Minimizing the Risk of Secondary Contaminations Through the Use of Isolators for Sterility Testing

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ABSTRACT

Today, isolators are nearly mandatory in every up-to-date sterility test laboratory. The risk of secondary contamination caused by the analyst or through the environment can be minimized by using isolators to perform sterility testing. The operator has to bear in mind that by using isolator technology no absolute safety regarding secondary contamination is guaranteed - a certain risk for secondary contamination during the performance of the sterility test still persists either through contamination derived from the environment, the surrounding air or through the sterility test itself.
The risk for secondary contamination can be circumvented by professional handling of isolator technology, by employing well trained operators and by gaining awareness about secondary contamination.
The following report summarizes the experience gained with isolator technology for the performance of sterility tests over a time frame of 15 years. The emphasis is especially laid on the development of isolator technology in the sterility testing environment regarding future developments.

1. Sterility Testing Isolators in Practice

1.1 Introduction to Sterility Testing Isolators

In 2009 two identical isolators have been in use to perform sterility testing at Novartis Pharma AG. The manufacturer of the isolators is SKAN AG, CH-Allschwil (Switzerland), which was not only responsible for the design of the isolators but for the complete commissioning and qualification as well.

These isolators are so-called „rigid-wall" isolators, contrary to „soft-wall" isolators, and consist of a rigid housing made from glass and stainless steel (see picture 1). They are equipped with a large front door that can be swung open vertically to allow for easy loading or unloading. This door includes 4 glove ports through which the analytical work inside the isolator can be executed. Another feature is the equipment with 4 HEPA filters to redundently filter the air (1x supply air filter, 2x recirculation air filters, 1x exhaust air filter). The exhaust air is ducted and directly discharged to the atmosphere.

The isolators are equipped with sensors to effectively control and monitor all relevant parameters: physical parameters as temperature, air flow velocity, differential pressure to the surrounding room, humidity and quantity of H_2O_2 (hydrogen peroxide) evaporated and monitoring particle and air

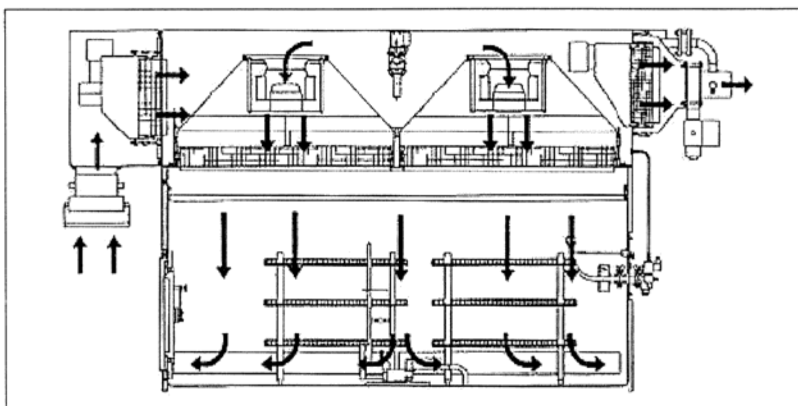
borne bacterial counts. All physical parameters are recorded, processed and stored in the monitoring system. Any deviation outside of given limits will generate an immediate alarm which either triggers an action by the isolator system software or asks for an interaction by the operator (e.g. aborting the sterility test process in case of below limit pressure loss in the isolator).

Decontamination of the isolator interior is performed with gaseous H_2O_2 .The isolator infrastructure holds a bottle with liquid 35% H_2O_2 on a balance. To start the decontamination cycle the previously validated quantity of liquid H_2O_2 is dosed by a peristaltic pump onto a hot plate in the upper plenum for evaporation. The gaseous H_2O_2 is entrained with the air stream of the recirculation fans and directed to the isolator work space through the terminal HEPA filters.

