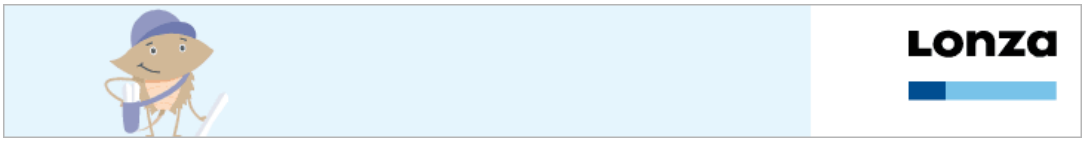

Microbiology in Filling and Sterility Test Isolators

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Isolators in the pharmaceutical environment are used to produce and test sterile drug products with a minimized risk of microbiological contamination from the surrounding. Isolators realize a separated aseptic core practically free of microorganisms, what offers maximized protection for production and testing. For the realization of such an isolated aseptic core, considerations in microbiology play a crucial role in the validation, maintenance and control of the asepsis of an isolator.

Isolators should be run with an adequately developed decontamination cycle to achieve a desired level of microbial reduction in the isolator chamber prior production or testing. After achievement of aseptic conditions by decontamination, the maintenance of the asepsis in the isolator chamber is controlled by the applied overpressure and other control mechanisms in place. A well-designed chamber, adequate maintenance and an established monitoring program play a decisive role in keeping an isolator under control. However, microbiological control starts much earlier in the conceptual design of the isolator with a well-accessible and well-designed chamber which is good to maintain and with surfaces and materials easy to clean and decontaminate. The development and qualification of an adequate decontamination cycle, using biological indicators of Geobacillus stearothermophilus [1,2] is essential, and should be verified for every routine cycle by a defined physical and microbiological monitoring program. Physical environmental monitoring gives indirect information on the microbiological status of the isolator, as some of the detected particles could be microorganisms (however, up to now no correlation is known on this matter). More significant is the microbiological environmental monitoring with nutrient media. Relevant for microbiological control is also the differential pressure of the isolator, as ingress of microorganisms from the surrounding is possible in case of downfall of the pressure to (or under) the level of the environment. Therefore, production isolators should be located in a microbiologically controlled environment (Zone C or D) to minimize these scenarios (and also to minimize the bioload of the material prior to decontamination) [3]. Other physical parameters liketemperature, humidity and air velocity do not have an impact on the microbiological status, if they are in normal working range.

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Even though the high reliance of microbiological reduction by the decontamination step and if all parameters of the decontamination cycle are within the validated range, a proper aseptic working technique is still required for testing and production. The area and surfaces in the isolator enclosure should still not be considered as "sterile", what is also expressed in the nomenclature that microbiological reduction in the isolator is achieved with a decontamination cycle and not with a sterilization process. Thus, operators should be aware that a good aseptic working is required for testing (e.g. sterility testing) and manufacturing of sterile

interventions. The material chosen for gloves and sleeves is thus essential for a proper aseptic working technique. Synthetic rubbers and elastomers like hypalon and EPDM (ethylene propylene diene monomer) offer these properties [4]. These materials provide a lot of capabilities in terms of stretching and elasticity for performing the desired intervention, however, they should be considered as one of the major weak points regarding integrity in isolators and a periodically performed integrity testing is fundamental. Checks should be applied prior to production or testing to eliminate leaky gloves and minimize the risk of compromising the quality of the manufacturing or testing. Integrity checks of gloves and sleeves can be performed visually (inspection by the operator), chemically (e.g. with ammonia, helium or peracetic acid) or physically (e.g. pressure hold test or flow test). Visual integrity checks can be very fast and reliable, if the operators are well-trained in the detection of holes and leaks. For chemical and physical checks, usually instruments are needed for testing and the testing periods are rather long. Additionally, these methods can show restraints in the localization of the hole and problems with false-negative results, as some leaks cannot be detected due to the dimension of the hole (e.g. tear-shapes holes are not easy to detect, [4]. Beyond period checks for integrity, gloves and sleeves should be replaced preventively.

Other parameters which could influence and impact the decontamination effect are deviations from the validated loading pattern or deviations in the amount of decontamination agent used, the air velocity, the time of decontamination and time of aeration [5]. Thus, all these parameters should routinely be checked prior to start of manufacturing or testing. The achievement of the desired level of microbial reduction by decontamination depends on the kind and amount of microorganisms typically found in the loaded isolator prior to the decontamination step. To verify the desired assurance level of microbial reduction, a regular check of the bioload prior to the decontamination should be performed, e.g. during yearly requalification.

The greatest significance in controlling the microbiological status of an operating isolator is produced with microbiological air and surface monitoring. Due to the nature of the methods used for microbiological monitoring, a continuous control is not possible for surface monitoring. Air monitoring can be conducted at relevant time points, a semi-quantitative monitoring is possible with settling plates, which are exposed to the environment for a defined (and validated) time span, e.g. 4 hours. Each sampling procedure only provides information of the particular sampling point at a particular sampling time. Additionally, there is a certain rate for the recovery of micro-organisms from the surface, so that microbiological monitoring can only be considered as spot checks of a vast area and it is possible that microbiological contaminations on surfaces or in the air in an isolator could go unnoticed. In sterility test isolators, contaminations in the environment do not have a direct impact on the significance of the test results, as long as they do not affect the sterility tests by secondary contamination. Nevertheless, each finding of microorganisms in environmental controls in isolators should be cause for major investigations, as their occurrence contradicts the efficiency of the developed decontamination cycle.

