Leak rate measurement for pharmaceutical isolators: Practical guidance for operators and test engineers

Tim Coles

Abstract

This paper starts by defining leaktightness of isolators and explaining the difference between leak rate measurement and leak detection. It then sets out the classes of isolator leakage rates from 14644-7:2004 and ISO 10648-2:1994 and gives recommendations as to which class of leak rate is applicable for which operation. Leak rate measurement is described in some detail starting with the different methods available including pressure decay, pressure hold and nitrogen dilution. Theoretical considerations on the pressure decay test are followed by practical guidance and examples of the different expressions for pressure decay. The paper then goes on to describe the relative merits of various leak detection methods including the use of helium, DOP (dispersed oil particulates), ammonia with proprietary bromophenol cloth, soap bubbles and ultrasonics. The paper concludes with sections on testing gloves and half-suits, when to test, the distributed leak test and induction leaks.

Introduction

Since isolators are designed to maintain a specialised environment, it makes sense to specify the nature, or performance, of the containment. This is done partly by defining the filters on the ventilation system and partly by defining the quality of the barrier. The leaktightness is a fundamental measure of the quality of the barrier.

This then means that the leaktightness of the isolator needs to be stated at the design stage and then quantified during validation at FAT, SAT, OQ and also during subsequent PPM.

Isolator operators and test engineers will often refer to leak testing. The correct description of the procedure is, in fact, leak rate measurement.

Leak rate and leak detection

It is important to understand at an early stage the difference between leak rate measurement and leak detection. The leak rate may be defined as the amount of air lost from a positive isolator or gained by a negative isolator per unit time. The most convenient way of expressing leak rate is probably as percentage volume loss per hour. By contrast, leak detection is applied when the isolator has failed a leak rate test, and detection is then used to find out where leakage is taking place in order to fix it.

A further important point to note is the fact that all isolators leak. The question is then by how much?

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Standards, guidelines and units

There are no rules on leak rate. However, we do have some guidelines. Both ISO 14644-7:2004 and ISO 10648-2:1994 specify four classes of leaktightness as follows:

Class 1: 0.05% volume loss per hour
Class 2: 0.25% volume loss per hour
Class 3: 1.0% volume loss per hour
Class 4: 10.0% volume loss per hour

It would therefore make sense for isolator manufacturers to specify which of these specified classes their products comply with. They may choose to meet a slightly different standard. For example; one manufacturer works to 0.50% volume loss per hour. This is quite acceptable since no absolute rules exist but, on the other hand, standards are developed to encourage consistency, so why deviate from accepted norms?

Leak rate can be expressed in a variety of terms and units other than percentage volume loss per hour. Pharmaceutical Isolators mentions reciprocal hours. Some manufacturers refer to specific pressure decay rates per unit time (e.g. 16 Pascals per minute), others express the leak rate the other way round, namely time to give a fixed maximum pressure decay (e.g. 6 minutes giving a maximum of 60 Pa decay). Tables comparing these alternative leak rate expressions are given later in the paper.

Once again, a more logical approach surely uses the established standards and their defined classes. So which class is appropriate for which operation? As previously noted, there are no rules, but the following broad advice is offered:

Class 1. Class III microbiological safety cabinets and very high containment isolators.

Class 2. Negative pressure aseptic isolators.

Class 3. Positive pressure aseptic isolators.

Class 4. Not appropriate for pharmaceutical isolators.

It can also be argued that the class of leak rate chosen should depend on the grade of cleanroom in which the isolator is housed; a better cleanroom permits a higher leak rate, especially in the case of a negative isolator used for aseptic processing, but this has to be decided on a case to case basis.

Leak rate measurement methods

How then should the leak rate of an isolator be measured in a practical and rational way? There are a number of methods, as follows:

a) Pressure decay
The majority of users will opt for pressure decay testing. This is easy to perform and does not need specialised equipment or highly trained personnel; indeed the isolator may be equipped to perform the test itself. It is essentially just a question of closing off all the ports and valves on the isolator, raising the isolator to a test pressure (or lowering to a test depression), and noting the change in pressure differential to ambient over time. However, there are a number of practical considerations and these are detailed later.

b) Pressure hold
In many ways, the pressure hold method is an ideal method to measure leak rate since it actually quantifies the leak rate directly. In this test the isolator is sealed off as if for the pressure decay test and is then supplied with air from a suitable pump, via an air flow meter. By careful adjustment of the air pump, it should be possible to raise the isolator to a suitable test pressure and then hold that pressure steady. The air flow required to hold that steady test pressure can then be read off the flow meter and that reading is, of course, the leak rate.

