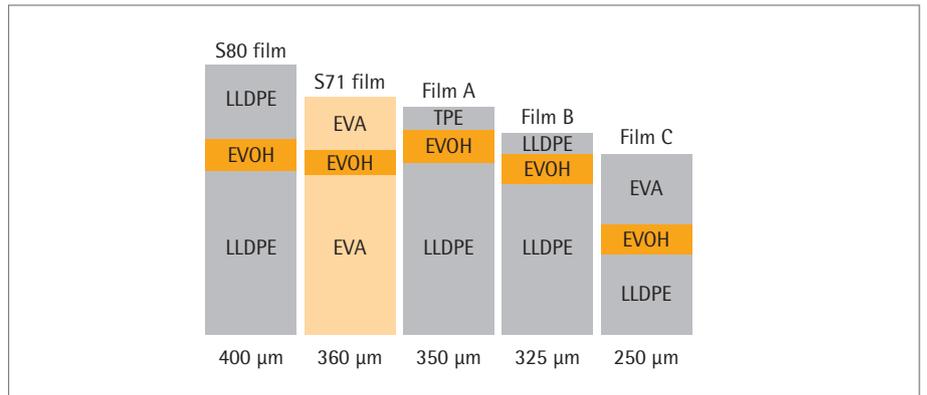


Figure 3: S71 and S80 film structures

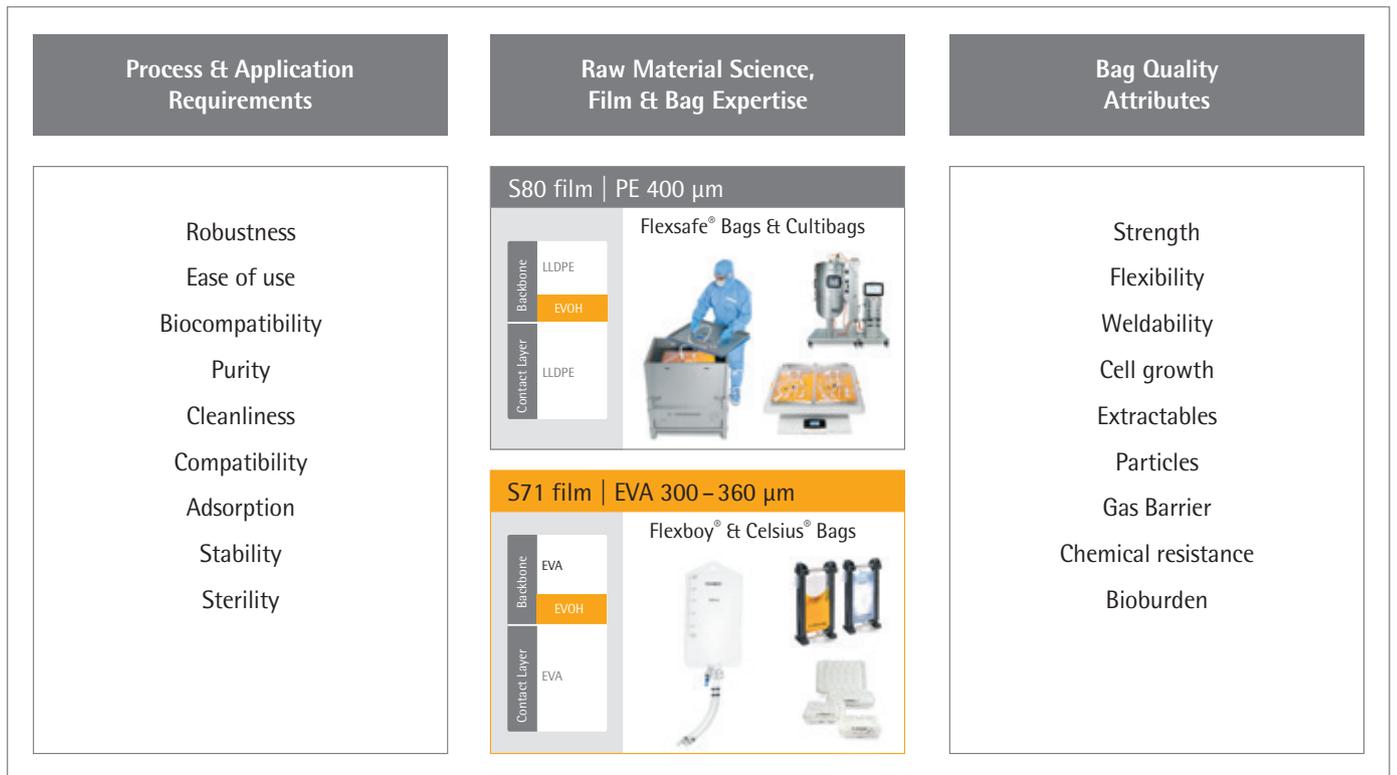


Figure 4: Achieving Bag Critical Quality Attributes (COAs) through Material Science and Film Expertise

