Sievers* Soleil Rapid Microbial Method Verification Testing for USP <1223>

White Paper

Introduction:

The Sievers Soleil Rapid Bioburden Analyzer is an innovative, at-line rapid microbial method (RMM) instrument designed to monitor bioburden at ultrapure levels utilizing fluorescence-based stains with results in less than 45 minutes. In addition to being rapid, easy to use, portable, and flexible, the Soleil provides near real-time testing that is equivalent to compendial plate count methods, as demonstrated by data presented in this white paper. Soleil offers significant improvements over existing agar plate methods by enhancing user capabilities and ease of use while not sacrificing essential characteristics of plate count and membrane filtration technology.[1] With the Sievers Soleil Rapid Bioburden Analyzer, manufacturers can make actionable decisions quickly. It allows users to quickly and easily monitor bioburden levels throughout the manufacturing process from raw materials to in-process samples to final product, alleviating risks and improving operations. With the 2023 publication of Annex 1, more manufacturers are adopting rapid alternative methods as a means of contamination control.

Background:

This white paper outlines and describes the Sievers Soleil microorganism verification testing aligning with parameters mentioned in United States Pharmacopeia <1223> “VALIDATION OF ALTERNATIVE MICROBIOLOGICAL METHODS” while also showing the comparison to traditional agar plating methods. The results are assessed against criteria described in USP <61> “MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: MICROBIAL ENUMERATION TESTS”, USP <62>“ MICROBIOLOGICAL EXAMINATION OF NONSTERILE PRODUCTS: TESTS FOR SPECIFIED MICROORGANISMS”, European Pharmacopoeia 2.6.12 “Nonsterile Products Total Viable Aerobic Count”, EP 2.6.13 “Nonsterile Products Specified Organisms” and Japanese Pharmacopoeia 4.05 “Microbiological Examination of Nonsterile Products”.

The following validation criteria described in USP <1223> were evaluated for the Sievers Soleil: Range, Linearity, Accuracy, Reproducibility, Robustness, Precision, Lower Limit of Quantitation (LLOQ), Lower Limit of Detection (LLOD), Upper Limit of Quantitation (ULOQ), and Ruggedness. Additionally, this white paper demonstrates the correlation of Soleil’s Total Viable Cell counts (TVCs, as the Soleil software reports) to colony forming units (CFUs) on agar plates. As such, the data here is reported in CFUs since they are comparable and equivalent to TVC using the Soleil.

A total of 11 microorganisms were tested, as well as a combination of organisms:

- *A. brasiliensis*
- *B. cepacia*
- *B. diminuta*
- *B. subtilis*
- *C. albicans*

- *E. coli*
- *P. aeruginosa*
- *R. pickettii*
- *S. aureus*
- *S. enterica*
- *S. maltophilia*
- *Mixture: B. diminuta, R. pickettii, S. maltophilia, & B. cepacia*

All samples were run at target concentrations in 100mL sample volumes. The target concentrations tested included:

- 0.05 CFU/mL (LLOD)
- 0.1 CFU/mL (LLOQ)
- 1 CFU/mL
- 10 CFU/mL
- 100 CFU/mL (ULOQ)

The concentrations tested under this study were specifically chosen based on industry standards. The 0.05 CFU/mL was selected because it is a common lower limit used in the pharmaceutical industry. For Water for Injection (WFI), the release limit is 0.1 CFU/mL. Testing 0.1 CFU/mL, 1 CFU/mL, 10 CFU/mL, and 100 CFU/mL provided a 3-log and 4-log set of concentrations to determine both linearity and the upper limit of quantification. For purified water systems, the FDA has mentioned that any action limit over 100 CFU/mL is not acceptable.

This testing also included the assessment of negative controls as part of the daily procedures during routine testing for microorganisms.

Methods:

Negative Control

As part of the daily start-up procedures within the Soleil software, a Flow Calibration, System Suitability Standards test, and a Negative Control were performed. Throughout the course of testing, a total of 85 Negative Controls were run using Water for Cell Culture (WFCC) over the course of several weeks by six analysts using 10 different instruments at two different locations.

