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Container closure integrity testing with High Voltage Leak Detection

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Abstract

Container closure integrity testing with High Voltage Leak Detection

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There are various methods that can be used for Container Closure Integrity Testing(CCIT). Each method has its pros and cons. The choice of the CCIT-method depends on various factors such as the material of the primary packaging and product properties.

High Voltage Leak Detection(HVLD) is an effective method. The sensitivity of the method is quite high but its is unclear whether it can detect all the hole sizes that may entails a risk for microbial contamination.

The theoretical calculations and practical experiments shows that HVLD can detect holes in different positions and it can detect holes that are a few micrometers. If HVLD detect hole size that are 5 micrometers, it means that alla sizes that can make risk for microbial contamination for products manufactured on AstraZeneca PET BFS can be detected with HVLD.

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Identifering av detektionsförmåga för High Voltage Leak detection

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Populärvetenskaplig sammanfattning

Container closure integrity testing (CCIT) är en viktig process inom tillverkningen av sterila läkemedel. Processen kontrollerar produktens integritet för att garantera steriliteten under hela hållbarhetstiden. Inom produktionen av läkemedel finns det alltid en risk för mikrobiell kontaminering. Ett läckage i produktens primärförpackning kan medföra en risk för mikrobiell inträngning, därför testas alla produkter innan de levereras.

Det finns olika metoder som används för CCIT. Alla metoder har sina för- och nackdelar och det finns ingen enskild metod som är tillämpbar för alla olika typer av produkter. Efter valet av metod valideras den för verifiering av dess prestanda såsom detektionsförmågan. För att verifiera detektionsförmågan används positiv kontroll. Det är en kontroll som görs genom att skapa artificiella hål i produkterna och testa de med den valda CCIT metod. Det finns även olika metoder för att skapa artificiella läckage i produkter. Valet av metoder beror på produktens egenskaper.

AstraZeneca PET BFS (Blow-Fill-Seal) är en produktionsenhet som tillverkar olika läkemedel såsom inhalationsampuller och lokalbedövningsmedel. Integriteten för alla produkter som tillverkas testas med CCIT-metoden High Voltage Leak Detection (HVLD). Metoden anses kunna detektera läckage ner till motsvarande några mikrometer stora hål, men metodens detektionsförmåga behöver undersökas mer ingående för att kunna garantera att den kan detektera alla läckage som potentiellt utgör en risk för mikrobiell inträngning.

AstraZeneca PET BFS har därför initierat projektet ”Optimering av integritetstestprocessen vid tillverkning av läkemedel” för att mer ingående undersöka HVLD-metodens kapabilitet. Målsättningen är att kunna visa att befintlig utrustning för läckagestest kan detektera alla läckage som potentiellt kan medföra en risk för mikrobiell kontaminering.

Litteratursökningen, alla tester och matematiska modellen som gjordes påvisar att HVLD är det mest lämplig metod i jämförelse med andra CCIT-metoder för produkter som tillverkas på AstraZeneca PET BFS. Försöken som gjordes visar att känsligheten för metoden är relativt hög men den visar endast resultat i form av ”fail” för en läckande ampull och ”pass” för en godkänd ampull. HVLD visar inte detaljerade resultat såsom vart hålet sitter eller vad hålet har för storlek. Med hjälp av en specialdesignad matematisk modell för flödeshastigheten kan gränsen för den minsta hålstorleken som ger en risk för mikrobiell penetration bestämmas. En serie av olika laserborrade hålstorlekar ska testas med HVLD för att ytterligare undersöka dess detektionsförmåga.

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List of Abbreviations

BFS Blow- Fill-Seal

CCI Container closure integrity

CCIT Container closure integrity testing

CCS Container closure system

FDA Food and Drug Administration

HVLD High voltage leak detection

PE Polyethylene

PET Process Execution Team

PLC Programmable Logic Controller

PP Polypropylene

1 Introduction

To make the drugs meet safety criteria, manufacturers must follow FDA guidelines for sterility of pharmaceutical products. Pharmaceutical companies have the responsibility to follow the requirements for pharmaceutical product sterility. The FDA issued a guideline in 2008 requiring the CCIT sterility test (Meissner and Gokhberg, 2018). CCI has always been of interest in the pharmaceutical and biotechnology industry to control and maintain the sterility of sterile products (Pti, 2018). The criteria for sterility mean that the maximum permissible leakage is 10^{-6} cm^3/s for very tight system, for water tight should be 10^{-2} cm^3/s and for bacteria tight should be 10^{-4} cm^3/s (Zapfe, 2016; Rottländer, Umrath and Voss, 2016).

There is always a limit for the maximum allowable size of leakage. Within this limit, there is no risk for microorganisms and other substances to move inside the product and cause contamination that ultimately affects product quality. Some studies explored the risks of packaging leakage with respect to microbial ingress. One of these studies shows that a hole with a few microns in diameter gives a risk of liquid passage and risk of microbial ingress (U.S. PHARMACOPIA, 2016). By selecting an appropriate interval such as the maximum allowable leakage limit, the risk of liquid leakage and microbial ingress will be minimized and ensures the validation of packaging integrity.

AstraZeneca is a global manufacturer of pharmaceuticals, including sterile products for inhalation and injection filled in plastic ampoules. Each manufactured product should be inspected and validated for its integrity to ensure that only products with verified integrity are delivered to customers. In this way, AstraZeneca can guarantee the sterility of its products throughout their shelf life.

AstraZeneca PET BFS tests all its products with respect to the primary packaging integrity with high-voltage leak detection (HVLD) technology. HVLD is a technology that is mainly used in the pharmaceutical industry for testing the integrity of containers. It works by applying a high voltage to the products and then measuring the electrical conductivity. In the presence of leakage, HVLD registers higher conductivity, i.e., higher voltage.

The project "Optimization of the integrity test process in the manufacture of drugs" was created to optimize the process for CCIT in AstraZeneca. The project concerns the evaluation and optimization of the current method (HVLD) to investigate whether it can achieve the highest requirements or how close to the requirement HVLD can detect (Flow rate for very tight system should be 10^{-6} cm^3/s, for water tight should be 10^{-2} cm^3/s and for bacteria tight should be 10^{-4} cm^3/s) to ensure that the product's sterility is maintained throughout its shelf life.

2 Background

2.1 CCIT and CCI

Container closure integrity (CCI) ensures the integrity of the product by preventing microorganisms from invading the sterile product packaging until use. Sterile products should not contain contaminants caused by microorganisms, gases, or other substances during the entire shelf life (Mahler *et al.*, 2017). CCI is also the ability of a container to protect the sterility of the product throughout its shelf life. CCS refers to the sum of the components present in the system and the protection that the system provides to the doses in this system.

Products are packed in various closed systems (CCS) to protect the product from contamination caused by e.g., microbial ingress (Mahler *et al.*, 2017). To check the safety of the product, the safety of the container should be checked by doing CCIT. This test is designed to detect the flow into and out of the container through a "leakage path". CCIT is usually very dependent on the leakage tester, i.e., the method of detecting holes/defects in containers. Some leak tests may also measure the size of the leak. The leakage test measures the leakage rate from the inside of the container to the leakage path. Thereafter, the measured leakage rate is usually converted to a flow rate. To facilitate expression, the flow rate is converted to the size of the hole. The sensitivity of a method can be expressed as the smallest hole size that can be detected with the method. The smaller hole a method can detect, the higher the potential sensitivity of the method.

The CCIT as mentioned earlier is important to perform during the manufacturing process to ensure the integrity of the products. This could be done by a visual inspection system that could be applied only to detectable hole sizes, larger defects like cracks. The smaller leaks cannot be detected visually, they can be detected by leak detection systems.

CCIT methods are used for inspecting product quality, therefore it is very important to choose a suitable leak tester. Not all leakage test methods are applicable to all types of product packaging systems. The choice of leakage test method depends on the material, design and contents of the container, the manufacturing process, and the configuration. These factors also include method type (destructive or non-destructive), product content (Small molecule or large molecules) and container material (Glass or plastic) (Chen, 2018).

CCIT methods can be divided into deterministic and probabilistic methods. A deterministic method does not involve elements of randomness and thus provide objective quantitative data which is invariable provided original conditions are maintained. A probabilistic method assumes some randomness and therefore may give random results depending on the case. The results are therefore assumed to be very uncertain and require a large quantity of samples to make the output results useful. Leakage test methods can also be classified as destructive or non-destructive. This affects their application and the choice of the CCIT-method (Mahler, Pelaez and Herdliteschka, 2019). It is desirable to use non-destructive methods because they are not going to destroy the product which means that the samples can be preserved. Destructive methods lead to potential loss of products, which means that it prevents other tests from being performed on the same samples. In some cases, destructive methods are the only option due to the limitations and requirements of the product (Mahler et al., 2017).

Although the non-destructive methods are made to be better than the destructive ones, there are pros and cons to each CCIT- method.

2.2 CCIT in AstraZeneca PET BFS

All products manufactured at AstraZeneca PET BFS should be tested for primary packaging integrity. The ampoules can be of the plastic-type PE or PP and are available in different formats (Figure 1). The ampoules are filled with a product which can be either a solution or suspension. The solution is conductive because it contains 0.9 % saline, corresponding to a conductivity of 16 mS/cm.

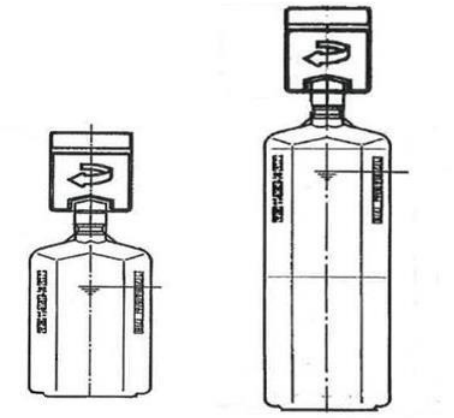


Figure 1 A: PP ampules filled with 10 ml and 20 ml product.

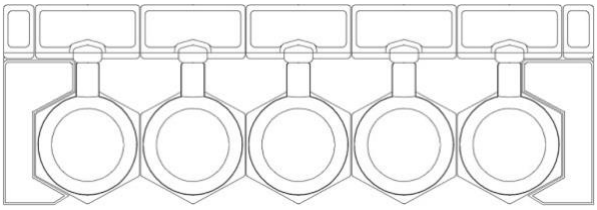


Figure 1 B: Block of PE filled with 2.5 ml in every ampule.

HVLD method is then used to check product integrity. Leak tests with the HVLD method are performed by generating a high voltage and placing it directly over each individual ampoule. The current flowing through the ampoule is converted to voltage that is displayed digitally as a measured signal. If the measured signal for an individual ampoule becomes lower than the set threshold value for an approved product, it means that the ampoule is empty. If the measurement signal becomes higher instead, this means that the container is defective. The PLC that controls the sorting will then decide if the product is empty (<1 V), defective (>4 V), or good (1-4 V) and then reject the empty and defective products. Limits differ depending on the machine used.