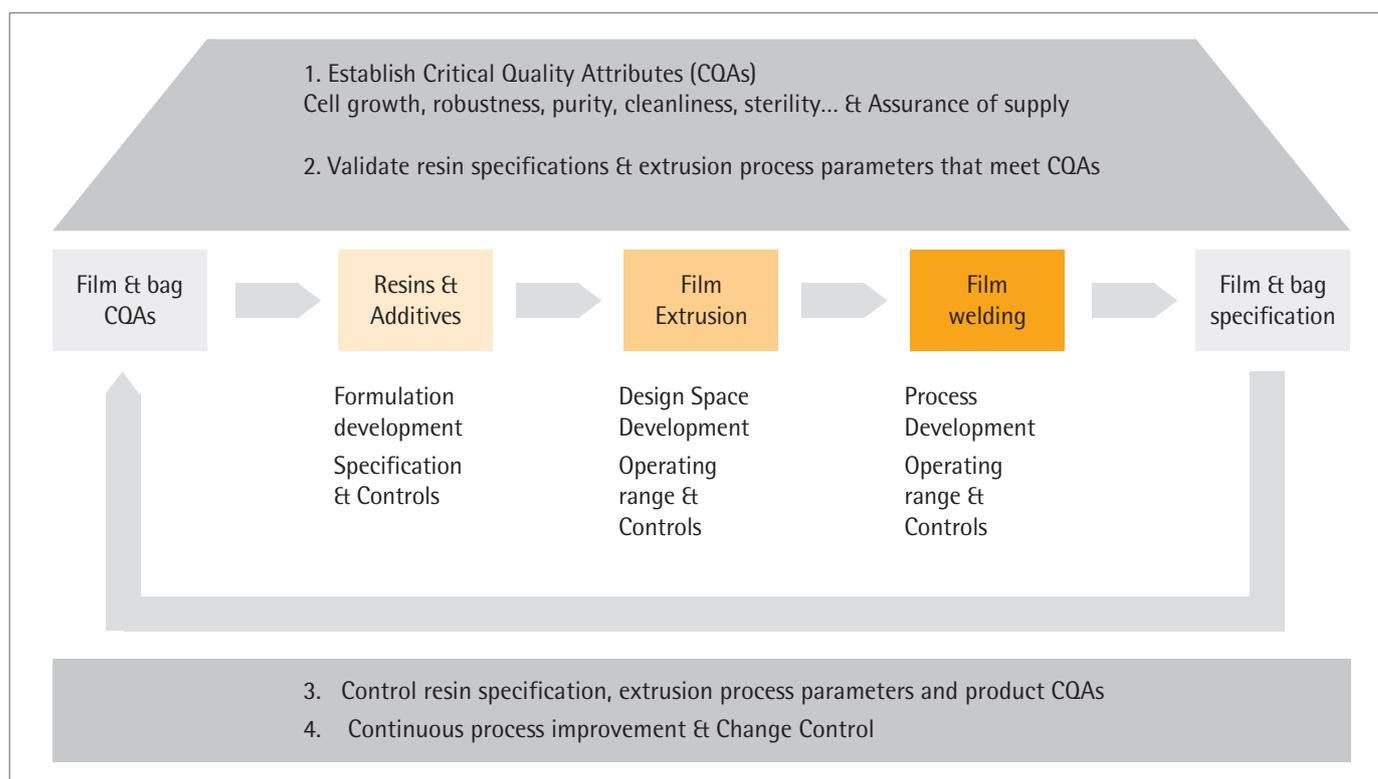
Figure 4 describes how we achieve the single-use bag COAs (Critical Quality Attributes) by applying material science and film expertise to ensure the bags meet the needs of the applications in which they are to be used.

The raw materials and thickness of each layer have been selected carefully to provide the optimal robustness and weldability. These layer combinations and formulations allow a balance to be struck between film stiffness, flexibility and barrier properties that ensures all the film COAs are met.

## Quality by Design (QbD) and Process Controls Help Achieve Consistent Film & Bag Robustness

A Quality by Design (QbD) approach, as described in ICH guidelines Q8, Q9 & Q10, was adopted to ensure that Sartorius single-use bags consistently meet the intended performance criteria.

The QbD approach was used to set the specifications and the design space for bag production. A set of CQAs for robustness were established prior to the start of development (see page 3).



**Figure 5:** Quality by Design (QbD) and Process Controls help achieve consistent film and bag robustness

### Quality by Design (QbD) and Process Controls Help Achieve Consistent Film and Bag Robustness

Resins, additives and process parameters were chosen to meet these quality attributes and the specifications set accordingly. The method for determining the film extrusion design space is provided in Figure 5. Once validated, specifications and process controls were established. We are providing an additional level of control through our continuous innovation and process improvement programs.

### Robustness Validated Across Entire Design Space of Flexsafe®, Flexboy® and Celsius® Bags

The design spaces and process controls for the extrusion of the films used in Flexsafe®, Flexboy® and Celsius® containers guarantee robustness and other consistent critical quality attributes.

Critical process parameters (3 CPPs) were defined based on the required critical quality attributes of both S71 and S80 films.

A design of experiments approach was used to determine the design space and film manufacturing window. Robustness was validated within the design space for Flexsafe®, Flexboy® & Celsius® bags with a full factorial  $2^3$  experiment consisting of 11 extrusion runs (3 center points and 8 variations of parameters).

**More than 3200 robustness tests performed in the entire design spaces of our S80 and S71 films**

**Flexsafe® – Co-extruded  
PE | EVOH | PE Film**

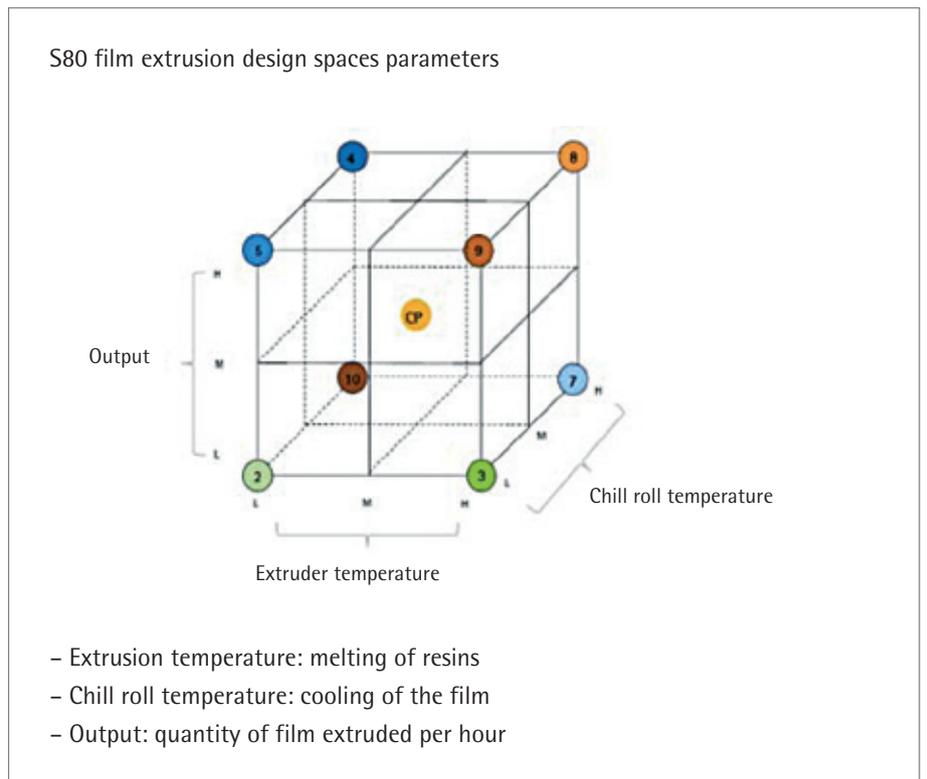
The design space parameters for the S80 film are provided in Figure 6.

