Summary overview of the design and use of Bioquell chemical indicators

Biological indicators

For many years biological indicators ("BIs") have been used to validate or confirm the efficacy of sterilization processes. For example, BIs are used routinely to validate autoclaves (i.e. steam sterilizers). One of the most common BIs used are 6-log *Geobacillus stearothermophilus* ("*G.stearo*") spores.

Although the 6-log reduction of *G. stearo* spores remains the 'gold standard' for the validation of sterilizers – and is mandated for the validation of a number of FDA-approved bio-pharmaceutical processes, there are a number of drawbacks to the use of BIs, including:

- **time:** it takes a minimum of 24 hours until even the first indication of BI kill is available – and hence the first useful indication whether the sterilization process has been effective; but note that for formal regulatory approval then 7 days of BI incubation are needed to get definitive bio-inactivation data; and

- **variability:** BIs can display significant inter and intra batch variability. There is increasing evidence that a large proportion of this variability is down to poor BI manufacturing techniques; however, there is also likely to be natural variability due to the 'biological' nature of a BI.

Notwithstanding the two issues summarized above, it is important to emphasize that the FDA (and other regulators) continue to insist on the use of BIs for formal regulatory validation purposes; and Bioquell is not claiming that CIs are formally equivalent to BIs.

The importance of micro-condensation

Bioquell has developed unique hydrogen peroxide vapor ("HPV") bio-decontamination technology. This HPV technology is validated in bio-pharmaceutical processes using a BI which comprises Tyvek pouched 6-log *G. stearo* spores.

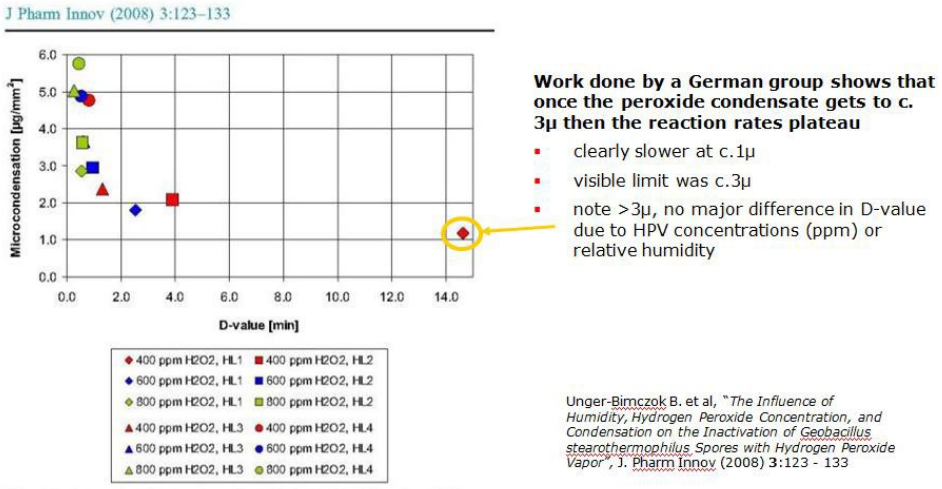

Fig. 12 Average microcondensation during first 15 min of bio-decontamination phase plotted against average *D* value

It is beyond the scope of this paper to explain in detail the mechanism and associated kill kinetics of Bioquell's HPV technology; however, it is important to note that for the process to work the HPV in the room or chamber needs to reach saturation or 'micro-condensation' conditions which effectively results in a thin layer - typically some three microns - of hydrogen peroxide solution being laid down on all surfaces. A paper published in 2008 in the Journal of Pharmaceutical Innovation by Unger-Bimczok *et al.* describes the optimization of the HPV

process around micro-condensation; key elements of this paper are summarized in the figure above.

Chemical indicators

Many sterilization processes are formally validated with BIs and then use color-change chemical indicators ("CIs") to provide indicative comfort that the sterilization process has worked. Moreover some sterilization processes use CIs to provide formal regulatory- approved assurance (cf. 'indicative comfort') that the sterilization process has worked. An example of this comprises autoclaves – which are used in hospitals to sterilise re-useable medical devices – where color-change CIs are used under regulatory norms to confirm that the sterilization process has worked, notwithstanding that the autoclave will also have been formally validated using BIs.

For some of Bioquell's HPV bio-decontamination technology clients, it has been clear for some time that an appropriately engineered CI would provide a real advantage due to the immediate indication (cf. 24 hours or even 7 days) that the HPV bio-decontamination process has worked – and hence provide appropriate comfort that the room or equipment can immediately be put back in service. Accordingly Bioquell has developed new CIs to provide immediate comfort that a HPV bio-decontamination process has occurred.

' Synthetic equivalence' / 'Calibration' of Bioquell's chemical indicators

Bioquell's CIs have been developed to change – and hence could be considered to have synthetic equivalence to - a single BI-related fixed point, namely the HPV conditions needed to achieve a 6 log inactivation of *G. stearo* assuming a D-value of 1.75 minutes.

By way of further explanation, the generally accepted 'log reduction' model for BIs suggests that it takes the same time to achieve a 1log reduction in the BI whether going from 6-log to 5-log – or 2-log to 1-log etc. – and this time increment is known as the BI's 'D-value'. (Note that theoretically D-values should only be considered on a system basis.) Third-party manufacturers of BIs typically try and bio-engineer their BIs to have a D-value of some 1 – 2 minutes.

Bioquell has developed its CIs to replicate a BI with a D-value of approximately 1.75 minutes, based on a 6-log change threshold. In other words, given that many regulators require a HPV bio-decontamination process to achieve a 6-log reduction on BIs, then a BI with a D-value of 1.75 minutes would require a '6 log proxy CI' to change color after 10.5 minutes (i.e. 6 x 1.75 = 10.5). Accordingly, Bioquell's CIs have been chemically engineered to change color after 10.5 minutes to replicate the HPV cycle conditions needed to achieve a 6-log reduction on BIs. However, it is important to note that Bioquell's CIs should only be treated as providing 'comfort' that the HPV process has worked and for the avoidance of doubt they must not be used to provide formal FDA- (or other regulator) mandated regulatory assurance.

(Note that Bioquell's CIs also show 4-log and 2-log reductions based on the D-value 'log-reduction' model, although the key Bioquell CI synthetic equivalence/calibration point is calculated by reference to a 6-log BI reduction.)

Please note that Bioquell's CIs only change color if micro-condensation has occurred and hence they have been designed to take into account, among other things, changes in relative humidity – which can affect the time taken to reach micro-condensation and hence can affect BI inactivation – which is a phenomenon effect which can be seen at facilities around the world depending on the time of day or time in the year.

****

This paper is only intended to act as a summary. If you would like more specific information on BIs and/or CIs please contact Bioquell.

Bioquell November 2012