Appropriate challenges for the validation of hydrogen peroxide vapour sanitisation cycles

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Abstract

Current convention requires log 6 reduction of spore-based biological indicators to demonstrate the efficacy of hydrogen peroxide vapour sanitisation cycles. Data is presented indicating that this is not an appropriate challenge and that a lower log reduction requirement offers significant advantages. A standard challenge of log 4 reduction is proposed.

Introduction

Hydrogen peroxide vapour (more correctly termed *micro-condensed hydrogen peroxide* or MCHP (Coles, 2016) is now widely used for the sanitisation or biodecontamination of enclosures used for aseptic processes, such as isolators. Note that the word "sterilisation" should not be used in this context, although MCHP meets the PICs criteria as being a sporicidal process (Pharmaceutical Inspection Convention /Pharmaceutical Inspection Co-operation Scheme, 2007).

In order to develop and validate MCHP cycles, biological indicators (BIs) are distributed around the aseptic enclosure. These BIs are made up with micro-organisms known to be resistant to MCHP, thus presenting a robust challenge to the gassing process. Currently, the BI favoured uses the spores of *Geobacillus stearothermophilus*. These are deposited on a small stainless steel disc, which is then slipped into a Tyvek envelope.

The development of the use of BIs in isolator technology has been described by Thorogood in his History of Isolator and Containment Technology (Thorogood, 2015).

The current BI challenge

Since the introduction of the MCHP process, a convention has evolved whereby a log 6 reduction is the applied standard for Hydrogen Peroxide Vapour (HPVP) sanitisation using the spores of *G.stearothermophilus* (Ref 3). The reason for the choice of log 6 reduction appears to be partly based on the mandatory requirement for steam sterilisation and also for Ethylene Oxide (ETO) sterilisation, and partly based on the fact that this is the highest practically-achievable density of spores on a carrier. Above this density, there is a tendency for the spores to form multiple layers and clumps, which then allow the more deeply-buried spores to survive the gassing process, however long it may be. Even with careful manufacture, at the log 6 level the issue of such "rogue" BIs may still occur. It has been suggested that in developing and validating MCHP cycles, a rogue rate of 0.3% should be factored into the gassing protocol (Byrne, 2009) and indeed some operators choose to accept a rogue rate of as much as 5%. The number of rogue BIs found at the high spore density required to demonstrate log 6 reduction, present very real problems to the operators.

The real challenge to the MCHP process

It would be good practice to present the MCHP process with a demanding challenge for validation purposes. However, it would be equally good practice to present a challenge of an appropriate robustness. Applying a test of unreasonable severity may generate side-issues, such as the rogue BIs described above, needlessly long aeration phases, peroxide penetration of product containers, and potential damage to internal equipment by heavy peroxide exposure.

This paper takes the view that the real challenge to the MCHP process, that is the typically resident microflora, should be examined, and that only from a careful review of this data can an appropriate challenge be developed. The actual, or base challenge, is evaluated as the number of colony-forming units (CFUs) per unit area, found on the surfaces inside the aseptic enclosure. The number of CFUs on a 50 mm by 50 mm area is normally quoted as the measure.

It is a primary rule of the MCHP process that it be used only on surfaces that are essentially clean. Unlike true sterilisation processes such as autoclaving or gamma irradiation, any significant degree of soiling before gassing may shield resident CFUs, and thus result in a failed cycle. For this reason, we should only consider the base challenge microflora found to be resident on surfaces which have been correctly cleaned, to a validated protocol. However, for comparative reasons, we have also examined the microflora present on surfaces which have not been fully cleaned.

Data on the resident microflora on isolator surfaces before and after cleaning

1. The following presentation of data on resident microflora represents a collation of information gathered over a time period from the early 1980's through to the year 2014. The data comes from 7 different countries.
2. A large volume of data has been condensed by presenting the highest count, the lowest count, and the mean count, for each study. This gives a clarified overview for the purposes of this paper.

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3. Each study relates to a number of test sites over the internal surfaces of an isolator.
4. Each study isolator had approximately 20 test sites. These sites are shown in Diagram 1 which illustrates a typical isolator, rather than any specific unit.
5. Testing was carried out using standard contact plates ("RODAC" plates)
6. For each study, the substrate material is noted since this can affect the MCHP process.
7. For each study, various cleaning agents were applied before the post-cleaning testing. These agents included diluted bleach with detergent, 70% ethyl alcohol and more recently, proprietary sporicidal solutions.
8. In each case, bioburden testing was carried out after the IOQ execution, but before the PQ execution for the isolator.