**S80 film extrusion design space parameters**

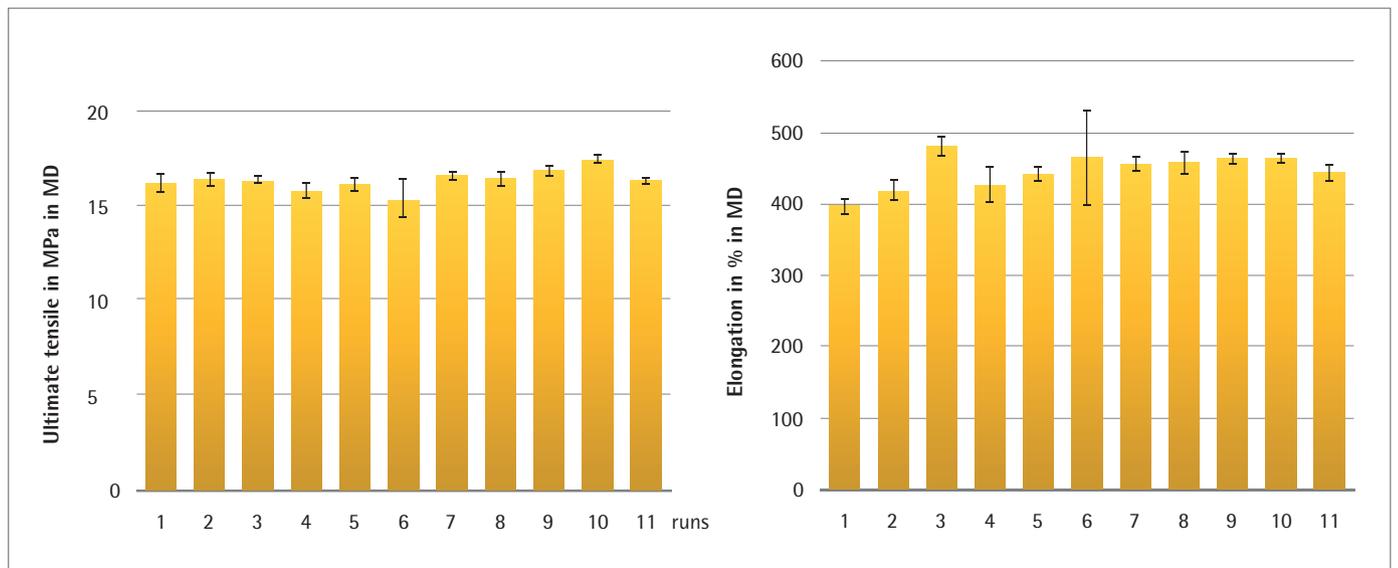
More than 3000 robustness tests were performed on samples from across the design space for:

1. Strength (UTS: ultimate tensile strength), 930 samples tested
2. Flexibility (Elongation), 930 samples tested
3. Puncture, 930 samples tested
4. Flex durability, 186 samples tested
5. Water burst, 26 samples tested

The results show lot-to-lot reproducibility & consistency of the target critical attributes of Flexsafe® bags (Figure 7a & b).



**Figure 6:** S80 film extrusion design space parameters



**Figure 7a:** S80 ultimate tensile strength results

**Figure 7b:** S80 elongation results

## Flexboy® & Celsius® – Laminated EVA | EVOH | EVA Film

The design space parameters for the S71 film are provided in Figure 8

~200 robustness tests were performed on samples from across the design space for:

1. Tensile Strength – 110 samples tested
2. Lamination Strength – 110 samples tested

The results show lot-to-lot reproducibility & consistency of the target critical attributes of Flexboy® and Celsius® bags (Figure 9).

In conclusion, Sartorius has validated robustness across the entire design space for Flexsafe®, Flexboy® & Celsius® bags

