

Pharmaceutical Container/Closure Integrity IV: Development of an Indirect Correlation Between Vacuum Decay Leak Measurement and Microbial Ingress

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ABSTRACT: *The rational development of a physical test method to evaluate the microbial barrier properties of sterile containers necessitates its correlation to microbiological exclusion. This can be accomplished by direct or indirect correlation. In the former, the proposed physical test is directly compared to microbial challenges using appropriate test units that stimulate container leaks at both high and low probabilities of microbial ingress. Previous work has demonstrated the development of a direct correlation using helium leak rate methods and microbial immersion challenges. An indirect correlation can be established by comparing the proposed physical method to well-defined leakage standards that represent various known levels of microbial ingress. Thus the quality assurance properties of a physical test method can be established by comparison to another physical test method that has been previously characterized. This approach has the distinct advantages of being faster, quantitatively rigorous, and less subject to the vicissitudes of microbial testing. This approach was demonstrated by comparing the helium leak rate method to vacuum decay testing. Additionally it was demonstrated that vacuum decay testing was a fast and reproducible method for detecting leaks of about 1 to 2 mm. Leaks were simulated by affixing micropipettes into glass vials.*

Introduction

The microbial barrier properties of sterile product packaging is typically determined by container/closure integrity testing. Integrity testing should be conducted during different phases of the product life cycle, e.g. product development, in-process control, and stability testing. Integrity testing includes both microbial and physical methods. Historically, sterility testing was used to demonstrate container/closure integrity as part of stability protocols. However, recent FDA guidelines and other regulatory agency edicts have questioned the appropriateness of sterility testing for container/closure integrity quality assurance. Microbial challenge tests have been used for package integrity quality assurance but are typically expensive, time consuming, and variable and may require simulated product in situations wherein the formulation does not support growth (1–4).

Physical leak detection methods do not directly measure microbial ingress, but rather, they measure some physical property of the leak that is related to microbial failure. The development of a physical method to characterize microbial product quality requires the establishment of a correlation, either direct or indirect, between microbial ingress and the physical method. A direct correlation is established by comparing the results of physical and microbial tests on the

same population of samples, e.g. in our previous studies we developed a quantitative relationship between liquid immersion microbial challenge and helium leak rate methods (5–7). An indirect correlation can be developed by comparing a physical test to a documented indicator of microbial ingress (2). For example, if a quantitative correlation is established between an alternate physical method and the aforementioned helium leak rate method then an indirect correlation between the alternate method and liquid immersion microbial challenge can be constructed. The purpose of this report is to illustrate the development of an indirect correlation between a physical test method, namely vacuum decay testing, and microbial ingress.

The general approach to indirect correlation development is shown in Figure 1. The process involves the quantitative correlation of helium leak rate and vacuum decay methods by testing the same population of samples which contain leaks of various sizes. The direct correlation is then used to indirectly relate the vacuum decay method to the probability of microbial ingress by invoking the quantitative, direct correlation between microbial ingress and helium leak rate. Thus the alternate physical method, vacuum decay testing, can be indirectly correlated to microbial ingress based on an *a priori* relationship between a standard physical method, e.g. helium leak rate and microbial ingress.

Vacuum decay testing is a non-destructive physical test method which involves placing a sample in test chamber, pulling vacuum to create a pressure differential across a

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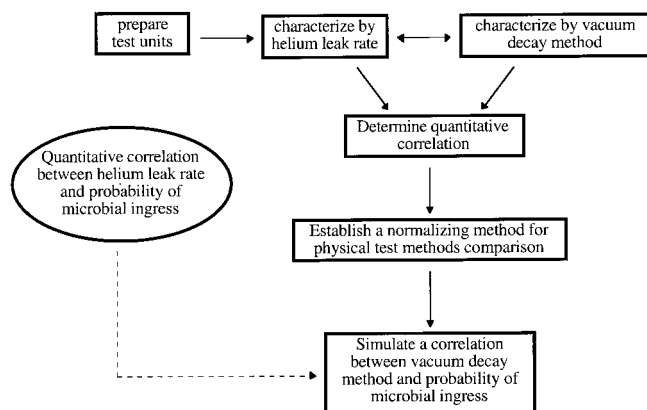


Figure 1—A process for the development of a quantitative, indirect correlation between a physical test method and the probability of microbial ingress for container/closure integrity.

container/closure and then measuring the pressure change inside the test chamber at a preset time interval. Pressure changes are primarily due to the leakage of the package contents: the larger the leak, the greater the pressure change in the chamber. This method of leak detection offers advantages which includes its ease of use, rapid testing time, in-process testing capabilities, and the ability to make quantitative measurements (2).