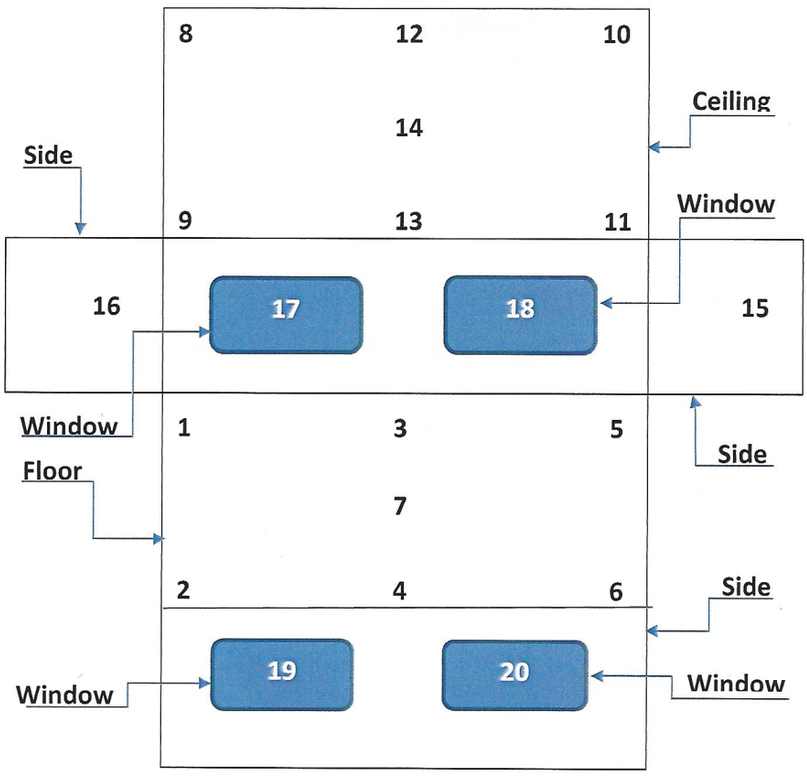

Figure 1 – "Exploded" diagram of a typical isolator from which surface microflora was quantified. The numbers identify the approximate test sites on the isolator internal surfaces. Sampling was carried out using contact plates, each representing a test area of 25 cm².

Table 1: Isolator surface-resident microflora before and after cleaning

			Before cleaning	Before cleaning	After cleaning
Study number	**Substrate material**	**Isolator process**	**CFU count range per 25 cm²**	**CFU count mean per 25 cm²**	**CFU count**
1	Plastic	R & D	12-241	55	< 10
2	Plastic	Aseptic processing	10-110	27	< 10
3	Plastic	Parenteral nutrition	12-42	25	< 1
4	Plastic	Sterility testing	12-57	23	< 1
5	Plastic	Sterility testing	20-132	49	< 1
6	Plastic	Sterility testing	21-122	44	< 1
7	Plastic	Sterility testing	25-97	52	< 10
8	Plastic/metal base	Aseptic processing	25-84	41	< 10
9	Plastic/metal base	Aseptic processing	22-74	40	< 10
10	Plastic & Metal	Aseptic processing	25-99	47	< 1
11	All metal/glass windows	Aseptic processing	28-94	49	< 1
12	All metal/glass windows	Aseptic processing	27-98	54	< 1
13	All metal/glass windows	Aseptic processing	20-89	56	< 1
14	All metal/glass windows	Aseptic processing	9-84	38	< 1
15	All metal/glass windows	Aseptic processing	42-102	63	< 1
16	All metal/glass windows	Aseptic processing	15-89	46	< 1
17	All metal/glass windows	Aseptic processing	26-112	57	< 1
18	All metal/glass windows	Aseptic processing	31-109	54	< 1
19	All metal/glass windows	Aseptic processing	29-58	42	< 1
29	All metal/glass windows	Aseptic processing	29-87	51	< 1

Data on the resident microflora on glove surfaces before and after cleaning

1. The tests were by finger dabs directly onto 90 mm culture plates, by Rodac contact plates on the palm and by Rodac plates on the back of the hand.
2. Counts are shown in Tables 2 and 3 as the range of values and the mean value for each set of tests.
3. 50 of each type of glove were tested.

