PRACTICAL INNOVATION

Leak Testing of Cryzotraq™ Cryogenic Vials: White Paper

Kay Pemberton, Ziath Ltd

Since the late 1990's biobanks and biorepositories have become a key resource, supporting many types of contemporary research such as genomics and personalised medicines. To ensure the integrity and viability of specimens stored in these sample storage respositories over long periods of time, the specimens are placed in a sterile glass or plastic vial and immersed in vapour phase Liquid Nitrogen (LN_2). Leak-free cryogenic vials are of utmost importance to ensure sample integrity. The closure should ensure the sample cannot leak from the vial and conversely that no contaminants can enter the vial. If the specimen is contaminated, it is rendered useless for further research.

Problems Associated with Leakage of Cryogenic Vials

If the seal of the cryogenic vial is not hermetic, evaporation of the sample can occur. This can cause the sample to be "lost" or for the pH of the sample to change and render it damaged. Additionally, should there be leak around the seal, as the vial is placed in ultra-low temperature storage (< -100°C), a vacuum is created inside the vial as the temperature causes the contents to contract. If the vial is accidentally submerged in liquid phase LN_2 and there is a leak around the seal, LN_2 will be drawn into the vial due to the vacuum. When the sample is taken out of storage, the LN_2 returns to room temperature, therefore expanding to 3000 times the liquid volume, causing the vial to explode. This creates three problems: 1) Shards of glass or plastic explode causing a safety hazard to the researcher. 2) The specimen is propelled around the room, causing a potential biohazard. 3) The specimen is lost and with it, potentially many years of priceless research. Lastly, but by no means least, if the seal is not intact, contaminants could enter the vial, rendering it useless.

Methods of Testing for Leakage of Cryogenic Vials

Evaporation Method

A cryogenic vial, containing a specimen is weighed and placed in LN2 storage. Over a long period of time, it is regularly removed to be weighed to measure any loss of sample due to evaporation. Whilst this method is a good indication, the time taken to gain worthy results is very long.

Pressure Method

The capped vial is placed inside a capsule, which is sealed and the vials are then subjected to a pre-set pressure. Pressure leaks from the vial are monitored and measured over a time period of time to measure the seal integrity.

Figure 1: To the left the pressure capsule can be seen, with an adapter for a particular tube size inserted. At the back (right) the lid to the pressure capsule can be seen. In front are other adapters for different tube sizes

Ziath Test Procedure – Pressure Method

Equipment

FOR TEST Model ET99W

This instrument of control in pressure decreases uses algorithms of advanced calculation in order to find with absolute precision, losses in lesser time. ET99W is adapted for tests in vacuum or pressure guaranteeing precision of measure of 100.000 points
on the end scale.

Figure 2: FOR TEST Universal Leak Tester Model ET99W

Method

The capped CryzoTraq™ Cryogenic Vials are placed inside a capsule, which is sealed and the vials are then subjected to a pre-set pressure (1000 millibars/100kPa). This pressure ensures compliance with IATA "receptacles and packagings filled and closed under an absolute atmospheric pressure lower than 95 kPa"[1]. The tubes are tested at room temperature, -80°C and +55°C. Pressure leaks from the vial are monitored and measured over a time period of time to measure the seal integrity. The maximum pressure drop allowed is 0.2mbar.

Results

Any pressure loss is recorded and any vials falling outside the acceptable limits are discarded.

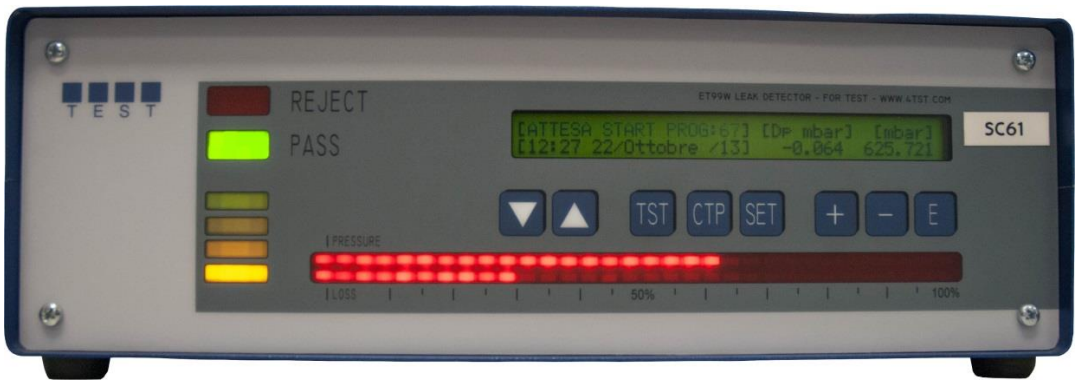

Figure 3: End of Leak Test result. The display shows the date and time of the test, the pressure loss recorded (Dp -0.064mbar) and the pressure at the end of the test (625,721mbar). The green "PASS" light shows the pressure drop value has fallen within acceptable limits (in this case <0.064mbar, maximum pressure drop = 0.2mbar)

Integrity of Ziath's CryzoTraq™ Cryogenic Vials

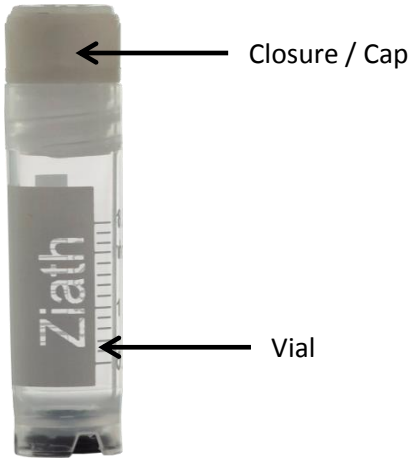

Figure 4: 2ml internally threaded cryogenic vial

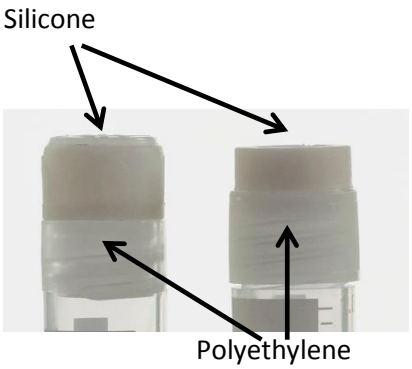

Figure 5: The caps have an innovative 2-step injection moulding process design

The compression ring (silicone) is moulded into the CryzoTraq™ screw-caps, in a unique 2-step injection moulding process negating the requirement for an o-ring. O-rings can constrict on freezing and potentially fall out of the cap, therefore challenging the leak-free seal.

Sample integrity means everything to Ziath. With many years' experience of sample management within the laboratory, we know how precious samples are.

With Ziath, our expertise and support come as standard.

References

1: IATA Packing Instruction 650, applicable to UN 3373 on passenger and cargo aircraft and CAO