The utility of a physical leak test depends on its sensitivity and limit of detection. Factors that effect the sensitivity of vacuum decay testing include the test chamber geometry and volume, pressure transducer capabilities, differential pressure across the leak, duration of testing, and environmental conditions (e.g. temperature, relative humidity, and atmospheric pressure) (8, 9). Vacuum decay leak detection is limited to pharmaceutical packages that can tolerate the application of a differential pressure and do not rely on tortuous path or barrier seal types as the primary mechanism of container integrity (2).

Materials and Methods

Test Unit Assembly

Standard 10 mL tubing glass vials (supplied by The R. W. Johnson Pharmaceutical Research Institute) were modified by drilling a 2 mm hole in the neck region (Iowa Laser Technology, Inc.). Micropipettes of various sizes (0.1, 0.2, 0.3, 0.5, 1, 2, 5, 10 μm nominal diameter, World Precision Instruments) were inserted into the 2 mm hole. A gastight seal at the micropipette/glass vial junction was formed with epoxy. The seals were allowed to dry for 0.5–1.0 hour. A tungsten carbide knife was used to cut the pipette so that 1–2 mm length extended from the vial. Butyl rubber closures (4405/50-gray, teflon coated) and aluminum flip-off crimps (20 mm, The West Company) were used as container seals (Figure 2). The test units were stoppered and sealed using a nitrogen-powered crimper (The West Company) at 40 psi in a helium-filled inflatable glove bag.

Helium Leak Rate Measurements

A test unit was placed inside a chamber which was attached to the inlet port of a helium leak detector (UL200, Leybold Inficon). The testing chamber was closed to form a

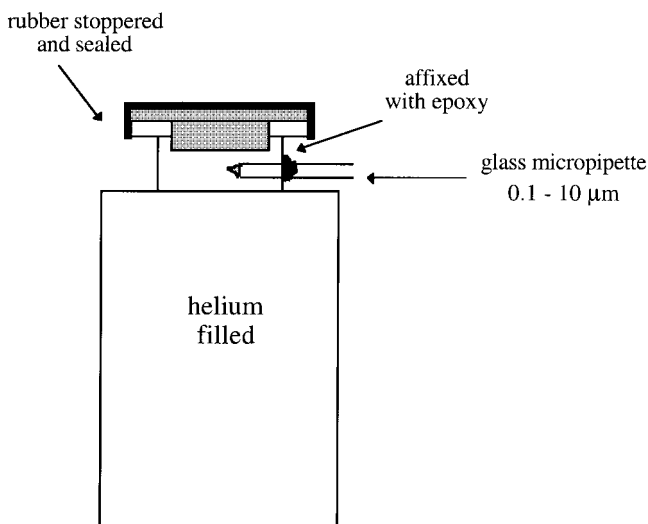


Figure 2—Diagram of test system.

gastight seal and leak testing was initiated. Leak rate measurements were recorded at 10, 15, and 20 seconds. If those readings were identical, then the reading was recorded as the test unit leak rate. If not, three additional readings were taken over 15 second intervals every minute until the identical readings were obtained.

Vacuum Decay Testing

Following helium leak rate characterization, the test units were transported from The University of Iowa (Iowa City, Iowa) to Packaging Technologies & Inspection (Tuckahoe, New York) for vacuum decay testing on a pressure differential leak tester (Model# WFX100, WILCO). The test lot was composed of intact and modified vials containing various leak sizes. The test units were randomized for each test run using JMP software (SAS Institute). Prior to vacuum decay testing, 10 pretests were performed on the same intact vial for stabilization of the transducer. The conditions for vacuum decay testing are shown in Table I.

