Case Study:
Isolator Sanitisation Cycle Development, Validation and Revalidation.

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(Abstract of a paper presented at the ISPE Isolation Barrier Forums in Washington and Prague 2005)

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Project Background

With its product Influvac™, Solvay Pharmaceuticals has been one of the world's leading influenza vaccine manufacturing companies for more than 50 years. Over the last decade sales volumes of influenza vaccines have significantly increased. To cope with these growing market demands and to provide a contract manufacturing service, Solvay began a strategic review of its vaccine production capabilities in December 2000. As a result of the strategic review Solvay invested in two state of the art filling lines utilising isolator technology. At Solvay's site in Olst, the Netherlands, liquid bulk vaccine is filled into syringes, packed and distributed worldwide (see figure 1). The isolator systems incorporate an automated sporicidal gassing cycle prior to commencement of production. The gassing cycle provides a controlled and reproducible decontamination of the internal surfaces of the isolators and the filling machines enclosed by them using hydrogen peroxide.

Figure 1: *Picture by kind permission Solvay Pharmaceuticals*

Overview

It was during the sporicidal gassing cycle development stages that irregularities were observed concerning the response of the biological indicators used. The intention of this paper is to give an insight into the inherent variability of biological indicator systems commonly used for assessment and validation of isolator gassing cycles. It provides an example of how this variability can show itself in the 'real life' situation. In order to better interpret the biological indicator data, a statistical approach was employed which enabled predictions to be made as to the likelihood of survivors after particular decontamination treatments and hence allow more accurate assessment of the effectiveness of the decontamination process. The methodology outlined in this paper can be used as the foundation for a compliant validation strategy and can help to ensure consistent revalidation results.

'Traditional' Cycle Development Approach

Typically most pharmaceutical companies employ what we shall term a 'single BI' approach to sporicidal gassing cycle development. The general consensus for this approach is to locate single BIs at: 'critical' locations such as gloves, stopper feed bowls, stopper feed tracks and transfer ports; potential worst case positions where gas distribution might be poor such as under open machine covers, sampling ports and recirculation plenums; positions exhibiting extremes of temperature and/or humidity; geometric positions chosen to indicate the overall distribution of the gas, for example isolator corners, walls, ceiling and base plate.

The biological indicators of choice are spores of Geobacillus stearothermophilus since these spores have been proven to be significantly more resistant to vapour phase hydrogen peroxide than common environmental isolates [1]. The spores are usually loaded onto stainless steel carriers at a minimum population of $1x10^6$. The stainless steel carrier is often sealed in a gas permeable primary pack. The entire sealed pack is exposed to the sporicidal process to allow easier handling and recovery of the indicator.

After evaluation of the isolator system a set of initial gassing process set points are chosen and a so-called fractional survivor study carried out at a convenient place in the isolator. This study involves the timed removal of BIs from the decontamination cycle at constant intervals, in order to determine the time point at which no further growth is observed after incubation of the exposed BIs in nutrient media. This time point can be used as the gas injection time for the first fully loaded cycle with single BIs at each previously determined position. The cycle parameters (injection rate and duration) are adjusted until all placed indicators are inactivated, in other words no growth is detected from any of the exposed indicators after the period of incubation in media. Finally, an arbitrarily chosen safety margin (say 20% additional time) is added to the cycle before starting the performance qualification. The performance qualification cycle is often the 'full

duration' cycle - already incorporating the 20% safety margin.

Expected Cycle Performance

The isolator lines in question were each 14 metres long, with 32 gloves and enclosing some quite complex filling equipment. On inspection of the enclosed equipment, a total of 280 locations were chosen for initial evaluation of the 21cubic metre internal isolator volume. The intention was to reduce the number of locations to be challenged in the PQ phase based on the data obtained from the development cycles. Drawing on previous experience of similar systems and the fractional survivor test results, the expected cycle performance included the following phase durations:

1. Dehumidification to <15% Rh: approximately 0.5 hours.

2. Gas injection time to inactivate BIs at the worst-case location: approximately 2 hours.

3. Aeration to less than 1ppm hydrogen peroxide: approximately 3 hours.

Results

The initial results did indeed follow the expected pattern with the fractional survivor study showing complete kill after 10 minutes and the recirculation plenum and various semi-enclosed locations requiring the longest time to inactivate the BIs located there. Modifications were made to improve the gas penetration in the enclosed areas. Cycle parameters (injection rate and time) were changed to account for plenum, wall and base plate positions. Once these modifications to improve the 'slower' locations had been completed it was assumed that a final cycle had been defined and therefore a series of full duration cycles were initiated to confirm acceptability of the cycle prior to PQ.