System Suitability Standards

The System Suitability Standards are a set of fluorescent beads with known spectral profiles mimicking those from viable microorganisms at two known concentrations per milliliter. Soleil has a built-in Pass/Fail system suitability test within the software to confirm the accuracy of counts, to show the flow calibration has been performed properly, and to show the analytics are accurately counting.The System Suitability Standards were run at the start-up of the instrument throughout the duration of the testing time frame. System Suitability 1 contained 10 beads/100mL and System Suitability 2 contained 20 beads/100mL. The acceptance criteria were as follows: System Suitability Standard 1 was ±5 beads/mL and System Suitability Standard 2 was ± 10 beads/mL.

Traditional Membrane Filtration

Throughout the testing period, samples were tested via traditional methods and on the Soleil. Organisms were added to 250mL of WFCC, gently swirled, and then aliquoted out for membrane filtration and for testing on the Soleil.

Lower Limit of Detection (LLOD)

LLOD is aimed at addressing whether or not the Soleil platform is capable of distinguishing between a blank (e.g., negative sample) and a low level of contamination, specifically 0.05 CFU/mL. To calculate the LLOD, 10 replicates from each microorganism species were tested at 0.05 CFU/mL and compared to 85 negative control samples during the testing period. This comparison was performed using an Analysis of Variance resulting in a p-value of 0.001, showing that the data is significant and the Soleil can reliably detect low levels of bioburden above the level of the negative control.

Lower Limit of Quantitation (LLOQ)

The LLOQ attribute was performed to confirm that the Soleil platform can properly quantify microorganisms down to a specific concentration. Ten replicates from each microorganism were tested at a target concentration of 0.1 CFU/mL. The acceptance criteria for the LLOQ was to recover the average concentration compared to plate counts >50% and a goal to be <200%. The acceptance criteria for the average concentration was determined because results from the Soleil can sometimes be higher than agar plates due to the health of the various cells. Some cells may be healthier or weaker when plated, which affects their ability to grow. The Soleil uses specific dyes that target organisms, regardless of their health status.

Upper Limit of Quantitation (ULOQ)

To determine the ULOQ, a target concentration of 100 CFU/mL was tested for each microorganism. There were six replicates tested for each organism in comparison to agar plates. The acceptance criteria was to have an average percent (%) recovery of >50% compared to traditional agar plate methods with a goal of <200%.

Linearity

For this testing, linearity is referred to as the correlation coefficient over a minimum of three logs of contamination. The linearity must be greater than 0.95 per USP <1223>. A total of five concentrations were tested across all organisms. Additionally, the linearity of agar plates was also assessed for the same microorganisms.

Accuracy

Accuracy is defined as the closeness of the test results obtained by the alternative test method (e.g., Soleil) to the value obtained by the compendial method.[1] To verify that the Soleil instrument was capable of measuring the quantity of microorganisms accurately, the traditional membrane filtration method was performed alongside the Soleil. Each sample was aliquoted evenly between Soleil and plate method to ensure both methods were testing the same sample, and the averages from each target concentration were compared to evaluate accuracy. The concentrations tested were 0.05 CFU/mL, 0.1 CFU/mL, 1 CFU/mL, 10 CFU/mL, and 100 CFU/mL.

Precision

Also known as repeatability, precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of the same inoculation. Precision was assessed across the test range (0.1 CFU/mL to 100 CFU/mL) on the Soleil by comparing the coefficient of variation (CV) of ten replicates assessed at each of the lower concentrations (0.05, 0.1, and 1 CFU/mL) and six replicates for the higher concentrations (10 CFU/mL and 100 CFU/mL).

Agar Plate

The concentrations for this study were prepared using the following method. Concentrations were targeted at 0.05 CFU/mL, 0.1 CFU/mL, 1 CFU/mL, 10 CFU/mL, and 100 CFU/mL in volumes of 125 mL in sterile PET bottles. Serial dilutions were performed in order to obtain the desired concentration. The solutions were added to buffered WFCC, in order to maintain the cell integrity. Agar plates containing tryptic soy agar (TSA) or sabouraud dextrose agar (SDA) as directed in USP <61> and USP <62> were used. Each solution was filtered through a manifold onto a sterile filter. The filter was then aseptically transferred to the agar plate. The plates were incubated in a cell incubator for a minimum of three days.

For the 100 CFU/mL samples, flood plates were used. The 100 CFU/mL stock solution was transferred to agar plates containing the appropriate media. A sterile cell spreader was then used to distribute the liquid across the surface. The plates were incubated for a minimum of three days.