Vacuum decay testing measures the loss of vacuum inside the testing chamber as a result of headspace gas leakage from the container. The procedure for performing the vacuum decay test involved placing the test unit into the test chamber and reducing the chamber pressure by 999 mbar. Then the test system was allowed to stabilize for 5 seconds prior to initiating the test. The measurements were recorded at the end of a predetermined test time as the differential pressure, defined as the pressure change inside the test chamber.

TABLE I
Vacuum Decay Test Conditions

Run #	1	2	3	4
Testing time (sec)	5.0	10.0	60.0	900.0
# intact test units	20	20	10	2
# modified test units	96	96	48	4
total # test units	116	116	58	6

Leak Rate Verification

Following vacuum decay testing, the test units were transported back to The University of Iowa for leak rate verification using the helium leak rate method. Leak testing was performed in the same manner as described above.

Results

Verification of Helium Leak Rates

It was necessary to verify that the test unit leak integrity remained the same during transport between testing sites. A comparison of the helium log leak rates before and after vacuum decay testing is shown in Figure 3. Overall there was good agreement between leak rates obtained before and after vacuum decay testing. Outliers were defined as test units with unusually large leak rate differences between before and after vacuum decay testing. Outliers were identified by computing the leak rate difference for each individual test units and evaluating the distribution of these differences values. The median and interquartile range (i.e. the range of values between the 25th and 75th percentiles) was computed. Outliers were test units with extreme difference values that did not fall within a range extending above and below the median error value a distance equivalent to four interquartile ranges (10). Twelve out of 116 test units used in this study were identified as outliers and eliminated from further analysis. No effort was made to identify the cause for outliers but past experience has shown that the test units are fragile and prone to damage by excessive manipulations (5).

Correlation between Vacuum Decay and Helium Leak Rate Methods

An exponential function was used to describe vacuum decay measurements at various testing times as a function of helium leak rates (Figure 4). The model, its parameter estimates, and coefficient of determination is shown in Table II. The vacuum decay measurements were accurately

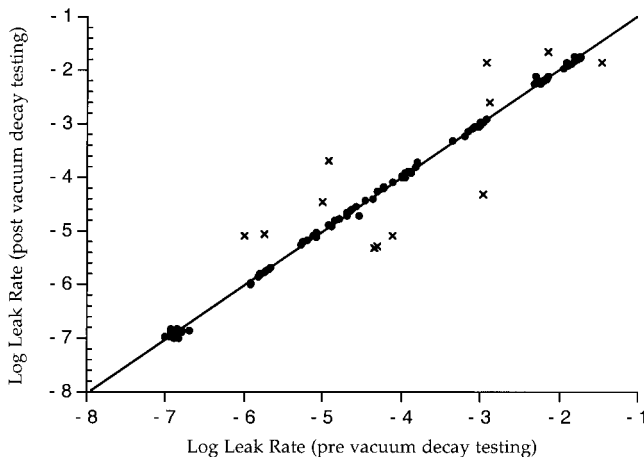


Figure 3—The correlation of log leak rate before (pre) and after (post) vacuum decay testing. Thirteen outliers were identified from a total of 116 test units tested and were eliminated from analysis. A linear regression model generated using measurements before and after vacuum decay testing had a slope of 1.0, y-intercept of 0.03, and a squared correlation coefficient of >0.99.

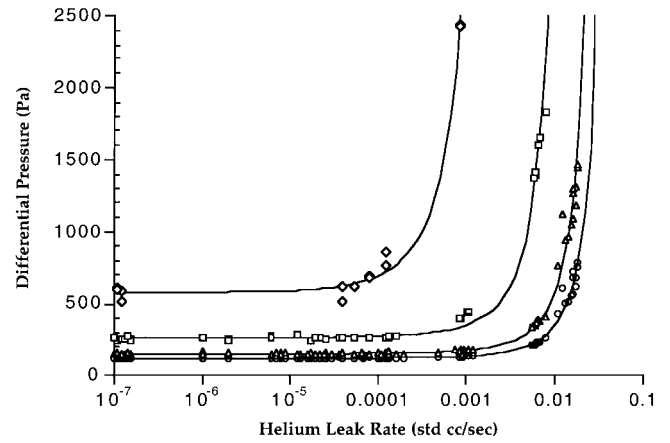


Figure 4—The correlation between differential pressure (Pa) and leak rate (std cc/sec) for intact and modified vials. The differential pressure was obtained by vacuum decay testing and the leak rate was determined by helium leak rate detection. The vacuum decay test times are indicated as follows; 5 sec (circle), 10 sec (triangle), 60 sec (square), and 900 sec (diamond). The exponential model for each testing condition is summarized in Table 2.

described in terms of helium leak rate by these mathematical models.