The BIs from the first three cycles were inspected daily over the incubation period and a low number of samples developed growth. As the results came through additional cycles were run with increasing gas injection durations in case the developed cycle had in fact been at the 'edge of failure'. However, it appeared that significant increases in gas injection time had no effect on the underlying frequency (1 - 2%) or random location of failures. Table 1 lists the BI positions by number, which resulted in growth from the series of sanitisation cycles (cycle numbers 13 to 17 inclusive), run prior to PQ. Cycles 13 and 14 used exactly the same cycle set points with a gas injection time of 210 minutes. Just prior to running cycle 14 the BIs from cycle 13 were inspected and growth was observed from the BIs at positions 102 and 173. Armed with this information it was decided to challenge these locations with more BIs and three were placed close together at each of these locations. Positions 177 and 239 developed growth slightly later. All four positions displaying growth in cycle 13 were rendered sterile in cycle 14, however, growth was now detected at positions 145, 185, 230 and 274. For cycles 15 and 16 the gas injection time was increased to 240 minutes. This additional time did not reduce the rate of failure and on each occasion failures were found at positions where the BI had previously been inactivated. Finally, cycle 17 was run for 270 minutes (60 minutes more than cycle 13 and 14). One can see from the results that at no time during the series of cycles did a failure occur at a particular position more than once.

Table 2 provides an indication of the very high levels of process lethality that the BIs appeared able to withstand. The BI positions from table 1 are listed again in the first column of table 2. If one takes position 72 as an example, column three ('Killed') gives the lowest gas injection time, which inactivated the BI during cycle development. All other things being equal, position 72 was normally inactivated after 40 minutes of gas injection. However, in cycle 17 it was apparently able to survive 270 minutes of exposure. Column four gives the number of previous cycles where the BI position was used, in the case of position 72, no fewer than 12 cycles. BI Position 72 was inactivated in all 12 of these previous cycles (see column 5 - 'No. Previous Failures'). Applying a crude 'rule of thumb', if 40 minutes gas injection delivers 6 log reductions at BI position 72, then in cycle 17 the BI appears to have survived a lethal process of more than 40 log reductions. The other

Table 1. Biological Indicator Failures in Cycles 13 to 17 Inclusive.

Failed BI Position	Failures By Cycle Number				
	13	14	15	16	17
64	-	-	P	-	-
69	-	-	P	-	-
72	-	-	-	-	P
79	-	-	P	-	-
102	P	- - -	-	-	-
117	-	-	-	P	-
140	-	-	-	P	-
144	-	-	P	-	-
145	-	P	-	-	-
173	P	- - -	-	-	-
177	P	-	-	-	-
185	-	P	-	-	-
198	-	-	P	-	-
230	-	P	-	-	-
239	P	-	-	-	-
248	-	-	-	-	P
267	-	-	-	-	P
274	-	P	-	-	-
Total Failures in Cycle	4	4	5	2	3
Gas Injection Time (Minutes)	210	210	240	240	270

Key: P = growth, - = no growth.

Table 1

Table 2. Biological Indicator Location Survival / Kill Profile (all cycles).

Failed BI Position	Cycle Time (minutes)		No. Previous Cycles	No. Previous Failures	Comments
	Survived	Killed			
64	240	40	10	0	
69	240	40	10	0	
72	270	40	12	0	
79	240	40	10	0	
102	210	90	8	1	Failed in Cycle 1
117	240	40	11	0	
140	240	40	11	0	
144	240	40	10	0	
145	210	40	9	0	
173	210	40	8	0	
177	210	40	4	1	Failed in Cycle 8
185	210	40	5	0	
198	240	40	10	0	
230	210	90	9	1	Failed in Cycle 1
239	210	90	8	0	
248	270	40	12	0	
267	270	40	10	0	
274	210	120	6	1	Failed in Cycle 1

NB: Cycle 1 = 40 minutes gassing
Cycle 8 = 90 minutes gassing

Table 2

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positive positions give similarly anomalous results. At this point performance qualification testing was postponed and attention turned to finding the route cause of the failures.

