Pressure Decay: The Container Closure Integrity Testing for Biologic Drugs

Salvatore Savino, PhD, Packaging Testing Laboratory Manager at Eurofins BPT Italy

1. Background

The assurance of the sterility of a parenteral drug product, prior to any human use, is a mandatory regulatory requirement. Sterile products should not contain contaminants caused by microorganisms, gases or debris, and the chosen Container Closure System (CCS) has to prevent the ingress of such substances during the entire shelf life[1].

The ability of the CCS to maintain the integrity of its microbial barrier, and hence the sterility of a drug product (DP), have nevertheless to be demonstrated[2].

The USP chapter <1207> extends the concept of Container Closure Integrity (CCI), encompassing the absence of all package leaks that could affect product quality.

The package integrity verification plays an important role throughout the product life cycle, starting with product development and continuing through marketed product stability studies[3].

The way to reach the product for the contaminants is to pass through a leak of the CCS.

Leaks are commonly conceptualised as holes of a defined diameter, or channels of distinctive diameter and length, although leaks that occur naturally are generally complex, multicavity tortuous paths and are rarely uniform in size or shape. The Container Closure Integrity Testing (CCIT) can be divided in two categories: probabilistic and deterministic tests. A probabilistic leak test method is stochastic in nature since it relies on a series of sequential and/or simultaneous events each associated with uncertainties. A deterministic leak test method is one in which the leakage event is based on phenomena that follow a predictable chain of events, and therefore it is intrinsically more reliable and recommended[3].

When selecting a leak test method, the first determining factor is the nature of the package content. For example, if testing liquid-filled packaging by vacuum decay, the test vacuum conditions may trigger some formulations to solidify inside leak paths[4].

The biologic drugs are products that are manufactured from living organisms or contain their components. Due to the nature of these products they often contain large molecule ingredients such as proteins, other biologics polymers and sometimes entire cells. The main challenge of the CCIT on this type of drug products is to ensure that these ingredients do not affect the defect detection capability of the method.

2. Study goals

This article reports the results of several pressure decay studies with the aim to demonstrate how this technique could be a valid choice for CCIT of biologics, such as liquid formulations with large molecules and polymeric ingredients or lyophilised product.

On the basis of the USP chapter <1207> Pressure decay testing is intended for integrity testing of the gas headspace region of the test sample, considering that small leaks below liquid-fill level would not be detected by this technique. This point needs nevertheless to be fully investigated and put into context of testing.

Pressure decay is based on the flow of a gas from the outside (test chamber; see the legenda for terms explanation and the Figure 1 and 2) to inside of the CCS object of the test. In light of this the hydrostatic pressure, which increases with the liquid volume, can affect the performance of the test, but there is no indication in USP <1207> that correlates the detectability under the liquid level and the nominal volume of the CCS.

Our R&D work has started from this observation, by testing containers with small nominal volume (2mL-6 mL-10 mL) with certified leaks placed under the product fill level, with the following objectives:

1. **An alternative for biologic drugs CCIT.** A significant inconvenience of CCIT, as mentioned earlier, is related to the potential clogging of the defect, obtaining false negative results during the test. A common feature is the leak blockage triggered by vacuum conditions: some formulations solidify inside leak paths when a vacuum is applied, especially for large

molecules based product. This paper will show that the use of the pressure instead to the vacuum allows to overcome this drawback.

2. **Multi-materials packaging testing.** The second goal of this research was the evaluation of pressure decay to detect packaging defects in multi-materials CCS which is a known limitation of the High Voltage Leak Detection (HVLD). This is a widely used CCIT technique for biologics but nevertheless presents limitations in the leak paths detection in the regions between the different materials.
3. **Powder products CCI testing.** The third objective was the setup of a reliable CCI test for powders products (e.g. freeze dried/lyophilised product), since not all the techniques are exploitable to test solid products. The HVLD for example works only with liquid products, while vacuum decay is often affected by the defects clogging due to the product powder interference. The applicability of pressure decay in leak detection for lyophilised product was also investigated.

3. Results and discussion

Five combinations of liquid formulation products with the related CCS (studies from 1 to 4; study 4 includes 2 combinations of DP and CCS) and one lyophilised product and own corresponding CCS (study 5) were tested by pressure decay and the analytical methods were successfully validated in accordance with the guidelines of the USP <1207> chapter. All the tests were performed on the LF-S11 leak testing machine for pharmaceutical package, supplied by Bonfiglioli Engineering.

A common approach of the validation was adopted for the different combinations, that includes the use of negative controls, certified positive controls, dedicated *test chambers*.

Each validation study (summarised in the Table 1) had taken in to account the location of certified defects under the level of the product (middle body of container or 3 mm from the bottom of the vial), using a worst case approach: challenging the potential clogging of the leaks generated. In addition, large leaks detection at closure level was evaluated.

