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This article describes procedures to test gloves, shortcomings of plastic glove behavior, and glove fixation. In addition, it presents a new impulse technique for the improvement of reproducibility and repeatability of a pressure decay test.

Safe Access using Glove Ports – Facts and Fiction

by Johannes Rauschnabel, Albrecht Kühnle, and Kuno Lemke

Introduction

Barrier systems, such as aseptic isolators, Restricted Access Barrier Systems (RABS) and glove boxes are being used more and more in pharmaceutical production, research, and laboratories. The purpose of these barrier systems is to separate a process area from a surrounding environment – either to shield the process from contaminants coming from outside, or to shield the environment from hazardous products inside. If manual operator access is needed either during processing, for maintenance reasons, or for environmental monitoring inside the barrier, glove ports are needed.

The integrity of these glove ports is crucial for maintaining sterility of the barrier system. Therefore, frequent inspection of the glove ports is required. The FDA guidance for industry on aseptic processing[1] asks for "*With every use, gloves should be visually evaluated for any macroscopic physical defect. Physical integrity tests should also be performed routinely,*" while EC GMP [2] requires "*Monitoring should be carried out routinely and should include frequent leak testing of the isolator and glove/sleeve system.*" Thus, some kind of leak testing has to take place in addition to visual inspection.

Challenge

Glove leaks have many reasons: mechanical damage through contact with sharp equipment, tools or broken glass, which cause cuts in the glove material. Clamping gloves in mechanical installations very often results in holes that are less discrete and hard to detect. The most frequent types of leaks come from heavy use of gloves and aging. The commonly used glove material "Hypalon" has a layered structure, which tends to flake off over time, therefore causing precarious perforations of the glove membrane. The glove membrane do not only show elastic behavior, it also has viscous properties, especially if it is stressed by tension or mechanical load. These stressed areas have a reduced membrane thickness and are more sensitive to mechanical impacts. Membrane rupture is very often the consequence of over stressing gloves locally. Chemicals can have a similar effect: some are capable of reducing the tensile strength of the glove.

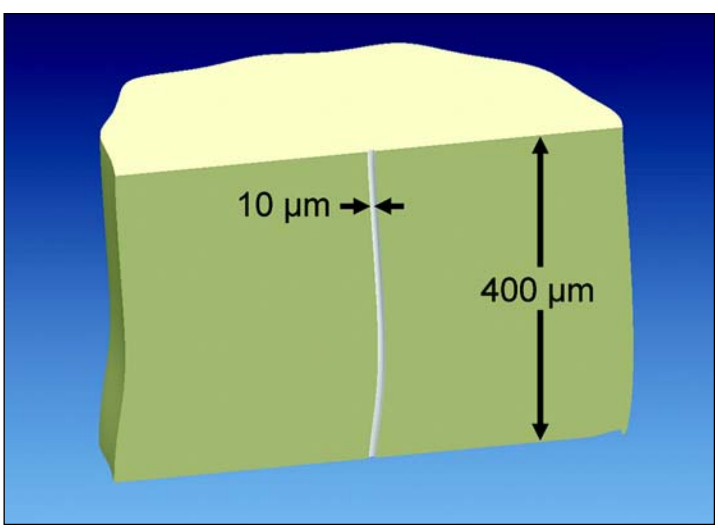

Figure 1. Proportions of a 10 μm leak in a 400 μm membrane.

Any type of leak should be detected with a physical glove testing procedure - at any position on the sleeve, cuff ring, or glove assembly.

The standard for operating isolator systems is to work with a second disposable glove (80% of all users)[3] – so that direct contamination is not very likely even in the case of a small leak. Additionally, in most isolator systems, an over pressure is applied inside barrier isolators, which prevents ingress of airborne contamination from out side to inside. But germs are able to grow through holes – against any pressure level. Bacterial spores have a diameter down to 1 µm, while vegetative bacilli and yeasts grow to diameters of 10 µm and more. By above mentioned diameters, the acceptance criteria for a glove tester seems to be given. Figure 1 demonstrates the proportions of a 10 µm hole in a 400 µm thick glove membrane (~15 mil).