The isolators are equipped with a RTP (Rapid Transfer Port) system to allow a direct and contamination-free transfer of autoclaved materials to the isolator work space from a RTP container docked to the RTP double door of the chamber. In the middle of the work surface a built-in peristaltic pump makes up part of the sterility testing equipment performed on the basis of membrane filtration. Additionally a magnetic stirrer is included (to help to dissolve products for some of the samples) as well as 2 racks to store all the materials used for the sterility testing within the work space.

Figure 1

Figure 2



Air flow in the „rigid wall" – sterility isolator

1.2 Instrumentation for Work with Sterility Test Isolators

Instrumentation of the isolators does not only include the above mentioned peristaltic pump and magnetic stirrer within the work space but a particle counter as well as an airborne sampler.

Furthermore, an autoclave is needed for the sterilisation of various materials (scissors, forceps, ampoule breakers) loaded in the RTP container.

A filter integrity test unit permits verification the integrity of the vent filter on the RTP containers and a glove integrity tester to detect leaks in gloves.

1.3 Key Figures

The work chamber of the isolators is about $1m^3$. This volume is held under positive air pressure of about 60 Pa. The pressure may vary during the performance of the sterility test because of the handling with the gloves. But an overpressure of minimal 15Pa must be maintained. Failure to do so leads to an alarm and measures are to be taken accordingly (e.g. potentially aborting the sterility test process).

The air flow velocity within the work space is set to 0.45m/s ±20% during the decontamination process. After decontamination, for the stages "at rest" and "in operation", the air flow velocity is reduced to 0.1 – 0.3m/s to reduce energy consumption and because of missing relevance of a higher air flow velocity to the sterility test result. The temperature within the isolator is only indicated but not controlled. Thus the sterility test process is run at ambient temperature, with a slight increase of 2 – 3°C during the decontamination process step.

During the conditioning phase of the isolator, prior to the proper decontamination phase,

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the relative air humidity is lowered to 20% to allow for an optimal take-up of the gaseous H_2O_2 in the decontamination phase.

For reasons of occupationnal health and safety the room in which the isolators are located is monitored for H_2O_2 emissions with the help of Dräger sensors in order to alarm at levels above a TLV (Threshold Limit Value) of 0.5ppm. Operators are thus alerted and can safely leave the room, eg. in case of an accident. The isolator inner volume is not monitored for H_2O_2 concentrations but the amount of gaseous H_2O_2 is controlled by the balance.

To initiate the decontamination process 70g of 35% H_2O_2 are evaporated. Then, at intervals of 3 minutes each, additional 10 doses of 7g H_2O_2 each are evaporated to maintain the decontamination effect and to obtain the required decontamination efficiency. After that process phase the isolator is purged with HEPA filtered air from the surroundding room in order to reach a final 1ppm H_2O_2 concentration within the work space of the isolator. This residual concentration allows the performance of sterility testing without any inhibiting effect.

Total decontamination cycle time is 2.5 hours.

1.4 Initial Validation of a Sterility Test Isolator

The acceptance inspection of the isolator by the user is followed by the IQ (Installation Qualification) realised by the supplier.

As part of the OQ (Operational Qualification) and previous to decontamination cycle development, a material qualifycation study [1] was established. This study investigated the suitability of various materials used in the construction of the isolator as well as for materials from consumables used for the sterility test as regards to the decontamination process. The study was elaborated in a common effort between user and isolator manufacturer. A few unqualified materials identified in the study were replaced by more suitable materials.

Decontamination cycle development has been executed by the isolator manufacturer according his procedures [2]. The objective was to assure a SAL (Sterility Assurance Level) of 10^{-6} after decontamination. Biological indicators (BIs) with spores of *Geobacillus stearothermophilus* were employed to make the proof. Then and with the same type of BIs, the user performed the subsequent PQ (Performance Qualification) to qualify the developed decontamination cycle for each loading pattern (see 1.5) in three independent repetitions. To determine the required purge time (to liberate the isolator from H_2O_2) the concentration of H_2O_2 inside the isolator is measured with the use of Dräger tubes at a number of intervalls during the purge phase. After a purge time of 90 minutes the maximal TLV of 1 ppm has been safely and reproducibly reached for all different loading patterns.

