The Fallacy of Proposed Annex 1 Revision Regarding Isolator Leak Testing
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In my opinion the concerns expressed in *GMP Annex 1, Draft Version 12, Example 4.23* regarding barrier technology and barrier glove integrity testing are excessive and do not consider actual risk.

I must assume that the hypothesis supporting proposed requirement 4.23 is that there is a critical level of risk associated with integrity of the isolator enclosure and with gloves used to conduct interventions during operations. To consider the actual risk associated with the use of gloves and isolator chamber integrity we must consider our history with isolators in terms of actual microbiological contamination risk. It is in my view inappropriate to initiate a regulatory compliance requirement based on a conclusion arrived at in the absence of actual data. Fortunately, such data exists, and studies have been done to evaluate the actual impact of gloves on microbial contamination risk.

There was information in the early days of isolator implementation circa 1990 that gloves installed in isolators were the primary mechanism for microbial ingress into isolator systems. The general cause of this problem was discovered to be the use of neoprene (polychloroprene) gloves. These gloves had two significant drawbacks: they were poorly resistant to puncture and prone to pinch damage and they deteriorated upon repeated exposure to vapor phase hydrogen peroxide (VPHP) used in isolator decontamination. The solution to this problem, which has proven to be extremely effective, was the use of chlorosulfonated polyethylene (CSPE) which was originally a product of DuPont Performance Elastomers and marketed under the tradename Hypalon.

Hypalon was both resistant to repeat exposures to VPHP and resistant to both penetration and pinch punctures. The switch to CSPE gloves in and of itself diminished glove leaks by well over 95%. The leaks occasionally observed in the mid-90s, when CSPE gloves were used nearly exclusively, were wear leaks at the glove gauntlet, where an O-ring seal attached the glove gauntlet to the sleeve. This minor risk has been obviated by a better attention to ergonomics in isolator design to avoid excessive stretching of the glove at critical access points and by ensuring that the glove was sized to fit the person assigned to an operation.

A further risk mitigation factor, introduced when neoprene gloves were common, was the use of a sterile clean room glove under the CSPE glove. This so-called “underglove” became a standard practice in the early 1990s, and while in my experience no studies were done to gauge its effectiveness it is important to note that glove integrity other than discarding obviously damaged gloves is not done at all in conventional clean room aseptic processing. It is safe to assume that the secondary clean room glove is as safe in an isolator operation as it is in aseptic processing in clean rooms.

In some operations single piece CSPE glove/gauntlet/sleeve assemblies have been used to eliminate completely the possibility of O-ring wear point leaks. This strategy is most often
implemented at glove locations where critical interventions are most likely to occur. As an additional risk abatement strategy, isolator operators were trained in aseptic processing and proper use of isolator gloves and emphasis was placed in not touching product contact surfaces or components with gloves, just as that good practice is emphasized in clean rooms. The final and perhaps most significant factor in reducing glove risk is the design of isolator-friendly production equipment. In the early days when leakage in gloves was a key concern, isolators were built over current aseptic processing equipment designs. These production line designs were not optimized for minimal reliance on intervention. However, over the last twenty years isolator-friendly designs have become the norm and the use of machine automation and robotics has reduced reliance on gloves for critical operations.

In summary, Annex 1 requirement 4.23’s concerns regarding gloves is something I would have expected to read in 1990 when experience with isolator operations in aseptic processing was limited, neoprene gloves were the norm, and the use of undergloves was not universal. An article by Gessler et al entitled “How Risky Are Pinholes in Gloves? A Rational Appeal for the Integrity of Gloves for Isolators” was published in 2011 (PDA J Pharm Sci. Technol. Jun 2011;65(3) 227-241). This article reported that pinholes are not necessarily predictive of contamination risk, that microbiological monitoring is not generally effective at evaluating glove risk and finally that a combination of “semi-automatic physical testing, visual inspection and control of bioload” has the effect of “minimizing risk” and assuring “maximum safety.” A key finding was that visual inspection is an excellent means of detecting leakage.

My experience aligns well with Gessler et al. I have not found isolator gloves in modern isolator installations, which I’ll characterize as early 2000s onward, to be a significant contamination risk. Certainly, firms should have detailed procedures for glove selection, testing and management. They should also use undergloves and consider single piece gloves where work requirements dictate. They should also routinely inspect the gloves visually with care and in good lighting and have procedures for periodically inspecting glove/sleeve connection points and the mounting points to isolator walls. In my experience visual checks, because they can be done frequently, are far more critical than physical leak testing, which has limitations in practical use. I believe the physical testing of gloves before and after shifts is unnecessary and potentially detrimental. Isolators are often used in a campaign approach, and I suggest my clients carefully inspect gloves and do physical tests between campaigns. In my experience isolator (or closed RABS) gloves are much more reliable than standard clean room gloves which in my experience rarely have a useful lifetime that exceeds two hours. Clean room gloves are fragile and easily damaged while CSPE isolator gloves are not.

The second point raised in 4.23 has to do with the leak integrity of the isolator enclosure. In my experience this is not a risk modality for microbial contamination at all! Isolators for aseptic processing are generally run at a positive pressure of 15-30 Pa relative to the surrounding room. The surrounding room is typically ISO 7 or 8 although in my experience this has no impact on risk relating to the microbial or particulate air quality within an isolator. I have worked with many isolators used for aseptic production of milk or low acid juices which are
typically done in unclassified spaces and in my experience the risk of contamination in these systems is as low as pharma isolators located in classified clean rooms.

Also, most continuous throughput operations rely on an opening often called a “mousehole” for transit of product in its sealed primary package to the labeling and secondary packaging operations. Thus, these isolators are reliant on an air seal for maintenance of integrity in operation. This is a zero-risk operation in properly designed isolators and although the mousehole has been the subject of much speculation there is scant evidence of it presenting any contamination risk at all. I have run studies of up to 30 days duration with clients in which we did both media fill testing and extensive monitoring using both growth and fluorimetric methods and have observed no contamination entering the isolator. We have done these studies in unclassified machine assembly halls and observed no contamination in any experiment. I have not observed ANY contamination entering an isolator through a mousehole or any other leak. even when we have purposely taken the pressure differential to zero for 30-60s in unclassified environments.

The integrity of the isolator is critical only from the standpoint of ensuring safety during VPHP decontamination if people are present in the area around the isolator during decontamination, which is most often not the case. Therefore, I find that the requirements in 4.23 are quite excessive and wholly unnecessary. In closed isolators used for batch operations, pathogen research, general R&D, sterility testing or cell processing the pressure differential is maintained typically in the 12.5-20 Pa range and there is no mousehole. All isolators read internal pressure in real time and control to an established set point. I have not seen an installation that did not alarm in the event of a pressure differential reading outside the set control limits. In some cases, they are interlocked to air handler or pressure alarms and the processing line is shut down immediately and automatically.

The recommendations in 4.23 may be well-intentioned but they betray a lack of awareness regarding modern isolator operations and harken back to a time 30 or so years ago when experience with isolator systems was limited, glove/sleeve materials were less reliable, and equipment was far from isolator-friendly or ergonomically installed. Thus, the excessive attention to integrity in 4.23 will not positively impact process safety which is already outstanding.

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