1.0 BACKGROUND AND SCOPE

Biological Indicators are used on site for the qualification of steam sterilisation processes. These Biological Indicators are challenge micro organisms presented either on a carrier or in a liquid suspension.

This review applies to the Receipt Verification of standard Biological Indicators used in the qualification of Moist heat Steaming and Sterilisation processes.

2.0 SPECIFICATION OF BIOLOGICAL INDICATORS.

These Biological Indicators (BI's) present a known biological challenge to the process to qualify the lethality and efficacy of the process. This challenge is defined as follows:-

2.1 Organism

The challenge organism currently used on site and most commonly used in the industry is *Geobacillus stearothermophilus* spores. This is the recommended challenge organism by both the USP and EP for steam sterilisation and moist heat sterilisation qualification. Other micro organisms may be used but are not currently used on site for this purpose.

2.2 Presentation

The spores are presented either by inoculation onto a carrier (e.g. paper, steel, thread) or by suspension in a liquid. There are many possible presentations and types of BI's that can be purchased. This document addresses the presentations that are used on site currently or are anticipated to be used.

2.3 Population

The number of viable spores per BI.

2.4 D value

The D value is the time taken in minutes to achieve a 1 log reduction in viable spores in the sterilisation conditions. This is defined at a reference temperature typically 121°C and written as follows:-

D_{121} = x minutes — where x is the time taken to achieve a 1 log reduction in viable spores at 121°C in steam / moist heat conditions.

The range for D_{121} values is typically between 1.5 and 3 minutes. Indeed this is the recommended range in the USP, whereas the EP recommended range is simply greater than 1.5 minutes.

2.5 Z value

The Z value is a measure of the temperature sensitivity of the micro organism. The Z value is quoted in °C as the change in temperature that delivers a 1 log change in D value. The Z value is important for sterilisation cycles run at temperatures that are different to the standard D value 121°C reference, also for fluids cycles where the overall cycle lethality is used in delivering sterilisation efficacy.

2.6 Compliance

The BI is a standard challenge, a medical device used for the qualification of the sterilisation process and therefor must also meet the following compliance requirements:-

2.7 Shelf life

The manufacturer will quote a shelf life of the BI based upon a time after manufacture, this is typically between 1 year and 2 years after manufacture. In addition the user site must define a shelf life on site based upon storage conditions and testing performed on site. This should be less than 1 year from receipt testing of each delivered batch of BI's.

3.0 EVALUATION OF BIOLOGICAL INDICATORS

3.1 Manufacturers Responsibility

The manufacturer is responsible for providing the following:-

3.1.1 Certificate of Analysis covering the items defined above in Specification of BI's.

3.1.2 Storage requirements (Temperature, Humidity)

3.1.3 Application details and instructions for use

3.1.4 Manufacturers Methods of Analysis for results reported on the C of A

3.1.5 Details of Regulatory approval for the devices (BI's) being manufactured and distributed.

3.2 Users Responsibility

The User responsibilities are as follows:-

3.2.1 Define in house acceptance criteria for the BI's specification and requirements just as for any other raw material or QC standard. Have procedures for QC checking suppliers Certificate of analysis with site specifications and acceptance limits.

3.2.2 Have a documented assessment / audit of the supplier establishing their suitability, approval of the devices they are selling (NOTE : Many BI manufacturers are situated in the US and have been audited and approved by the US FDA). USP asks for routine audits of the manufacturers facilities and procedures.

3.2.3 Upon receipt of **every delivered batch** the user shall:-

3.2.3.1 Identify the micro organism (to at least genus level)

3.2.3.2 Verify the population (EP and USP methods exist as well as manufacturers methods)

3.2.3.3 Verify the Moist Heat resistance of the BI.

The EP requires that the Resistance of the BI's is verified **either** by performing a sub lethal cycle **or** performing an independent D value verification.

The USP states that the user may consider conducting a D value assessment, or might want to perform an independent D value determination.

No pharmacopeia states that you have to perform an independent D value determination.

3.2.4 Move from quarantine into qualified storage conditions following successful receipt verification as described above. At this time set the storage life / shelf life of the BI's based upon the manufacturers shelf life or 12 months from receipt verification, whichever is the earlier.

3.2.5 Record the BI quantities and batch details into the BI log book to ensure fill reconciliation and control of BI's on site.

NOTE : This discussion document relates to point 3.2.3.3 above; Verification of the Resistance of the BI's to the sterilising agent. The other verification tests (identification and population) would still need to be conducted but would be performed on site rather than contracted out. No other change is to be made to these tests.