Additional microbiogical tests complement the PQ: e.g. the verification of integrity of all materials used against the decontamination process (nutrient media flasks, sample bottles), the influence of residual concentrations of H_2O_2 on the sterility test result, or to contact/settling plates routinely used for environmental monitoring.

Following PQ all the steps to undertake the sterility testing as well as the details of the environmental monitoring program have to be defined. Prior to startup operators have to be trained and qualified for the job at the isolator.

Routine, regular requalifications are required to guarantee proper operation. These include physical testing of all sensors (calibrations), inspecttion of the air handling system (qualification), verification of the decontamination effect using BIs, and retraining the operators. Upon modifications to the isolator or of the process, adequate requalifications will be necessary.

1.5 Loading Pattern of Sterility Test Isolators

The definition of one or more loading patterns, with all auxiliary materials and consuma-bles (samples, nutrient media, filter units) required for the sterility test, comes to the fore when the validation of the decontamination process is determined. Loading patterns, for which the decontamination process has proven to be effective for all exposed surfaces, have to be exactly observed in routine, to avoid any contamination risk from insufficiently decontaminated exposed surfaces.

For both isolators presented here, well defined and validated loading patterns are on hand. These include the empty isolator as well as two different maximal loading patterns: one for the sterility test by the membrane filtration method and one for the sterility test by the direct inoculation method. Both methods of sterility testing need different materials. Having a validation for a maximum loading pattern as well as for the empty isolator, loading patterns with less than maximum loading are included and therfore valid.

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2. Sterility Tests performed in Isolators

For both isolators approximately 4'000 sterility tests are analysed per year in so-called sterility test sessions. For each sterility test session the isolator is loaded according to the defined pattern and then decontaminated with the qualified decontamination cycle. Subsequently the sterility test session is held operating in the work space with the help of the 4 gloves. During and just after completion of the sterility tests, microbiogical environmenttal monitoring (air, surfaces) is performed to prove the perfect quality of all conditions within the work space. At the end of a session, the isolator is opened and all materials and tests are unloaded. Then the work space is cleaned and desinfected (by wi-ping) and the gloves tested for integrity. Upon that, the isolator is ready for the next sterility test session. For the isolators descrybed, up to a maximum of 12 sterility tests can be run at a time - depending on the size of the test samples to be analysed (1 – 100 ml). Depending the complexity and duration of the tests, 1 to 2 sterility test sessions are manageable per day and isolator. Typically the isolator is loaded in the afternoon and the decontamination cycle run over night. This allows the opening of the sterility test session the following morning.

The „initial failure rate“ is an important key figure for every sterility test lab. It defines the number of contaminations of the sterility test, calculated from the number of product contaminations as well as from the num-ber of secondary contaminations, as well. This rate has been found to be in the range of 0.05 - 0.3% for the system presented here.

3. Sterility Testing Isolators: Past and Present

Until 1995 the sterility tests at Novartis Pharma AG have been performed in a conventional clean room (class A, surrounded by class B). Various drawbacks (e.g.missing pass through with decontamination capability to decontaminate exterior surfaces of sterility test materials, only manual desinfection facilities for the clean room) and the fact, that operators have been „isolated“ in the clean room (not accessible for other collaborators) and that the „initial failure rate“ was relatively high, in the range of 1 – 1.5%, led to the decision to switch to isolator technology.

A „soft wall“ isolator (picture 3) has been set up in the sterility test lab in 1993. Due to the early stage of isolator technology in the field of sterility testing, only very limited qualification knowhow was available – neither from the manufacturer nor from the user side – and therefore final qualifycation has been finished in 1995 only and the first sterility test run the same year. The „soft wall“isolator had a large work space acessible through a half-suite in the middle of the work bench. Four gloves allowed for access to the work space from around the isolator. The work space as well as the two pass throughs could be decontaminated with peracetic acid independently. Shelves in-side the work space were used to stock materials for the sterility test (e.g.nutrient media).