3 Project aims

One of the aims of the present project is to recommend a suitable reference method "Container Closure Integrity Testing "for sterile drugs in plastic ampoules by performing

studies, theoretical calculations, and practical experiments. The project is also about investigating whether HVLD meets the requirements (Flow rate for very tight system should be 10^{-6} cm^3/s, for water tight should be 10^{-2} cm^3/s and for bacteria tight should be 10^{-4} cm^3/s) by investigating whether they can detect hole sizes up to the maximum permitted hole size corresponding to the flow rate for the water tight, microbial tight and very tight system that entail a risk for the microbial intrusion. The project also discusses the existing methods for detecting holes in ampoules and the suitability of these methods for products manufactured in AstraZeneca PET BFS. How effective is the HVLD method for detecting holes that are 1µm (Zebrasci) and what methods are available to investigate this? The project is divided into different parts to discuss these issues in order.

4 Method for proposing an appropriate CCIT

There are different methods for CCIT to be able to suggest which methods are most suitable. These methods include High voltage leak detection (HVLD), Vacuum/pressure decay, Laser Headspace Analysis, Helium Leak Test, Dye ingress and Microbial ingress. A comparison between these methods is required with the help of literature search. The literature search was based on published data concerning different methods.

5 CCIT methods

5.1 Non-destructive and deterministic methods

5.1.1 High voltage leak detection (HVLD)

The principle of the method is based on conducting electricity through materials with different electrical conductivity. This requires that the container be made of electrical insulation materials such as rubber, glass, or plastic. The solution in the container should contain conductive substances (Chen, 2018).

A high voltage is applied in a specific area on the container to pass through the inspection electrode. When a leak exists in a container, the flow (liquid) will move through the hole. The current that passes through the container and the conductive solution will then be collected at the detection electrode (Figure 2) (Chen, 2018).

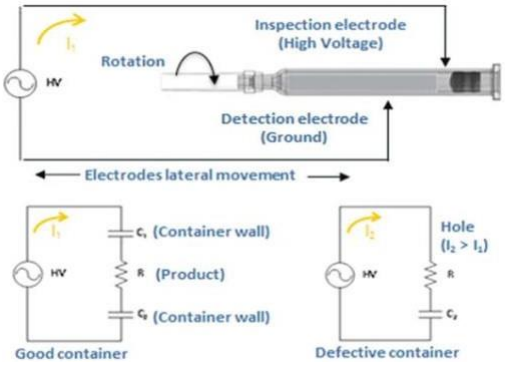


Figure 2: the principle of HVLD for electrical conductivity test (Chen, 2018)

The container that has a leakage loses resistance in the leakage area, which means that more current can accumulate on the detection electrode. It is important that the inspection electrode is placed close to the defect, therefore it is very important to study which area in the ampule is riskier for leakage (Chen,2018).

The studies show that high voltage used in the detection of leakage in the container have no proven effect on the effectiveness of the drug, which makes the method is non-destructive. Another study shows that the high pressure can cause the oxygen in the containers “head space” to be converted to ozone, which can affect the product (Chen,2018).

The HVLD method gives fast results and it is possible to design machines regarding the products' properties. In addition, the test parameters of the machine are very flexible and easy to adjust, which means that different products and types of containers (non-conductive) can be tested on the same equipment. HVLD is therefore usually used for continuous 100% online monitoring during manufacturing (Mahler *et al.*, 2017). On the other hand, HVLD applied only for liquid drugs because they are conducting current. The detection capability for HVLD will not be affected if the product contains large molecules as long the leak path is moist. The product should have a conductivity of >1 μS / cm to get this method to work (Meissner and Gokhberg, 2018). This would be requirement important to consider for the selection of the leak tester.

HVLD as mentioned earlier, is a local leak detection method, therefore the sensitivity of the method must be verified at different positions in the container. If this method is not placed in the right place and validated, it will not be able to detect all defects regardless of the position. The HVLD machines have a certain detection ability. The sensitivity of HVLD can be determined depending on which hole size it can detect. Smaller hole sizes (Measured in micrometers) show better sensitivity. For most HVLD machines, an empty product shows a voltage of about 0-1 V, for a good product it shows a voltage between 1-4 V. For a bad product (defective) the voltage should be higher than 4 V (Densok). It varies depending on the machine and only applies to products that have conductivity of > 1 μS / cm. PLC sorts out ampoules with respect to setting data for empty, leaking, or good ampoules that vary from one machine to another. The method is sensitive because it can detect small holes down to 1 μm (Zebrasci).

5.1.2 Vacuum/pressure decay

The method utilizes the change in pressure for leak detection. It is a non-destructive test used for both liquid and solid products as well as conductive or non-conductive containers. The system works by applying a vacuum in the test chamber which contains the product and then using a valve to isolate the test chamber from the vacuum source. Then the valve closes, and the pressure difference sensor measure the vacuum change in the test chamber. The degree of vacuum attenuation is related to the leakage rate, which determines whether there is a leak in the test sample (Mahler *et al.*, 2017).

The running time is usually short depending on the sensitivity and the amount of main space in the container. The accuracy of the measurement depends on the residence time, which means that there are limitations regarding the speed of the equipment that can affect the accuracy. The vacuum or pressure level in the chamber must also be determined to be able to measure the pressure change.

The method can detect holes in all positions in the container and the sensitivity of this method is ≥ 5 μm (Chen,2018). Vacuum/pressure decay is simple and reliable for positive control and is stimulated with a microflow meter to show the sensitivity. Another advantage of this

method is that it is non-destructive which makes it a candidate for the choice of CCIT method. Vacuum decay tests cannot be applied for products that contain large molecules because the leakage can be blocked by those molecules and no detection will take place. Due to the high-pressure differential that is applied to the samples, the method is assumed to be very sensitive for the detection of leaks that are ≥ 5 µm. In some cases, the high pressure moves on the large molecules in the solution against the leak and block it (Chen,2018).

A study with the vacuum decay method was done on fifty filling syringes with different hole sizes between 3.5-7 µm. The samples were tested every day for 10 days. A good product should show a differential pressure that is less than 15 pascals. Differential pressure for a product with leakage should be higher than 15 pascals. The results show that more than 97% of the tested samples had a value that corresponded to or slightly less than the standard (15 pascal) from the responses to the samples without leakage, which gives failed results (figure 3) (Chen,2018).

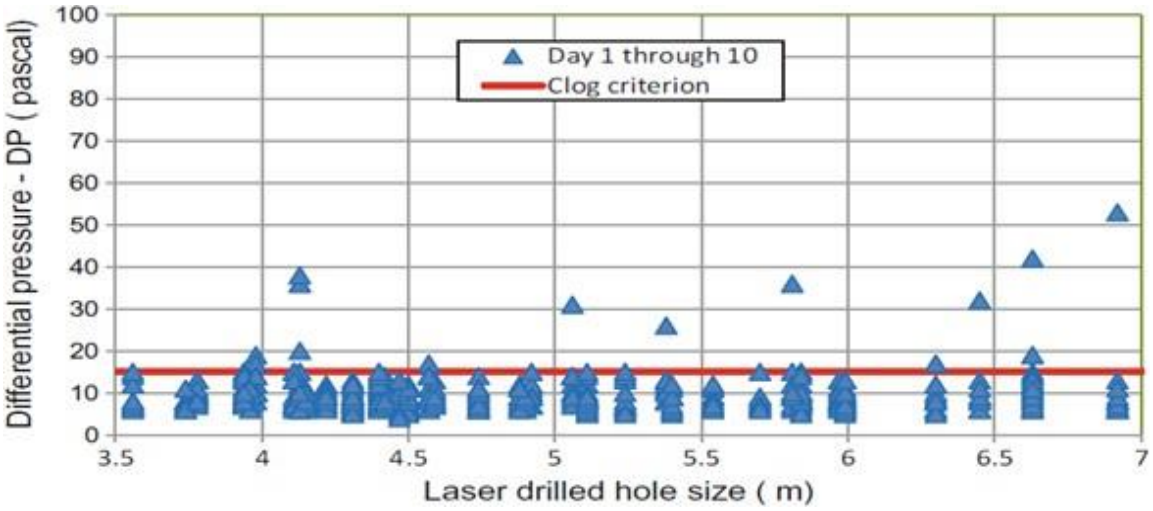


Figure 3: Laser- drilled products tested in vacuum decay (Chen,2018).

5.1.3 Laser Headspace Analysis

This method uses the low energy laser absorption spectroscopy technique to measure the gas space of the container for detection of the leakage. The frequency is set up with respect to the absorption frequency of the gas molecules to be detected. The laser passes through the container's "twist-off-breakpoint" and then the absorption is measured with a spectrometer. Packaging CCI is determined by comparing the concentration of the gas space in the leaking and non-leaking container. The technology has proven to be suitable for CCI testing of lyophilized drugs that are packaged under vacuum to minimize the moisture content of the container (Chen, 2018).

The method is non-destructive because the laser passes through the “twist-off-breakpoint” without affecting the contents of the container. The method is also fast, which makes it efficient to use for 100% online leak detection. On the other hand, it has shown limitations for leak detecting in the containers that are packaged in atmospheric pressure, this will not give

measurable changes in the concentration of oxygen, moisture, or pressure (Mahler *et al.*, 2017).
Another reason that limits the application of this method is the availability of "headspace", which risks that the laser passes through the liquid phase and gives false results. Liquid products can produce water droplets on the wall of the container that can interfere with the laser light (Chen, 2018). Depending on the volume of vacuum in the “headspace”, a longer waiting time may be required to achieve the required accuracy. The method has a sensitivity equivalent to 20 μm (Levac, Ramsey and Salsbury, 2019).

5.1.4 Helium leak test

The method is assumed to be one of the best methods with respect to sensitivity to detect leaks in all types of containers and even the leaks that are 2 μm. The method consists of a helium mass spectrometer, vacuum or pressure system, test chamber, and helium filling equipment (Mahler *et al.*, 2017). The container that has a part to be tested is placed in the chamber containing helium. Helium is used as a tracer gas to detect leakage and the helium mass spectrometer can detect the helium that is leaking from the leak and the rate of the leak (Chen, 2018).

Helium is non-toxic, non-reactive, and inexpensive. These properties make it possible for helium to pass through the smallest leaks without affecting the container and can later be measured with a mass spectrometer. However, helium permeability is believed to have effects on leakage tests therefore these effects were examined on plastic ampoules at two different temperatures (Chen, 2018).

Results have shown that the same container passed the leak test at -70 ° C and failed with the same pass / fail criteria at room temperature (Figure 4). This is because helium has a higher permeability in plastic materials at higher temperatures. This leads to the characterization of helium as an unusable tracer gas because products are usually not packed with helium and the filling step with helium makes it a destructive test. Another disadvantage with this method is that the test is carried out on non-product-filled containers (Chen, 2018).

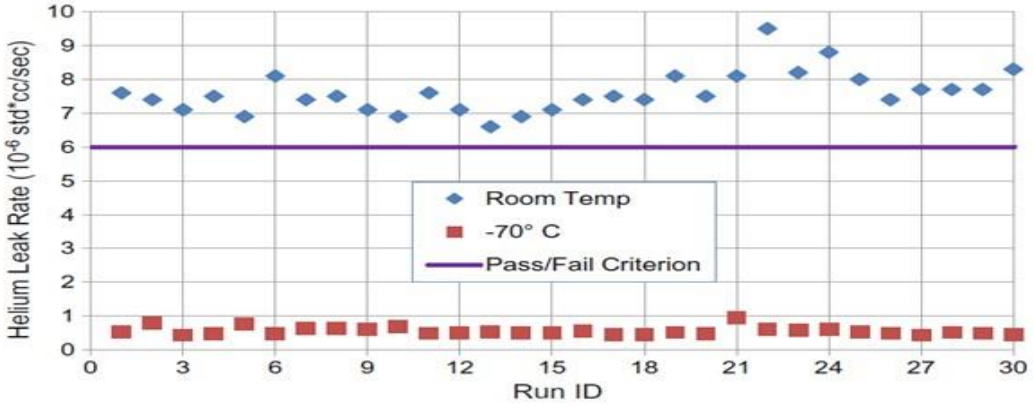


Figure 4: helium leak rates at -70C and rooms temperature in plastic container (Chen,2018).