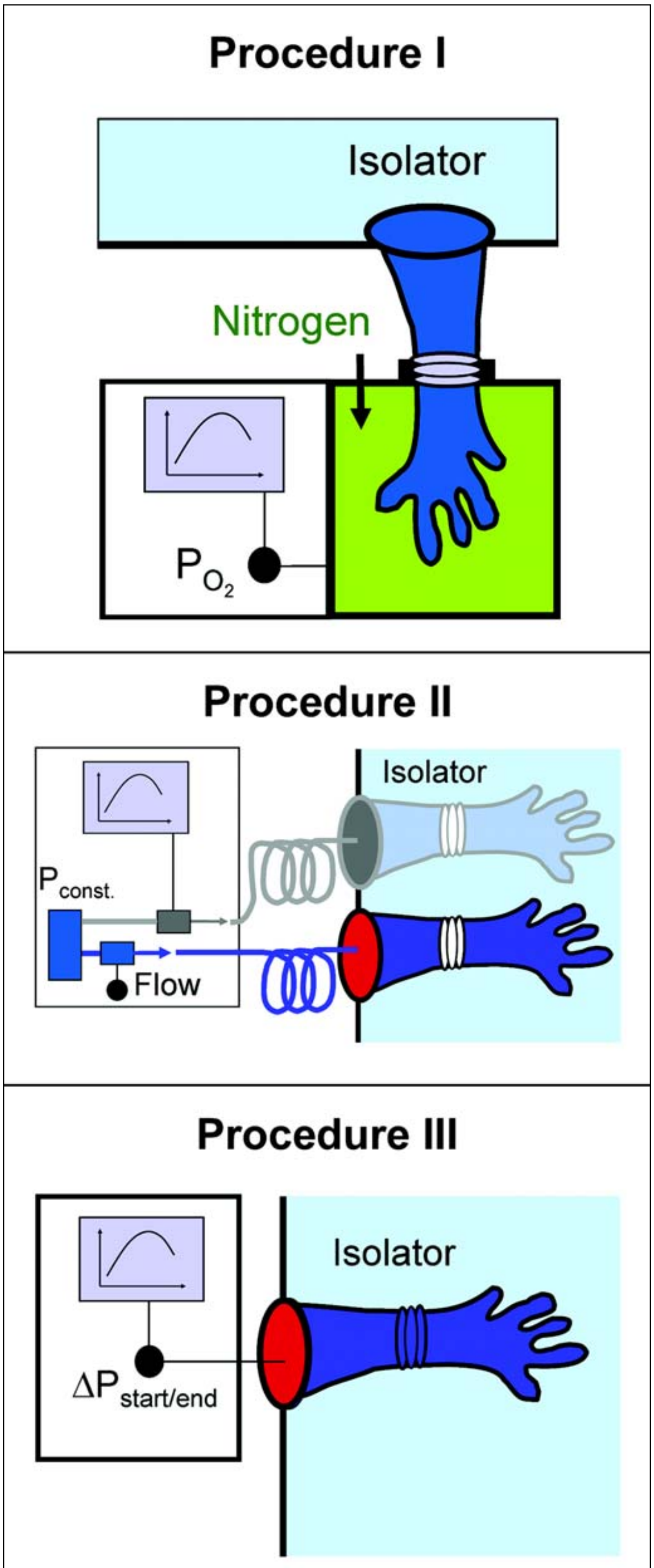

Figure 2. Procedures for glove testing applying pressure difference.

However, no existing physical test method is capable of detecting leaks in glove assemblies down to 1 µm diameter. What is worse: cuts of 100 micron scale may not be detected in every position of the glove assembly.

Existing glove test devices for the pharmaceutical industry apply different procedures utilizing pressure difference between inside and outside of the glove, including (I) oxygen measurement in a nitrogen chamber, (II) air flow measurement, and (III) pressure decay measurement - *Figure 2*.

Other techniques such as the bubble test, ammonia test, or Helium leak test are useful to detect the leak location, but do not give quantitative results, which help to decide 'passed' or not 'passed.' And conductivity measurement of a glove filled with electrolyte in a tub with distilled water can't be applied for in situ testing.