Results:

Negative Control

All 85 Negative Controls throughout this testing were below 6 CFU/100mL. The average CFU/mL was 0.0068 CFU/mL, which was below the internal requirements of <0.05 CFU/mL (Table 1 below).

System Suitability Standards

All System Suitability Standards passed the acceptance criteria set in the Soleil software. The acceptance criteria is as follows: System Suitability Standard 1 is ± 5 beads/mL and System Suitability Standard 2 is ± 10 beads/mL.

Lower Limit of Detection

The average biotic count for all microorganisms at a target concentration of 0.05 CFU/mL was 0.055 CFU/mL as illustrated in Table 1.

Table 1: Average of the Negative Controls and Results for the Lower Limit of Detection

	0.05 CFU/mL		
	Average	**Minimum**	**Maximum**
A. brasiliensis	0.063	0.030	0.09
B. cepacia	0.039	0.010	0.012
B. diminuta	0.078	0.040	0.015
B. subtilis	0.061	0.020	0.012
C. albicans	0.090	0.020	0.021
E. coli	0.052	0.010	0.090
P. aeruginosa	0.036	0.020	0.060
R. pickettii	0.037	0.0	0.080
S. aureus	0.058	0.020	0.090
S. enterica	0.066	0.030	0.011
S. maltophilia	0.041	0.010	0.090
Mixture	0.071	0.010	0.015
Avg of All Microbes	0.055	0.018	0.0113
Negative Control	0.0068	0.0	0.06

Lower Limit of Quantification (LLOQ)

The target concentration for the determination of the LLOQ was 0.1 CFU/mL across all organisms. The average percent recovery was 140.9%, which was >50% and achieved the goal of <200% set by the acceptance criteria. Results can be see in Table 2.

Table 2: Results for the Lower Limit of Quantification

	0.1 CFU/mL		
	Average	**Minimum**	**Maximum**
A. brasiliensis	70.6%	25.0%	113.0%
B. cepacia	97.6%	23.8%	238.1%
B. diminuta	180.8%	125.0%	271.0%
B. subtilis	172.0%	58.0%	484.0%
C. albicans	162.3%	77.9%	389.6%
E. coli	137.4%	78.9%	189.5%
P. aeruginosa	98.4%	62.5%	203.1%
R. pickettii	108.0%	50.0%	182.0%
S. aureus	262.5%	125.0%	625.0%
S. enterica	155.3%	78.0%	400.0%
S. maltophilia	155.4%	29.0%	400.0%
Mixture	90.1%	24.0%	213.0%
Avg of All Microbes	140.9%	63.1%	309.0%

Upper Limit of Quantitation

The average recovery for the upper limit at 100 CFU/mL was 136.7% across multiple organisms (Table 3).

Table 3: Results for the Upper Limit of Quantitation

	100 CFU/mL		
	Average	**Minimum**	**Maximum**
A. brasiliensis	82.7%	64.0%	112.0%
B. cepacia	74.7%	53.2%	97.3%
B. diminuta	272.0%	135.0%	453.0%
B. subtilis	313.3%	164.0%	508.0%
C. albicans	95.5%	77.9%	106.3%
E. coli	134.0%	117.0%	143.0%
P. aeruginosa	106.0%	100.5%	109.8%
R. pickettii	45.8%	37.0%	50.0%
S. aureus	152.8%	136.3%	164.5%
S. enterica	138.2%	92.0%	218.0%
S. maltophilia	115.2%	91.0%	146.0%
Mixture	109.8%	95.0%	129.0%
Avg of All Microbes	136.7%	96.9%	186.4%

Linearity

Least-squares regression calculations and corresponding coefficient of determination (R^2) values for a 4-log assessment and two 3-log assessments were determined. These values can be found in Table 4 (USP <1223> specification for R^2 is ≥0.95). The Soleil platform is capable of running a maximum of 4 logs. Linearity plots for Soleil and agar plates are presented in the Appendix.