5.2 Destructive and probabilistic methods

5.2.1 Dye ingress

Is the most common leak test method used in the pharmaceutical industry to ensure the integrity of the container. The container is immersed in a bath with coloured solution (Usually methylene blue) and then subjected to vacuum or pressure differences. Next, technicians check to see if the blue dye penetrates the package. This test is only performed on certain ampoules, i.e., not all ampoules to be delivered can be tested with this method. The sensitivity of the method is around 20 µm (Ewan *et al.,* 2018) and it depends on the difference between the vacuum and the atmospheric pressure. It is also dependent on the inspector's ability to detect dyes in the container, which means that there is a risk of human error (Mahler *et al.*, 2017).

5.2.2 Microbial ingress

The method is like dye ingress testing, but the samples immersed in the bacterial bath instead of stained solution. The samples should be filled with a nutrient-rich medium such as broth because bacteria thrive and grow in such an environment. The bacterial suspension must contain small and motile bacteria to do a worst-case scenario test (Test for most difficult circumstances). The samples are then incubated and checked visually by a technician or microbiologist to see if there is growth of microorganisms. The sensitivity of this method is around 0.3-20 µm (Mahler *et al.*, 2017). The method is stochastic and uncertain; therefore, it is not recommended as a CCIT method but only as a control for the sterility of the process (Aliaskarisohi *et al.*, 2019).

5.3 HVLD vs Vacuum decay

Some studies were done to test the sensitivity to HVLD and vacuum decay. For the first study, 1 ml and 2.25 ml glass ampoules filled with water for injection (3 samples) or albumin (5 samples) were used (Figure 5) (Pti, 2018).

The other study, 10 ampoules with three different hole sizes 15, 25, and 50 µm which are filled with product or placebo (Figure 6) (Guazzo, 2020).

Experiments were also performed to investigate the advantages of the HVLD method compared to the vacuum decay test method for CCIT. First, the vacuum decay method is used followed by HVLD method to test 20 syringes that contain biological drugs, having hole sizes between 5 and -100 microns (Figures 7 and 8). These results will be discussed in section 7.

Identified Positive Controls			#samples	VeriPac VP-455 Vacuum Decay Found positive		E-Scan 655 HVLD^{mc} Found positive	
5 µm	1 ml	Water	3	0	0 %	3	100%
		Albumin	5	0	0 %	5	100%
	2.25 ml	Water	3	0	0 %	3	100 %
		Albumin	5	0	0 %	4	80 %
10 µm	1 ml	Water	3	3	100 %	3	100%
		Albumin	5	0	0 %	5	100%
	2.25 ml	Water	3	0	0 %	3	100 %
		Albumin	5	0	0 %	5	100 %
20 µm	1 ml	Water	3	3	100 %	3	100%
		Albumin	5	0	0 %	5	100%
	2.25 ml	Water	3	3	100 %	3	100 %
		Albumin	5	0	0 %	5	100 %

Figure 5: Tests for ampoules with HVLD and Vacuum decay CCIT (Pti,2018).

Vial hole size (µ)	Packages tested (#)	# Packages ID'd as LEAKING DAY 1		# Packages ID'd as LEAKING DAY 29	
		Vacuum decay	HVLD	Vacuum decay	HVLD
PRODUCT-FILLED					
15	10	8	10	2	10
25	10	9	10	2	10
50	10	10	10	3	10
PLACEBO-FILLED					
15	10	10	10	10	10
25	10	10	10	10	10
50	10	10	10	10	10

Figure 6: Tests for product filled ampoules with HVLD and Vacuum decay CCIT (Gusazzo 2020).

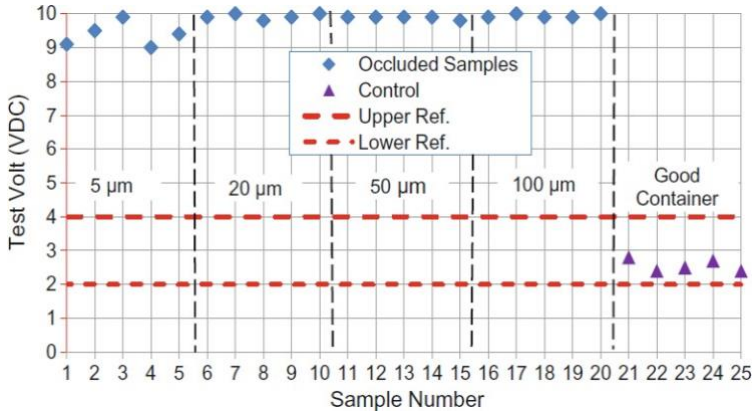


Figure 7: Tests for ampules with laser drilled hole by HVLD CCIT (Chen ,2018).

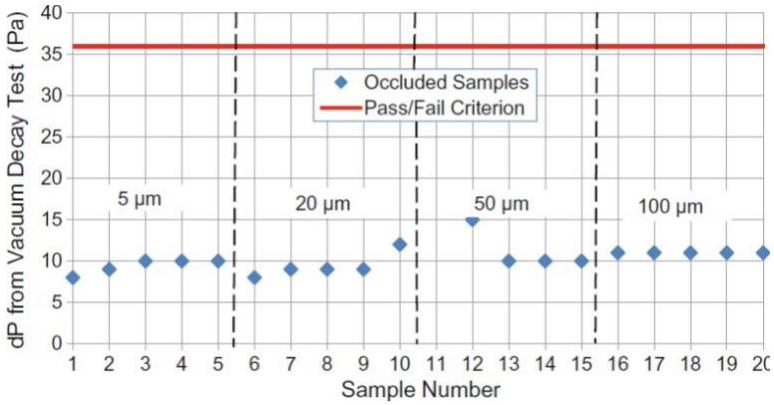


Figure 8: Tests for ampules with laser drilled hole by Vacuum decay CCIT (Chen ,2018).

6 Side-by-side comparison CCIT methods

Table 1: Comparison between different CCIT methods (Mahler et al., 2017).

Technique / method	**Principle of the measurement**	**Advantages**	**Disadvantages**	**Detection capability**
High voltage leak detection (HVLD)	The method is deterministic. It is based on conductivity measurement. In the presence of leakage, test resistance will decrease, which gives a higher signal (voltage) and this signal is compared with a predetermined limit for pass / fail.	● Non-destructive method. ● Fast method. ● Performed under normal atmospheric pressure. ● Accuracy depends only on the method, no human error. ● No special sample preparations are required. ● Useful for 100% online testing so that it is performed on site. ● Sensitive. ● Low risk of impact or contamination on the samples.	● Conductivity should be higher for samples than containers. ● Pass / fail result no detailed result. ● Ozone production ● For liquid products not the freeze-dried ones. ● Risk of product damage (protein degradation). ● It is difficult to detect holes after prolonged storage of the samples, as the leakage path can be blocked by the liquid residues. ● It becomes difficult to detect holes in the main area of the ampoules with HVLD due to. lack of product liquid contact (empty area)	>0.5 µm
Laser-based headspace	The method is deterministic. Controls "headspace" by laser-based analysis technology. Without destroying the sample, oxygen, nitrogen, carbon dioxide, water vapor or internal pressure in the main compartment can be measured.	● Non-destructive ● Can be used for 100% online testing. ● Fast method ● Quantitative, it is certainly not likely.	● Special container (transparent) ● The atomic colour of headspace needs to be adjusted. ● Time consuming for smaller leak sizes, it can take several weeks. ● Different container sizes require different spare parts. ● Due to the rapid balance of ambient air, large leaks cannot be detected. ● There is a risk of false results due to gas permeation. ● Is not accurate which makes it difficult to distinguish with the hole sizes. ● Works best on freeze-dried products.	>20 µm

			● It is very difficult to detect leaks in the liquid solution area, which makes the method unusable for liquid products.	
Helium leak test	The method is deterministic. The samples filled with helium are placed in the test chamber and the internal pumping instruments generate vacuum. Luminaires may be required to insulate specific areas of containers. Samples with leaks allow helium to escape and enter the test system and then be detected by the analyser unit. The helium ion current reaching the analyser is proportional to the partial pressure inside the sample.	● quantitative. ● Fast method ● Leakage rate can be calculated ● Accurate and repeatable results. ● Very sensitive method for detecting small hole sizes using a mass spectrometer.	● The method is destructive because it cannot be used on complete samples unless in an artificial helium atom. ● Low capacity ● Offline use, which is not recommended, then all samples must be tested. ● There is a risk that the leakage path is blocked by the product in the container, which gives incorrect results. ● It works best on freeze-dried products but not as well on the liquid products	>2 µm
Pressure decay	The method is deterministic. The container is placed in a specially designed test chamber that is subjected to overpressure. Indication of pressure passage means a leak.	● The method is non-destructive. ● Effective for online testing.	● It only shows pass / fail results, no detailed information. ● The product's 'clogging' leads to incorrect results. ● Low sensitivity compared to vacuum decay.	˷ 10 µm
Vacuum decay	The method is deterministic. The container must be placed in a specially designed test chamber which is then subjected to vacuum. The pressure change can be monitored using a pressure sensor. Increase in pressure is a sign of leakage.	● It is a non-destructive method. ● Can be used online. ● High speed (fast) ● Can be used on liquid and freeze-dried samples. ● Can be used on coloured CCS.	● The product 'clogging' can lead to incorrect results. ● The preparation of vacuum chambers is critical, and the humidity can affect the measurement. ● Less sensitive than HVLD, some hole sizes cannot be detected with Vacuum decay.	>5 µm
Dye ingress	The method is probabilistic. The container is placed in a water bath with dye that is usually blue and surfactants. These are then placed in a test chamber where a certain degree of vacuum is applied to the container. This method works by extracting air from the packaging cavity. The vacuum is then released from the test chamber. If the container leaks, then the paint will be pressed in. Then the operator will check if the packaging has any colour.	● Basic and efficient ● Flexible and can be used for several different CCS on the same run.	● The method is destructive ● Pass / fail results not detailed. ● 100% testing is impossible. ● The samples to be tested must be transparent for visual assessment. ● In samples with larger volumes, it becomes difficult to detect the colour. ● Detection is likely. ● It is suitable for liquid products but not lyophilized.	>20 µm
Microbial ingress	The method is probabilistic. The samples are filled with a sterile nutrient medium and on the outside of the container there must be an actively growing mobile microorganism to be able to then assess the sterility. After a certain storage period, all samples that are considered to have microorganisms (cloudy colour) are classified as leaking.	● Very well-known method ● Easily integrated into the medium filling ● The samples can be assessed directly.	● The method is destructive ● Pass / fail result, no detailed result. ● 100% testing is not possible. ● May take a long time until the samples are intubated. ● Only containers filled with media. ● Testing is due to human error, i.e., it is related to operator technology and knowledge. ● High probability when detecting small hole	>20 µm

Each CCIT method has its advantages and disadvantages. According to the literature, the pharmaceutical industry does not have a preferred CCIT technique (Meissner and Gokhberg, 2018). The choice of a method depends on the factors mentioned earlier. The method chosen must be able to meet closest to the requirements (Flow rate for very tight system should be 10^{-6} cm^3/s, for water tight should be 10^{-2} cm^3/s and for bacteria tight should be 10^{-4} cm^3/s) for the products (Chen, 2018). AstraZeneca wants to test the integrity of all their products to ensure that the products stay sterile throughout the shelf life. Therefore, the method that will be chosen must be able to test all the products and give safe results (Non-stochastic). Dye and microbial ingress are less suitable because they are less safe and give stochastic results. Microbial ingress is tested on products that contain a nutrient-rich medium, i.e., not on a regular product, which is not desirable for AstraZeneca. The leakage test must be done on ampoules that are filled with products that are then delivered to customers. It is difficult to see the blue colour at large product volume and small defects. The sensitivity of dye ingress is quite low compared to other methods. Although the sensitivity to microbial ingress is high, this cannot be guaranteed because there is no way to prove it, therefore these methods are excluded.

