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Non-destructive vacuum decay method for pre-filled syringes closure integrity testing

Current practices, challenges, new developments

Pharmaceutical Pre-Filled Syringes (PFSs) are currently used across a wide range of sectors such as biopharmaceuticals and vaccines; the market has moved in this direction for a number of reasons, including ease of drug administration, additional safety, lower risks of contamination and mix-up. One key aspects of PFS quality control is the assurance of closure integrity after filling and terminal sterilization. Leaking through the PFS may expose its drug product to the lack of sterility assurance. This article, serves to explore the validation of a non-destructive Container Closure Integrity (CCI) testing technique applicable to PFSs, filled in with vaccines manufactured by a global leading pharmaceutical company. Data and findings of a challenge test performed as stated in ASTM F2338, a FDA Recognized Consensus Standard referred to pharmaceutical package integrity, are provided. All CCI tests employed the equipment model "LF-S", manufactured by Bonfiglioli Engineering, located in Vigarano Pieve, Ferrara, Italy. The study demonstrates that LF-S is effective in detecting PFS leakages less than 5.0 µm in diameter by using the Vacuum Decay Method (VDM). A suitable test samples set was employed to determine the detection capability, the process sigma level and test efficiency indicators. Besides, an innovative system preventing possible PFS plunger movement during CCI testing execution is presented.

Case Study

The case study, investigated the capability of the LF-S equipment to detect simulated defects of different sizes and types on PFSs and to quantify the false-result rates. VDM process indicators as:

- Detection Rate of Positive Controls (DR_{PC});
- Detection Rate of Negative Controls (DR_{NC}),

are determined and:

- False-Negative Rate FNR = 1- DR_{PC};
- False-Positive Rate FPR = 1- DR_{NC}.

are obtained.

TEST SAMPLES PREPARATION

A set of glass syringes with luer lock plastic tip and rubber plunger (Figure 1) was arranged. Test samples were prepared according to the following criteria:

- Negative Control PFSs, having no leakages;
- Positive Control PFSs, having artificially created known defects,

in the same number of units.

The study was then arranged as a cycle showing two main scenarios:

I. Drug product PFS (fill level 0,4 ml);

II. Sterile water PFS (fill level 0,4 ml):

The reason for this was to establish a qualitative correlation in terms of drug product / sterile water vaporization capability.

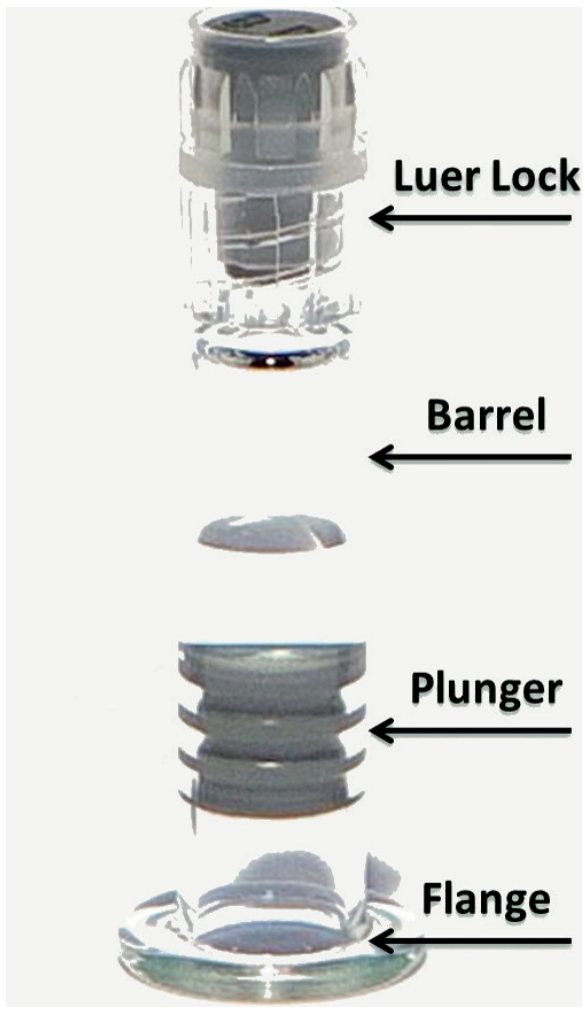

Figure 1 - Pre-filled Syringe – Container system used for the case study

Negative Controls

60 conforming PFSs were selected from a large initial set following the execution of microbiological test.