This method is used successfully by at least one isolator manufacturer but in practice it is really only suitable for large and / or relatively leaky isolators, such as industrial scale filling lines. With smaller isolators, it becomes hard to set an air flow rate which holds a steady pressure. The isolator tends to either rise or fall in pressure with small air pump adjustments. However, if you have a fairly large isolator, say larger than 3 cubic metres, give it a go. At least the maths is easy!

c) Nitrogen Dilution
This is a sophisticated test and, whilst the results may be excellent, the equipment required makes the method rarely used in the pharmaceutical industry. The test involves filling the isolator with nitrogen and holding it at a negative pressure. A sensitive oxygen meter inside the isolator then follows the increase in oxygen concentration as the isolator leaks air inwards. A plot of the oxygen concentration change gives the leak rate. The test is sensitive but not very practical and there may be some safety issues with handling nitrogen in this way as there is a risk of asphyxiation. (This is because there is no pre-indication to the human body of excessive concentrations of nitrogen as there is with carbon dioxide).

d) Parjo and Fosco Methods.
The nuclear industry is believed to have used the Parjo method for gloveboxes. The test equipment consists of a reference vessel inside the glovebox, with a glass tube connected into the top. The tube has a horizontal section into which a soap bubble is introduced. The soap bubble is effectively a frictionless piston. Any leakage of the sealed glovebox will cause the bubble to move along the tube. The speed at which it moves can be used to calculate the leak rate quite easily. The Fosco method replaces the bubble tube with a micromanometer. In practice, these methods only work where very low leak rates are involved and where conditions for testing are well controlled. Not ideal then for busy hospital pharmacies!

Theoretical considerations for pressure decay

Perhaps the first thing you need to know here is how to convert raw pressure decay data into percentage volume loss figures. The calculation is derived from the universal gas law and, in the easy-to-do form, the leak rate equation is as follows:

$$\text{Equation 1} \quad L = \frac{PD \times 6{,}000}{SP \times M}$$

Where:
L = Leak rate in % volume loss per hour
PD = Pressure decay in Pascals
SP = 101,325 + starting pressure in Pascals
M = Test time in minutes

Or if you want to know the decay allowed for a given leak rate, the leak rate equation looks like this:

$$\text{Equation 2} \quad PD = \frac{L \times SP \times M}{6{,}000}$$

As an example for Equation 1, suppose that an isolator is tested starting at 200 Pa for 5 minutes and the resulting pressure decay is 25 Pa. The equation tells us that the leak rate was 0.30% volume loss per hour, just short of achieving Class 2 (0.25%).

As an example for Equation 2, if you wish to test for Class 2 leak rate, you might choose a starting pressure of 150 Pa and a test time of 10 minutes. Equation 2 then tells you that the allowable decay is 42 Pa. In many ways, Equation 2 is more useful, but the two equations are the same thing, just with a different prime subject.

Having mastered the leak rate equation, the next decision is the starting test pressure. The four classes of leak rate may be assumed to be at working pressure and we could indeed measure the leak rate at working pressure; however, some added safety margin would be the norm and a quick test is generally preferred. Thus some multiple of working pressure would be rational. Some testers use pressures as high as five times working pressure but three times working pressure is probably adequate. Two times working pressure may also be considered adequate as long as the time of the test is such that the isolator does not actually drop below working pressure.

The next consideration is the arrangement of the isolator during pressure decay tests, more specifically, what do you do with the sleeves, gauntlets or half-suits? If these item move around in the test, the volume changes and the pressure changes accordingly. Perhaps they should be removed during the test and blanked off, but then the sleeves and suits are the parts most prone to develop leaks. The practical solution for sleeves is to evert them, i.e. allow the internal pressure of the isolator to push them completely out of the isolator in the case of positive pressure isolators or to pull them into the isolator in the case of negative isolators. In this condition they act as pressure compensators and hold up the isolator pressure, but at least they present a known and constant error; which is not ideal, but is at least practical.

For half-suits, the best plan is to agitate the suit gently while the isolator is above test pressure until the suit settles in a stable position.