Laser-based headspace testing is non-destructive which is good for being able to test all products. The sensitivity of the method is quite low compared to the other methods. The method is time-consuming as it can take up to several weeks to be able to detect the pressure difference for small hole sizes. Some products are controlled on both the “twist-off-breakpoint” and “bottom” positions, which then becomes impossible to do with the help of laser-based headspace because it only controls the "head space". The method is sensitive to air bubbles in the liquid, and it works best on freeze-dried products. AstraZeneca PET BFS manufactures only liquid products, that’s why this method is unsuitable. Helium leak test is the best method in terms of sensitivity, but it is offline which means that not all products can be tested. There is even a risk for leakage path to be blocked by the product, which makes it difficult to detect the hole and gives false results. The method works best on freeze-dried products therefore, it is not recommended as a CCIT method on AstraZeneca PET BFS. Pressure and vacuum decay are deterministic, they give safe results. They are non-destructive, which means that all the products can be tested with these methods before they are delivered. The methods are very effective on liquid products and have high sensitivity. On the other hand, the product's "clogging" and humidity influences the measurement, therefore the tests should be performed in a suitable environment. Despite its disadvantages, vacuum decay would work as a good alternative for CCI testing in AstraZeneca PET BFS.

HVLD is a non-destructive method and can be used for online testing for all products before packaging. The method has a low risk of impact on the product and it can be performed under normal atmospheric pressure. To be able to apply this method, the product needs to have a conductivity of > 1 mS /cm, and the container must be non-conductive. The products manufactured at AstraZeneca PET BFS have a conductivity of 16 mS /cm, and the ampoules are made of PE or PP. HVLD also works on liquid products, which makes it a very suitable CCIT method in AstraZeneca PET BFS. In addition, the method has high sensitivity compared to the other methods. On the other hand, the method does not give detailed results such as hole size or flow rate. It is also difficult to detect holes in the “twist-off-breakpoint” of the ampoules because there is no liquid flow there. The problem can be solved by putting a vibration machine in front of the leak tester to extend the liquid over the container. This

method is done on AstraZeneca PET BFS to enable HVLD to detect leaks in the “twist-off-breakpoint” position.

Further literature searches were done to suggest which method between HVLD, and vacuum decay is best to apply to AstraZeneca PET BFS products. Figures 5 and 6 show that vacuum decay's ability to detect holes decreases with smaller hole sizes as 5, 10 and 20 µm. When the same sample was subjected to HVLD CCIT, it showed higher detection capability. Vacuum decay is not a suitable method for detecting micro-leakage in containers if the product contains large molecules.

Results in Figure 7 show that HVLD managed to detect all holes with sizes 5, 20, 50 and 100 µm while Vacuum decay method failed, which means that HVLD has a higher detection capability. In addition, Figure 7 shows that the voltage value becomes almost the same for the tested hole sizes. This is a hypothesis that HVLD may be size dependent, i.e., above some sizes it is independent but below this size range it is dependent.

The conclusion is that HVLD can be used to detect micro-leakage and is believed to be a reliable CCI method for liquid-filled products. Since the products at PET BFS meet all the conditions for HVLD, it is assumed to be the most suitable method to choose.

7 Methods for evaluating CCIT technique

For evaluation of a leak test method, samples with artificial holes should be used as a positive control. This control can be done for all CCIT methods to provide the opportunity to evaluate the method before testing the products. A positive control should also be done once / several times a year to check the effectiveness of the CCIT-methods. There are various methods available for creating artificial holes, but it is necessary to choose a method that works best on the products in AstraZeneca PET BFS. A literature search was done to find artificial holes technologies. Various companies were also contacted for more information about the methods and their limitations.

8 Methods for creating artificial holes in containers

There are different methods for creating artificial holes in containers, which method to choose depends on the material of the container and the wall thickness (Mahler *et al.*, 2017).

8.1 Laser drilling

It is the most common method used to create holes of different sizes in CCS. Laser drilling has inherent changes due to the process such as it can expand over time and easily the create holes are blocked by particles in the air (Mahler *et al.*, 2019). The smallest hole size that can be created with this method is 0.5 µm. The limit varies depending on the material of the

container. The method is safe in that the hole sizes are calibrated against a known reference method and it is used to create holes in polymer, glass, and metal (Mahler *et al.*, 2017).
Laser drilled holes are not the same size all the way in the capillary. The figure shows a laser-drilled hole. The hole starts with the diameter of interest and then the hole becomes smaller when it goes deeper (Mahler et al., 2019).

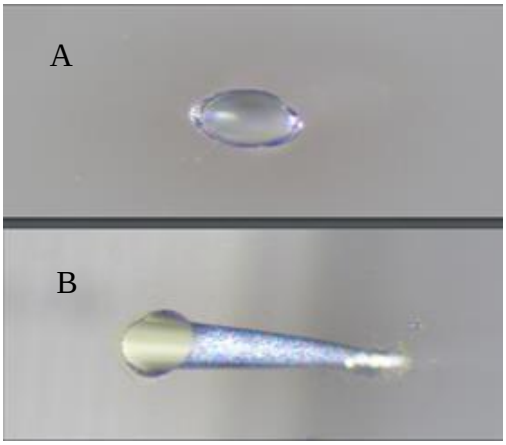


Figure 9: Laser drilled holes in glass ampoule. A is top view and B is side view (Mahler et al., 2019).

8.2 Micron wires

Copper wire is another method for creating artificial leakage. The wire is clamped between the "body" of the container and the stopper, in this way it will form a leakage channel (Figure 10) (Mahler, Pelaez and Herdliteschka, 2019). The method is cheap, it works best on glass containers with rubber stoppers but not on plastic containers because then it will be difficult to let the wire pass through the plastic. However, it is difficult to obtain the exact hole size for the artificial leaks, which is an important point for the validation. The limit for this method is 10 µm (Mahler *et al.*, 2017).

Figure 10: Artificial hole created with copper wire between rubber stopper and glass ampule (Mahler et al., 2019).

8.3 Micropipettes

The pipettes are in the glass form. It's inserted into the stopper for the container to create an artificial hole. The limit for this method is 0.2 μm (Mahler *et al.*, 2017).

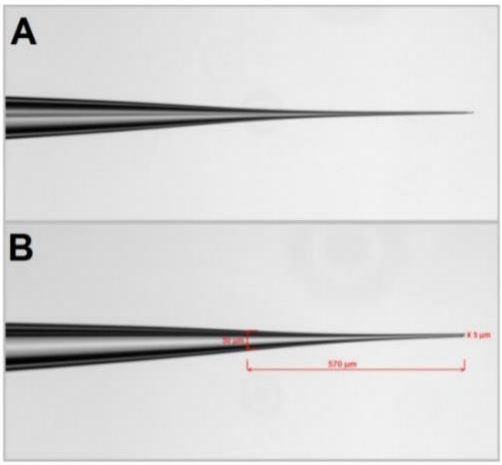


Figure 11: Micropipettes to make artificial hole (Nadezda A et al., 2014).

8.4 Capillaries

Leakage made by capillaries is carried out by nickel or glass capillary inserted into the “stopper” or “twist-off-breakpoint”. The capillaries should have a known length and inner diameter. For this, "epoxy" is used, which makes it easier for the capillary to break through the CCS. The exact diameter of the capillaries makes the possibility of the leakage repeatable quite high, but there is a risk that they will be blocked (Mahler, Pelaez and Herdliteschka, 2019). The exact diameter of the capillaries makes it possible to repeat the same type of leakage in several CCS, but there is a risk for clogging. It is possible to select a desired inner diameter and length of the capillary according to a measured flow rate. The minimum hole size that can be created by this method is 0.2 μm (Mahler *et al.*, 2017).

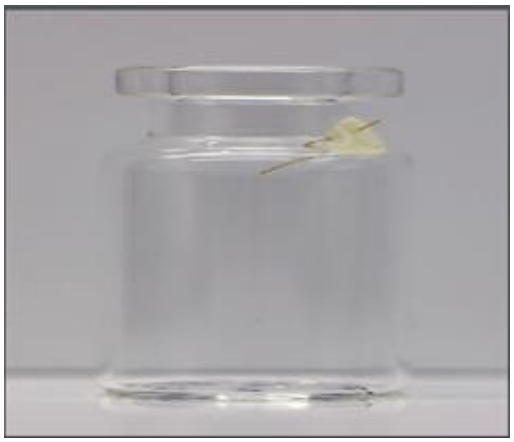


Figure 12: Artificial hole created by capillary using "epoxy" (Mahler et al., 2019).

8.5 Acupuncture needles

Test with acupuncture needles should be made at start and end of the filling process of the products. A machine operator creates a hole in the "twist-off-breakpoint" position and a hole in the bottom of the PP ampoules to check that HVLD is working properly. The same test is done on PE, but only for the "twist-off-breakpoint" position. The smallest hole sizes created with this method are between 120-200 µm and only in flexible ampoules such as plastic. This is because a smaller sized acupuncture needle is very hard to use, especially when the ampoule material is as compact as PP.

9 Side-by-side comparison for artificial holes methods

9.1 Comparison between artificial holes methods

Table 2: comparison between methods for creating artificial holes (Mahler *et al.*, 2017).