This test was based on the immersion of PFSs filled with a Tryptic soy broth in a bacterial suspension (solution with Brevundimonas Diminuta). All PFSs were then cleansed and incubated for 7 days at a temperature range of 25 to 30 °C. PFSs that did not show any growth following incubation were included in the Negative Control set.

Positive Controls

Laser-drilled holes

Holes ranging in size from 5 µm to 20 µm (Table A) were laser-drilled into the barrel of the PFSs (Figure 2) and positioned both above (position "A") and below the fill level (position "B"). The hole size lower limit was established to be coherent with the ASTM F2338; this was not intended to represent a possible limit of leak detection.

The reference PFSs were then individual-

Table A - Laser-drilled holes specification

Hole diameter (µm)	Position: A = above fill level B = below fill level	Serial Number ID range	Flow rate (sccm)	Number of units
5	B	1-10	0,213	10
5	A	11-20		10
10	B	21-30	0,855	10
10	A	31-40		10
20	B	41-50	3,41	10
20	A	51-60		10

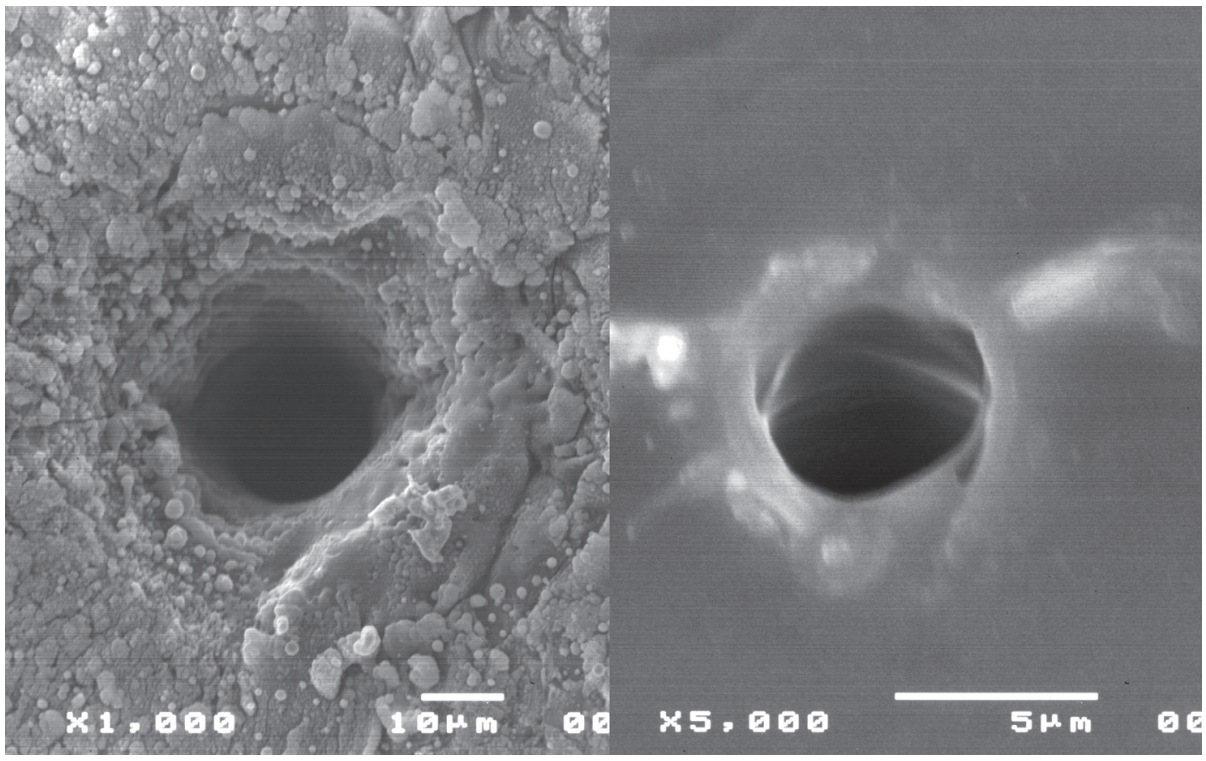

Figure 2 - Laser Drilled Holes – The picture on the left shows a 20 µm hole from interior side of PFS; the picture on the right shows a 5 µm hole from exterior side of PFS