The parameters validated were: System suitability; Accuracy; Precision; LOD; Specificity and Range[4]. The limit of detection (LOD) for the method is defined as the smallest leak in the positive controls that allows a consistent differentiation between leaking and not-leaking packaging.

The differentiation is provided by a *rejection limit:* pressure decay value above of which a leak is detected in a package. The *rejection limit* is the "pass/fail" limit of the method: the positive controls results have to be located above this limit (at least of 95% of times), differently the negative controls have to be located under or equal to this level the 100% of times.

The analyses were performed on different days by different analysts, to obtain a large number of replicates as reported in the scattering plots of Figure 3.

We can summarise in Table 2 the results obtained through a comparison of the advantages and limits of pressure decay with respect to the two CCI deterministic techniques widely used: vacuum decay and HVLD.

4. Conclusions

The present R&D work showed how pressure decay technique is able to detect leaks with a LOD of 20 µm under the liquid level of challenging products such as biologics with large-molecule ingredients.

This CCI testing provides a valid alternative to vacuum decay mode, significantly affected by the presence of polymeric ingredients that could lead to the clogging of the defects: the "false negative" results.

The article demonstrate how pressure decay is a good option to HVLD to overcome the following limits (see Table 2 for details):

1. Product must be present at leak site. **Pressure decay is suitable to detect leaks in the headspace.**
2. Moisture presence on the package can potentially trigger a false negative result. **Pressure decay is only affected by Temperature (easy to monitor and control).**
3. Impact of the product stability after test exposure is recommended. **Pressure decay is a non-destructive testing.**
4. The varying resistivity of the materials (e.g. glass vials stoppered with a metal crimp cap) may result in loss of sensitivity and reliability of a test method. **Pressure decay is exploitable to detect leaks in package with different materials including the leaks located into the material-change region.**
5. Test limited to liquid (with no combustion risk) more electrically conductive than package. **Pressure decay shows no limitations related to the physical state of the product.**

Despite the several advantages above indicated, in the choice of the right CCIT to be applied the following limitations of the pressure decay mode need to be also considered:

1. The ability to detect leaks below the liquid-fill level could be limited to the increase of hydrostatic pressure in large volume containers.
2. The LOD of pressure decay method is usually higher with respect to HVLD and vacuum decay techniques.

The leaks detection is an analytical procedure, not a standard method, and the type of CCIT that should be applied for this aim depends on the type of product-packaging coupling. There is no "one-size-fits-all" solution and a case by case approach need anyway to be considered. A proper risk assessment has to be performed each time for the most appropriate choice of the way of testing, in order to reach the most relevant and reliable analytical data.

5. Definitions

- **Test Chamber (Tooling):** A stainless steel (or polymeric material) chamber designed to hold sample, minimising the volume around it and in which the pressure/vacuum is applied.
- **Rejection Limit:** The pressure decay value above of which a leak is detected in a package. The rejection limit is the "pass/fail" limit of the method.
- **Negative Control:** not defective primary packaging, filled with drug product.
- **Positive Control:** primary packaging filled with drug product that contains a certified defect.
- **Large leak:** defect obtained in the closure in order reproduce a gross defect, simulating for example a fail in the closing process.

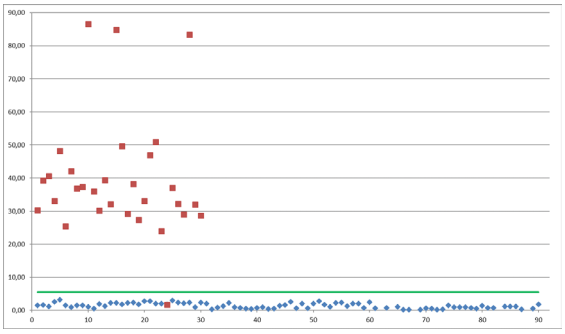
Figure 1. Test chamber for the 1mL AT-Closed vial® :

Figure 2. Test chambers for the 6mL AT-Closed vial® , 10R and 2R packaging respectively:

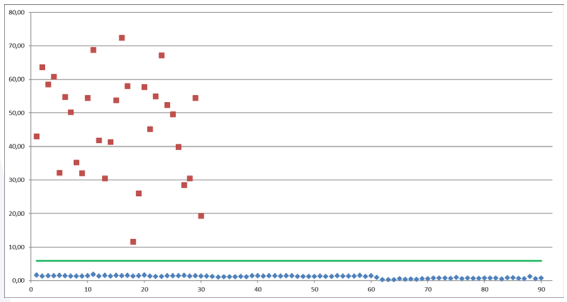
Figure 3.