Leak type	Advantage	Disadvantage	Range for artificial leak size
Micro-pipettes	● Easy to prepare samples.	● Difficult to handle the samples. ● It is not an accurate method which makes it difficult to determine the hole size. ● Difficult to detect broken tips. ● They are non-durable for routine use. ● There is a high risk of false sensitivity after widening. ● The material of pipettes plays a role depending on the material of the container	>0.2 µm
Laser-drilled holes	● The defects are like the natural defects in glass and polymer. ● Latest technology and most validated. ● Larger range of hole sizes. ● Calibration gives more reliable results. ● Positive control samples can be reused.	● Expensive ● The calibration is required to get the exact hole size. ● Small holes can be clogged. ● Large variations in hole size depending on material and wall thickness ● Irregular hole	>0.5 µm
Capillaries	● Easy preparation. ● It is possible to prepare positive controls for several products and packaging formats. ● Durable	● The normal diameter is 2 µm but there is a high degree of uncertainty compared to the actual diameter. ● It is not possible to compare the hole size with a corresponding flow rate. ● The length of the leakage path is significantly greater than at the actual defects.	>0.2 µm

Micron wires	● Cheap ● Durable	● The micron wires are difficult to handle; therefore, a calibration is required to define and represent the hole path. ● The holes can become clogged over time. ● No direct measurement of the size takes place.	>10 µm
Acupuncture needles	● Cheap ● Fast to create holes	● uncertain method. ● Not able to test small hole sizes. ● Done only for control. ● Depend on human error.	>100 µm

9.2 A comparison between laser drilled holes and holes created with acupuncture needles

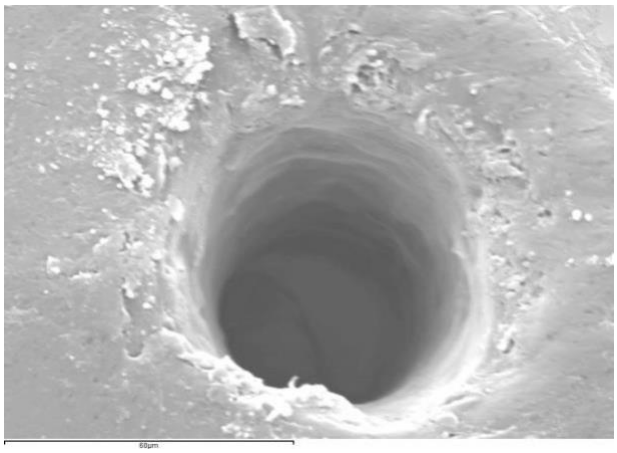


Figure 13: outside of a laser drilled hole (10 µm) in the "twist off-breakpoint" of PP ampoule.

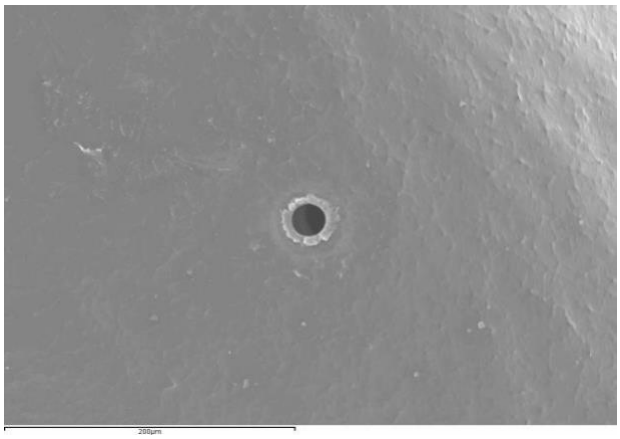


Figure 14: inside of a laser drilled hole (10 µm) in the "twist off-breakpoint" of PP ampoule.

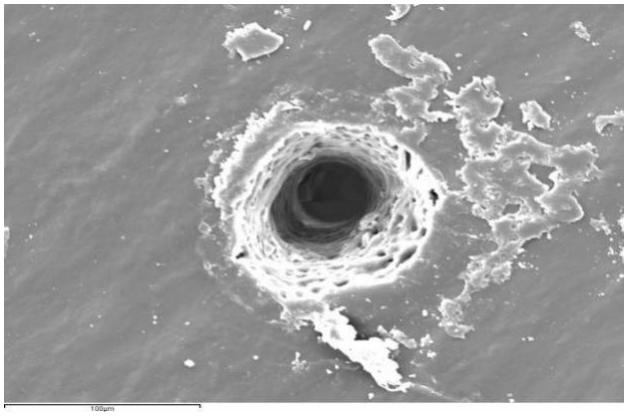


Figure 15: Outside of a laser drilled hole (10 µm) in the “bottom” of PP ampoule.

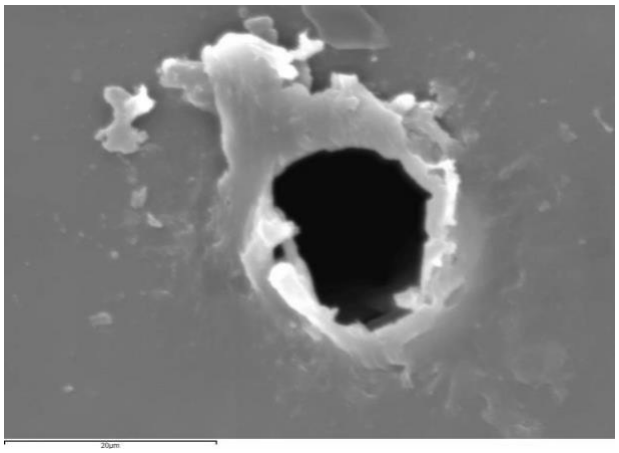


Figure 16: Inside of a laser drilled hole (10 µm) in the “bottom” of PP ampoule.

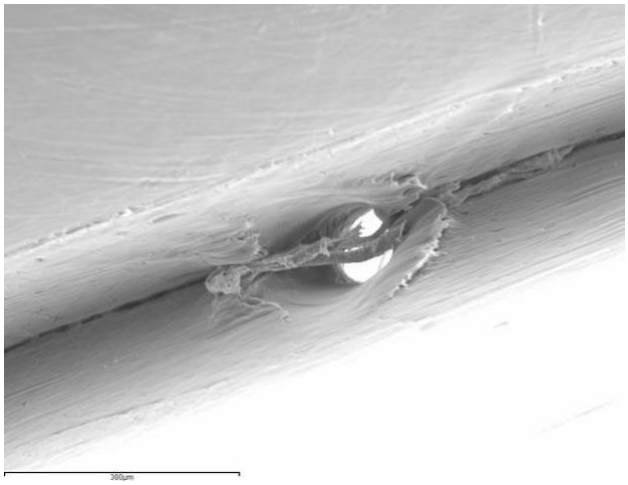


Figure 17: Outside of a hole made by acupuncture needle (200 µm) in the “twist off-breakpoint” of PP ampoule.

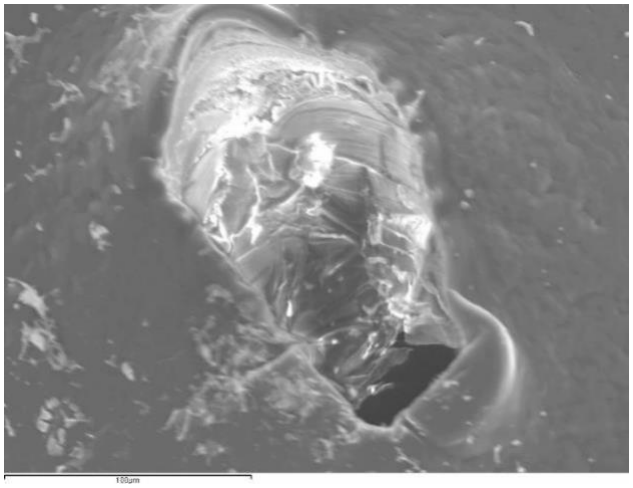


Figure 18: Inside of a hole made by acupuncture needle (200 μm) in the “twist off-breakpoint” of PP ampoule.

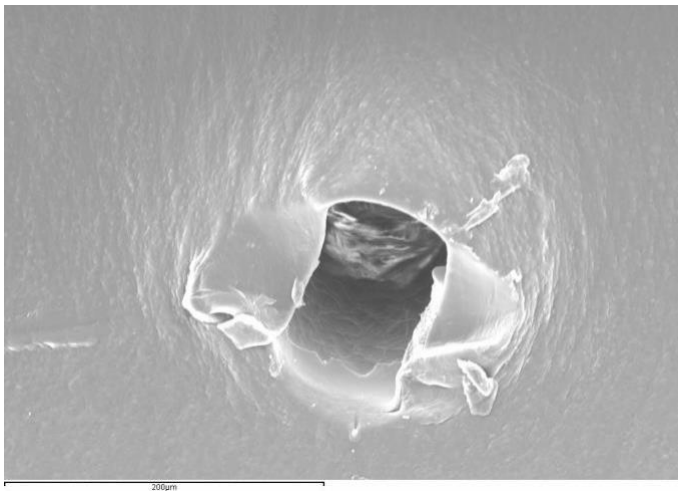


Figure 19: Outside of made by acupuncture needle (200 μm) in the “bottom” of PP ampoule.

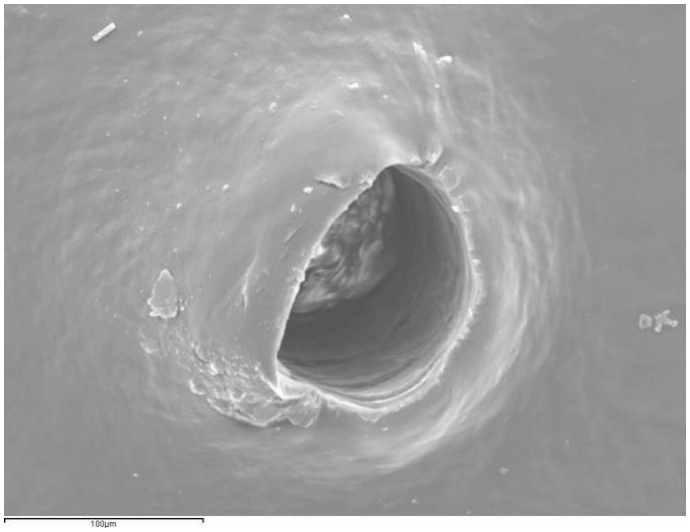


Figure 20: Inside of a hole made by acupuncture needle (200 μm) in the “bottom” of PP ampoule.

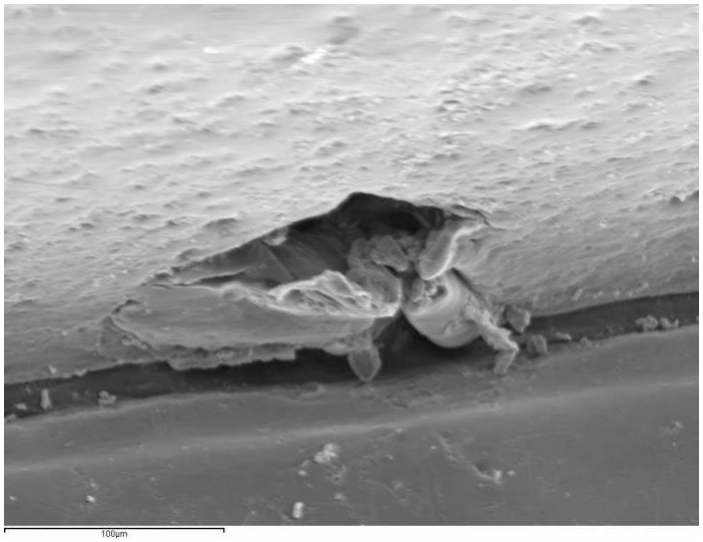


Figure 21: Outside of a hole made by acupuncture needle (200 µm) in the “twist off-breakpoint” of PE ampoule.

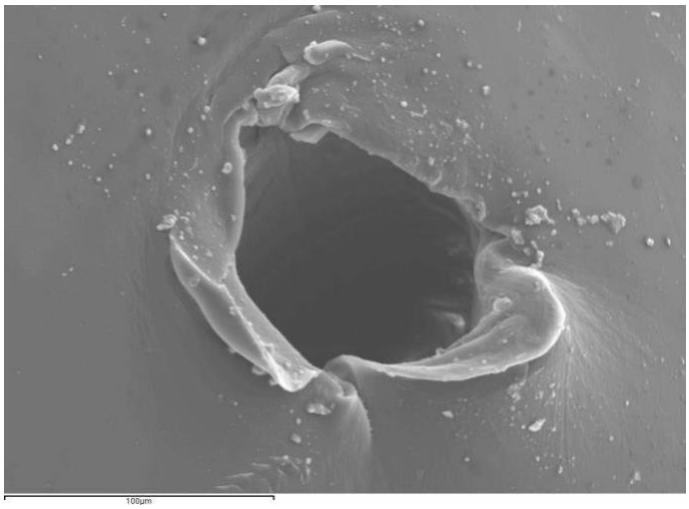


Figure 22: Inside of a hole made by acupuncture needle (200 µm) in the “twist off-breakpoint” of PE ampoule.