Table 4: Soleil Linearity results for 4 Logs and 3 Logs

	Linearity (R^2) of Soleil		
	4 Logs	3 Logs	
	0.1 - 100 CFU/mL	0.1 - 10 CFU/mL	1 - 100 CFU/mL
A. brasiliensis	0.960	0.956	0.954
B. cepacia	0.932	0.961	0.922
B. diminuta	0.992	0.992	0.991
B. subtilis	0.984	0.980	0.981
C. albicans	0.990	0.926	0.989
E. coli	0.993	0.979	0.992
P. aeruginosa	0.998	0.989	0.998
R. pickettii	0.963	0.984	0.957
S. aureus	0.989	0.987	0.987
S. enterica	0.996	0.957	0.996
S. maltophilia	0.963	0.993	0.957
Mixture	0.990	0.994	0.988
Avg of All Microbes	0.979	0.975	0.976

Linearity was also calculated over all organisms for the agar plates, Table 5 (next page). The average linearity for the agar plates for 4 logs was 0.952 looking at 0.1-100 CFU/mL.

Table 5: Results of Linearity of Agar Plates

	Linearity (R^2) of Agar Plates		
	4 Logs	3 Logs	
	0.1 - 100 CFU/mL	0.1 - 10 CFU/mL	1 - 100 CFU/mL
A. brasiliensis	0.996	0.989	0.995
B. cepacia	1.000	1.000	1.000
B. diminuta	0.756	0.937	0.723
B. subtilis	0.812	0.788	0.788
C. albicans	1.000	1.000	1.000
E. coli	0.999	0.997	0.999
P. aeruginosa	1.000	1.000	1.000
R. pickettii	0.986	0.994	0.983
S. aureus	0.996	0.954	0.995
S. enterica	0.928	0.969	0.917
S. maltophilia	0.984	0.826	0.981
Mixture	0.970	0.966	0.966
Avg of All Microbes	0.952	0.952	0.946

Accuracy

The acceptance criteria for accuracy for the Soleil platform is an average recovery of >50% and <200% compared to traditional plate counts from membrane filtration. The average recovery across all microorganism testing was 137.7%, as seen in Table 6.

Table 6: Accuracy Results for the Sievers Soleil

	Average Across all Concentrations (%)		
	Average	Minimum	Maximum
A. brasiliensis	76.7	46.6	111.8
B. cepacia	98.2	55.0	193.3
B. diminuta	206.6	103.8	355.6
B. subtilis	216.2	109.8	446.2
C. albicans	124.9	61.8	244.8
E. coli	155.4	85.9	214.7
P. aeruginosa	95.5	68.8	136.0
R. pickettii	80.1	41.8	126.6
S. aureus	192.4	119.0	303.7
S. enterica	147.2	89.6	294.8
S. maltophilia	134.4	54.8	386.0
Mixture	125.0	60.0	283.4
Avg of All Microbes	137.7	74.7	258.1

Precision

The Coefficient of Variation (CV) was calculated for each microorganism tested. Traditional methods typically have a CV % limit of 35% as microorganisms may grow differently on agar plates. From the data in Figure 1, it was determined that the LLOQ was 1 CFU/mL, as that was the lowest concentration that showed the best CV performance, with the average being below 25%.

