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WHITEPAPER

PRESSURE DECAY TESTS IN THE LIFE SCIENCES INDUSTRY

INTRODUCTION

Single-use systems have grown increasingly popular in the life sciences industry in the past three decades. Through the whole production chain, in both up-stream and downstream processes, these systems have been implemented successfully in areas such as bioreactors, mixers, buffer containers, media bags, and various others.

Single-use systems have numerous advantages compared to traditional stainless-steel solutions. Their application eliminates complex and time-consuming cleaning processes, yielding an increased flexibility, less needed space in clean rooms and a much faster time to market. Furthermore, they can help to minimize production costs. The list of advantages is long and the most recent years, accompanied by COVID-19 and the devel-opments in personalized medicine, have proven why single-use systems are be-coming more important.

New challenges for manufacturers arise alongside the implementation of such solutions. The two most important ones are

+ **Leachables and extractables that could migrate from polymers in-to its contents**
+ **The integrity of single-use systems**

According to the 18th Annual Biomfg report of 2021, leakage is one of the top three constraints for further implemen-tation of single-use systems (1). A risk-based integrity testing strategy is needed to enhance product safety and improve product quality. Integrity, being a critical quality asset, requires a close collaboration by end users and suppliers. The responsibility for system design, construction, and integrity is shared by all parties. An example of such a test strategy by Sartorius is provided in Figure 1 (2).

The Bio-Process Systems Alliance (BPSA) published a guideline in 2017 due to the importance of Integrity Assurance. By partnering with several manufacturers of the industry, e.g., the aforementioned Sartorius, Thermo Fisher, Millipore Sigma, Pall Life Sciences, GE Healthcare, an approach to assessing integrity of single-use systems was elaborated.

Figure 2 highlights the key aspects of a risk-based approach with a shared responsibility by end users and manufacturers (3).

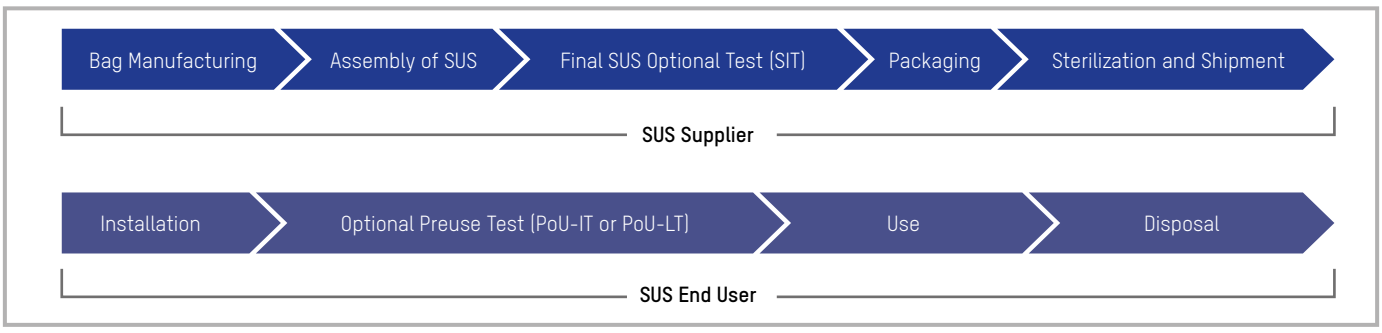


Figure 1: Example of a Single-Use System lifecycle by Sartorius (2)