Detection limit of Vacuum Decay Leak Test

The detection limit is defined as the instrument response, i.e. output signal, that is significantly different from the baseline signal. A set of intact vials were tested by vacuum decay at three different test times (5, 10, and 60 seconds). The coefficients of variation under each testing condition was 4.6%, 2.4%, and 4.6% at 5, 10, and 60 seconds, respectively or approximately 4% on average. Therefore, the detection limit was taken to be the mean value for a set of intact vials (DP_0) plus 12% of this value or about three standard deviations.

Using the empirical correlations illustrated in Figure 4 and Table II, vacuum decay values can be described in terms of the helium leak rate and therefore the limit of detection for vacuum decay testing can be expressed in helium leak rate units. Moreover, the vacuum decay detection limit can be described in terms of nominal pore sizes, since these have been previously correlated to helium leak rates (5).

Table III summarizes the detection limit for the vacuum decay test at various testing times in terms of helium leak rate (std cc/sec) and nominal leak diameter (μm). The increase in testing time from 5 to 60 seconds improved the

TABLE II
Summary of the Mathematical Model, Parameter Estimates, and Correlation between Vacuum Decay, in Terms of Differential Pressure (DP in Pa) and Helium Leak Rate (HLR in std cc/sec) at Various Vacuum Decay Testing Times, Where DP_0 and λ Are Constants

Testing Time (sec)	DP_0 (Pa)	λ	Squared Correlation Coefficient (R^2)
5	126	101	0.98
10	157	129	0.97
60	268	270	0.98
900	585	1714	0.99

Model: $DP = DP_0 * e^{(\lambda * HLR)}$

TABLE III

Estimated Limit of Detection for the Vacuum Decay Test in Terms of Helium Leak Rate and Nominal Pore Diameter

Vacuum Test Time (sec)	Detection Limit (std cc/sec)	Corresponding Nominal Pore Diameter (µm)
5	1.1×10^{-3}	2
10	8.8×10^{-4}	2
60	4.2×10^{-4}	1
900	6.6×10^{-5}	0.6

detection limit by >2.5 fold, whereas a test duration of 900 seconds increased the sensitivity limit >15 fold. The detection limit for vacuum decay testing in terms of the nominal leak diameter varied from 2.0 to 0.6 µm as the testing time increased from 5 to 900 seconds.

A Method for Comparing Physical Leak Test Measurements of Different Test Methods

The helium leak rate and vacuum decay test methods provide leak measurements in different units. The former describes leakage in terms of escaping helium mass per unit time. Measurement values can vary from 10^{-12} and 10^{-1} std cc/sec. The vacuum decay method measures leakage in terms of a pressure increase in the test chamber over a predetermined testing time. Values of differential pressure may vary from about 100 to 4000 Pa depending on testing conditions, equipment, and chamber design. In order to compare the utility of physical test methods with different measurement units, a method for the normalization of measurement values was developed.

The utility of a leak detection method is related to its ability to distinguish between a defective and an intact container in the presence of random test measurement error. A discrimination factor was defined as the measurement of the absolute value of the difference between defective and intact, non-defective test units divided by the standard deviation of the intact vial population. This discrimination factor describes the distance, in terms of the number of standard deviations, that separates the intact vial population from the population of defective vials. The measurement

units associated with specific methods are normalized in a manner analogous to the estimation of coefficient of variation.

Figures 5 and 6 illustrate hypothesized situations corresponding to low and high discrimination values. A test method with low discrimination capabilities is unable to distinguish between defective and intact glass vials. In Figure 5, a test method with a low discrimination value equal to 2 displays a large region of overlap between the defective and intact populations. As illustrated in Figure 6, higher discrimination values are associated with complete separation between intact and defective sample populations and thus are associated with greater assurance that the physical method can discriminate between intact and defective test units.