3.3 Current Approach to verification of the Moist heat resistance.

The current approach for BI's used on site is to send a quantity of each delivered batch of BI's to a contract laboratory for independent D value determination. The independently verified D_{121} value should be within 20% of the manufacturers Certificate of Analysis D_{121} value.

The Independent contract laboratory should use a BIER vessel (Biological Indicator Evaluation Resistometer) to deliver a very measured and accurate sterilisation dose to the BI's. To establish the D_{121} value the BIER vessel is run at 121°C +/- 0.5°C for a series of time points (time points are established through calculation based upon labelled D value). For each time point 10 BI's are exposed in the BIER chamber and then put into growth media and incubated following the exposure. A typical set of results is shown below:-

Example BIER vessel D_{121} value test results.

121°C Hold Time (mins)	BI's Positive / Growth after 7 days incubation.	BI's Negative / No Growth after 7 days incubation.
5.5	10	0
6.25	10	0
7.0	10	0
7.75	8	2
8.5	6	4
9.25	3	7
10.0	1	9
10.75	0	10
11.5	0	10
12.25	0	10

The contract laboratory will also perform a population verification and run positive controls with the tests requiring somewhere between 120 and 200 BI's for the performance of these tests dependent upon the laboratory procedures.

Based upon the population verification performed and the above documented results a D_{121} value will be calculated by the laboratory using one of two calculation methods; Limited Spearman-Karber or Stumbo-Murphy-Cochran procedure.

A key requirement for the contract laboratory is that it must not only comply with the standards for BIER vessels and D value determination but it must also recreate the manufacturers methods for population verification and D value determination. This test is less about identifying the actual D value and more about verifying that nothing has changed during transportation.

3.4 Proposed Approach

The proposed approach is to perform Resistance verification testing on site by running a sub lethal cycle on a sample from every delivered batch of Biological Indicators.

The Sub lethal cycle is described in the EP and should be applied to every delivered batch of BI's just as the independent D value determination is. The EP requirement for the sub lethal cycle is that exposing BI's to a sub lethal cycle of 121°C +/- 1°C for 6 minutes will leave revivable spores (i.e. when the exposed BI's are put in growth media and incubated as per manufacturers recommendations the exposed BI's show positive growth.

This sub lethal cycle relates to the guaranteed survival time which is detailed on the manufacturers Certificate of Analysis. It can be argued that either the EP 121°C for 6 minutes, or the documented 'guaranteed survival time' should be used for this verification.

The Guaranteed Survival Time is calculated by taking the log of the population, subtratcting 2 from it and multiplying by the D value for the BI's.

e.g. BI's with a population of 1 million, 10^6 and a D_{121} value of 1.5 min would have a Guaranteed Survival Time calculated as follows:-

Survival Time = (Log Population – 2) x D_{121}

= (6 - 2) x 1.5

= 6 minutes.

This sub lethal cycle will be run on site in the Laboratory Autoclave where a specific test cycle will be developed to achieve the EP requirements of 121°C +/- 1°C for 6 minutes. The heat up and cool down time should be as short as possible so as not to impact on the sterilisation dose delivered.

This sub lethal cycle is either a time at temperature controlled cycle (121°C +/- 1°C for 6 minutes), or an F_o controlled cycle where heating and sterilisation continues until a load temperature probe adjacent to the Biological Indicators has accumulated 6 F_o

10 BI's are located held in free space in the centre of the BI chamber with a load temperature probe adjacent to them to record the delivered steam temperature and time conditions. The sub lethal cycle is run as described above, the BI's are removed as quickly as possible, put into growth media and incubated as per manufacturers recommendations (media and incubation specifications).

A successful sub lethal cycle test shows all 10 BI's growing following the 7 day incubation period.

4.0 JUSTIFICATION FOR CHANGE

Having to send up to 200 BI's from every delivered batch away for testing can delay use of the BI's by up to 4 weeks and is costly in terms of the contract laboratory and use of 200 BI's. However, in addition to these cost and time implications there are several technical problems with the testing.

4.1 Recreating the manufacturers methods.

As with many tests in microbiology, it is not easy to create a standard or have a calibrated reference. Therefore the result obtained is not necessarily the correct result, it is the result obtained with the method, equipment and media, used. Change any aspect of the method, equipment or media and you may have a different result. For this reason it is essential that the manufacturers methods and media are used exactly as described. Often when there is a discrepancy between independently verified D value and manufacturers labelled claim it is due to the incorrect method or media being used.

4.2 Further transport and storage conditions.

One of the reasons for performing some receipt verification of the BI's in terms of population and resistance is that there is concern that transportation and storage conditions may have impacted on the spores viability and resistance characteristics. Therefore shipping 200 BI's off to another venue for independent D value determination has the potential to further impact on this shipping conditions and storage conditions to the BI's. If the independent laboratory reports a failure in low population or low D value there will be a potential question as to whether or not this was caused with the final shipment to the contract laboratory.