The pressure decay test procedure overall then consists of the following stages:

a. Decide what class of leak rate your isolator is required to achieve, on the basis of the design of the isolator, the process in the isolator, the transfer methods used and the room housing the isolator
b. Decide what test pressure to apply.
c. Decide how long the test should be and specify the maximum allowable pressure decay in that time.

d. Alternatively decide a specific pressure decay magnitude and specify the minimum time for that decay to occur. It should be noted that in the case of an isolator with good leaktightness, this method could take a very long time!

e. Ensure that the isolator is in stable thermal conditions, which means it should be turned off and equilibrated at room temperature.

f. Seal up all the ports, doors and valves.

g. Attach a suitable fan and valve, and a calibrated micromanometer.

h. Raise the internal pressure to around the test pressure and evert the sleeves and gloves, or shake down the half-suit.

i. Raise the internal pressure to above the test pressure and allow the pressure to decay back down to the test pressure. Note the time.

j. Run the time duration of the test and note the final pressure (or, if alternative d). is being used, allow the prescribed pressure decay to occur and note the final time).

k. Calculate the leak rate using Equation 1 above.

l. If the test fails to meet the chosen leak rate, repeat two or three more times to obtain consistent results.

m. If the test still fails, proceed to leak detection – see later.

A negative pressure test may be applied to negative pressure isolators, but a major concern with negative isolators, which are normally specified when containment is important, is that containment should be maintained if there is a pressure reversal and the isolator goes positive. This may occur due to due to exhaust fan failure, room pressure faults or even excessively rapid in and out glove movements. For this reason, some users will test negative isolators at positive pressure, arguing that this represents the worst case scenario for leakage.

Practical values and further considerations for leak rate measurement

So what test pressures and test times might be chosen by the isolator operator or test engineer? Three times working pressure has already been suggested and if an aseptic isolator is running at 50 Pa, then we have a comfortable test pressure of 150 Pa. As regards test time, we probably want a quick test, but we also want enough time to generate a repeatable result. 1 minute is a short test, 20 minutes is perhaps a long test, so 10 minutes may be a rational compromise.

What decay is allowed under this test? Using Equation 2, for Class 2 we get an allowable drop of up to 42 Pa.

However if we apply the same equation for Class 3, we get a drop of 169 Pa which is clearly unusable. We therefore need to shorten the test to 5 minutes, giving an allowable drop of 84 Pa.

Apart from problems with the movement of sleeves and suits during the decay test, we still have the universal gas law to contend with. If the air temperature inside the isolator changes during the test, then the pressure inside changes. Likewise if the ambient atmospheric pressure changes during the test, then the differential pressure changes. Is this significant?

The practical answer is a qualified "no", provided that the following are true:

a. The testing is only to meet Class 2 or Class 3 leak rates.

b. The test is short, not more than, say, 10 minutes.

c. The isolator is in thermal equilibrium with the surroundings which themselves are stable.

d. The outside weather is not changing quickly – no violent storms are passing by – and there are no changes within the building due, for example, to doors opening or HVAC systems readjusting.

Testing to Class 1 is not really relevant to pharmaceutical isolators but, for installations where it is has to be applied, more information on the necessary corrections is given in the Appendix at the end of this paper.

The more pedantic of critics will point out that the pressure decay test does not measure leak rate at a constant pressure, and that actual leakage will be more at high pressure, reducing steadily as the pressure decays. As mentioned, the pressure hold test overcomes this issue, however the decay test is still

Table 1. Leak Rates Compared.

ISO 14644 Class	% vol. loss per hour	Decay rate (Pa per hour)	Decay rate (Pa per minute)
-	0.03	30	0.51
1	0.05	51	0.85
-	0.1	101	1.7
2	0.25	254	4.2
-	0.5	507	8.5
3	1.0	1,015	17
4	10.0	10,150	169

Table 2. Maximum Time Allowable for a 42 Pa Decay Test

ISO 14644 Class	Decay (Pa)	Maximum Time (Mins)
1	42	50
2	42	25
3	42	2.5
4	42	0.25

Table 3. Maximum Decay Allowable for a 10 minute Test

ISO 14644 Class	Time (Mins)	Decay (Pa)
1	10	8.4
2	10	42
3	10	169
4	10	1,690

valid if the test pressure is above working pressure. A successful result will ensure that the leak rate at working pressure is better than the specified class. Most operators will simply accept this caveat for the decay test.