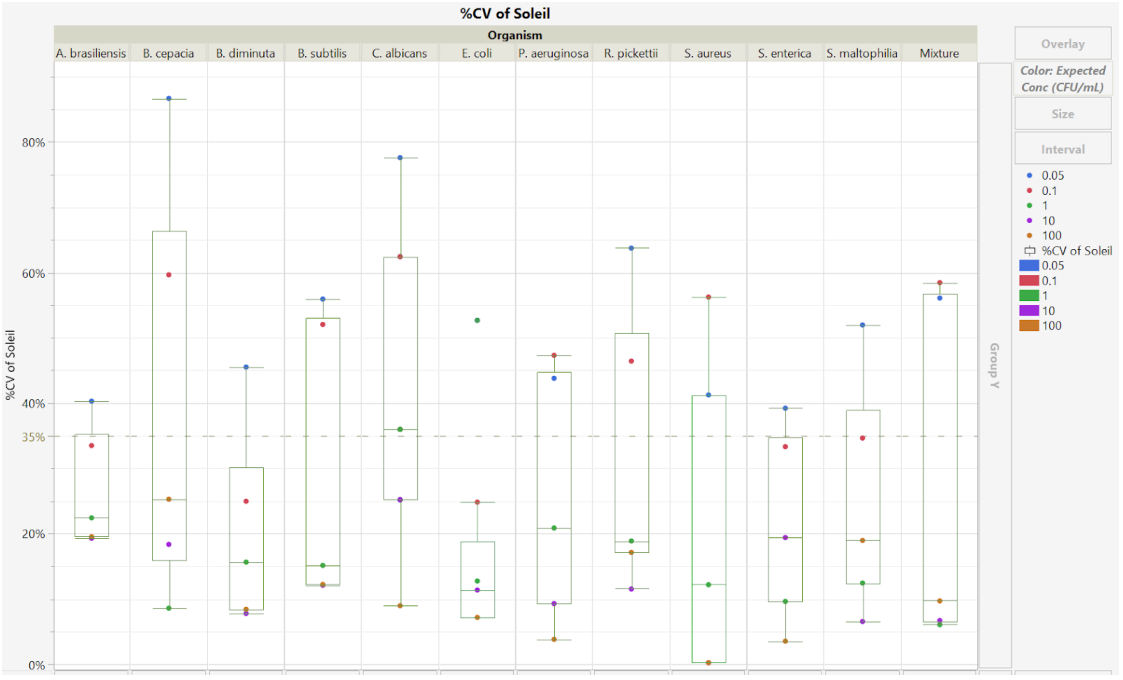


Figure 1

Figure 2 shows the Precision for agar plates at the various concentrations.

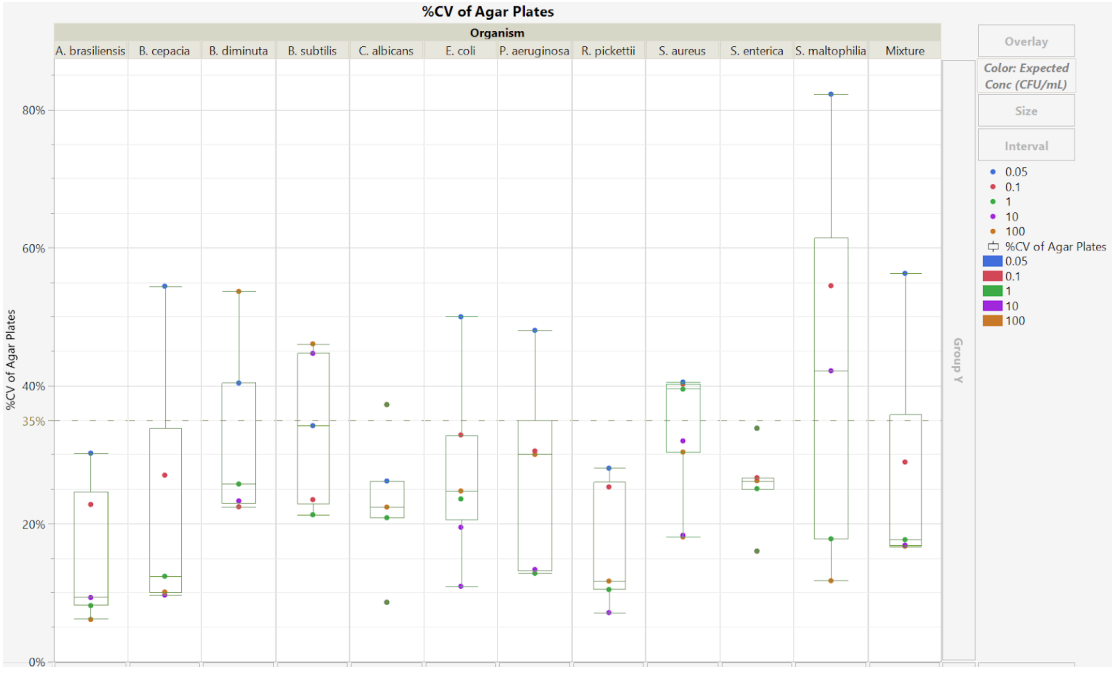


Figure 2

Conclusion

The Sievers Soleil Rapid Bioburden Analyzer has demonstrated the ability to detect and quantify bacteria (both Gram-positive and Gram-negative), yeasts, and mold in under 45 minutes with acceptable accuracy, linearity and precision as outlined in the USP <1223>. With a Limit of Detection at 0.05 CFU/mL and a Limit of Quantification at or below 1.0 CFU/mL, the Soleil shows suitability for all levels of high-purity water testing.