Half-suit surface resident microflora before and after cleaning

1. The tests were by Rodac contact plates on the visor, shoulders, sleeves and skirt.
2. Counts are shown in Tables 4 and 5 as the range of values and the mean value for each set of tests.
3. Number of half-suits tested was 34.

Table 2: Tests on gloves before cleaning

Glove material	Finger dabs CFU	Palm prints CFU	Back of hand prints CFU
Latex	2-90 Mean 27	12-87 Mean 34	10-23 Mean 18
Vinyl	8-76 Mean 15	14-56 Mean 27	23-56 Mean 34
Hypalon	10-67 Mean 34	12-34 Mean 14	10-29 Mean 16

Table 3: Tests on gloves after cleaning

Glove material	Finger dabs CFU	Palm prints CFU	Back of hand prints CFU
Latex	<1	<1	<1
Vinyl	<1	<1	<1
Hypalon	<1	<1	<1

Table 4: Tests on half-suits before cleaning

Face CFU	Shoulders CFU	Sleeves CFU	Skirt CFU
2–10 Mean 4	12-34 Mean 14	14-56 Mean 22	52-82 Mean 29

Table 5: Tests on half-suits after cleaning

Face CFU	Shoulders CFU	Sleeves CFU	Skirt CFU
<1	<1	<1	<1

Summary

This comprehensive array of data indicates clearly that the bioburden on the various internal surfaces of an isolator which has been cleaned is low. Indeed it might reasonably be described as very low.

The majority of sites indicated less than 1 CFU per test site, and the remainder indicated less than 10 CFU per test site.

It is not possible to extrapolate an overall bioburden for the cleaned isolator from this data. However, the indications are that the entire population of CFU's on the surfaces of a cleaned isolator is in the order of just 20 units.

Given that the real challenge to the sanitisation system presented by a cleaned isolator is of this order, the question has to be asked – is a reduction by one million (i.e. log 6) in any way justified?

It would appear that a reduction as low as 10,000 (i.e. log 4) is still very much an overkill, dropping the observed total surface CFU population to a hypothetical 0.002 units. The concept of 0.002 CFUs surely offers a low risk to an aseptic process inside an isolator.

Furthermore, the resident surface microflora tends to be of types which are not resistant to the effects of the bio-decontamination process, whereas BIs are made up using organisms that are specifically chosen as being resistant to the process.

The commonly-used BI has around 5×10^6 spores on a stainless steel coupon, placed inside a Tyvec envelope. The demand for the total inactivation of this device, as an analogue of the real surface bioburden of a cleaned isolator, does not stand up to scrutiny.

Conclusion

On the basis of the foregoing data, extensive in size and scope, we propose that log 4 reduction of *G. stearothermophilus* spores become the norm for the validation of bio-decontamination processes such as MCHP. This level of reduction will adequately prove the efficacy of the MCHP bio-decontamination cycle, but with the following advantages over log 6 reduction:

- Significantly decreased, and possibly eliminated, rogue BI incidence
- Shorter cycle times
- Lower overall exposure of the isolator and its contents to VPHP
- Less penetration of product containers by peroxide
- Less exposure of equipment and materials to the effects of peroxide
- Lower costs for cycle development and process cycles

'This comprehensive array of data indicates clearly that the bioburden on the various internal surfaces of an isolator which has been cleaned is low. Indeed it might reasonably be described as very low.'

This view is well supported by a number of other workers experienced in this field of study. (Akers & Agalloco, 2013).

References

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Tim Coles, BSc (Hons), M.Phil., Managing Director, Pharminox Isolation Ltd., 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 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.

Doug Thorogood, PhD, studied microbiology and virology in the UK, Belgium and the USA. He has many years' experience in the field of pharmaceutical and medical research as well as QA/QC Regulatory Affairs and Production. He started working in the field of containment in the late 1970s and from that point developed designs, validation procedures and operational systems for a variety of isolators for sterility testing and aseptic filling in 19 countries. He is a specialist in the cleaning and sanitation of enclosures as well as clean rooms and hospital environments.