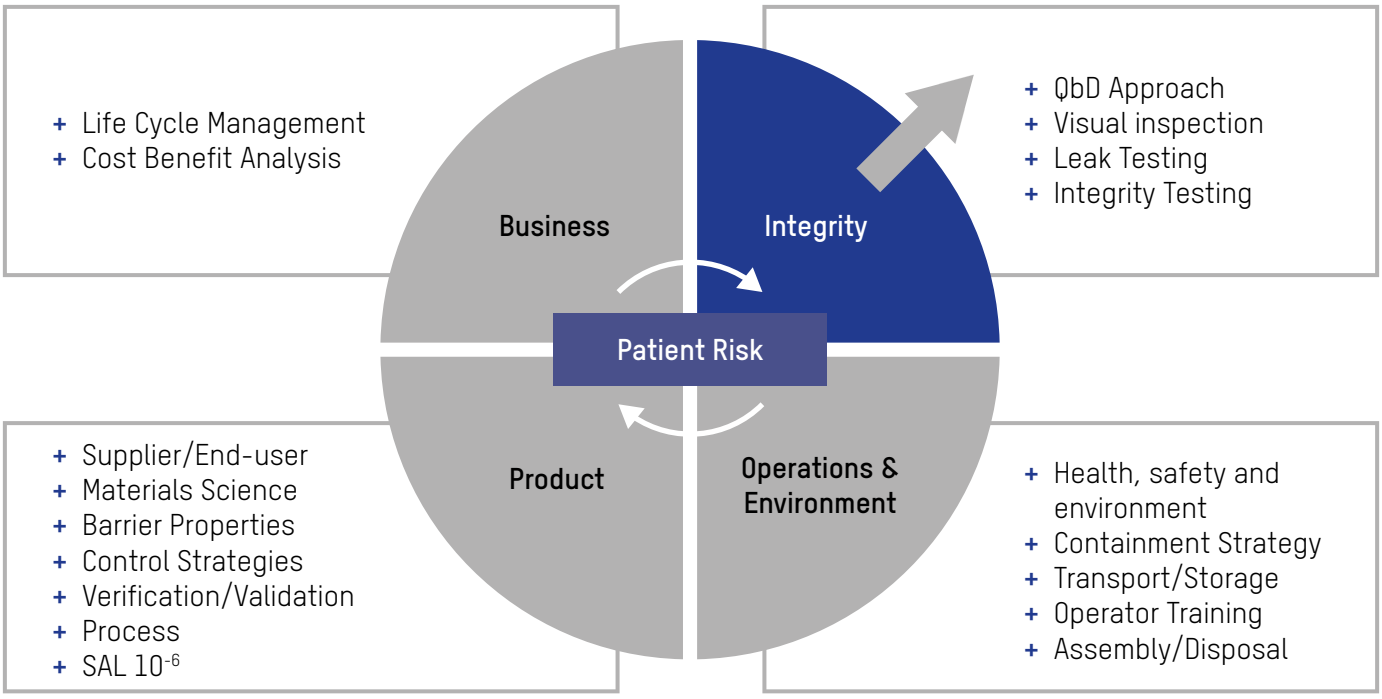


Figure 2: Risk-based approach according to BPSA guideline (3)

Assuring the quality of single-use products can be a challenge. Biopharmaceutical processes require ample solutions for all the various upstream and down-stream processes used in clinical trials as well as commercial production. A quality by Design approach is complemented by 100% tests of such products. Here, understanding the risks and potential defects associated with each step of the lifecycle is crucial. Manufacturers and end users have put much effort into defining a parameter called maximum allowable leakage limit (MALL). This limit differs depending on the context the product is being used for. The risk for liquid leakages as well as microbial ingress must be taken into account.

A test strategy has to be defined based on the above. According to the BPSA guideline, influencing factors for such a strategy are:

+ Destructive or non-destructive tests
+ Pre-use or post-use
+ Detection Limit
+ No addition of contaminants
+ Test time
+ Test cost
+ Ease of operation
+ 21 CFR Part 11 compliance (3)

MAXIMUM ALLOWABLE LEAKAGE LIMIT

"According to USP <1207> (4), the maximum allowable leakage limit is the greatest leak size tolerable that poses no risk to product safety and no or inconsequential impact on product quality" is a brief definition by Aliaskarisohi et al. (5).

Avoiding liquid leakage and microbial ingress are the key factors for defining the greatest leak size with regard to a potential product. The entire test strategy is based on this definition.

Various studies have been conducted that assess the MALL. One of these studies by Aliaskarisohi et al. presented the results highlighted in table 1 and table 2 (6). The probability of microbial ingress increases drastically with increasing pressure difference. Results such as these can be a promising starting point to defining the greatest defect size that does not oppose any risk regarding patient and operator safety.

Different application conditions require different MALL definitions. With regard to stationary solutions in factory sites, the main contributor for a pressure difference is the fluid column inside. As a result, the limit can be much higher compared to shipping conditions, where bags are transported by plane. Higher pressure differences lead to a MALL in the range of 2 – 10 µm according to the studies by Sartorius.

In addition to microbial ingress the liquid leakage is also part of many studies like the aforementioned study by Sartorius (5).

Defect Size (µm)	Bacterial Ingress / Total Samples	
	PE Film (400 µm)	EVA Film (300 µm)
2	0/18	0/18
10	0/30	0/30
15	0/30	0/30
20	1/30	0/30
25	0/30	0/30
30	0/30	0/30
40	0/30	1/30
50	6/30	1/30
80	15/30	14/30
100	22/30	14/30
Total Positives	44/288	30/288

Table 1: Results by Aliaskarisohi et al. (Sartorius Stedim Biotech GmbH).