Table B - Capillary tubes specification

Equivalent hole diameter (µm)	Capillary Tubes		Flow rate (sccm)	Number of units
	Length (mm)	Diameter (µm)		
5	9	25	0,213	20
10	13	40	0,855	20
20	28	75	3,41	20

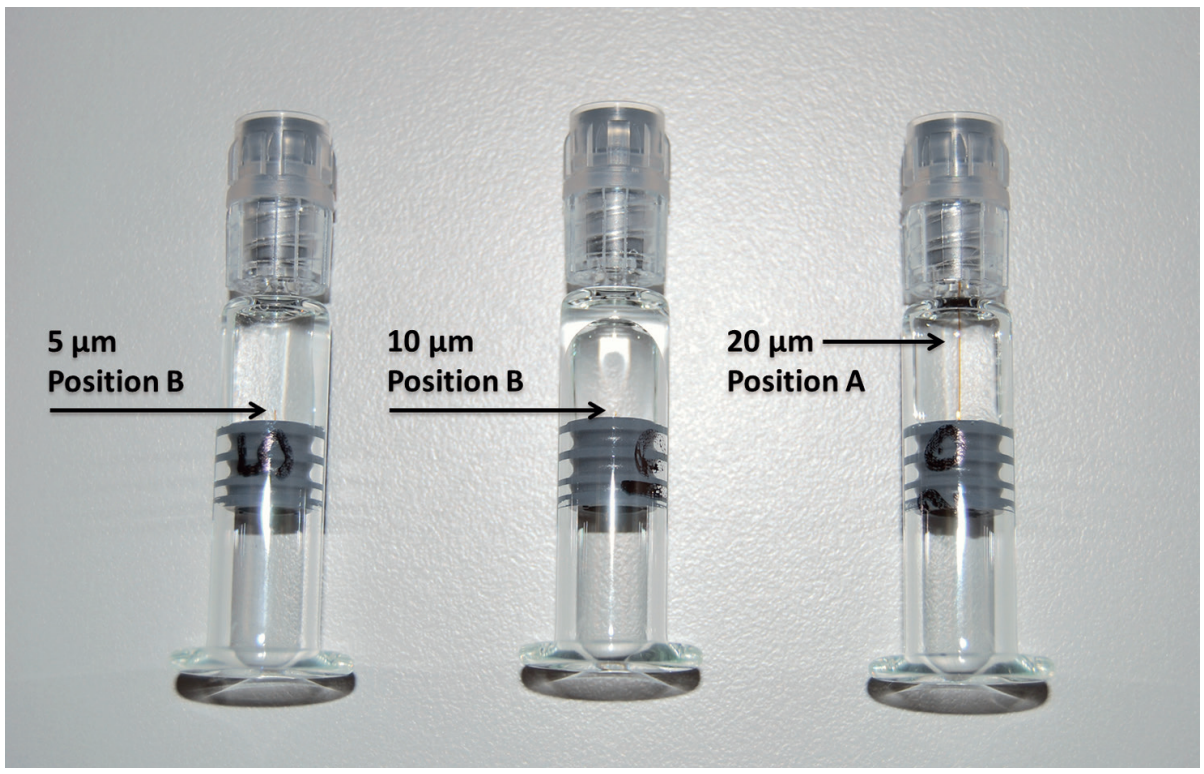

Figure 3 - Capillary tubes preparation – for each component carry out the following procedure:
Step a) Determine the diameter and length of the tube that will lead to the required flow rate until the reference differential pressure of 980 mbar is attained between the inlet and outlet.
Step b) Cut the selected tube perpendicularly on the desired length.
Step c) Set the reference differential pressure between the inlet and outlet sides of the tube and then measure the flow rate across it.
Step d) Place the tube in a needle, insert the needle in the plunger, adjust the position of the tube according the required height and remove the needle from the plunger in the end.

ly numbered from 1 to 60. The differential pressure across the PFS leak path was set to 980 mbar to be consistent with the intended vacuum level of the subsequent VDM tests. The following hole diameter tolerances were stated in the certificates of calibration: (5 µm ± 2 µm), (10 µm ± 3 µm), (20 µm ± 5 µm). Regarding the 5 µm position "B" group, the hole size of 3 drilled samples out of 10 was less than 3.5 µm.

