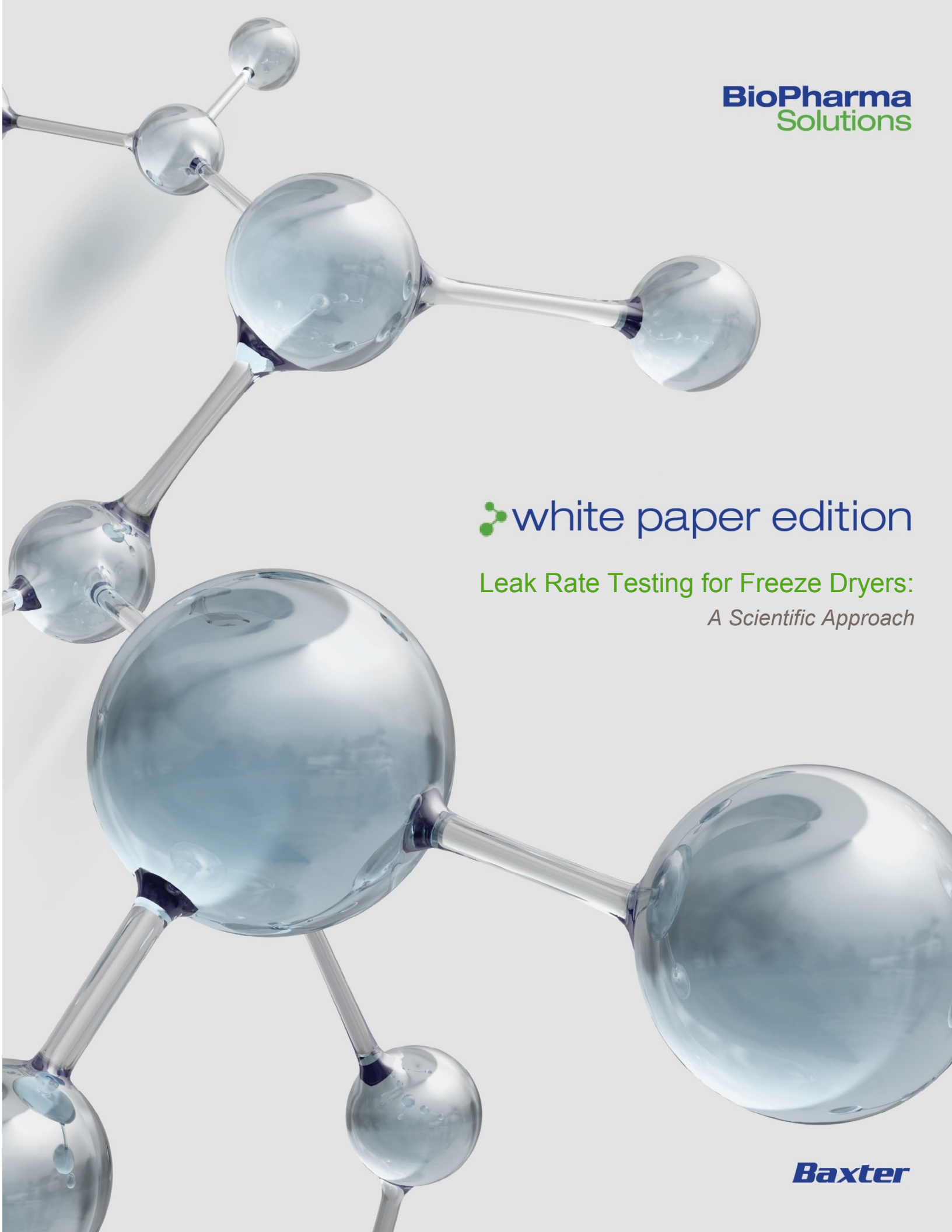

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white paper edition

Leak Rate Testing for Freeze Dryers:

A Scientific Approach

Baxter

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Leak rate testing for freeze dryers

Assurance of the vacuum integrity of freeze-dryers used for the manufacture of sterile pharmaceutical products is essential for GMP operations; however, there is currently no generally accepted scientific rationale for establishing acceptance criteria for such testing [1].

As it stands, current acceptance criteria are generally based on equipment capability – perhaps proposed by the manufacturer, or from data collected during qualification of a new freeze-dryer. The targeted specification is often one cited by The Parenteral Society, "A frequently specified leak rate for new, clean, dry, and empty freeze-dryers would be 2 x 10^{-2} mbar-liter/sec (15 µm (mTorr) liters per sec). The leak rate should not change significantly during the life of the freeze-dryer ..."[2].