Tested samples that have shown bacterial ingress for PE and EVA films at atmospheric pressure.

Defect Size (µm)	Bacterial Ingress / Total Samples	
	PE Film (400 µm)	EVA Film (300 µm)
1	0/30	0/30
2	2/30	0/30
3	2/30	9/30
5	10/30	17/30
10	20/30	15/30
Total Positives	36/150	41/150

Table 2: Results by Aliaskarisohi et al. (Sartorius Stedim Biotech GmbH).
Tested samples that have shown bacterial ingress for PE and EVA films at 300 mbar gauge pressure

NON-DESTRUCTIVE TEST METHODS

While researching the risks and defining the MALL make for pivotal first steps, this knowledge needs to be integrated into a test strategy that can be used throughout production. Based on the risk assessment, non-destructive testing plays a key role in ensuring the required quality. Here, two different test methods are used frequently:

+ **Pressure Decay Tests**
+ **Helium Vacuum Tests**

While tests with helium as a tracer gas have shown to have a higher sensitivity, they are also much more complex and expensive. As a result, pressure decay tests are also widely used to support the integrity test strategy. More so, both methods can complement each other in the lifecycle of single-use systems.

PRESSURE DECAY TESTS

This test method is based on the ideal gas law p * V = n * R * T. Keeping the volume and the temperature constant leads to a direct correlation between the pressure and the number of molecules. Therefore, a change in pressure directly correlates with the number of molecules exiting or entering the test object.

By pressurizing the test component, a pressure difference is induced. If there is a leak present, air will flow through a defect into the atmosphere and the pressure inside the test component will drop. A typical test procedure for such a test is shown in figure 3.