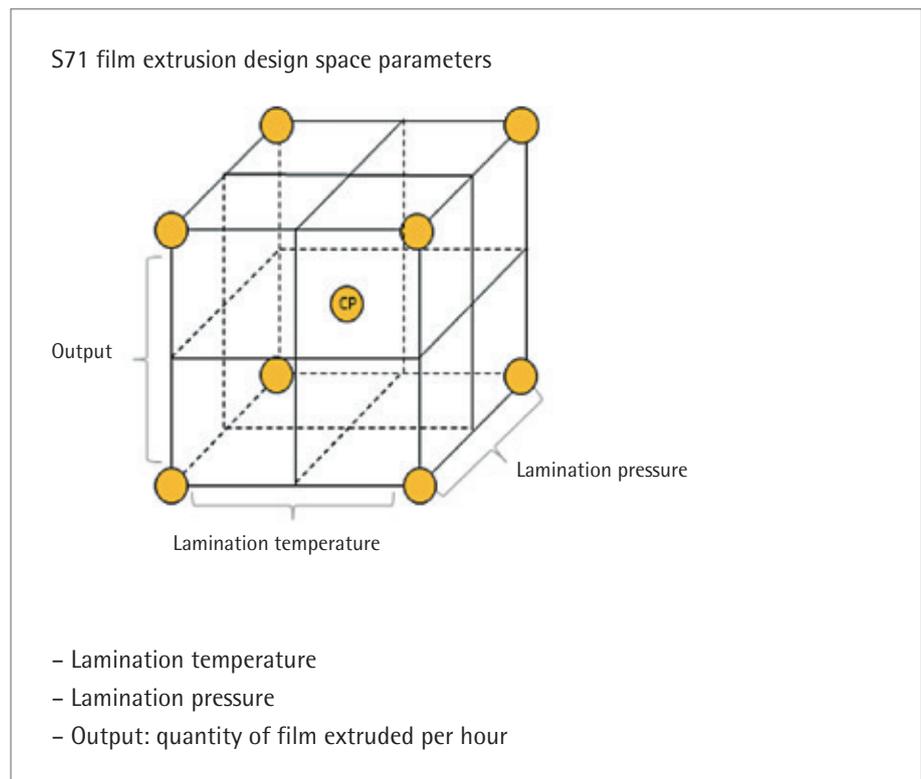


Figure 8: S71 film extrusion design space parameters

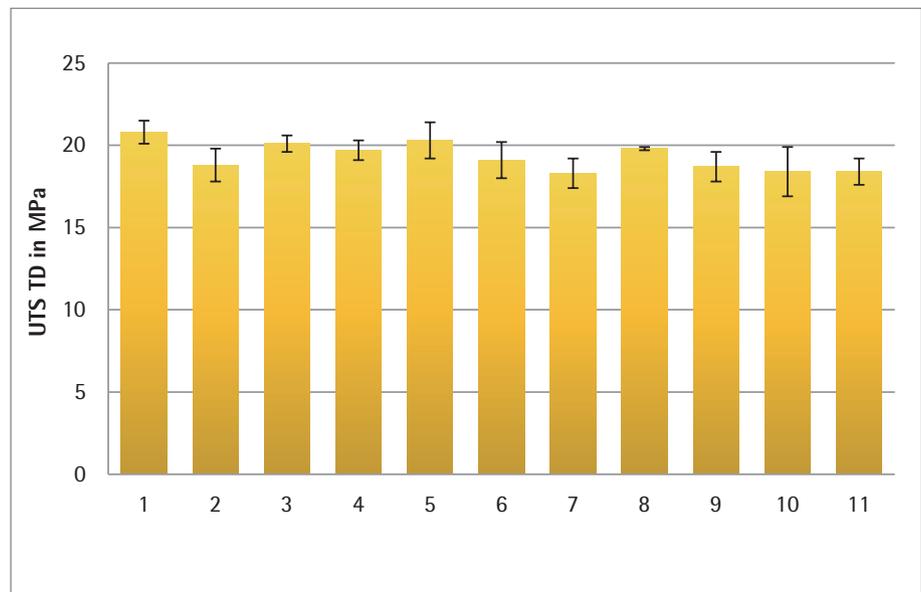


Figure 9: S71 ultimate tensile strength results

## Product Validation

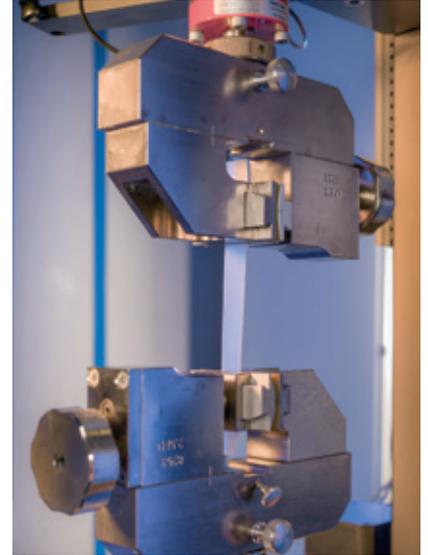
The mechanical strength of Flexsafe®, Flexboy® and Celsius® bags has been validated. In addition, application-specific testing has been performed on Flexsafe® bags for (i) liquid shipping and (ii) cell culture performance. This section outlines the test methods used and the results obtained.

### Standard Mechanical Testing for Flexsafe®, Flexboy® and Celsius® Bags

S80 & S71 robustness is qualified and routinely controlled by means of standard mechanical tests

Resistance to Uniaxial Traction is determined with a dynamometer until film rupture by measuring

- Tensile strength at break (maximum stress the specimen can withstand)
- Elongation at break (recorded when specimen breaks)

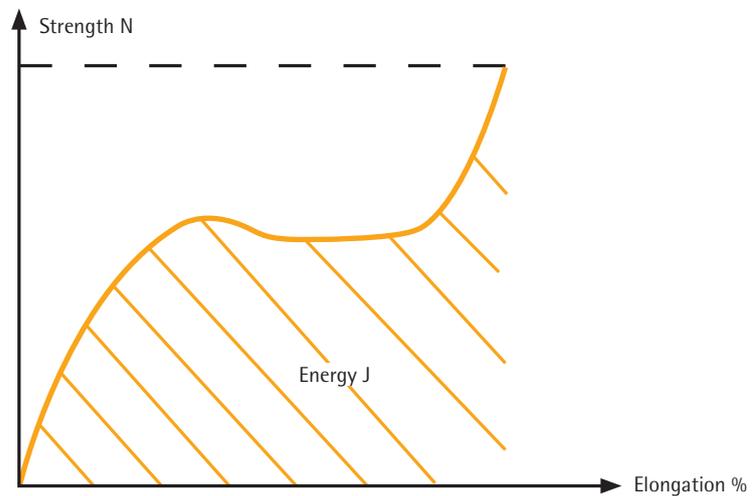


### Methods (Tensile Strength, Elongation at Break, Energy at Break)

**Tensile strength:** Tensile strength measures the force required to break a film. It represents the strength of the film.