For test procedure (I), the glove is placed with a special cuff ring into a vacuum chamber, which is evacuated and filled with nitrogen. If there is a leak, air (containing 20% oxygen) from inside the glove leaks into the chamber, where a gas sensor measures the oxygen level. This level can be correlated to a leak rate. The procedure is sensitive due to high test pressure of 4000 Pa and is very accurate (acceptance criteria is an oxygen concentration of 500 ppm, which refers to an artificial 40 µm hole). Gloves can be tested during production, but the test is not able to challenge the complete glove assembly.

Test procedure (II) pressurizes the glove over a certain time at about 600 Pa. The air volume per time needed to compensate pressure loss by leakage is measured with a flow meter and correlated to a leak rate. Theoretical calculations come to a minimum acceptance criteria of 2 ml/min, which would correlate to a hole diameter of approximately 66 µm. In practical testing, this method can only achieve results down to minimum 100 µm diameter, but reproducibility is poor. The 'history' of the glove/sleeve assembly plays an important role (see below).

Test procedure (III) 'pressure decay' is the most common physical testing method with pharmaceutical isolators. According to the ISPE 2004 isolator survey, [3] more than 70% of responses apply some kind of pressure decay testing. In this procedure, a positive pressure expands the glove (assembly). Either directly or passing a certain pressure level, the measurement starts. After a certain time, the end pressure is taken. From the pressure drop, a leak rate can be calculated. The principle of this test is very simple and can be performed with a pressure gauge and a stopwatch only. But the downside of this procedure could be lack of reproducibility.

All these procedures take as a basis the perfect 'virtually new' glove, which always behaves the same way during measurement. But this is fiction and not reality.

During pressure difference testing, the following two parameters can not be kept constant: air volume and glove elasticity. Air is compressible, which has to be accepted and will not vary with other parameters kept constant. A glove is not a fully elastic system, but shows some plastic behavior. The glove expands non-proportionally to the pressure level

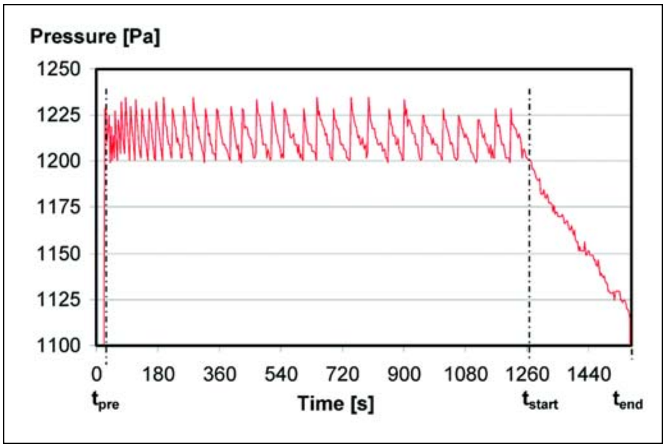

Figure 3. Measurement preparation utilizing impulse technique.

and does not keep its volume while keeping the pressure constant. This behavior is dependant on glove material, glove thickness, age, and history of the glove. Results differ with new gloves, heavily used gloves, freshly tested gloves, and aged gloves from stock, in addition to temperature fluctuations during measurement, which impact air compressibility and glove elasticity. From all these shortcomings, physical glove testing could be questioned principally.

Innovation and Results

There is one aspect, which gives a glimmer of hope for reproducible testing: the influence of this non-linear behavior decreases with stress level. This means that with higher pressure or by longer period of pressurization, the glove converges to a kind of 'equilibrium state,' from which glove behavior could be taken as constant.

Higher pressure levels should help, but have their limits. A 3000 Pa pressurization of a standard glove/sleeve assembly (400 µm membrane thickness) results in inflating the sleeve to balloon size while the glove keeps the shape. Therefore, the high pressure difference for test procedure I (4000 Pa) – which is principally useful – can only be applied for the glove alone.

The alternative – prolonging the period of pressurization – is feasible for complete glove/sleeve assemblies, but could take hours, which is not very practical.