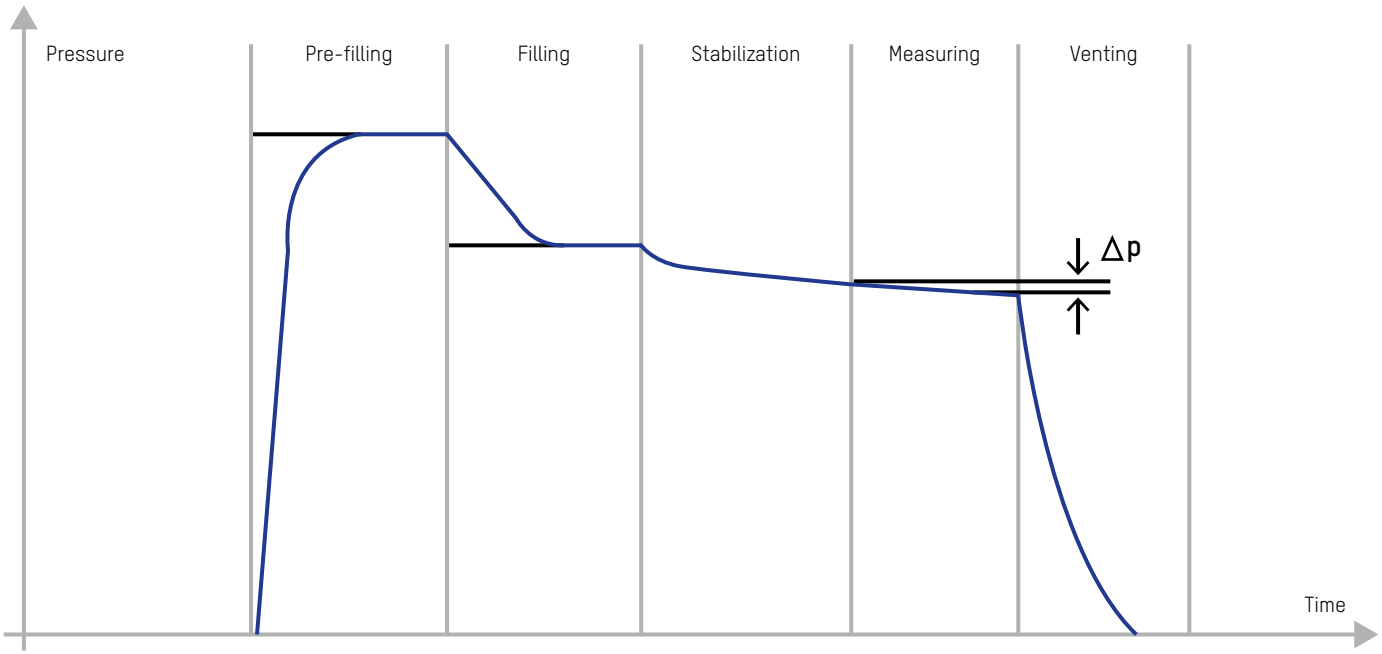


Figure 3: Typical test process for a pressure decay test including an optional pre-filling phase.
Pre-filling: The test component is pressurized at a pressure 10 – 20 % higher than the actual test pressure.
This option is typically used for flexible components and leads to a more stable measuring phase.
Filling: The test component is pressurized to the desired test pressure.
Stabilization: The test setup stabilizes itself.
Measuring: The pressure change is measured with the test system.
Venting: The component is vented until it is back to atmospheric pressure.

Increasing the pressure difference will in turn increase the sensitivity. More air will flow through the same defect, leading to an increase in pressure drop. Minimizing the test volume will further improve the sensitivity. The same amount of air going through a defect will lead to a bigger pressure drop. For ideal condi-tions, the sensitivity of a pressure decay test can go as low as 10^ (-3) mbar*l/s.

Measuring flexible components such as single-use systems can be a challenge. The volume is not constant and measuring times over a couple minutes up to half an hour contain risks regarding a stable environment. Changes in atmospheric pressure and temperature can lower the sensitivity. An optimization of the test procedure despite these challenges is crucial to achieving the highest sensitivity possible. Restraining 2D bags makes for an increase of the test pressure up to 300 mbar or more, depending on the test component, while 3D bags are typically tested below 30 mbar. Other factors, e.g., diffusion, have shown to be negligi-ble for pressure decay tests (3).

Maintaining the same conditions within the environment throughout the entire test sequence can improve the sensitivity as well.

The limit of what is achievable with a pressure decay test regarding the smallest, detectable defect always depends on the test component. However, already performed tests can provide an idea of what is possible. Minimizing the test time can lead to more stable test conditions throughout the measuring phase. Moreover, optimized sensors for detecting small pressure differences can lead to shorter measuring times and thus increase the sensitivity as well.

Multiple test series have been performed with different bags ranging from 10 L – 200 L to determine the smallest, detectable defect for each of these test components. The same bags were measured with and without fixed test leaks of different sizes, up to 25 times. The breaks in between tests were 2 h, in order to mitigate the effects of measuring the single-use bags multiple times. The tests were

performed under laboratory conditions, primarily overnight but without air conditioning.

The results of said tests are provided in figure 4 and figure 5.

All tests were performed without restraining plates in this test series. The test pressure was between 20 – 30 mbar. This research was primarily conducted for more complex assemblies where restraining plates could not be used. The test time ranged between 4 – 10 minutes.

For the 10 L bag assembly, it is shown that a 75 µm defect is detectable using 6 sigma intervals, whereas the 10 µm defect was not.

The results of a 50 L 3D bag assembly also tested in open space is shown in figure 5. Fixed leaks with diameters of 75 µm and 250 µm were used. The 75 µm leak was still detectable with a 6 sigma interval under these conditions.

