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ASIA EDITION

Get the best out of single-use bioreactors

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Singapore: The high value of growth media and the length of a typical cell culture process spur for highest scrutiny when setting up production facilities. A leaking bioreactor would generate financial loss and jeopardize the carefully timed production schedule in a GMP facility. In vaccine processes, it might also risk operator safety. Today, single-use technology is well accepted and the manufacturers' quality assurance ensures leak free single-use bags upon delivery, but what about the risk of installation and other handling errors? Training of operators is mandatory but should it be the only way to mitigate failures? A post installation pre-use test of the whole bioreactor including tubing, capable of detecting typical leaks that might have been introduced due to operator handling errors, would greatly improve risk mitigation capabilities in single-use production facilities. A critical example here is proper connection of different tubing when setting up the bioreactor and preparing for media and inoculum transfer where an operator error might immediately result in a leakage.

Selection of a suitable test method

More than 40 different test methods have been proposed for leak detection based on various technologies such as 'sniffers' for special gases, thermal imaging, flow measurement, pressure decay and pressure increase. Each technology has its own strengths and weaknesses in terms of sensitivity, feasibility and cost; all critical factors for a method that shall successfully make its way into routine bag testing in GMP production facilities of vaccine, monoclonal antibody and recombinant protein producing companies.

Risk assessment reveals that a predictive leak test for single-use bioreactors has to be performed at the point of use, pre-use but post installation. Therefore, testing a single-use bioreactor bag in a separate device such as typically used when applying gas sniffer technology and then installing it into its bag holder would hence not permit detecting operator handling errors. Pressure decay technology, on the other hand, can be used to test the single-use bioreactor bag after installation in its final configuration directly in its holder and presents therefore a reliable and predictive risk mitigation tool.

Reliable and reproducible methodology

Reliability and reproducibility of a test method have to be established before formally validating it. During the development of a method based on pressure decay measurement, it was identified early on that a leak in a plastic bag is totally masked when pressed against a smooth, hard surface due to the test pressure. Very little of the test gas can escape through the leak and the reliability of the pressure decay detection is nil. The use of specifically designed fleeces acting as a porous spacer

between the plastic film of the bioreactor and the holder prevents this masking effect. It delivers reliable and reproducible test values comparable to results obtained with the same test method on bag areas which are not covered by any hard surface. This was qualified by applying the same test method on bags with pre-defined leaks located on the upper part of the bioreactor bag that is not in contact with the holder and thus not presenting any risk of masking.

The leak test method

The objective of the test method is to identify potential damages of installed bioreactor bags (including bag seals, port welds, connections and bag surface) which would result in a loss of the bioreactor content or a biosafety risk.

The test method is based on ASTM F2095-01: "Standard Leak Test for Pressure Decay Leak Test for Nonporous Flexible Packages with and without Restraining Plates". The Sartocheck4 plus Bag tester (Sartorius Stedim Biotech) is used together with a specifically developed bag tester fleece preventing masking of any, during installation, potentially introduced leaks. It allows point-of-use leak testing of single-use bioreactor bags post installation and pre-use in its final bag holder. This pressure decay leak test can reliably detect defects in the Cultibag STR 50L of 50µm and the Cultibag STR 200L of 100µm in the bag walls, seals or connections of the entire flexible bag system including its tubing. The test method is non-destructive and enables the implementation of a reliable and reproducible point-of-use test in bio-production facilities. The pressure decay during the test is measured and compared to an acceptance criteria established during qualification of the method.

The validation approach was separated into two parts, an engineering study to establish the detection limit for different bag volumes and the formal qualification to verify the minimum detectable leak size and establish the test acceptance criteria (maximum allowable pressure decay). To determine the minimum detectable leak size, a simplified bag (only one bottom port connection for test gas application) was prepared with multiple defined defect patches representing different leak sizes. A defect patch is a circular sheet of film with a laser drilled and flow calibrated leak diameter that was welded to the bag surface. As second step, one standard bag without defect and one standard bag with a defect patch of the previously determined minimum detectable leak size were used to establish the final test parameters. The minimal detectable leak size in a CultiBag STR 50L bag was determined to 50µm and at the 200L scale a leak size of 100µm could reliably be detected.

Based on the results of the engineering study, the final test parameters for the qualification of the method were established. These parameters were used to generate an automatic test program for each STR bag volume in the Sartocheck 4 plus Bag tester.

Qualification study

The purpose of the qualification study was to verify the minimum detectable leak size for the different bag volumes on a statistically significant number of standard bags from different routine production lots. For the study, standard bags from production without further modification and a standard bag that was prepared with a single defect patch (according to minimum detectable leak size determined in the engineering study) of different lots per bag volume were used. All bags were gamma irradiated. Per bag, a minimum of 10 test repeats were performed.

All tested bags showed expected results, i.e. the non-defect, standard bags passed the test while standard bags prepared with a single defect failed. Hence, the test method using pressure drop combined with the fleeces was successfully qualified and proved to be a robust and predictive method for reliable detection of leaks.

This new single use bioreactor leak test which is qualified to be used post-installation but pre-use enables now the same level of risk mitigation and assurance as previously only known from conventional bioreactors that could easily be pressure tested prior use. It helps to mitigate project delays and offers a proper risk mitigation tool especially in biosafety critical applications typically found in vaccine production.