A new approach stresses the glove/sleeve assembly by repeated pressure impulses, which are applied as soon as the pressure inside the glove drops below the starting pressure level (1200 Pa). The equilibrium state is achieved within 10 to 20 minutes depending on glove/sleeve thickness and material. As shown in Figure 3, the impulse frequency reduces over time, which is an indicator for approaching the equilibrium state. All following results are achieved by that technique.

To demonstrate the influence of stressing time, glove/sleeve assemblies in two states (brand new, old/used) were performed - *Figure 4*. The results are the average value of three measurements. The longer the stressing time, the smaller the deviation. The same can be observed for the pressure level: the higher the pressure, the better the reproducibility; 1200 Pa showed to be the optimum pressure level for standard glove/sleeve assemblies.

To demonstrate the repeatable equilibrium state at the beginning of the measurement, a series of tests were performed with varied pause time between each run: from no pause to two hours - *Figure 5*. The results spread in a window of 20 Pa, which is sufficiently accurate for reliable detection of an artificial 100 µm hole with standard glove/sleeve combinations.

A prototype of reinforced Hypalon sleeve combined with a standard Hypalon glove was tested. The results were very positive: test pressure could be doubled (2400 Pa) without overstretching the sleeve. Stressing time could be cut in half and resolution doubled allowing reliable detection of 50 µm holes.

Practical Aspects

Holes of a 100 µm diameter or less can hardly be detected during visible inspections. Even cuts of a millimeter in size could remain undetected could remain undetected by visual check. Under unfavorable circumstances, the detection of these cuts also could be a challenge for physical glove testing procedures. Depending on the location and orientation of such cuts, the force induced by pressurization is either sufficient to open the leak or not. And it is not only the pressure level that affects opening probability, but also the direction of the cut in relation to the tension from pressurization - *Figure 6*. Cut locations in the sleeve can easily be detected, but cuts on the finger tip are much more difficult to be opened – this is because of geometrical aspects (see above), and also due to the higher glove thickness at the finger tip.

Another important aspect is the tightness of the complete assembly: port, sleeve, cuff ring, glove. The performance of the whole system is determined by the weakest link. Glove and sleeve fixations are critical points. The very common fixation by expanded o-rings clamping the glove (sleeve) on a ring is not the way o-rings should be used. They have there best sealing properties with being pressed between two faces of a connection. In case of oval shaped glove ports, the o-ring used as an expander shows a high contact pressure at the small radius sections compared with insufficient contact pressure at the big radius sections. What is more: Hypalon material tends to crawl under mechanical load – such as contact pressure by an o-ring – which has an effect on the tightness. Therefore re-adjustable fixations have advantages

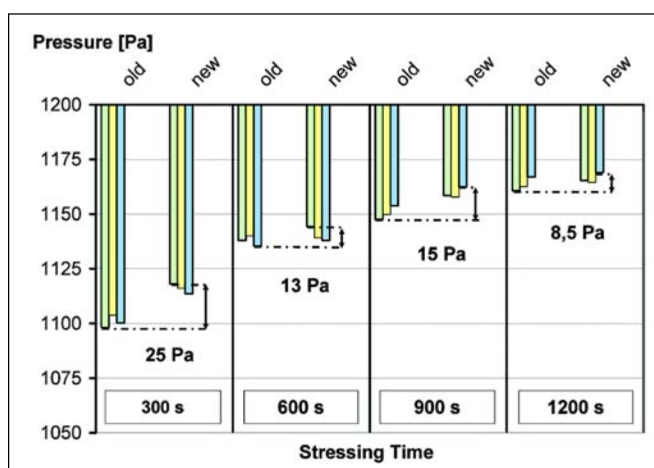

Figure 4. Variation of stressing time with set of old vs. new gloves.