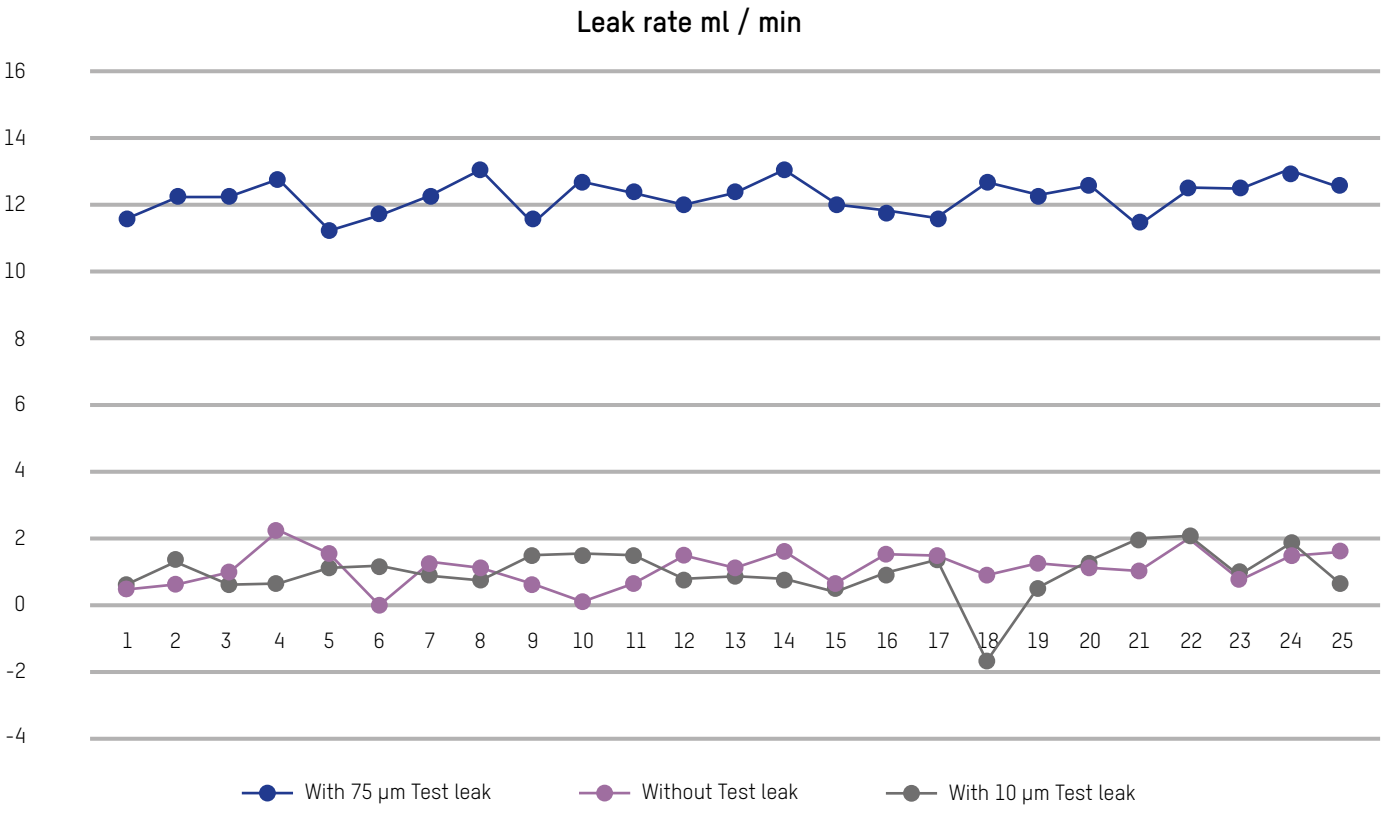


Figure 4: Results of a 10L 2D bag assembly.