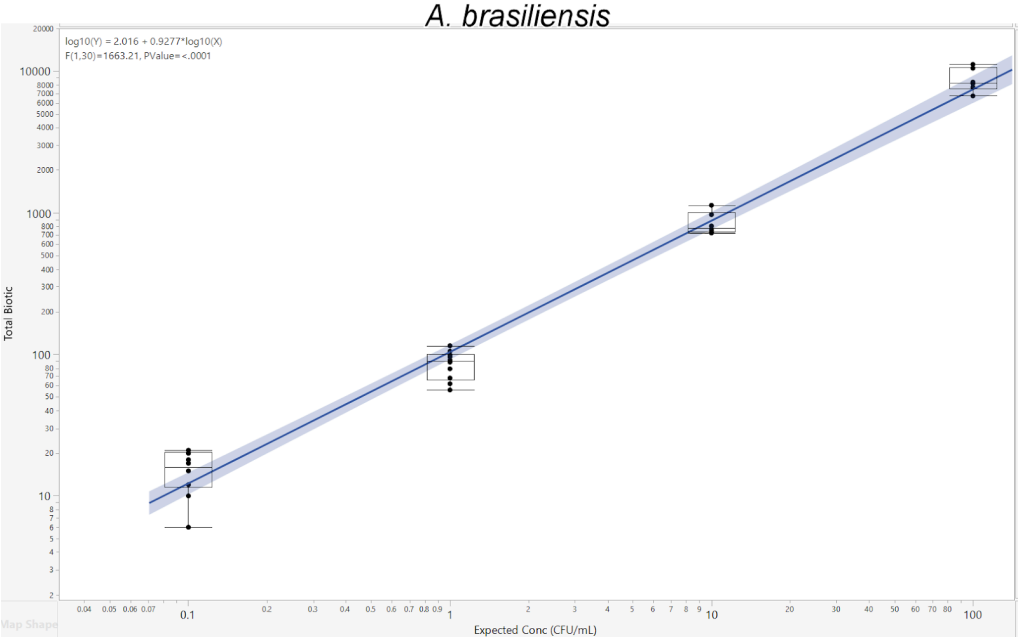
References

1.USP <1223> Validation of Alternative Microbiological Methods
2. EP 2.6.12 Nonsterile Products Total Viable Aerobic Count
3. EP 2.6.13 Nonsterile Products Specified Organisms
4. JP 4.05 Microbiological Examination of Nonsterile Products

