as it most closely simulates the product-package system. However, this may be impractical when validating a variety of products that use similar packaging. Verification of the media's growth-promotion capability at the completion of the package integrity test is important, especially if the test sample holding time is lengthy.

Test Package Preparation

Two approaches are possible for preparing sterile packages for testing. Either previously sterilized package components are aseptically filled with the growth-promoting vehicle, or media-filled packages are terminally sterilized. If feasible, the sterilization procedures and package assembly processes chosen should mirror those used for the actual product. Otherwise, the test package and seal may differ in some respect from the marketed product-package system. For example, vial package capped closures exhibit a certain amount of sealing force on the vial land seal surface. This residual seal force will noticeably decay upon terminal steam sterilization, thus potentially changing the seal quality (30,31). Similarly, plastic bag test samples exposed to gamma irradiation post heat sealing may not represent product bags normally sealed using ethylene oxide sterilized materials.

Microbial Growth Verification

Microbial growth as evidenced by cloudiness in the package may be detected visually or with instrumentation. In the case of product-filled packages, verification of nonsterility may require aseptic filtration and filter plating for microorganism identification. Any nonsterile package contaminants are generally identified to verify the challenge microorganism as the source of contamination.

Test Package Population Size

There is no guarantee of microbial ingress even in the presence of relatively large defects. Microbial ingress is a notoriously probabilistic phenomenon. For this reason, a valid test requires a relatively large population of test samples and positive controls.

Positive and Negative Controls

All leak test validation protocols, including microbial challenge tests, require positive control or known-leaking packaging in the test package population to demonstrate the test's leak detection ability. Because even significant leak pathways will not always demonstrate microbial leakage, a large database of samples is needed to minimize the risk of false–negative results. Despite the best efforts, microbial challenge tests may yield erratic results that do not reliably correlate to leak size or presence.

Residual Seal Force

Residual seal force (RSF) is not a leak test method, but it is included in this discussion since compendial and regulatory guidances reference RSF as a package integrity test method option, and because RSF is a valuable tool in parenteral vial package assembly optimization and verification.

RSF is defined as the compression force exerted by an elastomeric stopper or closure on the sealing surface of a container, typically a parenteral glass or plastic vial. This compressive force ensures package integrity at the stopper/vial interface. RSF is established when the stopper is crimped onto the vial finish, and is a function of elastomeric viscoelastic properties, capping machine head pressure, package component stack height dimensions, and aluminum seal skirt length. Because closures are viscoelastic in nature, the RSF will decrease somewhat as a function of time, processing procedures, and elastomer composition (30,31).

RSF values can be determined indirectly using a constant rate of strain stress tester, also called a universal tester. Genesis Machinery Products, Inc. markets automated residual seal force tester (ARSFT) that works according to the same principle. To perform a RSF test, a specially designed aluminum cap is placed on top of the sealed vial and placed on a compression load cell of the universal tester or ARSFT. The vial is then slowly compressed at constant rate of strain, and a stress-deformation response curve is generated. The RSF is the force where the slope of