Capillary Tubes

The second set of defective PFSs was built-up by inserting ceramic capillary tubes into plungers to contain external leakages (Table B). The capillary tubes length and diameter were sized and calibrated to measure the same flow rate as the laser-drilled holes groups in correspondence of a 980 mbar differential pressure across the flow path.

The position of the capillary tubes (Figure 3) was adjusted to get the leakages below (10 units – position "B") and above fill level (10 units – position "A"). PFSs with embedded capillary tubes were, in turn, filled with sterile water and drug product and used to establish their correlation with laser-drilled PFSs.

VDM (ASTM F2338)

Test Description

VDM is a non-destructive CCI testing practice applicable to most pharmaceu-

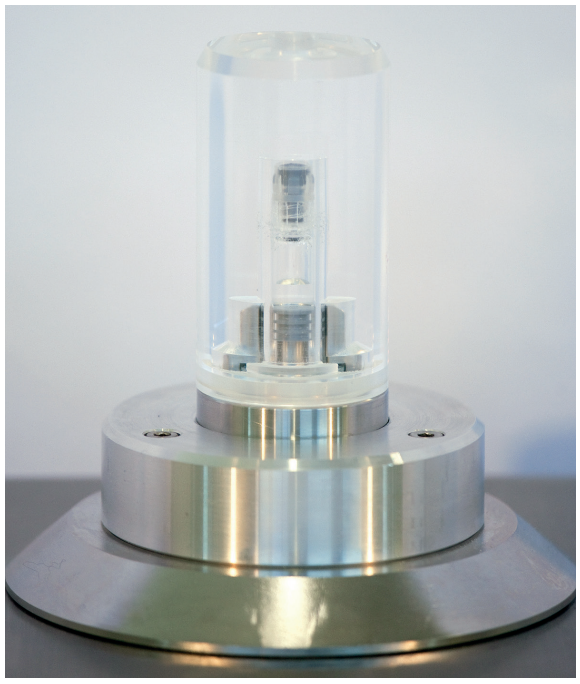

Figure 4 - Test Chamber of the LF-S equipment

tical packages. When dealing with PFSs, the CCI testing is performed while the PFS itself is held within an hermetically sealed test chamber (Figure 4). The principle underlying the VDM is that, as a consequence of the application of vacuum within the test chamber and hence of a differential pressure between the inside and the outside of the PFS, the air moves from high pressure zone (within the PFS) to low pressure zone (outside the PFS), causing a progressive pressure rise (that is a vacuum decay) outside the PFS. Vacuum decay can also result from the volatilization of liquid product that occludes the leak path. A vacuum decay greater than a given threshold at end of the testing phase, points out a PFS leakage. Once the test chamber is loaded with the PFS to be tested and hermetically closed, the VDM process comprises (Figure 5):

a) Vacuuming: test chamber evacuation period;

b) Stabilization: time needed to get a homogeneous vacuum distribution;

c) Testing: time frame in which the vacuum level is monitored by a dedicated transducer; two measurements are taken, at the beginning (1st reading) and at the end (2nd reading) of this phase.

The VDM decision-making is performed by means of comparing the vacuum decay "Δ" (Δ = 1st - 2nd reading) to a preset threshold THR:

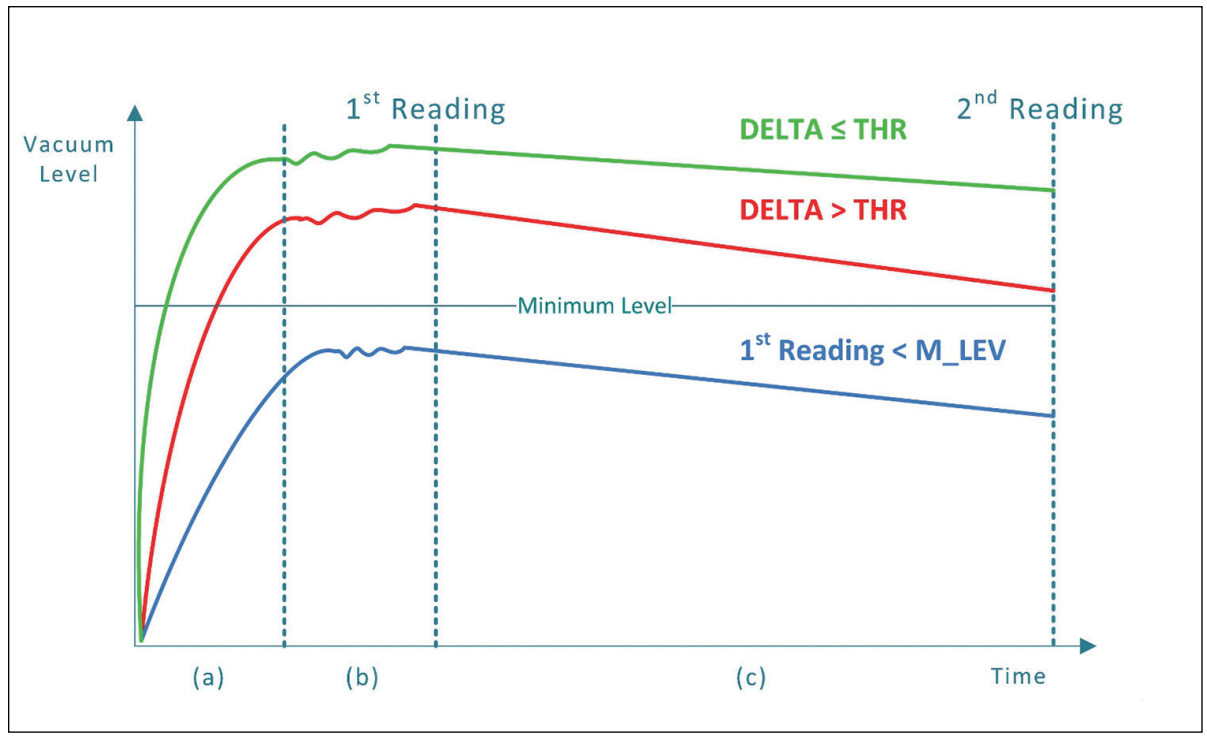

Figure 5 - Vacuum curves during VDM process execution
a) Green curve: PFS Conforming to closure integrity requirements (non leaking).
b) Red curve: Defective PFS (small-size closure integrity failure)
c) Blue curve: Defective PFS (large-size closure integrity failure)

• if Δ ≤ THR the PFS is classified as conforming (no detectable leakages);
• if Δ > THR the PFS is classified as defective (a micro leakage is detected).

Product Sterility Issues

The application of a differential pressure to a PFS, such as that of vacuuming phase, may cause the PFS plunger to move outwards and consequently to move back to the ordinary position once the testing phase has been completed and, therefore, the differential pressure has been removed.

This plunger movement, might induce entry of unwanted foreign matters and cause drug product contamination. PFSs, as every other parenteral package, must ensure drug product stability and sterility throughout the entire shelf life, hence any potential plunger movement is to be avoided.

Equipment Overview

The LF-S equipment employed is of the laboratory type to test PFSs by using the VDM (Figure 6). One test chamber is installed on the equipment frame and is made up of:

Figure 6 - LF-S equipment

• a fixed bottom part, connected with pneumatic actuators and vacuum transducer;
• a removable top part, needed to plug up the testing chamber before testing cycle start.

Test chamber fixed bottom part is equipped with a mobile piston "Plunger Stopping Device" (PSD) having vertical motion between two limit positions, respectively "High" and "Low" and having a section equal to that of the PFS plunger.

The test chamber removable top part used here is made of polycarbonate plastic providing full visibility of the PFS plunger dynamics during testing cycle. The vacuum generated in the test chamber during phase a) (Figure 5), produces an upward movement of the PSD.

Table C - Recipe parameters – LF-S equipment

Parameter ID	Value	Unit
Vacuuming	0,50	sec
Stabilization / 1st Reading	0,25	sec
2nd Reading	5,0	sec
THR	6,82	mbar
M_LEV	986,50	mbar

Table D - VDM (ASTM F2338) – Test results

Test Sample Kits	Hole diameter (µm)	DR (%)	FNR (%)	FPR (%)
Positive Controls	5 µm	100,00%	0,00%	-
	10 µm	100,00%	0,00%	-
	20 µm	100,00%	0,00%	-
Negative Controls	-	100,00%	-	0,00%

