Numerous studies have attempted to pinpoint the critical leak size that corresponds to risk of product sterility loss. Results vary, with some studies implicating leak paths as small as 0.2 μm, while others imply leaks 10 μm and larger. Regardless, and perhaps most importantly, all research shows that liquid presence in the smallest defects is a prerequisite for microbial entry. Therefore, research seems to encourage a shift away from direct correlation of a given leak test to microbial ingress, toward the comparison of a leak test method's ability to detect defects capable of liquid passage—a less probabilistic, more easily verified parameter.

Indirect Comparison of Microbial Ingress with Physicochemical Leak Tests

Two published works compared vacuum decay leak testers' ability to find leaks previously sized by helium mass spectrometry. As the same test package population had been previously tested for microbial ingress risk, these data were used to indirectly determine the ability of the vacuum decay testers of that day to detect such defects (15,16).

In the previous section, "Direct Comparison of Microbial Ingress with Physicochemical Leak Tests," those leak test methods capable of accurate and sensitive detection of liquid passage were shown to be more reliable and sensitive indicators of package sterility risk than microbial challenge tests performed under the same test conditions.

Another indirect comparison approach applicable for packages sealed under vacuum is based on the predicted flow of gas that would occur if a defect of a given size were present in an evacuated vial package. This concept is explained more fully under Test Methods Frequency Modulation Spectroscopy. Briefly, laminar gas flow theory can be used to predict the rise in pressure inside such an evacuated package, given leak paths of various widths. The change in pressure over time for such a package can be correlated to defect size, and therefore, indirectly correlated to microbial ingress risk.

LEAK TEST VALIDATION

Calibrated Leak Standards

Calibrated reference leak standards are an important validation protocol component when evaluating leak test methods that rely on tracer gas flow through leaks, for example, helium mass spectrometry. Calibrated physical leaks are designed to deliver tracer gas at a known flow rate. There are two main categories of such standard leaks: (*i*) reservoir leaks that contain their own tracer gas supply, and (*ii*) nonreservoir leaks that rely on tracer gas addition during testing. Calibrated gas leaks perform by one of two methods. Either the leakage rate depends on the permeation of specified materials by certain gases, or an orifice is present allowing specified gas flow rates under prescribed differential pressure conditions. Often tracer gas detection systems, such as helium mass spectrometry instruments, incorporate internal reference standards to verify test system functionality.

Other leak test instruments that rely on air movement for leak detection, for example, vacuum decay leak testers, may utilize either a calibrated variable rate flowmeter or a calibrated fixed size orifice to introduce air leakage into the test chamber during equipment qualification or start-up.

Whenever possible, leak test instrument performance should be challenged using such calibrated standards. The Nondestructive Testing Handbook, Volume 1 Leak Testing (17) is an excellent resource for precautions and limitations regarding calibrated leak usage. While calibrated leak standards provide valuable instrument functionality and sensitivity information, leak test method validation is not complete without studies verifying the method's ability to differentiate between known positive and negative control test packages.

Positive Control Test Packages

Proper leak test method validation requires a demonstration that the integrity test method can successfully detect leaks in positive control, with-leak packages. Often this seemingly simple and clear requirement has been misinterpreted. For example, a common misconception is that a media-filled package used for a growth promotion check in a microbial challenge test is equivalent to a positive control test sample. While a growth promotion test proves that the packaged media can support microbial growth, it does not prove that bacteria would or could