The value of Bioquell Room Chemical Indicator (Room CI) as an instant, quantitative evaluation of efficacy in biodecontamination processes

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Abstract

Bioquell Room Chemical Indicator (Room CI) offers an additional method for verification of hydrogen peroxide vapour decontamination efficacy in rooms and large enclosures. By extensive testing in varying room sizes, Bioquell Room CI was calibrated to a 6 log kill (*Geobacillus stearothermophilus*, 6 $\log_{10}$ CFU). Manufactured under rigorous quality control tests to reduce variability, Bioquell Room CI features a colour-changing reactive ink displaying an instant result immediately after decontamination is complete. Moreover, the degree of colour change is related to the extent of biodecontamination achieved. Bioquell Room CI delivers an instant, quantifiable result you can trust to give immediate assurance of decontamination process success. With less variability and no wait time for results, Bioquell Room CI is a fast and effective indicator that improves risk management and reduces your operating costs.

Introduction

Biological Indicators (BIs) are widely accepted and generally considered to be the industry standard validation tool for determining hydrogen peroxide vapour decontamination efficacy. The BI commonly contains spores of the microorganism *Geobacillus stearothermophilus* inoculated onto a stainless steel disc. The BI represents a heavy bioburden (6 $\log_{10}$ CFU) coupled with a highly resistant microorganism to provide cycle security. This ensures a wide range of equally and less resistant microorganisms are killed during the hydrogen peroxide vapour cycle.

Although BIs are commonly used as biodecontamination indicators, there are several limitations associated with their use. A key limitation is BIs require a seven-day incubation period. This can be seen as disadvantageous due to the length of time to wait for a final result, which induces a risk in resuming processes and delays product release whilst waiting for the BI result.

Additionally, even the most controlled BIs have an inherent variability. Bacteria, by its nature, can behave unpredictably and no one manufactured batch of BIs is identical. The variability in batches of BIs can lead to a phenomenon referred to as "rogue BIs"[1]. A "rogue BI" can be defined as a biological indicator that has a greater resistance to the decontamination process than the majority of the batch. Rogue BIs can occur if: "the spores form clumps or agglomerations; the spores are coated in debris; there are catalytic or protective substances present; the carrier substrate contains fissures into which some spores have become lodged."[2] This leads to a false positive BI result. Of note, Bioquell Biological Indicators are produced using processes and rigorous quality control tests specifically developed to limit these common variability concerns.

As a result of the inherent variability of BIs, there is a requirement for a more consistent and rapid method of determining whether 6 log kill conditions have been met by the hydrogen peroxide vapour decontamination cycle.

Chemical indicators offer a solution to the limitations of BIs. Bioquell's range of CIs are formulated and calibrated to a defined BI response to Bioquell hydrogen peroxide vapour technology, providing a visible colour change to indicate 6 log kill conditions have been met. This visible colour change occurs when CIs are exposed to a hydrogen peroxide vapour decontamination cycle and is due to the oxidative response of a specially formulated reactive ink on the CI card (see **Figure 1**).