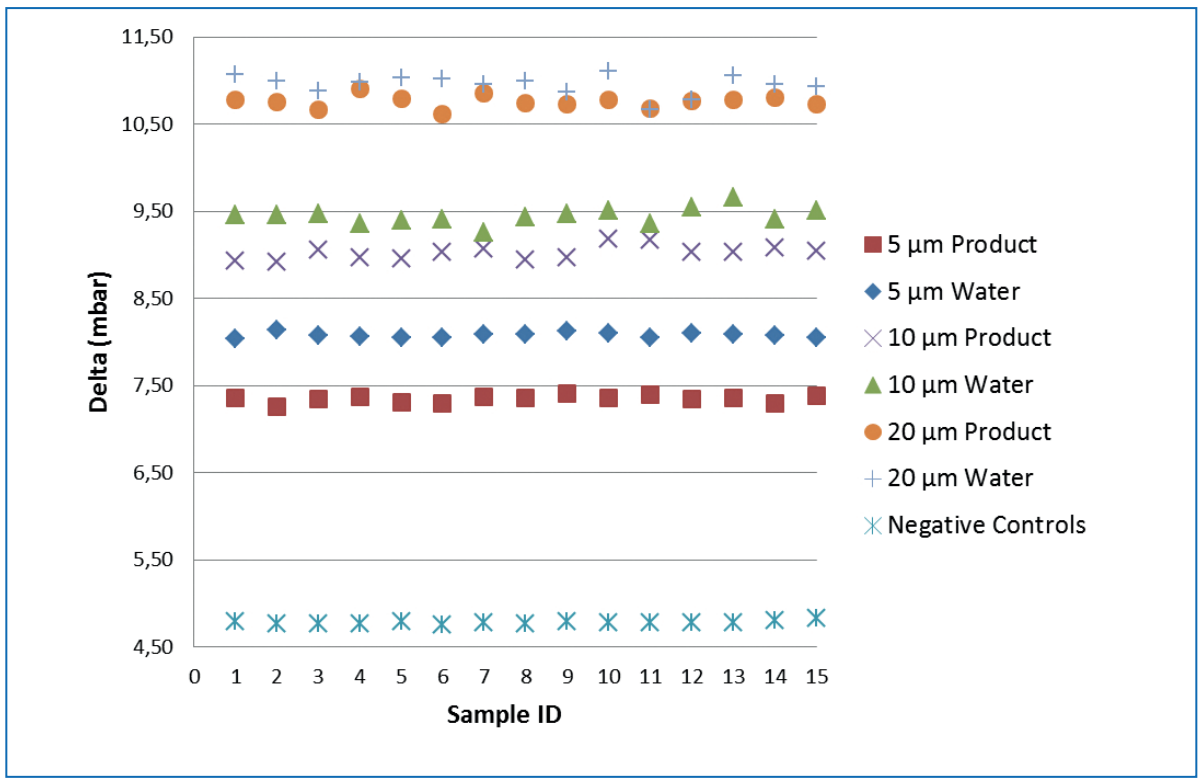

Figure 7 - Negative Controls vs. Positive Controls (Position B): VDM Test results for Negative Controls and Positive Controls (5/10/20 µm laser-drilled holes exposed to sterile water "Water" and to drug product "Product"). For each individual replication, on x-axis, the average vacuum decay values "Delta" are plotted on y-axis. Average Delta values are calculated on the 10 element sample as shown in Table A