Tables 1, 2 and 3 may be useful in comparing leak rate expressions.

Leak detection methods

If the leak rate test shows that the isolator does not meet the specified class, then detection is going to be needed to track down the source, or sources of the problem. All leak detection methods are time-consuming.

a. Helium is often the detection method of choice. In this case the sealed isolator may be carefully brought to 100 Pa or 200 Pa pressure with helium from a cylinder. A helium detector or "sniffer" is then used to search for points of leakage. The more expensive types of helium detector, not surprisingly, are more sensitive. The advantage of helium detection is that the gas is inert; however, the results are patchy and tend to be not easily repeatable. Even so, most isolator installations will have access to helium leak detection.

b. DOP (Dispersed Oil Particulates or "smoke") can be used for leak detection. A hot or cold smoke generator is used to put smoke into the isolator which is then sealed and taken to a test pressure. Either a particle counter or a photometer can be used then to scan the structure and locate leaks. In the case of negative pressure isolators the detectors may be inside the isolator which is at test depression, and smoke is applied to likely leak points around the outside of the isolator. Two disadvantages of this test are the potential deposition of oil and the likely activation of smoke alarms if they are not switched off.

c. Ammonia provides an excellent method for leak detection. It is sensitive, inexpensive and will trace small leaks with absolute repeatability. To carry out this test, a bottle of around 50 ml of ammonia solution is placed inside the isolator together with a pad of lint-free wipers and a polyethylene bag. The isolator is sealed up and using the gloves, the ammonia is poured out onto the wipers to disperse through the isolator volume. The isolator is then raised to perhaps 100 Pa pressure and proprietary bromophenol cloth is applied to the seals and joints of the isolator. Where ammonia escapes, the cloth turns from yellow to blue. At the end of the test the wipers are placed in the plastic bag and the isolator is ventilated, ideally to atmosphere. Ammonia is of course pungent, but the quantities used are small and the real hazards are small. This method is highly recommended to isolator users.

d. Soap solution and ultrasonic detection. The soap solution test is exactly the same as is applied to pneumatic tyres. The method is cheap, sensitive but messy and laborious. Ultrasonic detection may detect large leaks from isolators under high test pressure but is generally not a useful test for isolator leaks.

e. Progressive elimination. When all else fails, progressively sealing off the various sections of the isolator, where practical, may help to pin-point leaks.

Component leak testing

Gloves, sleeves and half-suits are the most likely source of leaks in a well-built isolator since they are subject to much wear-and-tear. They are essentially consumable items. They can be leak tested as part of the complete isolator, or they can be tested separately.

Many isolator manufacturers offer test devices which close off the sleeves or gauntlets of the isolator and then apply a manual or an automatic leak test by pressure decay. Similar devices may be available for half-suits.

One manufacturer offers a device which leak tests sleeves and gloves, or gauntlets, in situ, using nitrogen dilution, but this is an expensive and time-consuming method.

Leak test rationales

When should leak rate measurement take place? The following occasions would seem to be logical:

a. FAT (Factory Acceptance Test)

b. SAT (Site Acceptance Test)

c. OQ (Operational Qualification)

d. PPM (Planned Preventative Maintenance)

e. Following any maintenance or repair which has breached the sealed structure

The distributed leak test

Pharmaceutical Isolators mentions a 'distributed leak test'. This is really only a leak detection and repair exercise carried out immediately prior to an overall leak rate measurement test. The objective is to set a baseline for subsequent leak rate measurement tests. Whilst there might be benefits in carrying out this exercise, it is recognised that leak detection tests may not detect all leaks due to inaccessibility. Therefore the objective of setting a baseline might be unrealistic.

Induction leakage

Pharmaceutical Isolators also mentions induction leakage. This may occur where local air velocities within the isolator are such that the pressure within these flows is reduced by the Bernoulli effect. Thus a positive pressure isolator could locally generate a negative pressure. Such an effect could be significant when operators are leaving the sleeves, gauntlets or half-suit. Generally-speaking, this theoretical leakage is not an issue.

Training

Leak rate measurement is a relatively simple process but even so, some training for test engineers will give quicker and more reliable results.

Training can be obtained from the isolator manufacturers, from organisations such as the PHSS, and from a variety of specialist training companies and consultants.