Each method has its pros and cons. Micron wires are made of a durable material, which means that they can be reused and are also cheap. It cannot be used to create holes in PE and PP ampoules because the copper wire must penetrate between a "rubber stopper" and the "body" of the ampoule. The ampoules used on PET BFS consist of either PP or PE and are plug-free. In addition, the method does not provide an exact measurement of hole size and the minimum hole size that can be achieved with this method is 10 µm, this is large compared to the smallest hole size obtained by corresponding methods. Capillaries provide the opportunity to create holes that are 0.2 µm but they usually have very large variations in diameter which can give false hole sizes. The method works best on glass with a stopper but also on a plastic. It does not give exact hole sizes because the holes are so different that they cannot be compared with a corresponding flow rate. Micropipettes are usually made of glass, so they are difficult to handle because they can easily break. The PE and PP ampoules have a thick wall thickness, which makes it difficult to use micropipettes because they are very fragile. The method does not provide an exact hole size as it depends on the material of the container and

pipette. Acupuncture needles is not a certain method and it is not effective because it's impossible to create holes smaller than 120 μm in PP and PE.

The laser drilled holes are more regular compared to those created with acupuncture needles (see figures 13-22). Figure 13-16 shows that the laser drilled holes start with that diameter of interest and then the diameter decreases the further into the hole. A hole of interest corresponds to the hole is on the outside and becomes smaller on the inside. This means that with laser drilling technology it is possible to get smaller hole size than the desired ones.

The method that is recommended by most companies and is suitable to work for PP and PE is Laser drilling. The defects created by this method are like the natural defects, giving reliable results for positive control for CCIT methods. Laser drilled holes can be calibrated against a known reference method, which means that it is possible to guarantee the size of the created hole. The recommendations from the companies support the results from the literature study that Laser drilling is the best method for creating holes in PP and PE. However, not all companies could offer certificates. Only Lenox laser and Oxford laser provide certificates and can create holes that are approximately 5 μm. The other companies can create holes that are 10-30 μm and cannot offer a certificate. Therefore, laser drilling was chosen as the method to perform positive control for HVLD and Lenox laser was chosen as the supplier to create laser drilled holes in ampoules which are then used for evaluation of HVLD.

10 Method and material for examination of HVLD detection ability

AstraZeneca did validations for the leak tester earlier which showed that HVLDs are effective, but the detection limit is still indefinite. A limit for minimum hole size where the microbial intrusions can occur should be found. Furthermore, this must be tested with methods available on AstraZeneca PET BFS. The CCIT method available on AstraZeneca PET BFS is High voltage leak detection (HVLD). Since it is difficult to find a limit, one hypothesis is that to prove that HVLD can detect holes regardless of size and in this way, there is no risk of microbial intrusions. Investigation of HVLDs' detection capability will be done by doing tests with different laser drilled hole sizes. An order was placed to Lenox laser to create a series of 6 laser-drilled hole sizes 5, 10 ,25 ,50 ,100 and 250 μm (Table 3,4,5). The hole positions were determined by starting from the scientists' own experiences and the validation. There are some critical positions where real holes can occur. For PP there are 2 positions, the "twist-off-breakpoint" and the bottom (Table 3,4) and for PE there is only one position, the "twist-off-breakpoint" (Table 5). They are designed in such a way that there is no risk of them becoming defective at the bottom due to the extra small plastic cover that lies around the edge of the ampoule.

Table 3: Size, number, and position of the holes in PP ampoules in the middle of the “twist-off-breakpoint”.

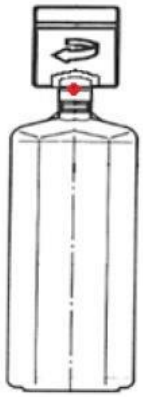
Hole size (µm)	Quantity	Hole position
5	10	
10	10	
25	10	
50	10	
100	10	
250	10	

Table 4: Size, number and position of the holes in PP ampoules in the middle of the bottom weld.

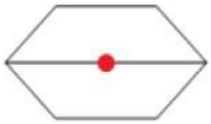
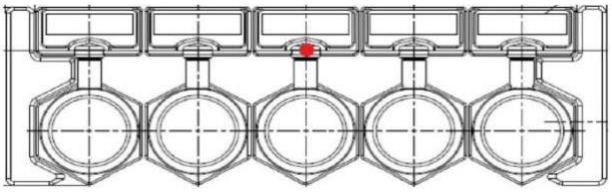
Hole size (µm)	Quantity	Hole position
5	10	
10	10	
25	10	
50	10	
100	10	
250	10	

Table 5: size, number and position of the holes in PE ampoules in the middle of the “twist-off-breakpoint”.

Hole size (µm)	Quantity	Hole position
5	10	
10	10	
25	10	
50	10	
100	10	
250	10	

11 Methods for technical and practical experiments

Before testing ampoules with laser-drilled holes, ampoules were tested with holes made with acupuncture needles (120 and 200 µm) with HVLD. The voltage was measured with an oscilloscope which was connected to the HVLD. The settings for HVLD used for these experiments are 1.0-2.7 V for a good ampoule “twist-off-breakpoint” and 0.5-1.8V for the bottom.

These experiments were made as a start to check the HVLD's detection ability and to be able to see from the diagrams the differences in voltage for the respective hole sizes. The ampoules with holes were tested several times with HVLD to investigate how the result is affected when the samples tested several times.

Another technical experiment was performed to test the detection capability of the leak tester. Ampoules with laser drilled holes created in 2017 were used for this technical experiment. The holes are placed in different positions as shown in the figures below. Holes that are 200 µm were created with acupuncture needles in the same positions as for 10 µm.

Before the tests began, a technician checked that the parameters were set correctly. The ampoules with laser drilled holes were cleaned with a cloth and ethanol to remove the salt crystals that were on the outside of the ampoules. The ampoules were then dried with a cloth to avoid the wrong signal. First, 12 "good" ampoules were placed on one pallet and then 12 more were placed on the next pallet, where 11 of them were "good" and one ampoule with leakage. Another 12 "good" ampoules were placed on the third pallet. A total of 14 runs 7 of them were made for 10 µm in different positions on the ampoules. And 7 for the hole size 200 µm with different positions (Attempt 1, table 9-15).

Additional tests were performed to examine how detection ability changes depending on where the hole is located, and this was done in the same way as previous tests (Attempts 1 and 2 in Table 9-15 and table 16-21). The experiments provided a better understanding of HVLD.

12 Results for the technical and practical experiments

12.1 Results for the tests for 120 and 200 µm holes in PP ampoules filled with 0.9% saline

Table 6: The voltage at different attempts for 120 and 200 µm holes made in "twist-off-breakpoint" position.

Hole size (µm)	Attempt 1	Attempt 2	Attempt 3	Attempt 4
120	7 V	7 V	7.2 V	8 V
200	7.6 V	7.7 V	7.7 V	8 V

Table 7: The voltage at different attempts for 120 and 200 µm holes made in the bottom.

Hole size (µm)	Attempt 1	Attempt 2	Attempt 3	Attempt 4
120	4.2 V	4.5 V	4.2 V	4.2 V
200	4.1 V	4.2 V	4.1 V	4.1 V

The results in Table 6 give a standard deviation of 0.48 V for 120 µm and 0.17 V for 200 µm. The average value is 7.3 V for 120 µm and 7.75 V for 200 µm. For the bottom, results in Table 7 show that the standard deviation will be 0.15 for 120 µm and 0.05 for 200 µm. The average value is 4.275 V for 120 µm and 4.125 for 200 µm. The results show higher signal

than the set (> 1.8 for bottom position and> 2.8 for the "twist-off-breakpoint") for HVLD used for these experiments.

12.2 Results for the tests for 120 and 200 µm holes in PE ampoules filled with 0.9% saline

Table 8: The voltage at different attempts for 120 and 200 µm holes made in “twist-off-breakpoint”

Hole size (µm)	Attempt 1	Attempt 2	Attempt 3	Attempt 4
120	7 V	6.4 V	6.8 V	6.7 V
200	8 V	5.6 V	7.5 V	6.9 V

The results in Table 8 give a standard deviation of 0.25 V for 120 µm and 1.03 V for 200 µm. The average value is 6.725 V for 120 µm and 7 V for 200 µm. Results show quite large variations in voltage, but this must be proven with the help of several technical experiments. The results show higher signal than the set voltage (> 1.8 for bottom position and > 2.8 for the "twist-off-breakpoint") for HVLD used for these experiments.

12.3 Results for the tests for 10 and 200 µm holes in different position in PP ampoules filled with 0.9% saline

Table 9: The voltage for 10 and 200 µm holes made in “twist-off-breakpoint” in position 1

Position 1	Attempt 1	Attempt 2	Attempt 3	
10 µm	7.9 V	-	-	
200 µm	12.5 V	6.8 V	6.6 V	

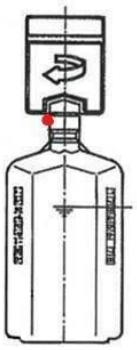


Table 10: The voltage for 10 and 200 µm holes made in “twist-off-breakpoint” in position 2.

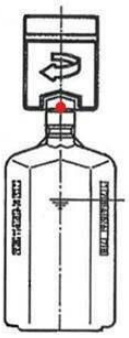
Position 2	Attempt 1	Attempt 2	Attempt 3	
10 µm	8.0 V	-	-	
200 µm	11 V	7.5 V	7.6 V	

Table 11: The voltage for 10 and 200 µm holes made in “twist-off-breakpoint” in position 3.

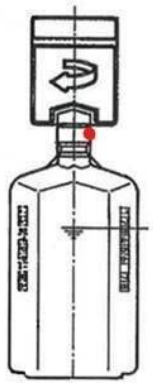
Position 3	Attempt 1	Attempt 2	Attempt 3	
10 µm	7.8 V	-	-	
200 µm	9 V	7.5 V	7.6 V	

Table 12: The voltage for 10 and 200 µm holes made in the back of PP ampoule.

Position 4 (back of the ampoule)	Attempt 1	Attempt 2	Attempt 3
10 µm	8.0 V	-	-
200 µm	12 V	7.5 V	7.8 V

Table 13: The voltage for 10 and 200 µm holes made in the bottom in the position 5.

Position 5	Attempt 1	Attempt 2	Attempt 3	
10 µm	8.1 V	-	-	
200 µm	10 V	6.0 V	5.5 V	

Table 14: The voltage for 10 and 200 µm holes made in the bottom in the position 6.

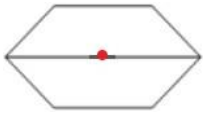
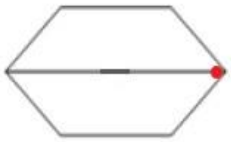
Position 6	Attempt 1	Attempt 2	Attempt 3	
10 µm	8.4 V	-	-	
200 µm	9.6 V	6.0 V	7.5 V	

Table 15: The voltage for 10 and 200 µm holes made in the bottom in the position 7.