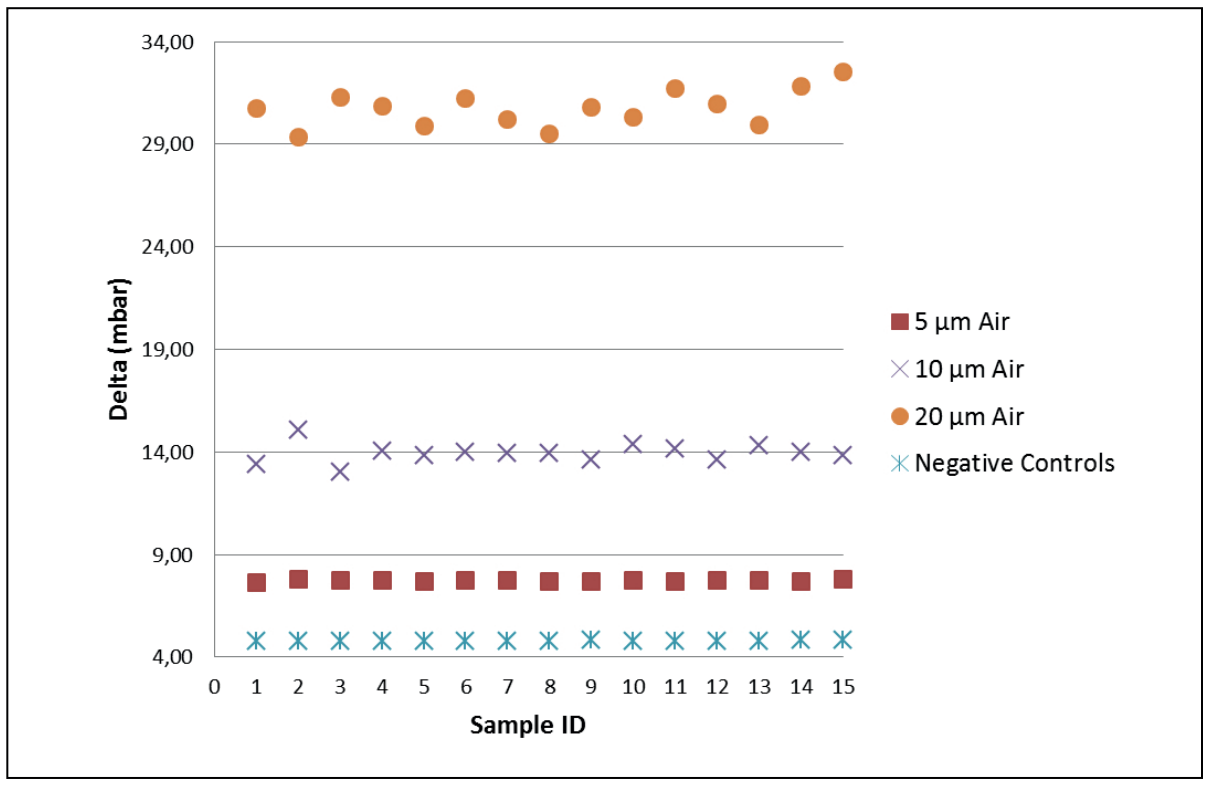

Figure 8 - Negative Controls vs. Positive Controls (Position A): VDM Test results for Negative Controls and Positive Controls (5/10/20 µm laser-drilled holes exposed to air "Air"). In this investigation Positive Controls are filled with drug product.

Once the PSD comes in contact with the PFS plunger ("High" position), this exerts a force equal in magnitude and opposite in direction to that of the plunger itself. The same "High" position is kept until the 2nd reading; at that time the action of a dedicated pneumatic actuator provides for exhaust of vacuum in the test chamber bottom area and produces the PSD downward movement towards the "Low" position. Therefore, the original position of the plunger within the PFS is maintained during the entire VDM testing cycle. Importantly, this system demonstrated that the execution of CCI testing based on VDM does not cause any negative impact on the product sterility and safety.

Case Study Execution

Recipe parameters used are shown in Table C. Test samples were subject to VDM

Figure 10 - Complete set of laser-drilled Pre-filled Syringes used for the case study

15 times. 1st reading and Δ resulting raw data were gathered, mean and standard deviation values were calculated. The Kolmogorov-Smirnov test confirmed the normality of raw data, allowing for process sigma level calculation. Resulting data were then organized to document (Table D) the percentage of:

- Positive Controls, which were indeed found positive by VDM (DR_{PC});
- Negative Controls, which were indeed found negative by VDM (DR_{NC}).

FNR and FPR values are reported as well. As shown in Figure 7, including the mean of the multiple measurements, the detection of drug product filled Positive Controls resulted in lower vacuum decay values than those referred to sterile water, by virtue of slight reduction in the vaporization capability. Note that, by focusing on the worst case scenario (drug product), the detection of Positive Controls was found significant at the 7.2 sigma level, therefore demonstrating negligible false-results probability. Regarding the Positive Controls with the holes in Position A, a growing spread of the groups is observed (Figure 8); this confirmed that laser-drilled holes exposed to air led to higher vacuum decay values, which was foreseeable due to the small density of air as compared to drug product / sterile water.