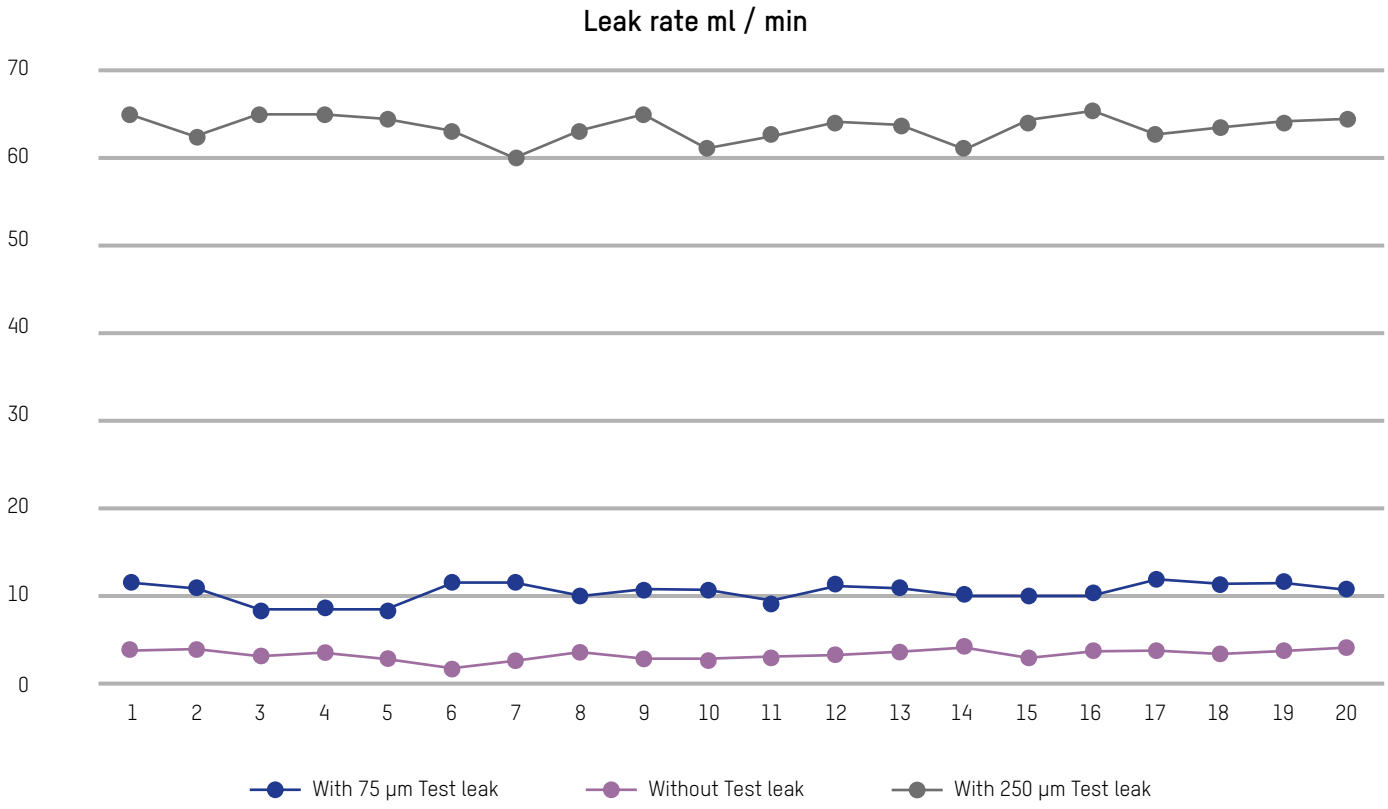
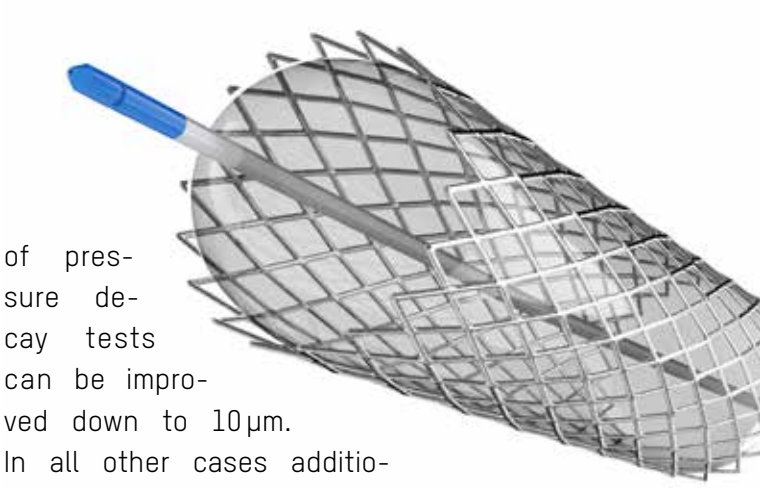


Figure 5: Results of a 50L 3D bag assembly



Other bag assemblies of various sizes have provided similar results. A defect of 75 µm was detectable up to 50 L 3D bags within these studies. The results of the smaller assemblies have shown that potentially even smaller limits can be reached without restraining plates, which makes those tests viable for more complex assemblies.

Comparing those results to typical values for the MALL reveals that a pressure decay test alone is not sufficient to ensure the integrity of single-use systems. Nevertheless, they can complement quality by design approaches together with helium vacuum tests when needed. Being aware of the limitations of every approach is crucial to finding the right test strategy. For especially small com-ponents, this can be restrained, and the limit of pressure decay tests can be improved down to 10 µm. In all other cases additional tests at different stages in the lifecycle of single-use systems can further increase product quality and efficiency. Specifically, handling single-use assemblies can lead to damaging the film and thus a compromised integrity. Here, having a point-of-use pressure decay test, when the com-ponent is already in place right before use, can mitigate such risks. The biggest advantages of pressure decay tests are comparatively low costs and an easy implementation into the existing processes.

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Dominic Hofer is the industry manager for Medical Technology at ZELTWANGER Leaktesting & Automation GmbH in Dußlingen. He started to build his foundation of technological knowledge in this industry early on by studying Medical Technology in Tübingen and Stuttgart. He lives in Reutlingen and supports the ZELTWANGER team worldwide with his extensive knowledge in medical leak test applications.

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