Investigation Of Inconsistent Results

A detailed and wide-ranging investigation was initiated in order to determine the cause of the random failures observed during the cycle development phase. After all the positives were identified as *G. stearothermophilus* the investigation could focus on three main areas; the reproducibility of the sanitisation cycle; the robustness of the BI handling and media inoculation technique including verification of the quality of the media itself; and finally the potential variability in the response of the BIs.

All the sanitisation cycle data was collated and reviewed to establish any difference in the performance of the isolator and generator from one cycle to another. Fan speeds, airflow velocities, instrument calibrations and valve damper positions were all re-checked. Checks were also made during each functional mode of the isolator to ensure the software and hardware was correctly installed. Chemical indicators (as supplied by Steris Corporation) exposed during the sanitisation cycles at various fixed locations were examined for variation in response. The hydrogen peroxide solution used in the VHP generators was chemically tested to confirm strength and identity. The efficacy of the later stages of the gas injection phase i.e. beyond the 270-minute time was confirmed by delayed exposure fractional negative studies where the BIs were exposed to the cycle after 270 minutes. The efficacy after 270 minutes was found to be much greater than at the beginning of the cycle. No discrepancies could be found between sanitisation cycles and the isolator and associated systems were all found to be functioning correctly.

Another possible cause of the random failures observed could have been the technique used to harvest and inoculate the BIs or the growth media might have been pre-contaminated from the supplier. To test this, a sanitisation cycle was set up where the 6-log BIs were replaced by 5-log BIs in all 280 locations. The cycle parameters were identical to cycles 15 and 16 (240 minutes gas injection). The same harvesting and inoculation procedure was followed. All 280 BIs were killed. A further 100 unopened tubes of growth media from the same batch were incubated under the same conditions. No growth occurred. Therefore the quality of the media could be eliminated as a possible cause and the harvesting and inoculation procedure was shown to be robust.

Meanwhile, a different supplier of BIs was identified (for the purposes of this paper named supplier 'B'). A few hundred 6-log BIs (all of the same batch) were purchased from supplier 'B' for a comparative test against the original batch. For this comparative test 300 BIs from each of the suppliers would be exposed in the same cycle. The cycle had the same parameter set points as all the previous cycles. At the location where the BIs were to be exposed a fractional survivor test had demonstrated complete kill of 3-replicate BI samples after just 10 minutes of gas injection. The BIs were arranged in rows, each row alternating the 'original' supplier 'A' with the new supplier 'B' product as close together as possible. One hundred of each supplier's BIs would be removed at 60, 90 and 150 minutes. At the final time point, the BIs sampled would have been exposed to a massive 90 logs of process lethality. A summary of the results obtained from this test is given in table 3. Whilst BIs from supplier 'B' were completely inactivated at each time interval, a low, consistent percentage of the original BI batch survived regardless of the process lethality applied just as in the full-scale isolator trials.

Table 3. Results of 'rogue' detection test (all BIs exposed in same cycle).

Exposure Time	Batch A Log 6	Batch B Log 6
60 minutes	1 of 100	0 of 100
90 minutes	2 of 100	0 of 100
150 minutes	2 of 100	0 of 100

Table 3

What Causes Rogue's?

Here then, was the reason for the random underlying failures in the full-scale cycles, a low percentage of highly resistant 'Rogue's'. Whilst so called 'tailing' of survivors (for instance to ethylene oxide treatment) is relatively well known, the word 'tailing' gives the impression of a graduated resistance which can eventually be overcome. It would be somewhat misleading to describe the failures found in the Solvay project as 'tailing' since the D-value exhibited by these 'Rogue's' bore no relation to the rest of the batch. Even very large increases in process lethality did not reduce the percentage of survivors. Other researchers have carried out investigations of this phenomenon in an attempt to determine how some spores can survive very aggressive sanitisation treatments. Rogue's seem to be prevalent where: the spores form clumps or agglomerations; the spores are coated in debris; there are catalytic or protective substances present; the