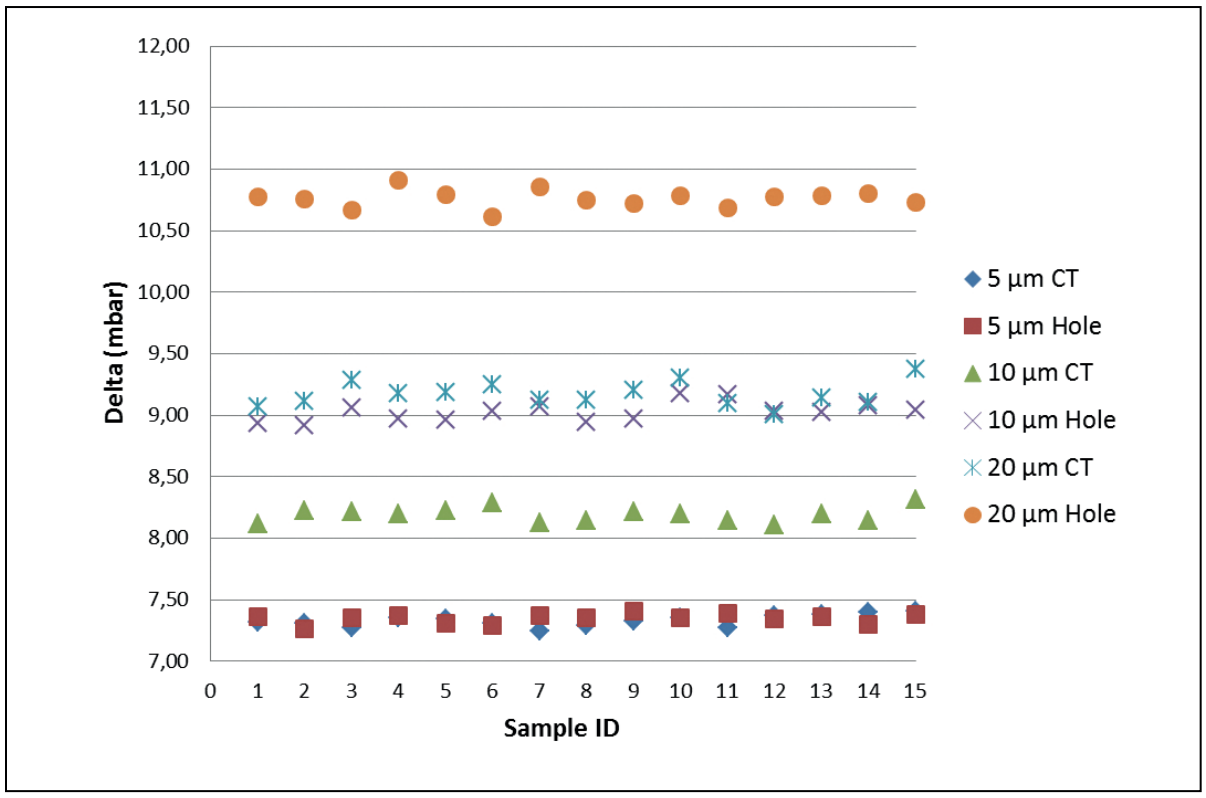

Figure 9 - VDM test results for Positive Controls and correlation between:
a) 5/10/20 µm laser-drilled holes ("Hole") exposed to drug product
b) 5/10/20 µm capillary tubes ("CT") in Position B.

Laser-drilled Holes versus Capillary Tubes

An excellent correlation (difference in vacuum decay values below 0,2%) was found between laser-drilled holes and capillary tubes in the 5 µm group Figure 9), whether the defect was exposed to sterile water and to drug product (position "B") or to air (position "A"). This all points out how the two different methodologies developed for Positive Controls implementation produced parallel results, even though the leakage took place in two channels having length and diameter different from each other (Table A and Table B).

Conclusion

This study provides important contributions to foster a better understanding in the adoption of a non-destructive CCI testing method for ensuring the integrity of PFSs. The investigation was carried out on suitable sets both of conforming and known defective glass syringes (Figura 10) filled with a vaccine and sterile water. The main purpose was to focus specifically on gathering evidence of known defective samples detection capability and of false-results rate. Results in Table D, highlighted certain advantages of VDM which proved its effectiveness in combination with an innovative system preventing PFS plunger movement during the CCI testing phase.