The mathematical relationship between vacuum decay and helium leak rate measurements (Figure 4 and Table II) was used to calculate the predicted helium leak rates associated with vacuum decay measurements at various testing times. Using the predicted helium leak rate values and the logistical regression model correlating the probability of microbial ingress and helium leak previously developed (2), the probability of microbial ingress associated with vacuum decay test was determined. Thus an indirect correlation between the probability of microbial ingress and vacuum decay testing was obtained.

In order to use a physical method as an indicator of the microbial barrier properties of a container system, the relationship between a physical leak test and the probability of microbial ingress should include a region of relatively high discrimination values (5–10) wherein the probability of microbial ingress is zero or near zero. Moreover, the test should be capable of detecting containers defects associated with a high probability of microbial ingress at a high discrimination level. This idealized relationship is consistent with the helium leak rate values presented in Figure 7. For vacuum decay testing, discrimination factors of 5 to 10 correspond to relatively high microbial ingress probabilities (>0.4). Thus this method does not have the sensitivity required to demonstrate the maintenance of microbial barrier properties of the container system.

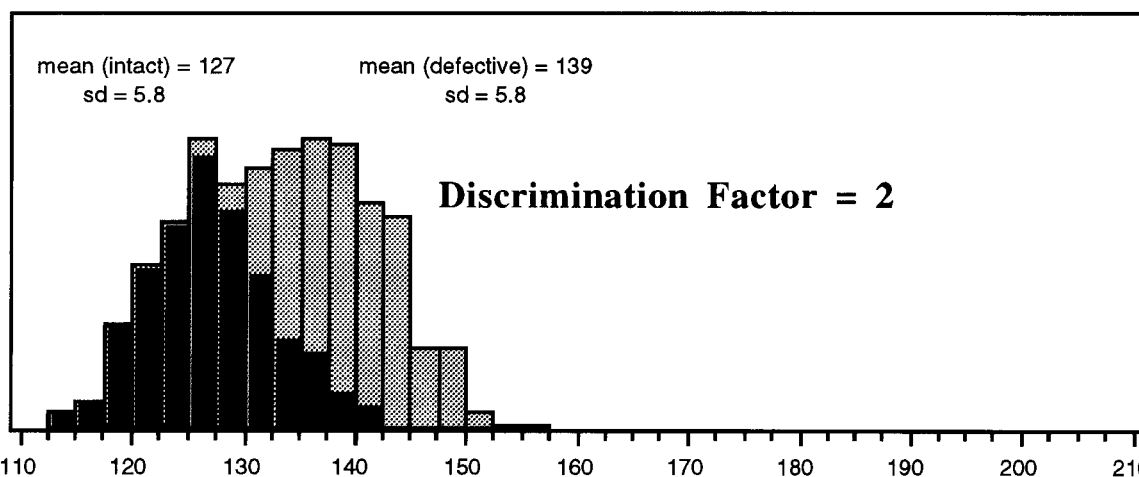


Figure 5—Population distributions of intact and defective measurement using a physical leak detection method with a discrimination value of 2.