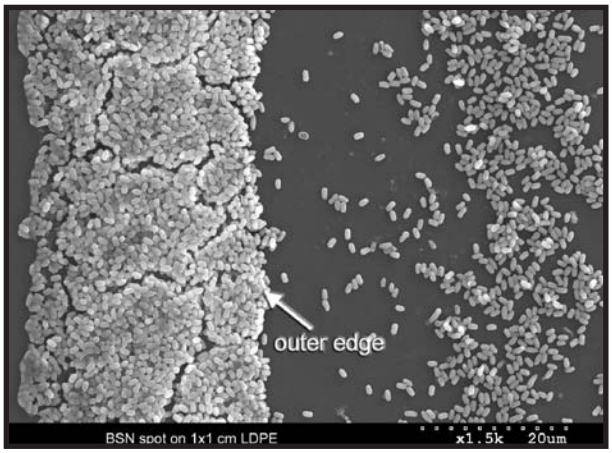

Figure 2: *With kind permission of Baxter Healthcare.*

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carrier substrate contains fissures into which some spores have become lodged. However, potentially protective effects can even occur when using very clean spore suspensions and defect free carrier surfaces. Examples of these are shown in figures 2 and 3. In both cases the carrier substrate is clean and continuous LDPE. In figure 2 one can see how spores begin to pile upon one another at the edge of the droplet of inoculum as it dries. Spores at the carrier surface may be partially protected by the layers above. A spore at the centre of the agglomeration shown in figure 3 is unlikely to receive the same process lethality as discretely dispersed individuals.

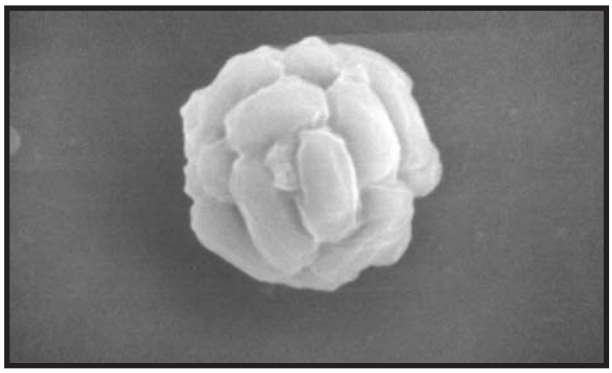

Figure 3: *With kind permission of Baxter Healthcare.*

Reliability Of BI Data

Whilst manufacturers of BIs obviously do everything possible to ensure a consistent product the inherent variability of biological systems is likely to give rise to variation in BI response both batch to batch and occasionally within the same batch. To expect a complex manufacturing process using a biological system to result in hundreds of thousands of product units that always react the same is hopeful to say the least. Sanitisation cycle developers and validators are therefore left in a difficult situation in that they must use an inherently variable 'sensor' to measure the effectiveness of a critical lethal process. **One must therefore call into question the wisdom of interpreting the efficiency of lethal processes based on the use of single BIs at each location.**

A discussion of the biological indicator's response to an applied decontamination process begins with establishing the baseline model of death kinetics. It is true that even now, one hundred years after it was first reported the log-linear model of death kinetics is still critically discussed, however, in practical terms it must be regarded as an excellent tool and basis for investigating process lethality. The main feature of the log-linear model is the approximation to a straight line obtained on transformation of survivor numbers data to log10, a 'curve' with which all undergraduate microbiologists will be familiar (see figure 4). The classic method of deriving such plots involves exposing known starting populations of, in this case spores, to a decontamination process of consistent lethality and counting the number of survivors at regular spaced intervals. The key point is that when the applied lethality is constant the log reduction in survivors is found to be the same for equal intervals of time. In such an experiment spore inactivation or viability becomes predictable and quantifiable.