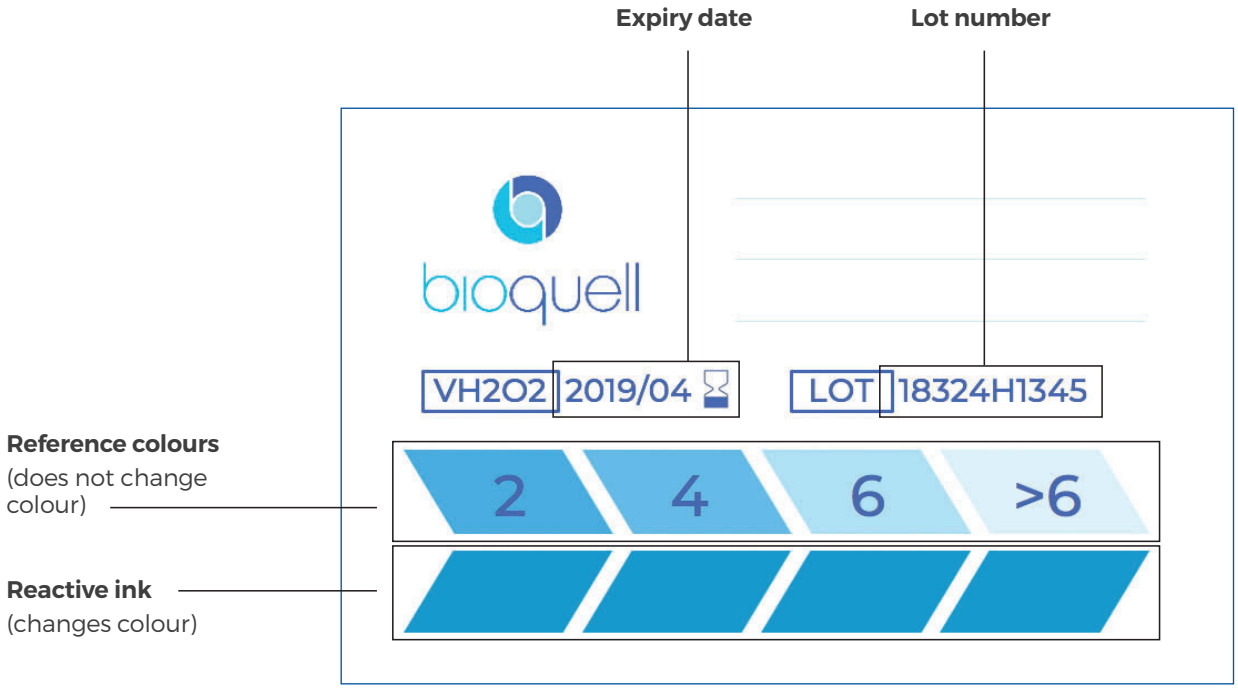

Figure 1. *Description of the Bioquell Room CI card highlighting key information (lot number and expiry date), reference colour areas and reactive ink areas. When the reactive ink colour matches the reference colour, it indicates the level of biodecontamination relative to a 6 log biological indicator.*

The use of Bioquell Room CI

Bioquell Room CI can be used in conjunction with BIs or as standalone indicators to show that 6 log kill conditions have been met after a hydrogen peroxide vapour cycle.

Chemical indicators show a 6 log bioburden reduction relative to *Geobacillus stearothermophilus*. The extent of the visual colour change, from blue to white (see **Figure 2**), of the reactive ink has been calibrated to give an instant, quantifiable result. A range of static reference colours printed on the CI card are compared to the colour of the reactive ink to instantly read the result.

Biological indicators are typically used to show one of two results (growth or no growth) by incubation in nutrient broth. When used in this way, the biological indicator result will show if 6 log kill conditions were met or not met. However, it will not give information about the extent of biodecontamination efficacy or how far away the decontamination cycle was from achieving successful 6 log kill conditions.

With Bioquell Room CI, the extent of the colour change can be compared to the reference colours (see **Figure 2**) to give a quantifiable result relative to 6 log kill conditions.

Biological indicators and enzymatic indicators (an alternative type of chemical indicator) require sample processing and/or incubation for results to be obtained, extending the time for confirmation that 6 log kill conditions were achieved. In the worst scenario, a positive result can be obtained after decontamination, which can lead to further downtime, resulting in product not being released and subsequent loss of revenue.

With Room CIs, a truly instant result can be obtained with no sample processing – the result on the Room CI card can be checked for 6 log kill conditions when the hydrogen peroxide vapour concentration has reached safe levels to enter the room and retrieve the samples. Using Room CIs in hydrogen peroxide vapour decontamination cycles provides an instant indication that 6 log kill conditions have been met. As well as providing instant results, Room CIs are relatively inexpensive, making them cost-effective in comparison to other indicators. A decontamination cycle using both BIs and Room CI will give an instant result for 6 log kill conditions, which can be confirmed using BIs after seven days, and eliminates doubt from rogue BI results by using the less variable Room CI indicator.

Challenges in the calibration of a chemical indicator

Bioquell CI technology is based on the reaction of a specially-formulated ink with hydrogen peroxide vapour. This reaction will be different to the interaction of hydrogen peroxide vapour with a biological spore, as found in BIs, which presents challenges in calibrating CIs to BIs.