4.3 BIER Vessel variability.

BIER vessels are designed to deliver a very accurate dose of saturated steam to the BI's with a short heat up and cool down time (<10 seconds). This is a very difficult standard to achieve and many contract analytical laboratories struggle to achieve reliable performance in this area.

Even with two compliant BIER vessels, the acceptable tolerance and the instrumentation calibration tolerance could equate to a 50% lethality variance.

The BIER vessel specification is 121°C +/- 0.5°C. Measured with temperature instrumentation which usually has a calibration acceptance criteria of +/- 0.5°C.

Therefore two fully compliant BIER vessels could be operating at two extremes ;

BIER vessel A with an indicated 120.5°C and a temperature measurement reading 0.5°C high. Therefore an actual Temperature of 120°C.

BIER vessel B with an indicated 121.5°C and a temperature measurement reading 0.5°C low. Therefore an actual Temperature of 122°C.

In terms of lethality, this would equate to BIER vessel B being over 50% more lethal (due to the temperature effect). This in itself would be sufficient to cause a failed D value verification.

4.4 Media variability

Media growth characteristics vary dependent upon media type, media recipe, sterilisation conditions etc. Although QC testing and growth promotion studies will be performed on all media used both at the manufacturers works and at the contract analytical laboratory, this variability will still exist. The recovery of damaged / stressed spores following a steam cycle is likely to challenge the performance of the media. Again, it is not necessarily which media is best, but ensuring it is representative of the manufacturers media. The best way to ensure this is to purchase the media from the BI manufacturers but many contract laboratories do not do this because of cost of this media.

4.5 Steam Quality Impact

On site the steam quality is controlled to be wfi quality condensate with physical properties in compliance with EN285. Many contract analytical laboratories will use Plant Steam for supply to their BIER vessels with potential chemical contamination that could have an impact on the resistance of the spores.

4.6 Population method variability

The Contract Laboratory performing the D value determination will also perform a population verification. Again, it is important that the manufacturers methods are applied rigorously otherwise a higher or lower population count will be obtained. This population result will be used in the Laboratories calculation of the D value in the Limited Spearman-Karber calculation, therefore variability here will further add to variability in the calculated D value.

Due to all of these variables any discrepancy between Labelled D value and independently verified D value rarely results in a definitive conclusion with regard to which result is correct. Many sites have moved away from trying to perform independent D value verification for this reason. A discrepancy has resulted in a batch of BI's being rejected and the manufacturer claiming that there is no problem with the batch as their repeat testing is satisfactory.

5.0 IMPACT ASSESSMENT

5.1 Business Impact.

The Business Impact is positive both in terms of cost, risk and time.

The BI verification work can be performed on site in the Micro biology laboratory. This will be population verification, identification and a sub lethal cycle on 10 BI's from every delivered batch.

The Contract Analytical laboratory are no longer required to perform this work.

Far fewer BI's will be used for QC testing from each delivered batch.

The receipt verification testing could be performed on site within a 2 week period rather than the time taken with a contract analytical laboratory which is typically 4 to 8 weeks.

The risk of a false failure due to the variability's described above is far less. Such a false failure impacts further time delays, QA and QC investigation effort and a repeat purchase and retest of a further batch of BI's.

5.2 Technical Impact.

The BI's purchased for use in the qualification of Steaming and Moist Heat Sterilisation processes on site will be used exactly as they are now. Therefore no Technical Impact.

5.3 Regulatory.

This approach complies with the requirements of the US and EU pharmocopea's. In addition, clarification was sought from the HPRA some years ago who confirmed verbally to the author that either a sub lethal cycle or independent D value determination were satisfactory.

This approach is regarded as cGMP as many companies apply this receipt verification method for standard Biological Indicators.

NOTE : It would be very unlikely if there were details of performing independent D value determination in any product license, however this should be verified.

6.0 CONCLUSION AND RECOMMENDED ACTION

Based upon the review and discussion above, the receipt verification of BI's should change to the Sub lethal Cycle method rather than independent D value verification. This change and approach is regulatory compliant, has no Technical Impact and has positive business impact in terms of cost, time and risk.

The work required to implement this change is as follows:-

6.1 Raise Change Control documentation

6.2 Check Product Licenses and Corporate Guidance Documents / Standards for any specific requirements for BI verification

6.2 Develop a cycle on the Laboratory Autoclave capable of running a compliant Sub Lethal Cycle.

6.3 Write Procedure for Running Sub Lethal Cycle. Review all aspects of this SOP with current requirements and best practice as part of this review.

6.4 Implement Change