Graphics of scattering plots of the 5 studies.
X-axis: number of tests collected on different days by different analyst
Y-axis: pressure decay (mbar)
■ positive controls with a defect size equal to the LOD validated
◆ negative controls
— rejection limit

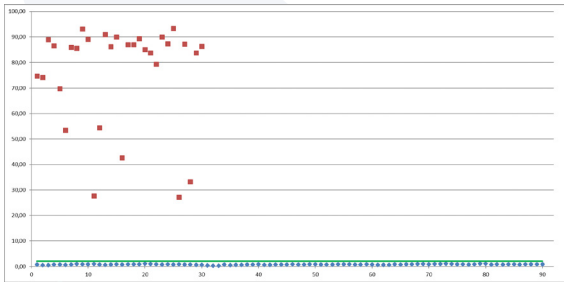
Study 1:



Study 2:



Study 3:



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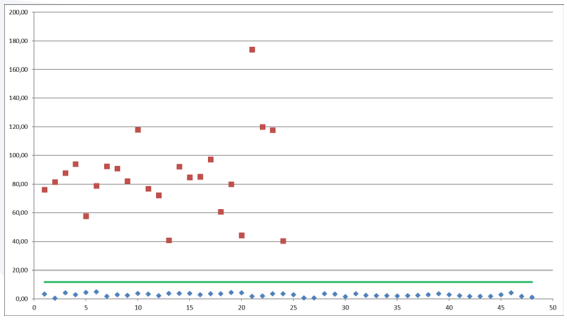

Study 5:

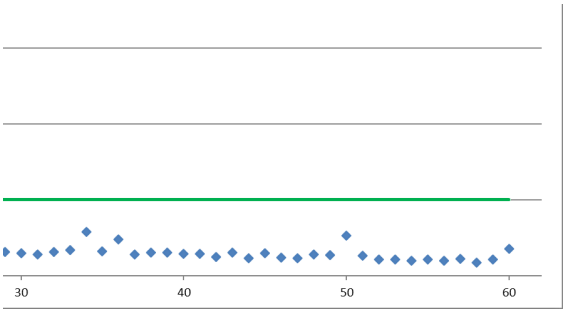

Table 1. Container Closure Integrity studies details.

Study (Combination of DP-CCS)	CCS	Product physical state	Controls tested	Product	Validated LOD
1	Aseptic Technologies closed vial® of 6 mL: 1. Body in COC 2. Stopper in TPE 3. Top ring 4. Cap	Liquid Biologics	• 30 negative controls • 10 positive controls	Human Solution Albumin 40 mg/mL	20 µm
2	Aseptic Technologies closed vial® of 1 mL: 1. Body in COC 2. Stopper in TPE 3. Top ring 4. Cap 5. Base ring	Liquid Biologics	• 30 negative controls • 10 positive controls	Human Solution Albumin 40 mg/mL	20 µm
3	1. 2 R glass vial, Nuova Ompi 2. Stopper Flurotec® 4110/40 B2-40, West 3. Flip-off seal 13mm, Datwyler	Liquid Biologics	• 30 negative controls • 10 positive controls	Recombinant Interleukin-7 solution 2mg/mL	20 µm
4	1. 10 R glass vial, Supleco 2. Crimp seals with PTFE/silicone septa, Sigma Aldrich 3. 20 mm rubber stopper, Omniflex™	Liquid Biologics	• 24 negative controls • 12 positive controls	• Albumin solution 50 mg/mL • Casein solution 50 mg/mL	20 µm
5	1. 10 R glass vial, Nuova Ompi 2. Stopper 87-J grey (4416/50), West 3. Cap 20mm Flip off green, Capsulit	Lyophilized Biologics	• 20 negative controls • 10 positive controls	Recombinant form of human alpha-mannosidase	10 µm

Table 2. Deterministic CCIT: Advantages and limits.

Features	Technique		
	Pressure decay	HVLD	Vacuum decay
Advantages	• Non-destructive[3] • Deterministic[3] • Good tolerance to environmental condition (Temperature easy to control and monitoring) • Exploitable for large molecules products (e.g. protein based) • Exploitable for lyophilized product • Exploitable to detect leaks in multimaterial packaging	• Deterministic[3] • High Sensitivity[3] • Exploitable for large molecules products (e.g. protein based)	• Non destructive[3] • Deterministic[3] • High sensitivity for liquid products composed by small molecules • Packages ranging in volume from a few milliliters to several liters may be tested[3]
Limits	• Lower sensitivity compared to HVLD or vacuum decay[3] • Affected by the temperature variations (necessary a monitoring) • For large volume containers the leaks below the liquid-fill level could not be detected by this method	• Product must be present at leak site[3] • Moisture presence on the package can potentially trigger a false negative • Impact evaluation of test on product stability is recommended[3] • The varying resistivity of the materials (e.g. glass vials stop-pered with a metal crimp cap) may result in loss of sensitivity and reliability of the test • Test limited to liquids (with no combustion risk) more electrically conductive than pack-age[3]	• Package surfaces below the liquid fill-level may be tested only for those liquid products that volatilize at test vacuum without solidifying and blocking leak paths[3] • Product–package gas headspace must be at atmospheric pressure or at a pressure greater than test vacuum conditions[3]

6. Bibliography

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5. USP NF 2021 Issue 3<1207.2> Package Integrity Leak Test Technologies
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