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Appendix

Correcting the results of pressure decay tests for Class 1 leak rate

It would seem that the only way to make a pressure decay test of this sensitivity supportable is to apply the appropriate corrections to the decay figures. The corrections are made to the pressure reading at the end of the test thus:

- If atmospheric pressure rises, add 10 Pa to the final pressure reading for every 0.1 mb rise during the test.
- If atmospheric pressure falls, subtract 10 Pa from the final pressure reading, for every 0.1 mb fall during the test.
- If the internal temperature rises, subtract 3.5 Pa from the final pressure reading, for every 0.01°C rise during the test.
- If the internal temperature falls, add 3.5 Pa to the final pressure reading, for every 0.01°C fall during the test.

A two-place or even a three-place thermometer will be needed inside the isolator. A barometer resolving to 1 Pa will be needed outside the isolator.

In practice, the theoretical correction for temperature and pressure is difficult. Measurement of temperature at a single point in the isolator is by no means representative of the entire volume. Local heating may not be registered by the thermometer but will increase pressure. Measurement of atmospheric pressure to within a Pascal is not easy since rapid fluctuations take place at this level.

Measurement of very low leak rates by pressure decay, or indeed any other method, is difficult and the results must be regarded with some degree of scepticism.

i. BS EN ISO 14644-7:2004, Cleanrooms and associated controlled environments, Part 7: Separative devices (clean air hoods, gloveboxes, isolators and mini-environments)

ii. ISO 10648:1994, Containment enclosures, Part 2: Classification according to leak tightness and associated checking methods

iii. Pharmaceutical Isolators, B.Midcalf, W.Mitchell Phillips, J.Neiger, T.Coles, Pharmaceutical Press, 2004

iv. Full derivation from the universal gas law can be obtained from the author.

Tim Coles, BSc, MPhil, has worked in the field of isolator technology for over twenty years. He was a founding member of the UK Pharmaceutical Isolator Working Party that produced Pharmaceutical Isolators, Pharmaceutical Press, 2004, and more recently of the PDA committee that has just produced Technical Report No 51: "Biological Indicators for Gas and Vapour Phase Decontamination Processes" [for the validation of isolator sanitisation]. His book "Isolation Technology - a Practical Guide", CRC Press Inc. 2004, is now in its second edition.

Q and R are for...

Quality

(ICH Q9*) The degree to which a set of inherent properties of a product, system or process fulfils requirements.

ISPE Glossary of Pharmaceutical and Biotechnology Terminology (GAMP 5, A Risk-Based Approach to Compliant GxP Computerized Systems)

Qualification

(ICH Q7*) Action of proving and documenting that equipment or ancillary systems are properly installed, work correctly, and actually lead to the expected results. Qualification is part of validation, but the individual qualification steps alone do not constitute process validation.

ISPE Glossary of Pharmaceutical and Biotechnology Terminology (ISPE Baseline® Guide: Vol. 6, Biopharmaceutical Manufacturing Facilities)

Requalification

Execution of the test sequence specified for the installation to demonstrate compliance with ISO 14644-1 according to the classification of the installation, including the verification of the pre-test conditions.

ISO 14644-6:2007: Vocabulary

Editor's note: This is the only definition that I could find and is specific to ISO 14644-1. My own definition would be "The qualification process repeated at prescribed intervals throughout the lifetime of an installation".

Risk

Combination of the probability of occurrence of harm and the severity of that harm.

ISO 14644-6:2007: Vocabulary

Hazard

Potential source of harm

ISO 14644-6:2007: Vocabulary

Risk assessment

(ICH Q9*) A systematic process of organizing information to support a risk decision to be made within a risk management process. It consists of the identification of hazards and the analysis and evaluation of risks associated with exposure to those hazards.

ISPE Glossary of Pharmaceutical and Biotechnology Terminology (GAMP 5, A Risk-Based Approach to Compliant GxP Computerized Systems)

Rapid transfer port (RTP)

A transfer device in the form of a double-door transfer port system used to move items from one isolator to another without contamination entering or escaping from the system.

Pharmaceutical Isolators, Pharmaceutical Press, 2004

**ICH is the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH publishes a series of Quality Guides. The subject of ICH Q7 is Good Manufacturing Practice and of ICH Q9 is Quality Risk Management. These are available as free PDF downloads on the ICH website: http://www.ich.org*