A study was performed to use a more scientific approach to establish leak rate acceptance criteria, by answering the question: What is the maximum volume of air that can leak into the freeze-dryer without violating the Class 100 / Grade A microbial specifications? A method was developed to measure the exact void volume of a dryer, that is, the total volume of air space present in chambers, condensers, and connecting ducts. A worst-case bioburden value for air that could leak in was determined, and then the maximum allowable leak rate was calculated. The leak rate was then converted to the maximum allowable pressure rise over the duration of a leak test.

Background

Class 100 (FDA) and Grade A (EU) specifications concerning active microbial counts are similar - an action level of one colony forming unit (cfu) for each cubic meter of air space for Class 100 [3] and an average limit of <1 cfu/m^3 , with "appropriate alert and action limits" for Grade A [4].

FDA states, "Facility design should ensure that the area between a filling line and the lyophilizer provide for Class 100…"[3]; however, it does not make reference to requirements for the inside of the dryer. EU guidance is clearer: "Partially stoppered freeze drying vials should be maintained under Grade A conditions at all times until the stopper is fully inserted"[4].

Process control is demonstrated with media fills, but regulatory guidance is sparse, and sometimes seemingly contradictory. FDA states, "Media fill studies should closely simulate aseptic manufacturing operations… For lyophilization operations, FDA recommends that unsealed containers be exposed to partial evacuation of the chamber in a manner that simulates the process. Vials should not be frozen, and …ensure that the medium remains in an aerobic state..."[3]. Although these recommendations simulate the air turbulence associated with freeze-drying, while still maintaining the viability of the growth medium, they create conditions that do not simulate lyo operations, per the FDA definition: "Lyophilization - Drying in which the water vapor sublimes from the product after freezing"[5].

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EU media fill guidelines contain the following recommendation for all media fills (although nothing specific for lyophilized products), “The process simulation test should imitate as closely as possible the routine aseptic manufacturing process and include all the critical subsequent manufacturing steps “ [4].

For vacuum integrity (leak rate) testing of freeze dryers, no official limits are dictated per FDA. A general discussion states, “Leakage into a lyophilizer may originate from … the atmosphere into the vessel itself...It is necessary to monitor the leak rate periodically to maintain the integrity of the system…Should the leak rate exceed specified limits, determine the actual leak site for purposes of repair. Thus, it would be beneficial to perform a leak test at some time after sterilization…The time and frequency… will vary and will depend on the data developed during the cycle validation. The pressure rise found acceptable at validation should be used…during production. [6]”.

EU guidance makes one leak test reference (pertaining to moist heat sterilizers), “There should be frequent leak tests on the chamber when a vacuum phase is part of the cycle.” The only indication that specifications should be established is this guidance, “The efficacy of any new procedure should be validated, and ...verified at scheduled intervals based on performance history…” [4].

For this study, the testing was performed and results evaluated under the following three assumptions:

1. Although leaks can occur in numerous places in a freeze-drying system, to create a worst-case scenario the leak is considered to be from a source(s) large enough to admit microorganisms, and located in a part of the system housed in an unclassified mechanical room (with no set specifications and no routine monitoring performed).
2. Risk to the product from a system leak is limited to the time from the end of primary drying to stoppering of the vials (i.e., all of secondary drying). During primary drying product risk is minimal, because the vigorous rate of mass transfer of water vapor from the product out through the open slot in the lyo-stopper during sublimation creates a positive pressure in the vial headspace. Matter that might enter the system from the outside will likely travel to the area of lowest pressure, the ice on the condenser, and stay there. Since it is difficult to determine when exactly positive headspace pressure ceases, duration of risk is defined as the entire time the product dwells in the chamber from the end of primary drying to stoppering of the vials.
3. The leak rate remains approximately constant over the time course of secondary drying.

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Materials and Methods

The dryers in this study were manufactured by IMA Edwards and have the same external condenser design. Each have identical footprints but contain shelf area of either 98 ft^2 (5 shelves) or 215 ft^2 (11 shelves). The chamber is loaded and unloaded via a slot door in the main chamber door, which opens into a Class100/Grade A area of the production suites. The condensers, at the rear of the units, are housed in unclassified mechanical rooms. Three dryers were installed in 2001, and three in 2006.

Each system was initially verified, after a typical production sequence of cleaning, steaming, and drying, to achieve leak rate test results of not more than 0.027 mbar (20 mT) pressure rise over 30 minutes. Post-cycle leak tests, performed to ensure that aseptic integrity was not lost during the cycle, continue to require the same results for a passing test.

Measurements and calculations based on the Ideal Gas Law, $\Delta pV = \Delta n\, RT$, were performed to arrive at the maximum allowable pressure rise (leak rate) value that would maintain Class 100 / Grade A conditions for each dryer.

