Nelson Labs
A Sotera Health company

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MEMORANDUM # 26093

DATE: 18 Sep 2021

TO: Whom It May Concern

FROM: Daniel Loverro

RE: Updated Calculation of Bioburden: Effective 14 Oct 2021

To Whom it May Concern,

This memo is to address changes in the calculation of bioburden results according to the global, harmonized bioburden procedure, STP0036: Bioburden and driven significantly by the ISO bioburden standard 11737-1:2018. USP bioburden testing is not included in the scope of this memorandum.

The method Nelson Labs Fairfield (NLF) is currently performing for ISO bioburden testing is STP0036: Bioburden. This STP instructs to perform calculations differently than was performed under Gibraltar Laboratories (GBL). These changes will become effective starting 14 Oct 2021. This time will allow NLF to make necessary preparations as well as allow our customers time to prepare themselves for the changes. These changes include:

1. Recording of Volume In/Out and Calculation of Plating Factors
The Plating Factor (PF) is calculated as the (volume in / volume out) and is a measure of the portion of total extract that is plated and enumerated for growth. The legacy GBL data forms did not adequately drive the recording of the volume out. This has been corrected with the adoption of the STP forms, which require the volume in and volume out to be recorded with greater precision. This may manifest in sample results as a higher PF than previous lots of the same product, due to loss of volume that was not being adequately captured (such as absorption into the product). In those cases, the bioburden may have been slightly underestimated.

2. Application of the Limit of Detection (LOD)
In bioburden testing, the LOD is determined by the PF. Historically, the LOD was not applied to results when the PF was 1; results of 0 CFU would not be counted towards the total bioburden. The final result would be reported as <LOD if the average was <LOD. According to STP0036, the LOD is to be applied in all scenarios. Counts of 0 are to be treated as <LOD for calculation. Application of the LOD in this way will result in a higher calculated bioburden average in cases in which the plating factor is 1 and the total CFU is less than the number of samples. For example:

Results for 10 samples: 0, 0, 2, 0, 1, 0, 3, 0, 0, 0. PF (LOD) = 1

Calculation Method	Sum	Average	Result Reported
GBL Method	0 + 0 + 2 + 0 + 1 + 0 + 3 + 0 + 0 + 0 = 6	6/10 = 0.6	**<1 CFU/sample**
NLF/SLC Method	<1 + <1 + 2 + <1 + 1 + <1 + 3 + <1 + <1 + <1 = <13	<13/10 = <1.3	**<1.3 CFU/sample**

3. Use of Efficiency Factor Versus Percent Recovery
Once the raw average bioburden is calculated, the efficiency factor (EF) is applied, if applicable. Historically, GBL calculated the average EF as 100/(Percent Recovery) for application to future studies and expressed it on final reports as an integer rounded to one decimal place. NLF has adopted a validated spreadsheet for calculation of bioburden results, and the spreadsheet requires input to be the Percent Recovery. As changes are implemented and processes improved, this will reflect on final reports as a change from EF to Percent Recovery where applicable. Due to rounding, these changes may result in minor (if not negligible) differences between sets of identical raw results calculated under different guiding procedures.

4. Replacement of "Adjusted Average Bioburden" with "Bioburden Estimate"
NLF will begin expressing the Adjusted Average Bioburden as the Bioburden Estimate. The calculation is identical, except for omission of "<" in the final result. This omission is largely because the absolute value of the result is used to calculate appropriate dosing and Nelson wants the reported estimate to reflect that. Using a hypothetical set of data in which the Percent Recovery is 85.0% (EF = 1.2):

Calculation Method	Average	Adjusted Average	Bioburden Estimate
GBL Method	<1.3 CFU/sample	<1.3 * 1.2 = **<1.6 CFU/sample**	N/A
NLF/SLC Method	<1.3 CFU/sample	N/A	<1.3 * (100/85) = **1.5 CFU/sample**

As noted in section 3: due to rounding, these changes may result in minor (if not negligible) differences between sets of identical raw results calculated under different guiding procedures.

5. Application of LOD to Repetitive Recovery Results
When performing repetitive recovery studies, NLF will apply the LOD to all washes other than the first wash. Washes in which 0 growth is recovered will be treated as LOD when performing the Percent Recovery calculation. NLF will retain all previously obtained data and EFs, but it may be recommended that studies significantly impacted by this (typically studies that have low natural bioburden) be assessed for alternative test methods, i.e. inoculated product recovery efficiency. As an example for a study in which the LOD is 1:

Results: W1 = 12; W2 = 4; W3 = 0; W4 = 0

Calculation Method	Method	Calculation	Percent Recovery
GBL Method	*W1 result / sum of all washes * 100* = 12/(12+4+0+0)*100	(12/16) * 100	**75.0%**
NLF/SLC Method	*W1 result / sum of all washes (including LOD values) * 100* = 12/(12+4+<1+<1)*100	(12/18) * 100	**66.7%**

The above is an example of a low bioburden repetitive recovery result. A higher bioburden repetitive recovery result would be less impacted due to the LOD being insignificant when the first number and/or total bioburden is sufficiently higher (e.g., ~100 CFU or greater).

Results: W1 = 120; W2 = 18; W3 = 5; W4 = 0

Calculation Method	Method	Calculation	Percent Recovery
GBL Method	120/(120+18+5+0)*100	(120/143) * 100	**83.9%**
NLF/SLC Method	120/(120+18+5+<1)*100	(120/144) * 100	**83.3%**

6. Cumulative LOD When Performing Re-incubation

For calculation of the Bioburden Estimate, NLF will treat the LOD as cumulative when performing re-incubation studies. A re-incubation study is when test articles are incubated at one incubation parameter (such as 30-35°C) and after incubation and enumeration, transferred to a second incubation parameter (such as 20-25°C) for an additional incubation and enumeration. This type of incubation scheme represents the majority of studies at NLF. The harmonized procedure and validated spreadsheet treat the LOD as cumulative. This means that for calculation of the Bioburden Estimate, the results of the first incubation will be added to the results of the second incubation. When calculating under the GBL SOP, the total counts would be added and averaged for reporting.

As an example, where the LOD (PF) is 1 and the validated Percent Recovery is 85.0%:

Incubation Temperature	Sum of Results For 10 Samples at Noted Temperature	Average	Bioburden Estimate
30-35°C	14 CFU	1.4 CFU/sample * (100/85) = 1.6	1.6 + <1.2 = **2.8**
Re-incubated 20-25°C	10 CFU	<1 CFU/sample * (100/85) = <1.2	

According to the GBL procedure, Adjusted Average Bioburden would have been reported as (14/10) * 1.2 = 1.7. In cases where no growth is observed, this change will manifest as an ostensible increase in product bioburden. To correct for this change when comparing results calculated under different guiding procedures, attention should be paid to the total counts obtained and the incubation scheme to determine if an increase in the Bioburden Estimate is to be attributed to a mathematical difference or to an increase of microbial populations.

7. Adoption of Customer Specification Sheets (CSS)

NLF has identified an area of improvement potential in the process used to reference validated methods for routine work. NLF has adopted the CSS process to capture validated methods and assure adherence for future work. The implementation of these documents has already begun at NLF, and customers will be contacted regarding validation records and CSS introduction as needed.

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Nelson Labs is very committed to delivering you the highest quality results and the above process improvements are in line with that commitment. We aim to provide you with results that are aligned with regulatory requirements and that help us achieve our own company goal of Safeguarding Global Health. If you have any questions or comments on the contents of this memorandum, please do not hesitate to reach me by phone (973-582-1621) or email (dloverro@nelsonlabs.com).

Respectfully Submitted,

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