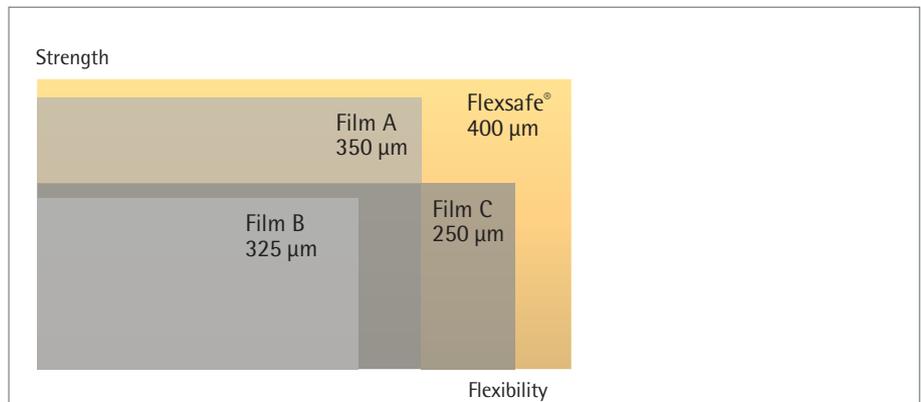
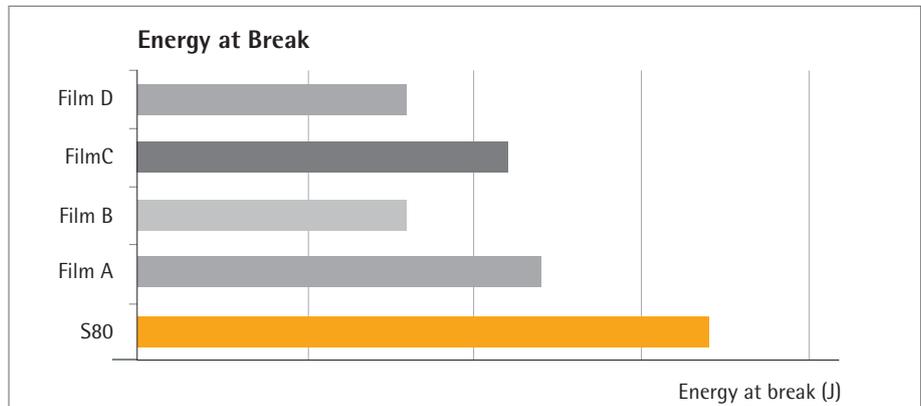
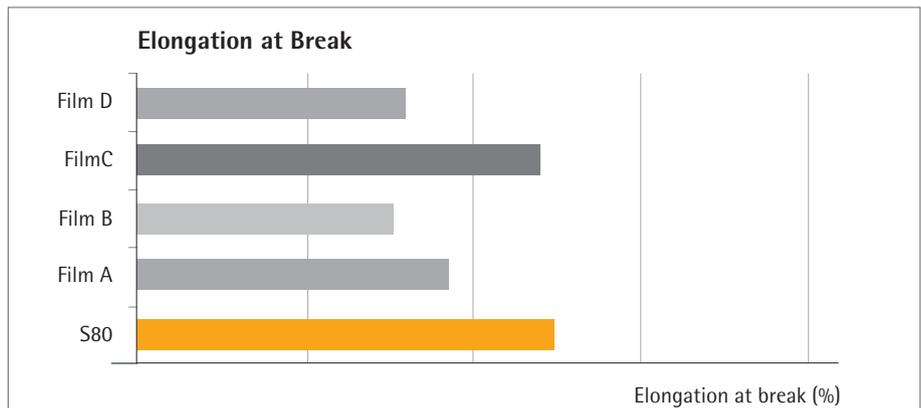
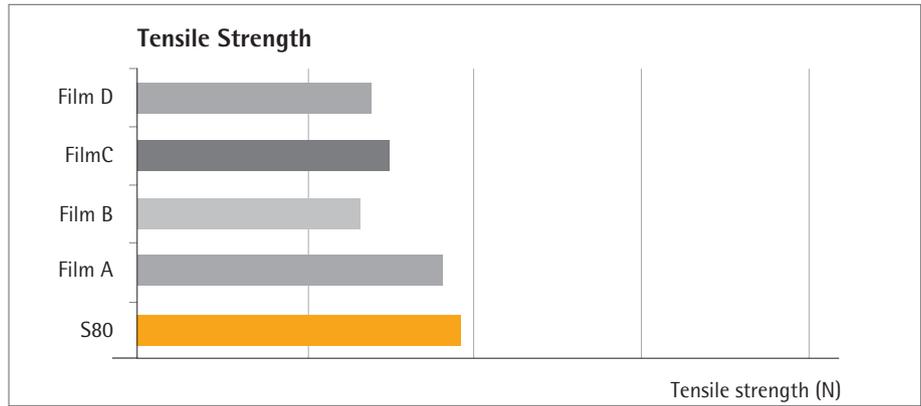
**Elongation at break:** Elongation at break is the maximum elongation that a film can withstand before breaking. It characterizes the behavior of the film with regard to deformation & resistance to breakage. It represents the flexibility of the film.

**Energy at break:** Energy at break is the total energy required to break a film. This combines the maximum strength and maximum flexibility of the film in one direct measurement thus characterizing the general robustness of a film.