Therefore, the particular utility of the log-linear response is in providing decontamination cycle developers and validators with a quantifiable interpretation of biological indicator data. The model allows one to assess whereabouts on the 'curve' a particular decontamination process resides and make any necessary adjustments before retesting. In addition, the log-linear plot makes it clear that microbial death is not instantaneous and therefore individual organisms or spores appear to have more or less resistance to the lethal process when compared to each other. For this reason one cannot be precise about predicting the 'time of death' of a particular spore. If an individual spore could be selected from a total population of 1 x 10^6 (a common BI population acceptance criterion) and its viability monitored throughout a decontamination process one could not know before hand exactly when it would be inactivated. But it is possible to calculate the likelihood or probability of the spore being inactivated in a particular time. Looking at the log linear plot it can be seen that during the first 2 minutes, the viable population is reduced by 90%. The likelihood that the chosen spore was part of that 90% is therefore 90% or in probability terms 0.9. Hence the chance of our spore surviving the first 2 minutes is 0.1. In this example the 2 minutes interval is also the D value or time to achieve a single log reduction. The phrase 'log reduction' will be substituted for time for the rest of this discussion. The chance of a spore surviving a single log reduction is always 0.1, so if one asks at the very beginning of the experiment what is the chance of a spore surviving 2 log reductions the answer is 0.1x 0.1 = 0.01. In other words if a spore is chosen at random from a population of 1x10^6 it is 99% certain that it will be inactivated after 2 log reductions. In fact the starting population and number of log reductions delivered by the decontamination process determines the likely number of viable spores remaining at the end of the decontamination process. Although a kill certainty of 99% appears very high, when starting with 1x10^6 spores there would on average still be 10,000 viable spores remaining after 2 log reductions.

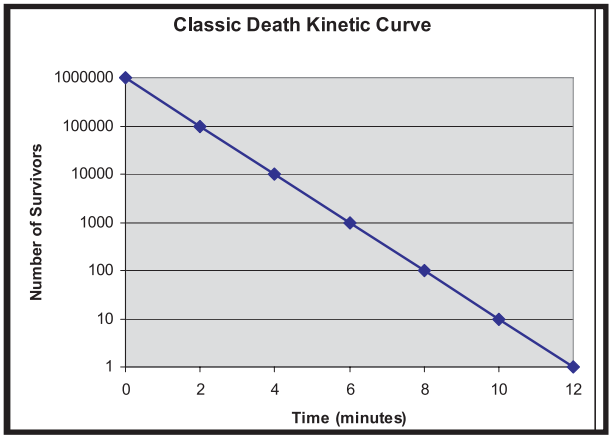

Figure 4

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What is the correct interpretation of biological indicator data like this? If at the start of a decontamination process there were $1x10^6$ viable spores and at the end only 10^4 spores remain viable, the number of viable spores has been reduced by a factor of 10^2. It would be fair to claim that on the occasion of this single experiment the lethal treatment applied, reduced the starting number of viable spores by a factor of 10^2. The previous sentence is carefully worded! It is important to note that no claim is made concerning the magnitude of the decontamination process itself.

Would it be inappropriate to make a claim also about the process lethality delivered by the decontamination process? Surely the statement can be made that this decontamination process has a magnitude of 2 log reductions? Unfortunately, things are not so simple and in fact to make such a claim from this single trial is indeed inappropriate.

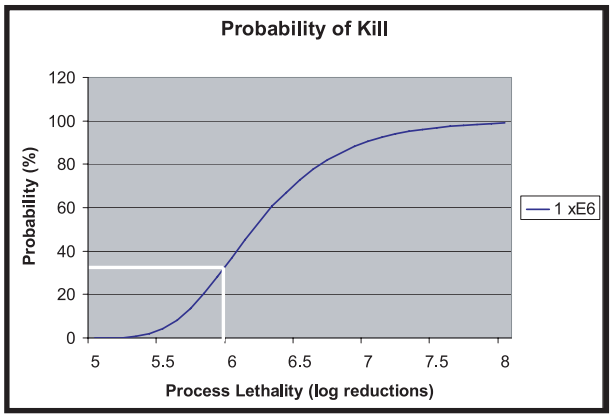

Figure 5

Why should this be so? It is because when a predetermined number of spores are exposed to a lethal process the number of remaining viable spores is not exactly the same every time the trial is performed. The interpretation of the data must account for the fact that one is dealing with averages and probability and this in turn is due to the inherent variability of the 'sensor'. Hence the outcome in terms of log reductions achieved by a particular process appears uncertain. If the probability of inactivating the spore loading of $1x10^6$ is calculated for various process lethalities the result is the probability curve in figure 5. This curve provides critical insights for the correct interpretation of biological indicator data. First, is that a process with lethality less than 6-log reductions still has a significant chance of inactivating the spore loading of $1x10^6$. For example, a process lethality of 5.8 logs has a 20% chance of inactivating a spore challenge of $1x10^6$. Conversely, a process lethality of 6.8 logs will fail to inactivate the same spore challenge on 18% of occasions.