Bioquell Room CI cards were calibrated to Bioquell HPV-BI (6 $\log_{10}$ CFU; *Geobacillus stearothermophilus*) by testing the indicators in rooms of different volumes. In these rooms, the Room CI cards and HPV-BIs were subjected to hydrogen peroxide vapour decontamination cycles, yielding measurements of both indicators near the limit of 6 log kill conditions. The Room CI and HPV-BI indicators were placed side-by-side in various locations in the test rooms and a hydrogen peroxide vapour cycle was initiated. Once the hydrogen peroxide vapour concentration reached safe levels to enter the room (<1 ppm, UK working exposure limit of 8 hours), the Room CI reactive ink colour was measured and the HPV-BIs were incubated. The HPV-BI results were

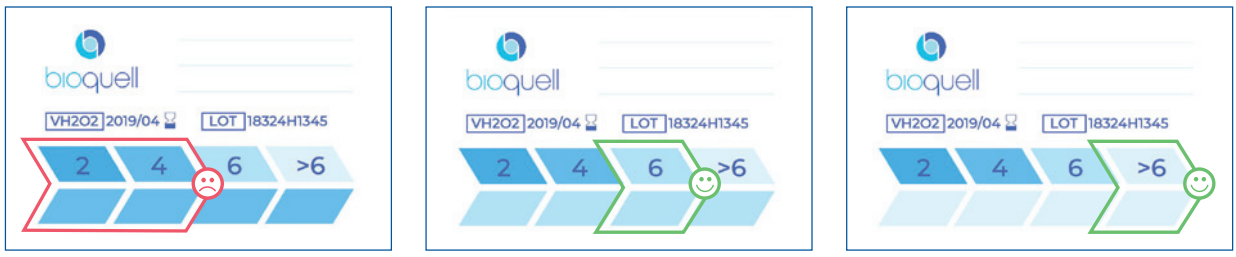

Figure 2. *Examples of Room CI cards exposed to hydrogen peroxide vapour, indicating a variety of 6 log kill conditions. Left: less than 6 log kill conditions were achieved; Centre: 6 log kill conditions were achieved; Right: 6 log kill conditions were exceeded.*

obtained after seven days, where a positive read is growth and a negative read is no growth. The biodecontamination cycles and sample measurements were repeated several times for each room to increase the sample size of the dataset, reducing the impact of indicator and cycle variability on the results.

The measured colour of each Room CI card was plotted against the corresponding HPV-BI result (growth or no growth). A representative sample of data points for measured Room CI colour versus the HPV-BI result is shown in **Figure 3**, where a corresponding positive HPV-BI read is displayed as red and a negative HPV-BI read is displayed as green. The representative sample shown includes variability from cycle conditions and sample placement in different positions in the room. From the whole dataset, the optimum 6 log reference colour (dashed light blue line, **Figure 3**) was determined by calculating the best agreement between the measured Room CI colour and the HPV-BI result.

A safety margin was applied to the optimum 6 log reference colour, resulting in the set 6 log reference colour (dark blue line, **Figure 3**) as presented on the Bioquell Room CI card (see **Figure 2**). This ensures a reliable biodecontamination efficacy result is shown on the Room CI card. The Room CI 6 log reference colour provides a low occurrence of false positives and false negatives i.e. Room CI eliminates the risk of a rogue result, as can be experienced when using BIs alone.

Room CI colour intensity for positive BI
Measured Room CI colour intensity for HPV-BI growth (shown as **red** in Figure 3).

Room CI colour intensity for negative BI
Measured Room CI colour intensity for HPV-BI no growth (shown as **green** in Figure 3).

Optimum 6 log reference colour
Reference colour giving the best agreement between room CI and HPV-BI result (shown as **light blue** in Figure 3).

Set 6 log reference colour
The 6 log reference colour as presented on Room CI cards (shown as **dark blue** in Figure 3).