Position 7	Attempt 1	Attempt 2	Attempt 3	
10 µm	9.2 V	-	-	
200 µm	9.8 V	3.3 V	3.5 V	

The results for attempt 1 in Table 9-15 show that the differences in signal between 10 µm and 200 µm are small in comparison with the difference between the hole sizes. Which means that a hole of 5 µm could also be detected by HVLD, to state this, further experiments with different laser drilled hole sizes are required. Results for attempts 2 and 3 in table 9-15 show a small variation in the signal for the different hole positions. The signal for attempts 2 and 3 in table 15 is quite low, the reason is that this position is close to the leak tester, which means that the signal does not have time to be registered completely. Signals are still higher than 1.8V, which shows that the ampoules are leaking. There are also differences in signals between experiments 1, 2 and 3 and this is because another equipment is used for experiment 1 which may have affected the results.

12.4 Results for holes in different positions on PE ampoules filled with 0.9% saline

Table 16: Hole made in position 1(Red point)

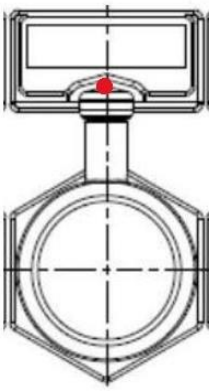
Position 1	Attempt 1	Attempt 2	
120 µm	8.36 V	6.85 V	
200 µm	8.97 V	6.96 V	

Table 17: Hole made in position 2 (Red point)

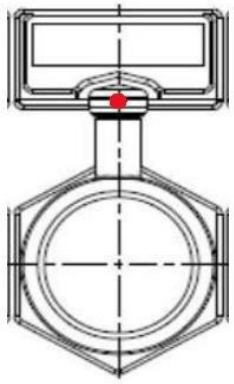
Position 2	Attempt 1	Attempt 2	
120 µm	8.06 V	8.00 V	
200 µm	7.68 V	7.55 V	

Table 18: Hole made in position 3 (Red point) in PE

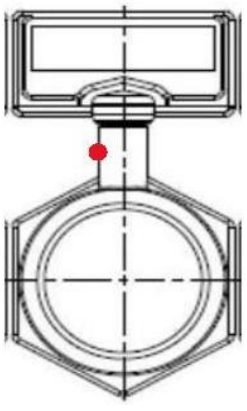
Position 3	Attempt 1	Attempt 2	
120 µm	5.80 V	5.40 V	
200 µm	6.94 V	5.55 V	

Table 19: Hole made in position 4 (Red point) in PE

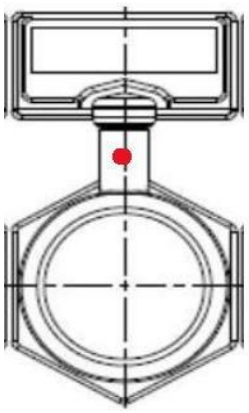
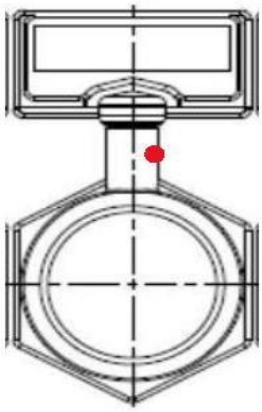
Position 4	Attempt 1	Attempt 2	
120 µm	8.82 V	7.09 V	
200 µm	8.37 V	6.17 V	

Table 20: Hole made on the back of the PE ampoule

Position 5	Attempt 1	Attempt 2
120 µm	8.14 V	7.14 V
200 µm	7.01 V	6.97 V

Table 21: Hole made in position 6 (Red point)

Position 6	Attempt 1	Attempt 2	
120 µm	6.85 V	5.59 V	
200 µm	5.45 V	5.52 V	

The results in Table 16-21 show small variations in the signal, which means that HVLD can detect holes in different positions. It also shows that the signal does not vary much between the hole sizes 120 µm and 200 µm, this needs to be investigated further with several technical experiments for different hole size.

13 Method to set a limit for microbial ingress.

The smallest hole size that can be created by laser drilling is 5 µm in PE and PP ampoules. It is impossible to prove that HVLD can detect holes smaller than 5 µm because there are limitations in creating the smallest leakage with laser drilling. It is possible to prove the detection ability of HVLD for the hole sizes smaller than 5 µm by theoretical/mathematical

equation. Equations that could help to find a relation between the detection ability and the hole size was found. Another equation to convert flow rate and hole size was also founded. This is used to calculate the flow rate for each hole size and compare it with the tightness limit presented in (Table 22), (Zapfe, 2016) and with the limit for water (Saline) and microbial tightness (Rottländer, Umrath and Voss, 2016).

The principle of HVLD described in following equation for container without defect (Good) (Densok):

$$I = \frac{V}{R+\frac{1}{2\pi fC1}+\frac{1}{2\pi fC2}} \quad (1)$$

Where I is the current, V is the voltage, f is the frequency of high voltage, C1 and C2 are capacitance, 1 / 2πfC2resistive capacitance and R is resistivity.

Following equation for containers with defect (bad) (Densok):

$$I = \frac{V}{R+\frac{1}{2\pi fC2}} \quad (2)$$

Relationship between flow rate (Gas) and hole size is described in the following equation (Lenox, 2015):

$$Q(Gas) = 0.01749 \times \frac{P1}{29.7} \times n \times d1 \times \sqrt{\frac{528}{Temp}} \quad (3)$$

Where P1 is the inlet pressure, n is 1 when ΔP/P1 is higher than 0.5, d1 is the hole diameter and temp is temperature in Rankine.

Relationship between flow rate (Liquid) and capillary radius is described with following Poiseuille's law (Pfitzner, 1976):

$$Q(Liquid) = \frac{Pr^4\pi}{8\eta l} \quad (4)$$

Where P is the pressure, and it is gauge pressure calculated by following equation:

$$\mathrm{P} = \text{Ƥ} * \mathrm{h} * \mathrm{g} \quad (5)$$

where g is 9.82 m / s2 and h is the height of the liquid and Ƥ is the density of water, 1000 kg / m3. l is the wall thickness in meter and Ƞ is dynamic viscosity of water is 8.9 * 10-4 at room temperature.

The flow rate that will be calculated with these equations will then be compared with the theoretical value of flow rate which is the limit of microbial tight and very tight system (table 22).

Table 22: The flow rate (Liquid) limit that describes the tightness for CCS

Systems tightness	Flow rate in mbar l/s	Flow rate in cm^3/s
Very tight system	10^{-6} mbar l/s	10^{-6} cm^3/s
Watertight	10^{-2} mbar l/s	10^{-2} cm^3/s
Bacteria tight	10^{-4} mbar l/s	10^{-4} cm^3/s

13.1 Results for the theoretical part by using equation 3,4 and/or 5.

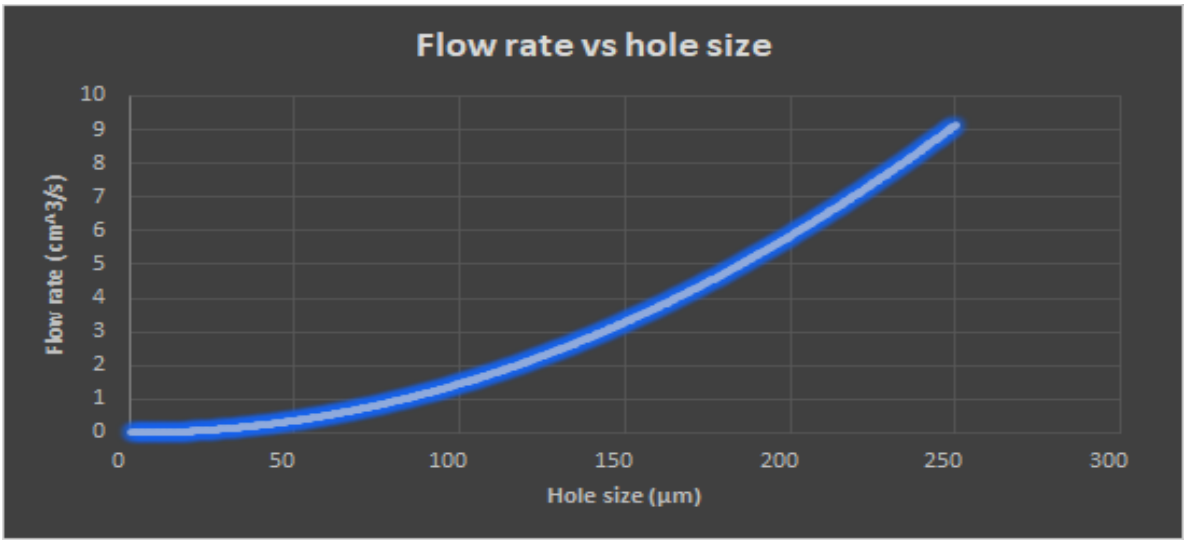


Figure 23: Flow rate of gas though capillary with diameter 0-250 µm calculated with equation 3.

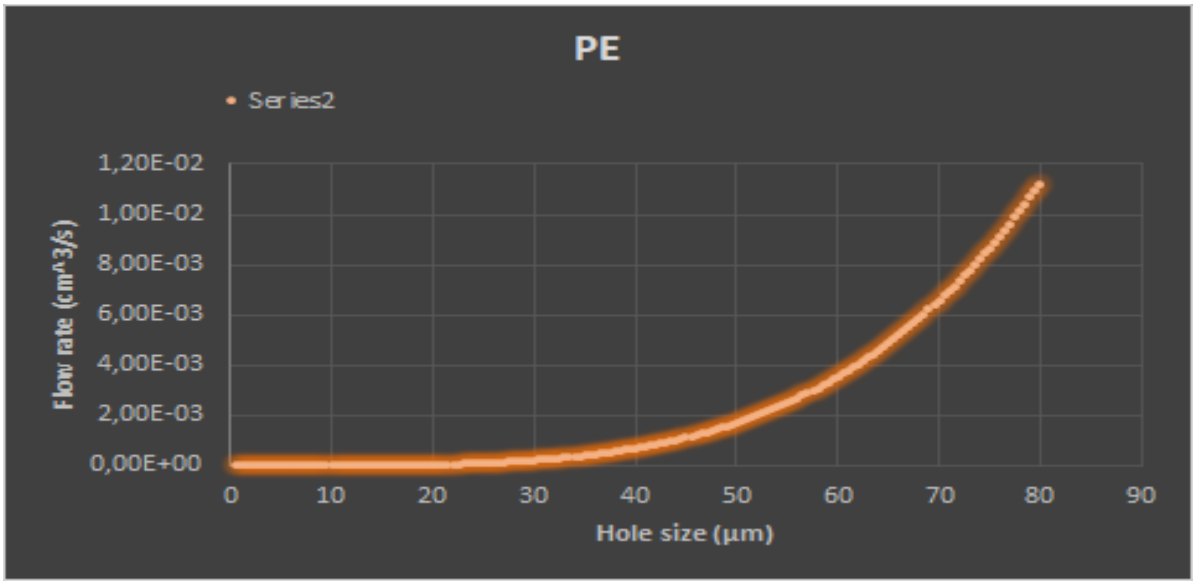


Figure 24: Flow rate (Liquid) through capillary with diameter 0.5-80 µm in PE ampoules filled with product and calculated by equation 4 and 5.