What then could be reasonably claimed when a spore challenge of $1x10^6$ is inactivated? Referring again to figure 5, one can see that a 6-log process has a 37% chance of inactivating the challenge and therefore on average 63% of trials would result in viable spores remaining. To put it another way, if the biological indicator were inactivated in a single trial the most that can be claimed is that one is 63% certain that the process lethality delivered was at least a 6-log reduction. This level of certainty seems very low compared to that generally required in experimental design, where the scientific community would be looking for certainties of say 90 or 95%. Despite the very low level of certainty provided by data showing the inactivation of a $1x10^6$ spore loading it is commonly used in decontamination cycle development, decontamination cycle performance qualification and decontamination cycle revalidation.

Can the traditional method of placing single biological indicators with a minimum loading of $1x10^6$ at a number of locations throughout the isolator prove that the decontamination cycle has a capability of 6 log reductions? If 'prove' means being 63% certain then the acceptance criteria has been met. But when it is considered that one is using this method to 'prove' the final and most critical process step before aseptic manufacture is started in an isolator, the methodology looks particularly weak. In the case of a decontamination cycle revalidation, where typically only a single trial is performed on a six to twelve monthly basis, can a reliable conclusion be made?

Recognising the limited worth of the process lethality data generated by the single BI approach, a more statistically reliable methodology was developed for the Solvay project. In order to establish greater confidence in the BI results a larger sample size was required at each location in the isolator. This was easily achieved firstly by placing three BIs next to each other at each location. When using three BIs there are four possible outcomes of the test: all the BIs are inactivated; two BIs are inactivated; one BI is inactivated; none of the BIs are inactivated. The probability of each outcome can be calculated using the binomial distribution. If each BI is loaded with $1x10^6$ spores, the probability of inactivating all three BIs with only a 6-log process calculates as 0.05. In other words if one runs a sanitisation cycle and inactivates all three BIs then one is 95% certain that a process lethality of greater than 6-logs has been achieved at that location. Remember that the kill of a BI or BIs in one location says nothing about the process lethality at other locations within the isolator. The effective sample size is also increased if the BIs are each loaded with more spores. With a spore loading of 2×10^6 the probability of inactivating all three BIs using only a 6-log process is further reduced to 0.0025. In other words one would be 99.75% certain that a process lethality of greater than 6-logs has been achieved at that location. Interestingly when the spore loading is at least 2×10^6 if only two of the three placed BIs are inactivated the probability is such that one is still 95% certain that a process lethality of greater than 6-logs has been delivered at that location. This fact is crucial since it allows cycle development and validation to continue with statistically reliable results even when the BI batch contains a low number (say up to 5%) of highly resistant Rogue's.

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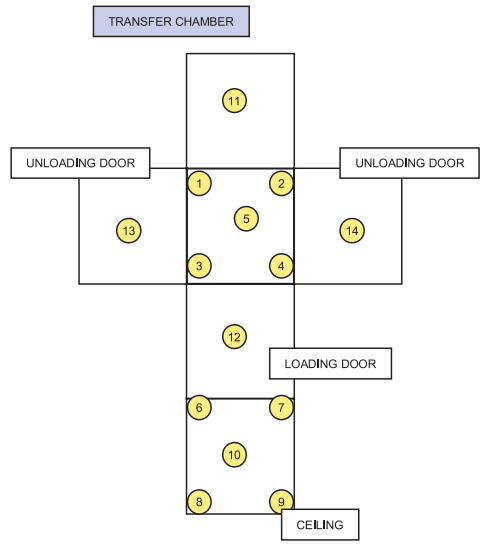

Cycle Number	001	002	003
Time (minutes)	3	4	5
BI Location 1	+--	---	
2	---		
3	---		
4	---		
5	+++	+++	---
6	---		
7	---		
8	---		
9	---		
10	+- -	---	
11	---		
12	---		
13	---		
14	---		

Figure 6

How Should BIs Be Used?

Having established that the data obtained from single BIs is statistically invalid and unreliable, clearly triplicate BIs provide a way of reliably quantifying process lethality. During cycle development the best strategy is to run a series of cycles where locations are gradually eliminated from the test as all three BIs are inactivated. The transition from all three BIs surviving to all three BIs being inactivated can be observed allowing the process lethality at each location to be quantified and the influence of a low percentage of 'Rogue's' (if present) is eliminated. The location which is seen to require the longest for inactivation of all three placed BIs is defined as the worst case location. Figure 6 illustrates this technique used on a small rapid transfer chamber.