The worst-case potential bioburden for leaked air was determined by using an air IDEAL™ unit, operating according to the ISO/DIS 14698-1 recommended impaction principle, to measure the microbial load of air in the two mechanical rooms behind the freeze dryers. Twice the standard deviation was added to the average worst-case room results, for a bioburden maximum with a 95% confidence level. The calculated limit was doubled for an extra margin of safety, and this was the value used as the maximum potential bioburden load from the mechanical rooms.

A canister made from a closed section of stainless steel tubing was attached to a spare port on the chamber of each freeze dryer. The pressure inside the chamber was decreased and stabilized, then a valve inside the canister was opened, and the air in the canister entered the chamber. The change in internal chamber pressure was measured and used to calculate the void volume of the freeze-dryer (that is, the volume not occupied by shelves, condenser coils, and other hardware).

The following page provides an overview of all measurements and calculations performed.

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Measure		Calculate	Measure		Calculate					
Barometer "Hg	Room Temp	Moles of air in canister	P init	P final	Δ P	Dryer Void Volume	Liters 300 cfu/m^3 air that may enter	n=moles of air that may enter	Max. passing flow rate	Corresponding pressure rise

Measure pressure and temperature of air, and use it to calculate # of moles of air in a canister of known volume

By measuring the change in pressure of the chamber/ condenser when the air in the canister is introduced to the chamber, the # of moles of air that causes that pressure change can be used in a calculation to determine the void volume of the chamber/condenser

Calculate how much air at worst-case condition (300 cfu/m^3) could enter chamber/ condenser and still keep it below the maximum allowable value for cfu/m^3

Calculate how fast the air flow from the leak could be over the entire course of 2° drying to keep the total microbial load below the maximum allowable value for cfu/m^3; Pressure rise result is then calculated from the flow rate and void volume of the dryer

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Results and Discussion

Calculations were performed over a range of possible secondary drying times for all dryers tested, with representative results for one large dryer and the smaller dryer shown in the table. On the days of testing, all dryers meeting the leak rate limit of no more than 0.027 mbar pressure rise over 30 minutes would have been able to maintain Class 100 / Grade A microbial standards for up to 64 hours of secondary drying, even with an elevated assumed air bioburden of 300 cfu/m^3.

Note that, using this approach, the allowable pressure rise is independent of the size of the dryer, despite the fact that the allowable leak rate is a function of dryer size.

Calculations were made to matrix hypothetical results, using a range of room temperatures (16 – 27°C), and the extremes of barometric pressure that were recorded during the previous 12 months at the study site (979 – 1043 mbar. [7]). The calculations showed that room temperature has no significant impact on the maximum allowable leak flow rate; however, the barometric pressure does, with higher pressures allowing higher leak rate results for maintenance of Class 100 / Grade A microbial standards.

The approach can be put to practical use by establishing or justifying current acceptance criteria, and by developing a reference tool that lists maximum calculated acceptable leak rate results as a function of the length of secondary drying, using the worst-case conditions of lowest expected barometric pressure and an inflated room bioburden load. This tool can be used as an aid to assess the potential microbial impact to any cycle that exceeds the established post-test leak rate acceptance criteria.

Hours 2° dry	Max. allowable leak flow rate to maintain Class 100/Grade A (L mbar/min)		Pressure rise that corresponds to max. flow rate (mbar per 30 min)	
	215 Ft^2	98 Ft^2	215 Ft^2	98 Ft^2
1	740.6	488.2	1.681	1.696
2	370.3	244.1	0.840	0.848
5	148.1	97.6	0.336	0.339
10	74.1	48.8	0.168	0.169
20	37.0	24.4	0.084	0.085
30	24.7	16.3	0.056	0.056
40	18.5	12.2	0.043	0.043
50	14.8	9.8	0.033	0.033
60	12.3	8.1	0.028	0.028
64	11.6	7.6	**0.027**	**0.027**
65	11.5	7.5	0.025	0.027
66	11.2	7.4	0.025	0.025

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References

[1] C. Dern, *The Vacuum Integrity Testing of Lyophilizers*, Pharmaceutical Engineering, 25(1): 2005

[2] The Parenteral Society, *Technical Monograph No.7: Leak Testing of Freeze-Dryers*, (Wilshire, England: The Parenteral Society, 1995), 9

[3] FDA Guidance, *Sterile Drug Products Produced by Aseptic Processing — Current Good Manufacturing Practice*

[4] EU Guideline, *Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use – Annex 1*

[5] FDA draft Guidance, *SUPAC: Manufacturing Equipment Addendum*

[6] FDA *Guide to Inspection of Lyophilization of Parenterals (7/93)*

[7] http://www.wunderground.com/cgi-bin/findweather/hdfForecast?query=Bloomington%2C+IN MADIS Weather station (KINBLOOM4)

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