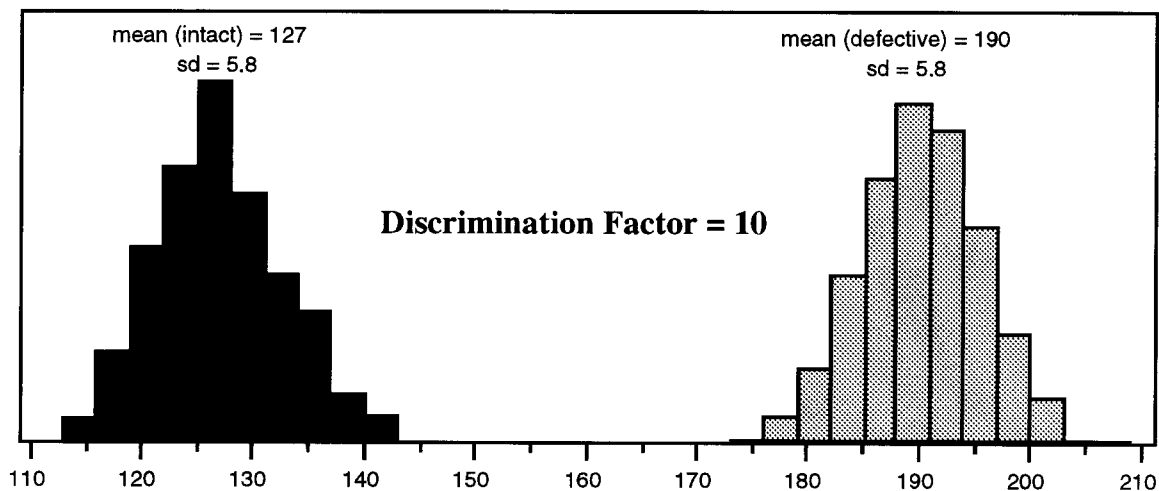


Figure 6—Population distributions of intact and defective measurements using a physical leak detection method with a discrimination value of 10.

Summary

The primary objective of these studies was to demonstrate the development of an indirect correlation between a physical leak detection method and microbial ingress. We believe that a successful demonstration of the process, as described in Figure 1, was achieved. This work suggests the future possibilities of comparing a variety of physical leak tests (e.g. spark, dye, bubble, and headspace gas methods) to the probability of microbial ingress in such a way that the relative utility of all the methods can be compared simultaneously.

Two caveats must be pointed out. First, as with all analytical methods, the interpretation of the method utility is limited by the conditions of analysis. For example, many factors can affect the sensitivity of the vacuum decay method such as the transducer design, test chamber, void volume, ambient temperature and humidity, test unit surface and volume, and testing duration. In our studies, only the latter was investigated as a controllable variation source. Thus instrumental improvements or methodological changes

may require a continuing re-evaluation of this or any other physical test method that is being re-engineered.

The second issue that must be considered is the method of microbial testing which is held as the standard for physical test method evaluation. Our previous work has involved the comparison of liquid immersion microbial challenges and physical leak rate methods. For pharmaceutical container systems intended to present a barrier to airborne sources of microbial contamination, our model may not be appropriate. Here again, further work is needed to define leak rate limits that are associated with airborne microbial contamination.

Finally, the utility of vacuum decay testing should be emphasized. Our tests found it to be a fast and reproducible method for detecting defective vials containing leaks of about 1 to 2 μm diameters. For extended testing times, the detection limit approached 0.6 μm diameter leaks. For many applications, this type of performance is very appropriate, for example, the on-line detection of gross leakers.

The selection of an appropriate leak detection method depends in part on product quality factors such as product package design, component materials, and performance criteria. The assessment of container/closure quality assurance by physical methods depends also on characteristics of the test method, i.e. its sensitivity, reliability, and reproducibility. From the work previously presented (5–7), the feasibility of a direct correlation between physical leak measurements and microbial ingress was demonstrated. Herein we have demonstrated a procedure for indirectly correlating alternate physical test methods, e.g. vacuum decay testing, to microbial ingress.

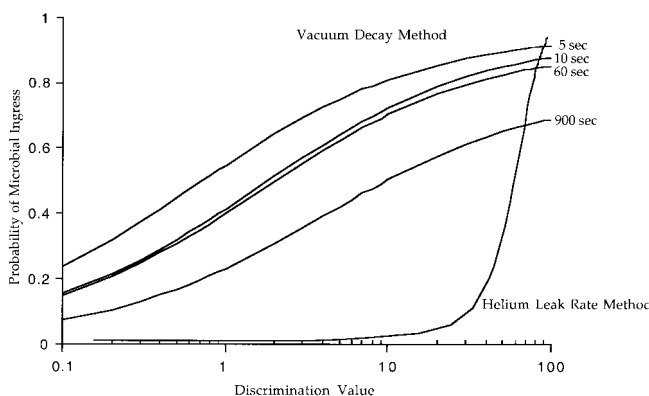


Figure 7—Simulated correlations of the probability of microbial ingress to discrimination values for helium leak rate detection and vacuum retention testing. The plots were constructed by utilizing the mathematical relationship established between vacuum retention and helium leak rate at various vacuum decay test times of 5, 10, 60, and 900 seconds and the logistical regression model that established the correlation between microbial ingress and helium leak rate.

Acknowledgments

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