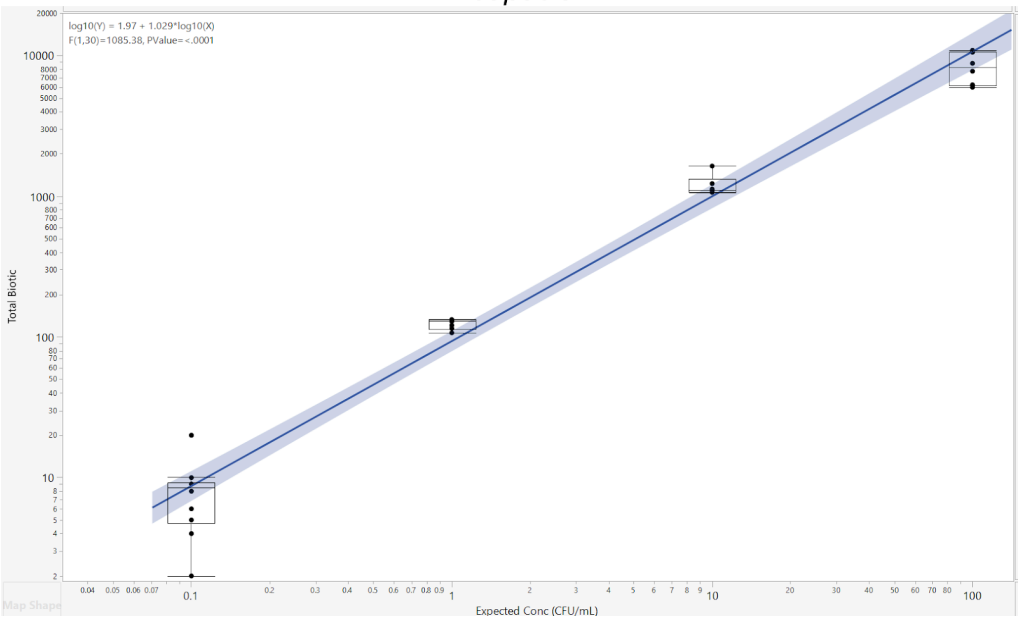
Appendix

Linearity

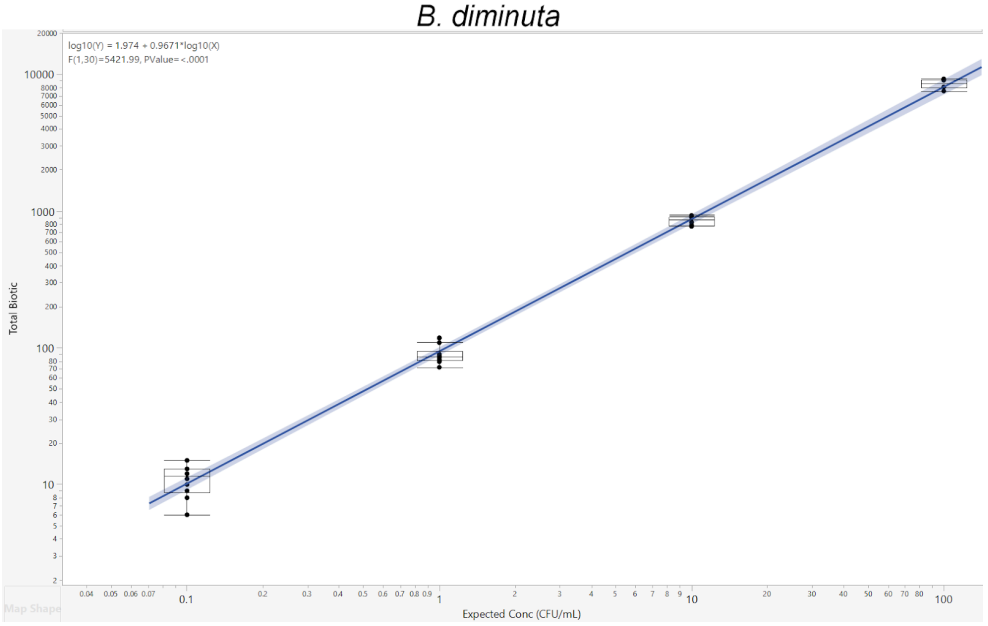
A. brasiliensis



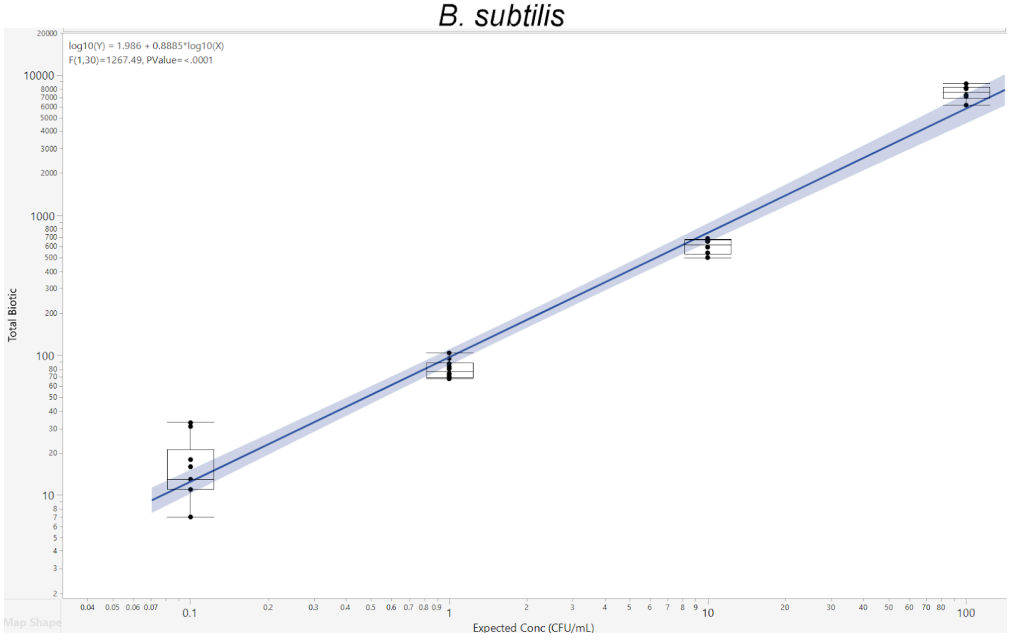