Compared to the sterility testing in a conventional clean room, the handling in the isolator demonstrated some distinct advantages. Thanks to the validated and thus reproducible decontamination procedure, the sterility tests have been performed in a microbiologically well defined and controlled environment. The acceptance of the isolator by the operators has been very high after a short change-over, especially the fact of being personally less isolated than in a clean room. The „initial failure rate“ could be lowered down to appr. 0.5%. During inspections the isolator was the „epicenter“ of the microbiological lab. The acceptance from the various authorities was also very good.

Still, this technology had a few drawbacks. The decontamination process was not automated, meaning, that valves had to be shut/switched prior to decontamination.

Figure 3

„Soft-wall“ sterility isolator

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From time to time the peracetic acid vapours from the external evaporator were therefore directly passed over to duct instead into the work space. Misleadingly, the work space was considered to be decontaminated. The monitoring at that time was very simple, with no alarm management, and manipulation errors as described have not been recognised automatically. Another drawback was the persistent smell of peracetic acid in the room surrounding the isolator. This was even more a source of irritation because of the known carcinogenic potential of that chemical. Bear in mind that peracetic acid is inflammable, and a few incidents have happened due to the fact of absorbing spills of peracetic acid with towels that started to burn later on in the waste bin. To work in the half-suite was not everyone's job. Operators with claustrophobia were not able to use that suit. It was considered a disadvantage too, that it was virtually impossible to integrity test either the half-suite or the isolator as a total, only visual checks were feasible. Over the years the material of the soft wall and the half-suite became untight. Although leaky areas could be temporarily sealed with tapes, the material itself became turbid upon the attack of the peracetic acid. All of this led to inflammation and irritation of the eyes of the operators that had to work under these conditions. A regular replacement of the soft wall was considered too expensive.

Besides attacking of the soft wall, the peracetic acid was further responsible for strong corrosion of all other materials that came in contact with it. After 3 years the isolator looked pretty rough.

A few contaminations in the work space of the isolator revealed another drawback. Whereas the pass throughs with the test materials have been decontaminated regularly, the work space of the main unit has been decontaminated every month only. A contamination present in the isolator work space could therefore survive and be carried over to sterility tests over a period of several days.

Summarizing the general advantages of isolator technology and the distinct disadvantages of the soft wall isolator resulted in the decision to continue to perform sterility tests in isolators but to switch over to a new design of isolator.

The previously described „rigid wall“ isolator with integrated H_2O_2 decontamination system was thus taken in operation in 1999. A major advantage was the fully automated H_2O_2 decontamination process. Operating errors which could adversely affect the decontamination effect are excluded. H_2O_2 as the decontamination agent is much less harmful.

Because of the modified operational mode – the work space is decontaminated before each sterility test session – potential contaminations can be contained to that one session. This allows for more flexibility too. The „initial failure rate“ could be further reduced to 0.05–0.3%.

The new technology introduces other drawbacks. Due to the higher complexity of the equipment failures can't be self eliminated easily, an external support from the manufacturer is needed. This may prolonge shutdown time. A major weak point – leaks – has definitely been minimised by the use of glass and stainless steel. Gloves and sleeves still require focus with regards to leaks. But these disadvantages can be handled: to control eventual leaks in gloves and sleeves a preventive maintenance program has to be established before leaks develop. Additionally a routine program covers the integrity test of gloves and sleeves.

The advantages of this isolator technology definitively overbalance the disadvantages.

4. Sterility Test in Isolators: Future Developments

As a result of the advantages described beforehand and in view of the handling of the drawbacks of isolators, this technology will still be the technology of choice for the performance of the sterility testing.

Nethertheless users expect and ask for further developments and improvements. Mostly higher flexibility and higher performance are specified to get a better throughput time – simply more and more complex sterility tests will have to be analysed in the isolator in a shorter time. This can be accomplished with the introduction of fast transfer pass throughs which allows the transfer of materials for the sterility test in a much shorter time.

When handling highly active and potent substances the specific features needed for the appropriate protection of the operator and the environment have to be built in, e.g. with the use of specially adopted filter systems. Ergonomic aspects, as optimal positioning within the work space of the isolator, optimal illumination of the work zone, or reducing noise are receiving more emphasis in the design of new isolators.