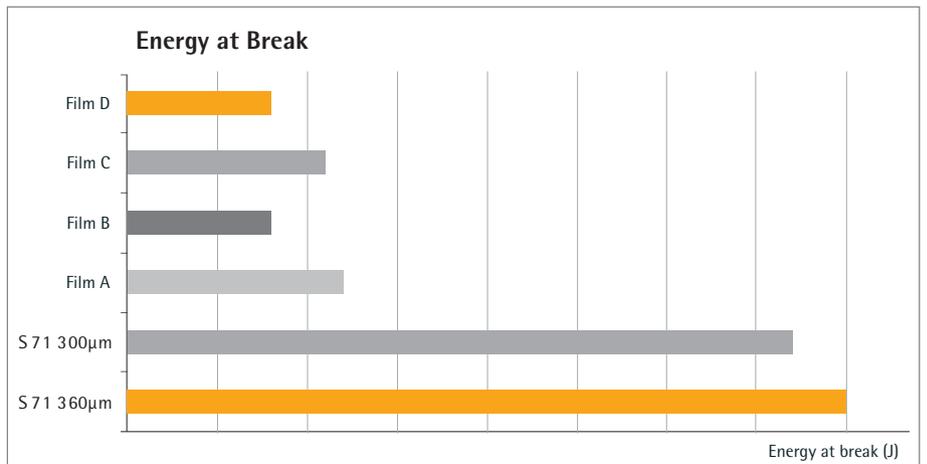
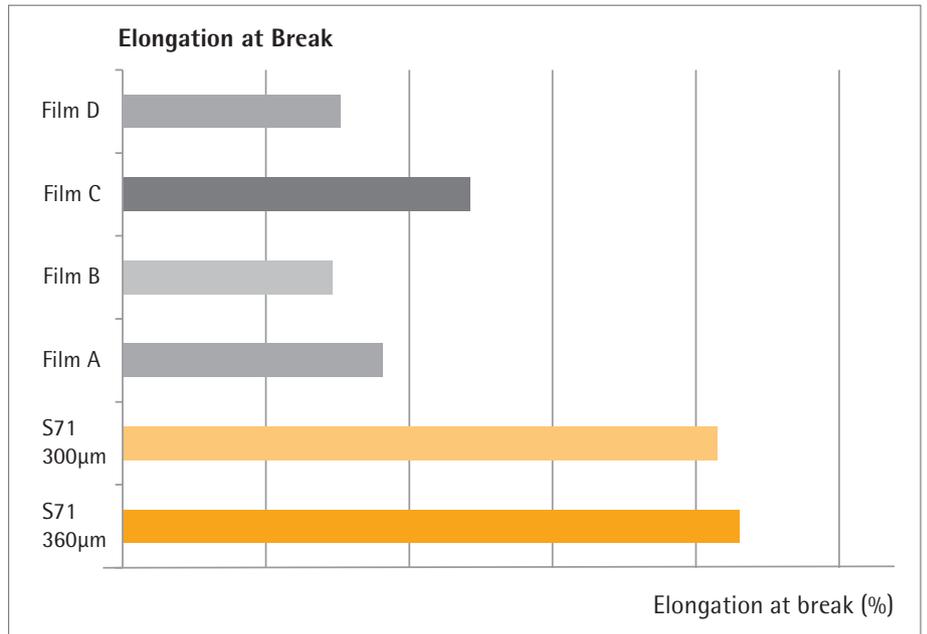
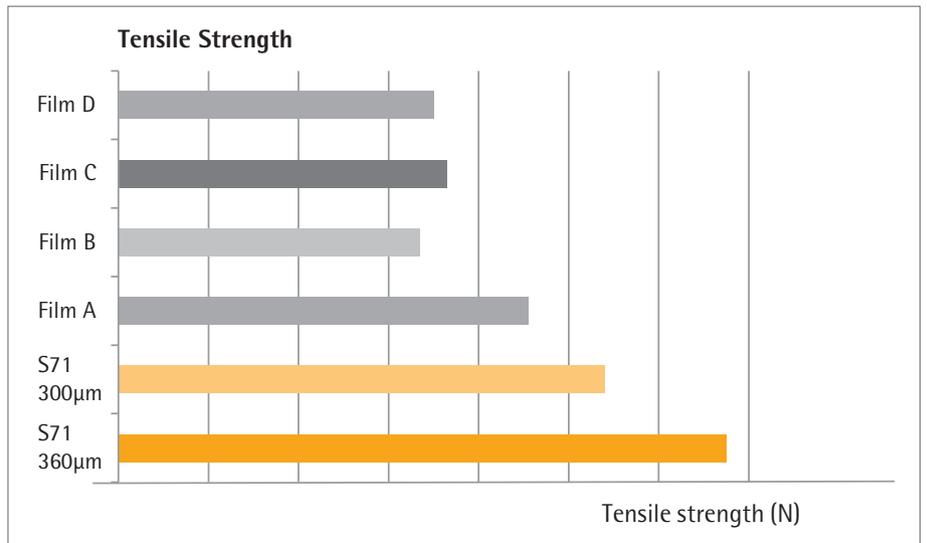
**Results (Tensile Strength, Elongation at Break, Energy at Break)**

Figure 10 shows the tensile strength, elongation at break and energy at break data for the S80 film used in Flexsafe® bags. Equivalent data generated from other available films is provided for comparison. While competitor films provide either strength or flexibility, the S80 film combines strength & flexibility for greater robustness & ease-of-use.



**Figure 10:** A characterization of the S80 film and comparison with competitor films showing how the S80 film combines strength & flexibility for greater robustness & ease-of-use

Figure 11 describes the tensile strength and elongation at break characteristics of the S71 film used in the construction of Flexboy® & Celsius® bioprocess containers. Data from other available films is provided for comparison. The S71 film also combines outstanding strength and flexibility and outperforms other films on the market, making Flexboy® and Celsius® extremely robust and safe bags.



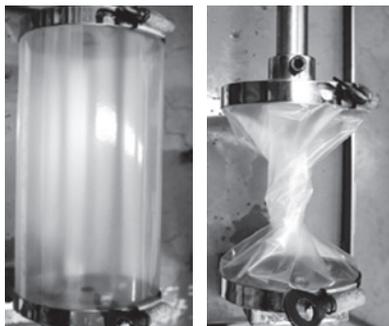
**Figure 11:** A characterization of the S71 film and comparison with competitor films showing how the S71 film also combines outstanding strength and flexibility

Furthermore all Sartorius bags are validated with the following mechanical tests.

- Flex-durability measures the resistance of the film to fatigue
- Puncture resistance predicts the resistance of the film to damage by penetration
- Pressure resistance
- Water burst test on scale-down test bags confirms superior mechanical properties of the film & the welds

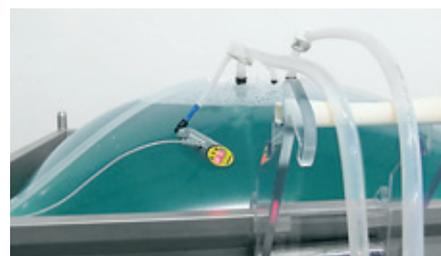
#### Flex Durability

Resistance to Flexion & Fatigue is measured by counting the number of pinholes formed in the film at the end of the Gelbo Flex Test (repeated twisting & horizontal pushing motion)



#### Puncture Resistance

Puncture testing predicts the resistance to damage by penetration of another object. The puncture test creates a multi-axial stress which is more representative of the stress imposed on bags in use. Puncture resistance evaluates the film strength and extensibility properties.



### Application-specific Validation of Flexsafe® Bags

#### Liquid Shipping Testing for Flexsafe® Bags

The increased use of disposable bio-processing bags in all critical process steps of the biopharmaceutical drug production and the international context in which biomanufacturing is operated has driven the increased demand for robust & safe shipping systems for drug substances.

The S80 film used in Flexsafe® 3D bags for Shipping has been proven to be robust with the most stringent ASTM standard D4169 liquid shipping test and real shipping conditions.

We have demonstrated experimentally that the ASTM D4169 shipping test is the worst-case regime even compared to real shipping tests & the ISTA protocol.

The real shipping tests were performed as follows: Hazards encountered during two different shipments of 100, 200 & 500 L filled Flexsafe® 3D bags were recorded. Two different shipments were studied (i) a truck transportation through Europe (>3,000 kms/ 1,900 miles) and then (ii) an international transportation by airplane (>30,000 kms/19,000 miles).

During these shipments the Flexsafe® shipping systems were equipped with data loggers to record accelerations, horizontal and vertical shocks (collision of two objects), shakes (vibrations inducing shocks) and vibrations (small amplitude displacement). Data was then analyzed and compared to that generated from ASTM D4169 testing and testing using the ISTA protocol.