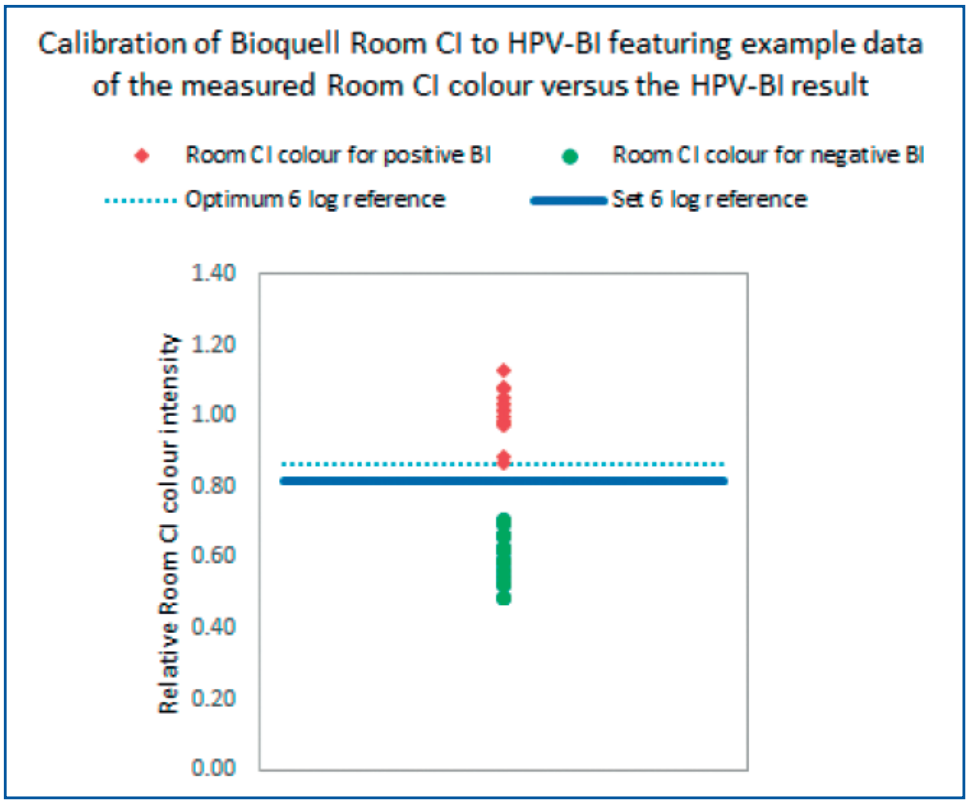

Figure 3. *Calibration of Bioquell Room CI to HPV-BI, showing a sample of the measured relative colour intensity of Room CI according to the HPV-BI result, the calculated optimum 6 log colour and the set 6 log reference colour.*

With its calibration to 6 log bioburden reduction relative to *Geobacillus stearothermophilus* and instant read result, Room CIs are undeniably a useful tool for determining the efficacy of a hydrogen peroxide vapour biodecontamination cycle. However, Room CIs and BIs will never be perfectly aligned due to the unpredictable nature of the BI. For example, Room CIs are less variable than a BI and will not suffer from rogue results such as a BI rogue[1]. Conversely, the Room CIs may indicate growth but the BI will show no growth, which can arise from the safety margin applied to the Room CI 6 log reference colour calibration (see **Figure 3** for details).

Room CIs can be used as cost-effective indicators to quantitatively determine 6 log kill conditions have been met; for example, as an indicator of 6 log kill conditions on a regular basis for validated cycles or to study hydrogen peroxide vapour distribution in a room or enclosure. Using Room CIs and BIs together makes use of two indicators whose advantages complement each other's drawbacks.

Conclusion

Biological indicators are commonly used for determining biodecontamination cycle efficacy and are necessary as they present a viable organism challenge equivalent to 6 log bioburden reduction. Nevertheless, they are an added expense to biodecontamination cycle verification. Moreover, biological indicators have drawbacks due to their very nature such as a seven-day incubation period and variability (e.g. "rogue BIs"[1]). Bioquell Room CIs offer a reliable, cost-effective indicator for decontamination cycle verification and mitigate the drawbacks of BIs to yield instant and reliable results.

To summarise, Room CIs provide:

- A valuable tool for verifying the efficacy of hydrogen peroxide vapour decontamination cycles.
- An instant result for 6 log bioburden reduction, relative to *Geobacillus stearothermophilus*, with no processing required and no seven-day incubation time.
- Less variability than biological indicators and will not suffer from "rogue BI"[1] results.
- A quantifiable result, relating to the extent of biodecontamination achieved.
- A cost effective indicator when compared to other chemical indicators, such as enzymatic indicators.
- Reliability using extensive calibration data to support the kill relationship between Room CIs and HPV-BIs for varying room sizes.

References

1. Biological indicators for hydrogen peroxide vapour technology (2012), Dr Lynne Murdoch: Bioquell document number CRP001-MKT-041 (available from Bioquell).

2. Case Study: Isolator Sanitisation Cycle Development, Validation and Revalidation. (2005): pp. 4-5.

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