The further reduction of the "initial failure rate" is another design topic for new isolators. Especially secondary contaminations have to be eliminated as more and more highly valuable sterile products will be on the market, for which a lot rejection upon unproven secondary contaminations represents a intolerable financial risk.

5. Operating Experience with Sterility Isolators

On the basis of many years use of isolators for sterility testing, ample experience with microbiogical contaminations has been gathered. In spite of good planning, of well qualified isolators handled by qualified and well trained operators, microbiogical contaminations are not fully excluded. These microbiogical contaminations in the isolators can be detected either by microbiogical environmental montoring during or after a sterility test session, or the microbiogical contaminations are discovered through contaminations of the sterility test itself. Vulnerabilities that led to microbiological contaminations in the isolators within the last 15 years experience with sterility test isolators have been identified. The following case studies do not represent a complete listing of all microbiogical contaminations.

5.1 "Ring of contamination"

With the use of RTP systems for contamination-free transfer of autoclaved materials from the RTP container to the work space of the isolator the so-called „ring of contamination" gets of special interest. This is a small circular area at the double door of the RTP system that will neither be sterilised on the RTP container by autoclave on one side, nor be decontaminated by the decontamination process of the isolator on the other side. The „ring of contamination" thus represents a potential contamination source when transferring materials from the RTP container to the isolator and may lead to contaminations of the work space. It is therefor required to manually disinfect both docking areas of the RTP container and the port at the isolator first, and second, to disinfect the „ring of contamination" from within the isolator before transferring any materials into the work space.

5.2 Surfaces not decontaminated

The decontamination with H_2O_2 is a method applicable to surfaces, means that surfaces not exposed to the gaseous H_2O_2 will not be decontaminated. Well defined loading patterns with well defined positions for all of the materials are thus essential. Any contact areas have to be minimised. As some contact areas can not be fully avoided (e.g. bottom side of nutrient media bottles) it must be attempted to reduce any contact area to its minimum, for example to place materials on large gridded supports. Bioload on these surfaces have to be kept very low already before decontamination. This can be accomplished with the use of gloves when loading the isolator, and previous disinfection of all these materials.

5.3 Unclean Surfaces

Any surface to be decontaminated has to be clean and free of residuals. „Unclean" surfaces lead to the situation that gaseous H_2O_2 will not develop sufficient decontamination efficiency to effectively reduce the bioload. Less resistant vegetative germs may be inactivated by the decontamination process but more resistant spores will survive on these surfaces.

5.4 Use of Materials unsuitable for Isolator Processes

Sticker

Experience has shown that stickers used to label sterility tests are often contaminated with spores on the adhesive side which are then disseminated within the isolator by gloves.

Silicon Oil

Silicon oil that helped to more easily dock/undock RTP containers to the isolator port was heavily contaminated with *Propionibacterium acnes*. It was virtually unpossible to eliminate the contamination, and only upon change of all seals the contamination problem was solved.

5.5 Seals of RTP Containers

A number of contaminations within the isolator could be attributed to unsufficient sealing of the RTP containers. The cover seal lost its sealing function after a few autoclavings. Therefore contaminations found their way into the RTP container through that gap during cooling (in the sterility test lab). The contaminations were subsequently found within the isolator after docking.

5.6 Leaks in Gloves

Currently leaks from gloves are believed to be the main source of contaminations in isolators. In our personal experience no correlation could be demonstrated between leaks in gloves and contaminations in isolators. In fact internal investigations have proven that the loss of integrity of a glove will not lead to a migration of microorganisms into the isolator – due to the low bioload on the external side of the glove (provided regular disinfection).

6. Conclusion

Reflecting 15 years of isolator technology the statement can be made: it was a correct decision to perform sterility testing with isolator technology solely.

To comply with future challenges in this field users rely on isolator technology and further developments associated with it.

Isolator technology has gained a very high acceptance from authorities and is considered as state-of-the-art.

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