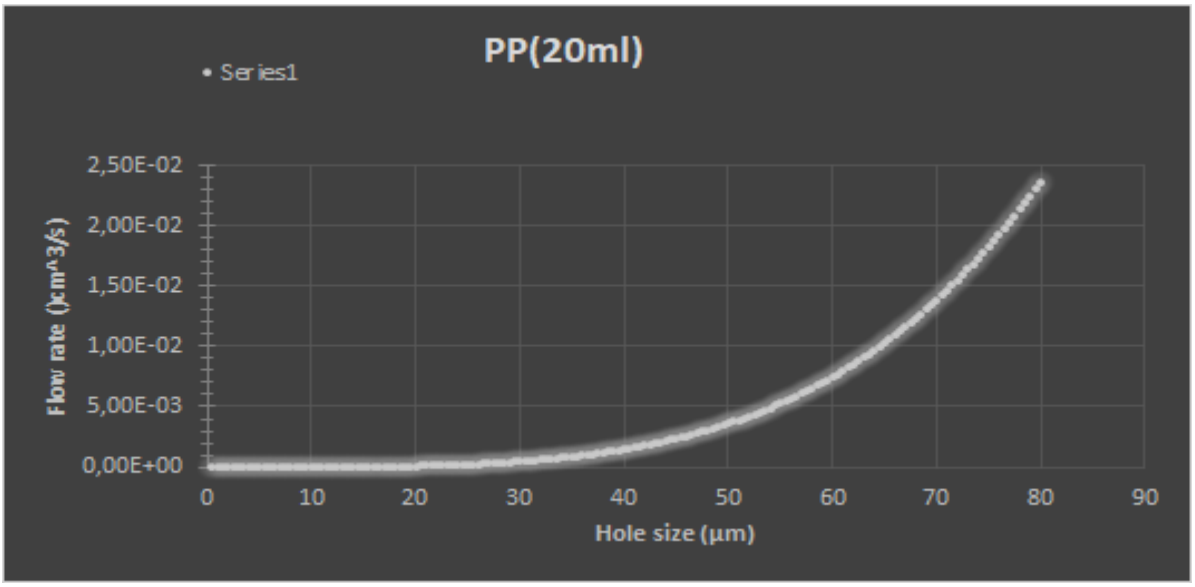


Figure 25: Flow rate (Liquid) through capillary with diameter 0.5-80 µm in PP filled with 20 ml product and calculated by equation 4 and 5.

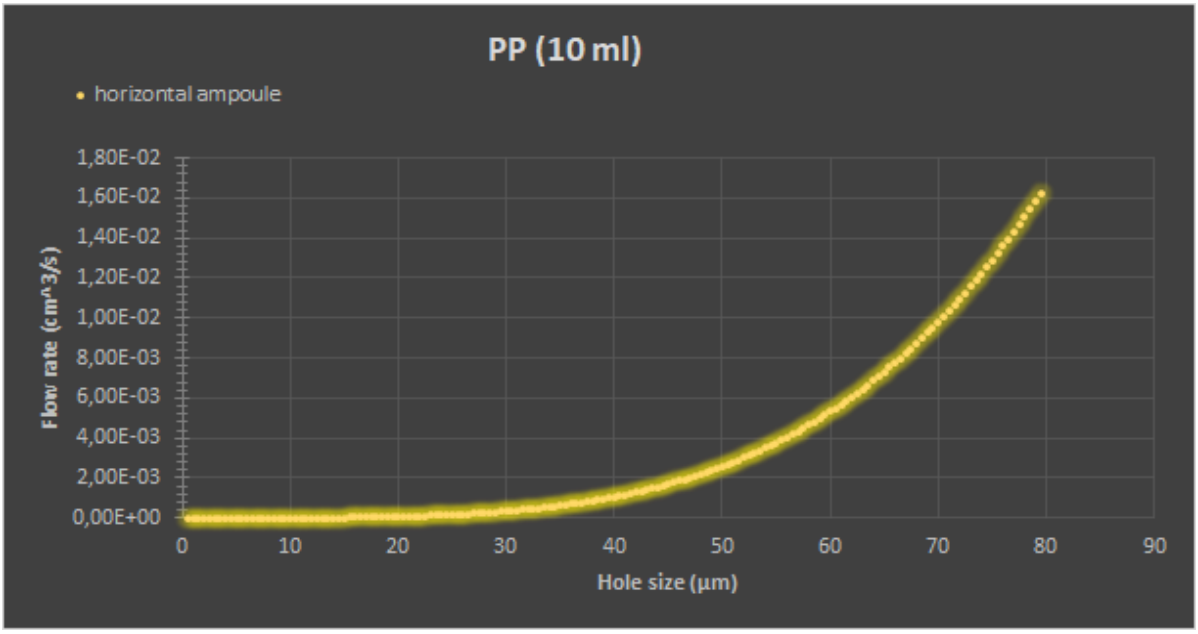


Figure 26: Flow rate (Liquid) through capillary with diameter 0.5-80 µm in PP filled with 10 ml product and calculated by equation 4 and 5.

14 Discussion of the technical and practical experiments

The samples with different hole sizes were ordered, but due to the current circumstances (Covid-19 pandemic), they could not be delivered. These samples are important to be able to

test HVLD's detection capability for different hole sizes. Because the samples were delayed, some experiments were made instead with 120- and 200- µm holes. The results show that there are small differences in voltage for the experiments for PP (Attempts 2 and 3 in table 9-15). This applies to both the "twist-off-breakpoint" and the "bottom" positions; it detects leakage and reports it as a fail or good product. According to the results, HVLD is independent of the hole size, and it can detect the leakage when the "capillary" is moist. To assert this, a broader investigation is needed with a wider range of hole sizes that are laser drilled and calibrated. For PE, the results were the same as in PP that the voltage is not affected by the hole size (10, 120 and 200 µm) so much (Table 9-21). This also needs to be proven by testing several hole sizes.

According to validations, there are two critical areas where leakage can occur in PP and one area in PE. The tests that were done for leakage in different positions show small variations in the voltage that are within the limits of a leaky ampoule (>1.8 for "bottom" position and >2.8 for "twist-off-breakpoint" position). This means that HVLD can detect leakage no matter where it is on the ampoule and this is an advantage as it can guarantee that the holes can be found. This can also be confirmed by looking at the results (Table 9-21). Two different types of leakage and hole sizes were tested. The results show high voltage for the different types of holes and for different sizes. Hole size 10 µm is quite small and in addition the holes are quite old since they were created in 2017, which mean that there would not be a risk of recrystallization. Despite this, the results have not affected and HVLD managed to sort out all leaking ampoules. These tests are important because they have shown that laser drilling is a good method, because the ampoules can be reused without having any effect on the results. There are differences in voltage between 10 and 200 µm, this difference is small compared to the differences between the hole sizes.

Leakage of different hole sizes gives different flow rates (Gas); larger holes give greater flow and smaller holes give less flow. Hole size can always be converted to flow rate, but the result can vary vary depending on the viscosity of the liquid and thus needs to be considered for different products separately. Viscosity can vary also with temperature. Flow for gases (Air) was calculated by equation 3. The result shows that it goes through very little flow at small hole sizes (Figure 23). Hole sizes that are smaller than 5 µm do not present a risk for microbial ingress. To investigate this further, Equation 4 was used to calculate the flow rate for liquid (0.9 % saline) for the "worst case". Equation 4 is written according to the properties of the product. The length of the liquid is measured for a horizontal ampoule because this is the closest to reality, the samples are tested when they are horizontal. The length of the capillary(l) is the same as the wall thickness of the ampoules.

A system is assumed to be watertight when the flow is $<10^{-2}$ cm^3/s (Table 22). This limit corresponds to the hole size of 77.5 µm in PE. In PP (20 ml) this limit corresponds to 64.5 µm and in PP (10 ml) 70.5 µm. The system is watertight, there is no risk of microbial ingress because microorganisms thrive in humid environments. When it is only gas molecules, the bacteria cannot move into the product, therefore the system is assumed to be sterile at the limit where it is watertight. To make even higher demands, we looked at the limit of very tight systems (Table 22). The results for horizontal PE ampoules show that the risk of microbial ingress can occur at hole size > 7.5 µm, which corresponds to the flow rate $8.68 * 10^{-7}$ cm^3/s. This flow rate is less than 10^{-6} cm^3/s which is the limit for a tight system (Table 22). At hole sizes <7.5 µm, the system is very tight and there is no risk of microbial

penetration. For the horizontal PP ampoule (20 ml), the flow rate was calculated to be 7.48 * 10^{-7} cm^3/s, which corresponds to a hole size of 6 µm. The system is assumed to be very tight for hole sizes smaller than 6 µm. Lying PP ampoule (10ml) was shown to be very tight at hole sizes 7 and smaller, this corresponds to a flow of 9.53 * 10^{-7} cm^3/s Leakage corresponding to smaller hole sizes gives no risk of microbial penetration. These calculations are assumed to be the worst-case because as mentioned earlier, the holes are not cylindrical, they are more irregular, the flow is therefore assumed to be even smaller than the calculated one and the tightness of the system can be confirmed for larger hole sizes. This conclusion is very important in this report, because it supports that if HVLD can detect a hole that is 5 µm in PE and PP, it means that it can detect probable hole size that give risk of microbial contamination in PE and PP products manufactured on AstraZeneca PET BFS.

The diagrams (24-26) show that tightness can be obtained at hole sizes between 6-7.5 µm. If HVLD detects 5 µm laser-drilled holes, they will be able to detect all leaky systems that pose a risk of microbial contamination. Holes that are smaller than those mentioned do not give a risk of microbial contamination and systems are assumed to be very tight already at 6-7.5 µm and without risk of microbial penetration at 64.5-77.5 µm.

As mentioned earlier, it is difficult to create holes that are smaller than 5 µm in PP and PE with a wall thickness between 0.6-0.8 mm. With a laser, it is possible to drill holes that are 5 µm, but this is time-consuming. In addition, due to the current circumstances, it takes extra time to send and get back an order. These perforated ampules are important for creating a series that shows which hole sizes can be detected by HVLD. Being able to detect 5 µm hole means that the method has passed the worst case. Unfortunately, this will not be able to be tested until the degree project ends but there is a plan on how to proceed.

The perforated ampoules should be tested one by one. The voltage and current must be measured for each individual PP product and for each PE block. The data must be entered in equation 1 to calculate resistive capacitance which is calculated from measured voltage for each hole size. These measurements must be functionally compared with those values obtained by the idealized model of a plane with variable hole sizes. The model for capacitance as a function of hole size will be modified by scientist from AstraZeneca. In this way, it is possible to examine HVLD's detection ability for all hole sizes both theoretically and practically.

15 Conclusions

HVLD can be recommended as an effective reference method "Container Closure Integrity Testing" for sterile drugs in PE and PP ampoules. To investigate what is the minimum size that can be detected using HVLD recommenders Laser drilling as an artificial hole method for positive control. The calculations show that there is no risk of microbial contamination and the system is perceived to be very tight at a hole size of 6-7.5 µm and water tight at a hole size of 64.5-77.5 µm.

HVLD can detect holes in different positions and could also detect holes of 5 µm as the results do not show large variation in signal between 10 and 200 µm respectively. Evidence of the assumption is needed by making several experiments with different hole sizes.

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