The fractional negative data relating to the worst case location can be used to calculate a mean process D-value at that location using the limited Spearman Karber procedure [2]. This is unlikely to be a true D-value since the efficacy of the sanitisation cycle is hopefully increasing as it progresses over time but nevertheless it enables an estimate to be made of the process lethality delivered and quantifiable levels of safety margin to be added to the final production cycle (expressed in log reductions rather than arbitrary time or percentage units). At this stage the cycle can be considered sufficiently defined to begin performance qualification testing.

Performance Qualification Strategy

In formulating the PQ strategy reference is made to cGMP guidance. Most guidance now indicates that the minimum target should be a 6-log reduction. Importantly documents such as the PICS guidance on isolators [3] recognize that a 6-log process does NOT necessarily result in complete kill of all the BIs placed in the isolator. Indeed PICS goes on to say that 'If there are $2x10^6$ spores on the BI to start with then there will be 2 surviving spores at the end of a 6-log reduction. 'Whilst a 6-log sanitisation process will more often than not render the internal surfaces of an isolator sterile when low levels of starting bioburden are present, it cannot be considered in the same way as an overkill terminal sterilisation process delivered for example by an autoclave. Sporicidal gassing can however be considered as a controlled, repeatable and quantifiable surface sanitisation. The cycle duration used during PQ of the Solvay isolator systems was therefore calculated based on the time point when all three BIs located at the worst case location were inactivated. Additional time can be added to account for batch to batch variation in D-value and population which together influence the 'kill time' [4].

By using three BIs each loaded with a minimum 2×10^6 spores per BI, growth of 1 of the 3 BIs can be accepted because >6-log reduction has been achieved and **the routine production cycle is at least 4 logs more effective.** PQ can therefore continue when a small percentage (say <5%) of Rogue's is present. It is not necessary to re-challenge every location that was evaluated in the cycle development phase. Representative numbers of BIs should be placed at a range of locations including the 'worst case', 'critical' and 'geometric'. This approach is the most practical method to comply with PICs.

Revalidation Strategy

The first consideration is the actual purpose of the revalidation effort, which primarily would be to demonstrate that the time point at which the cycle achieves a minimum 6-log reduction has not changed since initial PQ.
Therefore the reduced cycle time used in PQ must be employed again.
Cycle efficacy throughout the operational cycle duration must be reconfirmed.

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The BI as a 'Sensor'

The BIs used for revalidating the system must have a similar kill time to the BI batch used for the initial PQ. BI batches with kill times significantly lower than that used for the initial PQ will not detect system changes / faults at revalidation. Conversely BIs with very high kill times compared to that used for the initial PQ may give rise to genuine survivors and therefore 'failure' of what is actually a perfectly working system. It is also useful to know if the BI batch contains any Rogue's. Effectively the BI is being used as a critical instrument or sensor and normally such a 'sensor' would be 'calibrated'. Clearly calibration is not possible but screening of potential BI batches for population, D-value and Rogue's can be achieved using a suitability screening test.

The suitability test uses a specifically designed cycle, which includes a 'plateaux' phase. This phase must have a constant efficacy such that D-values obtained at the beginning and end of the of the plateaux are the same. A large sample of BIs (say 100) are exposed to the full plateaux phase in order to detect Rogue's.

Summary

The validation methodology used for sporicidal gassing cycles includes a number of in-built safety margins. *G. stearothermophilus* has a much greater resistance to hydrogen peroxide in comparison with other spore forms [1] and the large spore loading used in validation is many times greater than the normal microbial challenge. Additional apparent resistance and therefore challenge is also conferred by the Tyvek® primary packaging. Since BIs are biological systems their use as the primary 'sensors' for evaluating gassing cycle effectiveness must account for their inherent variability. Some batches may contain a small number of rogue BIs capable of withstanding treatments of more than 90-logs. By using three BIs at each location quantifiable data for process lethality can be generated. When the BIs are loaded with sufficient spores PQ acceptance criteria can allow growth of 1 out of 3 BIs for a reduced cycle duration. An additional calculated safety margin is then added for routine operational cycles. A pre-screening programme is required to ensure the appropriate quality and resistance of BIs used in subsequent revalidation testing which also uses the reduced PQ cycle.

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