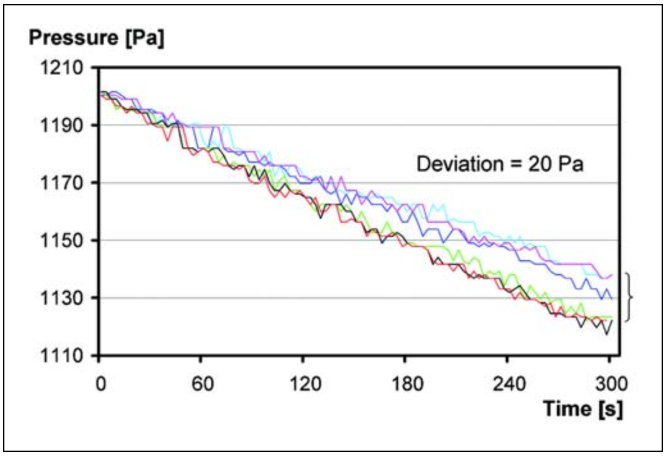

Figure 5. Repeated measurement with variated pause time between the runs.

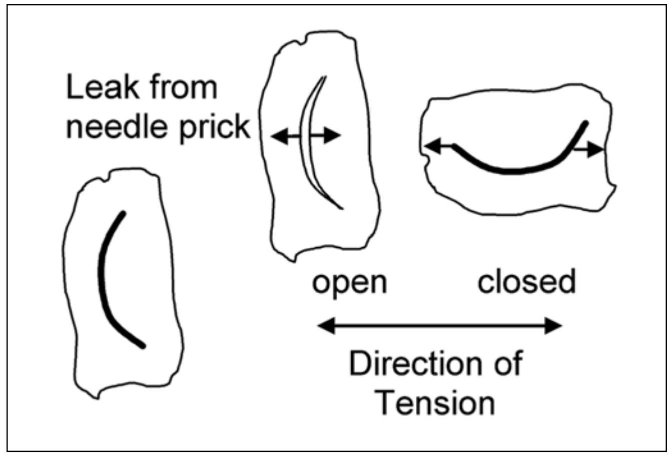

Figure 6. Behavior of leaks from needle prick in relation to leak orientation.

over expanding techniques involving o-rings.

Experience on filler isolators show that many perforations or leaks in glove/sleeve assemblies are caused by interventions with stopping machinery. Routine work – such as environmental monitoring in the isolator, transfers, and routine adjustments do not have impact on glove integrity as often. Leaks in the sleeve are very common, typically coming from overstretching (bad ergonomics), wear through leaning on the glove port ring, and untrained or inappropriate handling by the user.

Hypalon is the favorite glove material for use in isolators because of its stability against oxidizing agents, such as hydrogen peroxide vapor. In case of Restricted Access Barrier Systems (RABS), which are installed inside cleanrooms of high air quality (at least ISO 7), the gloves have to be sterilized prior to transfer into the operations room. The cost of glove/sleeve assemblies is very high – so for economic reasons, being able to sterilize glove/sleeve assemblies multiple times can be an important requirement although autoclavability of Hypalon gloves is poor. The mechanical properties change after 6 – 8 autoclave cycles and result in leakage after 12 – 15 cycles. Alternative glove materials could be an option to overcome that disadvantage.

With containment systems, the risk of operator contamination require a different glove approach. The need for mechanical stability and leak tightness comes from GMP and HSE requirements. For aseptic containments with positive pressure, leaks could blow contaminants into the operator area. Therefore, thicker gloves or two layered types can be the better choice. In addition to gloves with improved mechanical properties, single piece types can be recommended to reduce interfaces.

For containment operation in general, glove testing is an important part of health and safety precautions. The measurement procedures described above are applicable, but thicker glove membranes require higher pressure levels to detect small leaks.

Summary

Physical glove integrity testing is required by regulatory guidance. Investigations demonstrate that gloves to be tested applying pressure difference should be prepared in order to achieve reproducible results. Increasing the pressure level or prolonging the stressing time shows improvement in reproducibility of the test results. A new impulse technology helps to save time. Recommendation from practical experience emphasizes the need for a reliable glove fixation/sealing technique. Utilization of gloves in RABS, isolator, and containment systems requires a slightly different approach, but it should be performed in conjunction with integrity testing.

Physical glove testing with a reliable procedure helps to reduce dependence from adherence to visual inspection SOPs.

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