The results show that the ASTM D4169 shipping test is the worst-case regime (Figures 12)

**Our Flexsafe® 3D bags pass the most stringent ASTM D4169 test regime for liquid shipping applications**

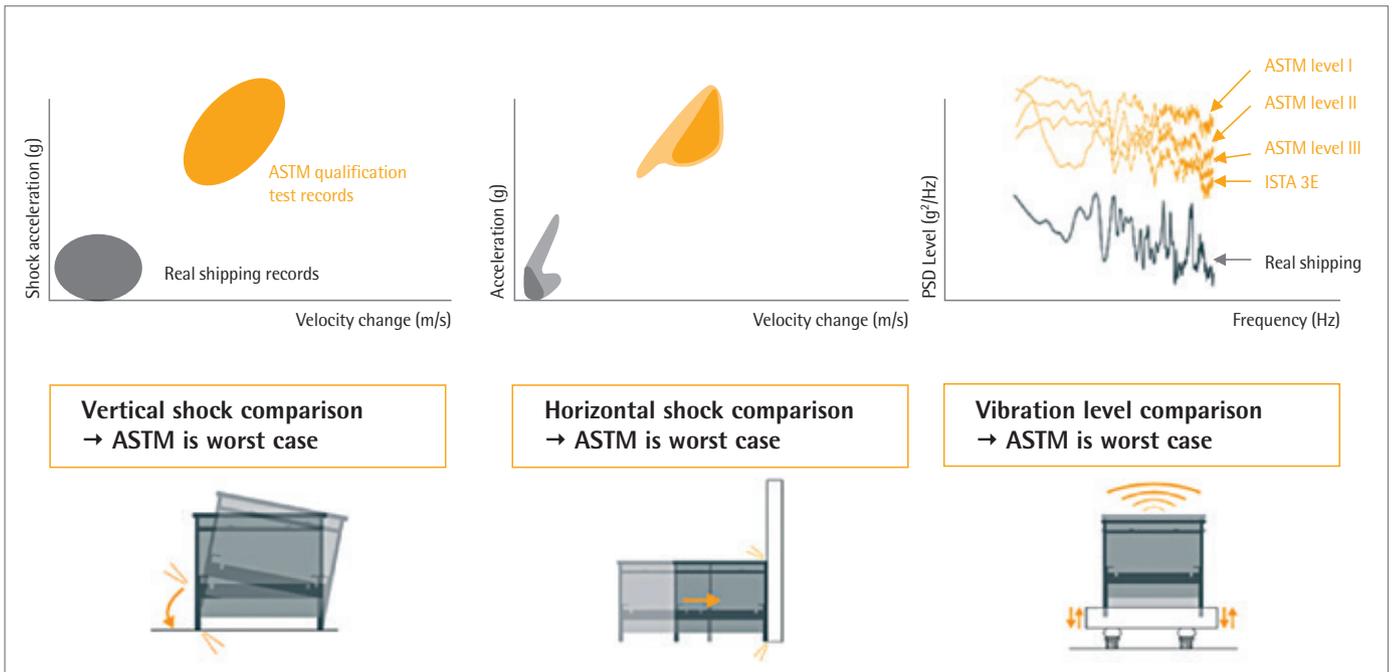


Figure 12: Data showing that ASTM D4169 represents the worst-case testing regime for single-use bag shipment

For this reason, Flexsafe® 3D Bags in shipping Palletank have been validated using the ASTM D4169 standard liquid shipping test regime for truck and air shipment for:

- Vertical & horizontal shocks
- Vibrations
- Handling
- Compression

From the results of these experiments it was decided that Flexsafe® 3D bags should be qualified according to the ASTM D4169 (distribution cycle 12 & 14) at assurance level II.

Comparison Trip   Random Vibration		Rack-1
All trip	Number of shake	73
	Shock maxi (g)	1.9
ASTM D 4169-09 Truck Level III	Number of shake (during 1 min.)	178
	Shock maxi (g)	21.0
ASTM D 4169-09 Truck Level II	Number of shake (during 1 min.)	285
	Shock maxi (g)	31.0
ASTM D 4169-09 Truck Level I	Number of shake (during 1 min.)	463
	Shock maxi (g)	69.0

500 L Flexsafe® 3D Bag in Palletank for Shipping

During ASTM level II qualification, bags experience  $285 \times 180 = 51300$  shakes based on a vibration duration lasting  $2 \times 90$  minutes. This is 700-times more than during the real truck shipment through Europe with a maximum shock intensity that is more than 16-times higher.

The testing sequence was designed to reflect worst-case conditions for air and truck shipment and was performed at several temperatures. In addition to the handling test, the truck transport behavior of the bags was tested by investigating horizontal impacts or rotational shocks. Air shipping behavior was tested by exposing the shipper to vibrations, compression or low-pressurized environments.

The testing sequences is provided in Figure 13.

Test Sequence		Test Reference – ASTM D4169 – Assurance Level II
1	Pre-conditioning	4°C (39.2°F) or 40°C (104°F) during 72 hours
2	Mechanical handling	SCHEDULE A
2.1	Truck Handling	ASTM D6055 Method A   5 cycles round trip
2.2	Horizontal impact	ASTM D880 Method B   each side 1 shock (total 4)
2.3	Rotational flat drop test	ASTM D6179 Method C   2 drop tests (short and long edge)
3	Vehicle vibration	SCHEDULE E
3.1	Truck spectrum	ASTM D1728 Method A – 90 minutes truck spectrum
3.2	Air spectrum	ASTM D4728 Method A – 90 minutes airplane spectrum
4	Low pressure	SCHEDULE I ASTM D6653 40°C and 4°C – 595.73hPa – 60 minutes
5	Mechanical handling	SCHEDULE A
5.1	Truck Handling	ASTM D6055 Method A   5 cycles round trip
5.2	Horizontal impact	ASTM D880 Method B   each side 1 shock (total 4)
5.3	Rotational flat drop test	ASTM D6179 Method C   2 drop tests (short and long edge)
6	Warehouse stacking	SCHEDULE B – ASTM D642 – 1297daN during 3s

Figure 13: Testing Sequence

### Conclusion

In conclusion, our new Flexsafe® bags successfully passed the most stringent ASTM D4169 test for air and truck shipment at assurance level II with a high safety margin (Figure 14).