> “If environmental monitoring is performed by manufacturing personnel, an “environmental monitoring surprise” by QA personnel should be considered to verify significance and the validity of the data produced in the delegated monitoring.”

Sampling points for microbiological monitoring should be defined after the qualification of the decontamination cycle on points representative for the filling or testing operation. Because of the significance of the monitoring for aseptic processes, each sampling point should be laid down in a written, QA-approved assessment, in which the rationale for this sampling point is demonstrated. Of particular interest are points with proximity to the open product, to mouseholes (a direct connection out of the aseptic core, e.g. used for the exit of filled vials) and transfer systems (like Rapid Transfer Ports or airlocks for the introduction of sterilized and decontaminated material into the aseptic core, e.g.). Others are areas of frequent interventions, and points with higher bioloads prior to decontamination, as well as gloves and sleeves. As the used material of surfaces directly impacts the resistance of micro-organisms to the decontamination cycle by an alteration of the D-value [6], materials which are harder to decontaminate should also be considered as sampling points. The sampling points should be re-evaluated periodically on the basis of trending data obtained from the regular microbiological monitoring results, changes on processes and equipment, and the knowledge of (new) weak points in the manufacturing or testing. If environmental monitoring is performed by manufacturing personnel, an “environmental monitoring on surprise” by QA personnel should be considered to verify the significance and the validity of the data produced in the delegated monitoring. QA should also be involved in an

quantitative air sampling method, e.g. by the impaction method [7]. Quantitative sampling can be applied whenever feasible (e.g. beginning, middle and end of filling, or after special interventions). Surface monitoring of manufacturing isolators should be performed at the end of batch filling. A monitoring of surfaces during production is not recommended, as additional interventions during the filling are needed for sampling and residuals of nutrient medium can endanger the asepsis of the interior of the isolator. If batches are manufactured in campaigns with several batches during several days, the monitoring can also be done at the end of the campaign production. However, it has to be considered that satisfactory monitoring results at the end of a campaign might not be representative for the first batches of the campaign due to a decline of count numbers. Additionally, unsatisfactory monitoring results might question a whole campaign production and not just a particular batch. Hence, a batchwise monitoring is recommended and preferable not just due to quality, but also to business reasons.

Usually Tryptic Soy Agar (TSA) is used for environmental monitoring. The nutrient media should be terminally sterilized (by radiation) and wrapped in a gastight foil to prevent penetration of the decontamination agent. It is also crucial to use agar supplemented with adequate neutralizers against the decontamination agent (and disinfectants), this neutralizing effect has to be shown and validated. The growth promoting properties of a nutrient media batch has to be checked batchwise and preferably including in-house isolates. Additionally, radiation certificates ought to be reviewed prior to use. The media supplier should be audited periodically.

Microbiological Monitoring is usually performed aerobically. Nonetheless, on a regular basis, an anaerobic microbiological monitoring should be performed to produce data on the anaerobic status of the filling line. This is of particular interest in case of a positive result in the sterility test from the concerned filling line with anaerobes. Only a solid knowledge on the occurrence of anaerobes on the filling line and in the sterility test isolator can support the investigations for the cause of the positive testing result with anaerobes.

It is generally known and accepted that operators in a clean room and conventional filing lines are the major contamination source (8). As a consequence, isolators should be “free of micro-organisms”, as the personnel is completely separated from the production process – in contrary to conventional filling lines, where the Zone A is frequently exposed to the Zone B surrounding (where micro-organisms in low-levels are tolerated) for interventions in the filling process. In fact, the incidence rate of microbiological findings in isolators is usually lower than in conventional filling lines [8]. However, up to now in guidelines no differences regarding a “conventional zone A” and an “isolator zone A” appear. Nevertheless a deviation in the microbiological monitoring in an “isolator zone A” should lead to more thorough investigations as a deviation in a “conventional zone A”, since the major contamination source is barred out, the cycle for the decontamination of zone A is validated and the entire system is closed and mostly run in an overpressure. Hence, contaminations on sampling points must be considered as major deviations which might – depending on the time and point of sampling – as a possible cause for batch rejection. If a weak point is found during the investigation, it should be eliminated by suitable measures.

The microbiological validation of a filling line for aseptic processes is done with Media Fills. Media Fills simulate the manufacturing process and include compounding, filtration and filling of the primary packaging material with a suitable nutrient medium, usually Tryptic Soy Broth (TSB). Included in this process simulation should be the maximum time period and maximum amount of batches (for campaign production) and major interventions. The procedure of Media Fills is essentially the same and the requirements are identical for isolator and conventional filling lines [7, 9]. As interventions during the production in isolators are less critical than interventions on conventional filling lines, media fill validation in isolators is rather focused on the validation of the process and the manufacturing steps than on interventions. Important in this case is e.g. the replacement of gloves after detection of a leak during production. Media Fills are also necessary to qualify personnel in performing a defined kind and number of interventions in the process under supervision.

In summary, isolator technology offers an elevated state of product and testing protection and tangible advantages over the operations in a conventional Zone A/Zone B setup. The consideration of conceptual design aspects, adequate maintenance procedures and a properly developed and qualified decontamination cycle are crucial to bring an isolator under microbiological control. The qualification of isolator filling lines with an appropriate media fill process

testing without risk of secondary contamination.

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