Figure 14: Comparison of real shipping record with ASTM qualification test records showing high safety margin

## Cell Culture Application-based Testing for Flexsafe® Bags

S80 film robustness was qualified using tests based on worst-case cell culture applications.

Extensive robustness trials have been performed under worst-case cell culture application conditions with rocking motion (RM) bioreactor bags (1, 5, 10, 25 & 100 L working volume) and stirred bioreactors (STR) bags (50, 200, 500, 1000 L & 2000 L working volume). The bags were filled with water to the maximum working volume and either rocked at the maximum rocking rate or stirred at the maximum stirrer rate for 21 days at a temperature of 40°C under the maximum operating pressure (after 21 days, the pressure is increased from 30 to 50 mbar).



A full description of the method is available from Weber, A., Husemann, U., Chaussin, S., Adams, T., De Wide, D., Gerighausen, S., Greller, G. & Fenge, C. (2013) Development and Qualification of a Scalable, Disposable Bioreactor for GMP-Compliant Cell Culture. BioProcess International 11(4)S April 2013.

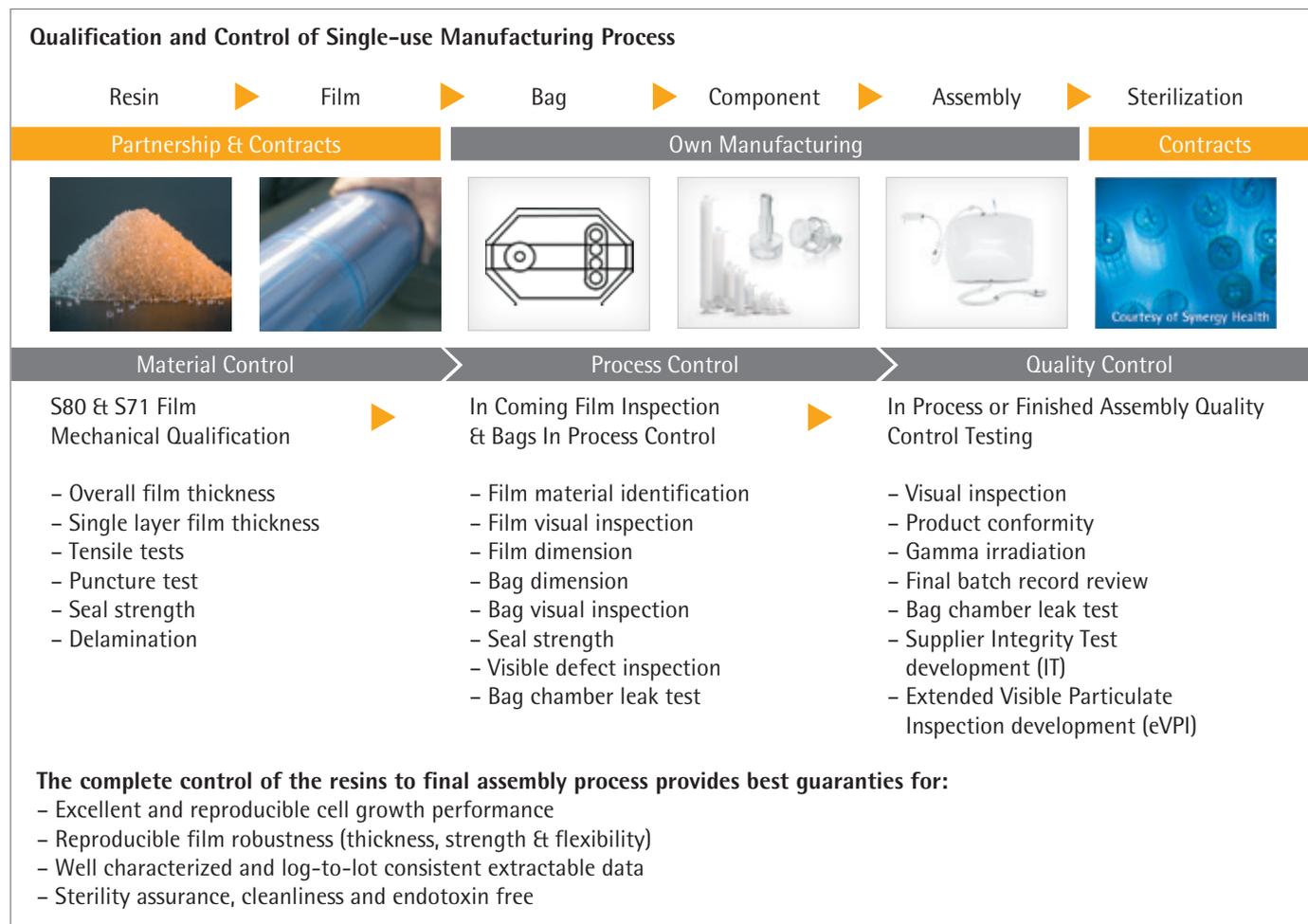
- 30 STR bags made of new S80 film passed worst-case robustness trials
- 40 RM bags made of new S80 film passed worst-case robustness trials
- ▶ 100% of STR & RM bags tested passed worst-case tests



## Process Control

Sartorius ensures consistent lot-to-lot robustness of bioprocess containers through the complete control of our manufacturing process. Raw materials & process parameters were selected after several materials & film compositions were tested against pre-defined COAs. These ensure the bags are suitable for use in all process steps and applications. Critical

process parameters were identified by a QbD approach to ensure bag COAs are achieved. The COAs of the films themselves must meet pre-defined specifications and the bags and films have undergone robust qualification regimes. Finally, QC controls, such as bag chamber leak testing, are performed on the film and the bag chamber during production.



## Summary

Sartorius achieves the robustness of its Flexsafe®, Flexboy® and Celsius® films by working closely with its suppliers and applying its material science and extrusion expertise to select the most suitable raw materials & film layer combinations for film robustness and weldability. Design of experiments has been used to establish the extrusion design spaces and the critical welding parameters for both our films.

Robustness has been demonstrated in the design space & qualified with standard mechanical tests and worst-case shipping and cell culture application tests. Consistent robustness has been ensured by the control of raw materials, the extrusion & welding process parameters and by routine QC testing on films & bags such as leak tests for bags.

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