B. cepacia



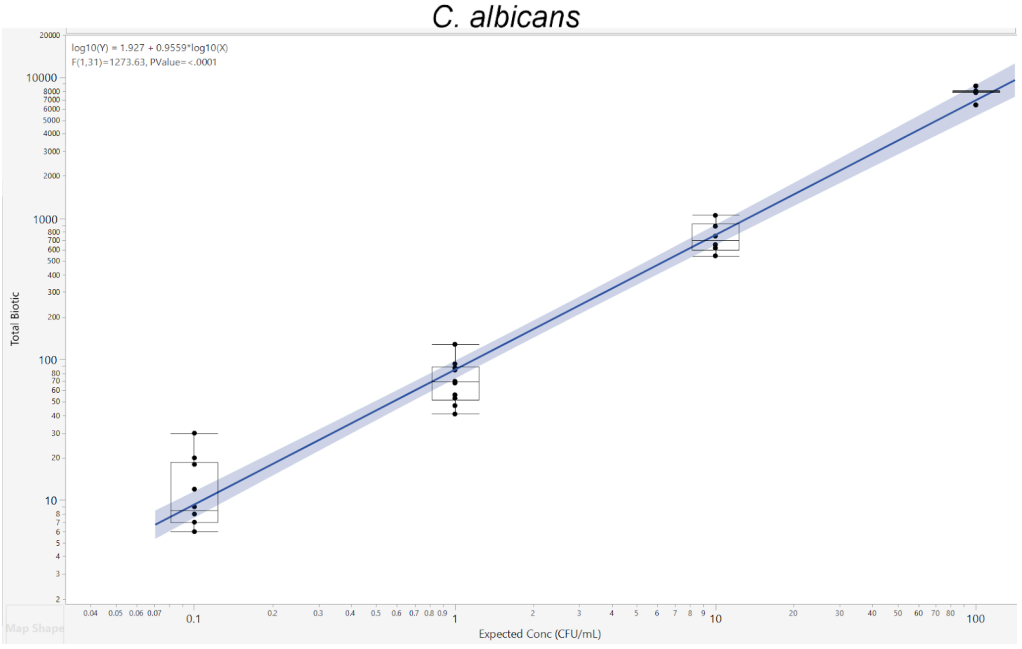
B. diminuta



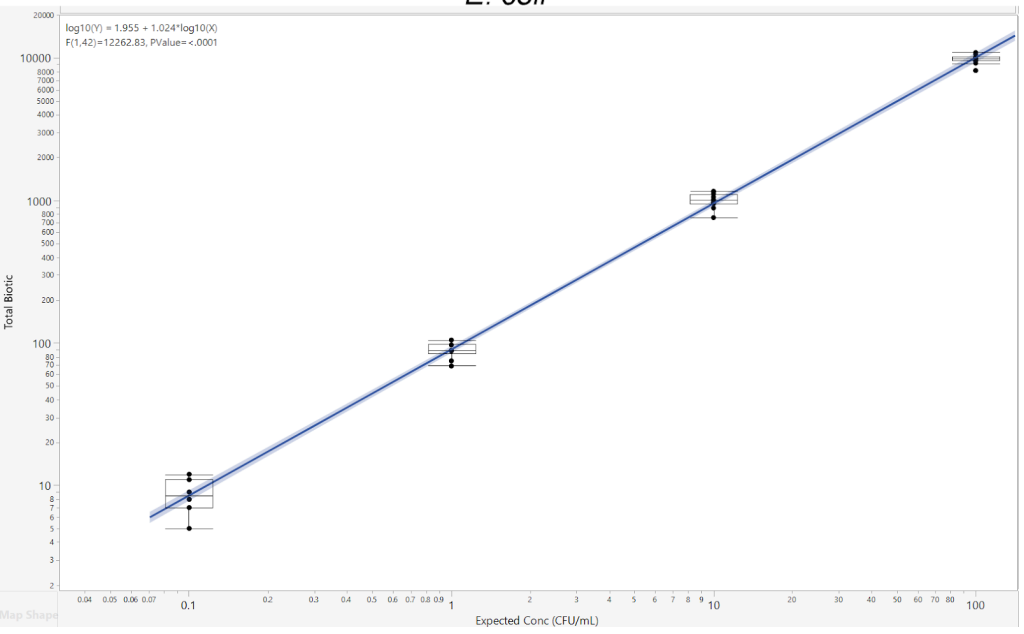
B. subtilis



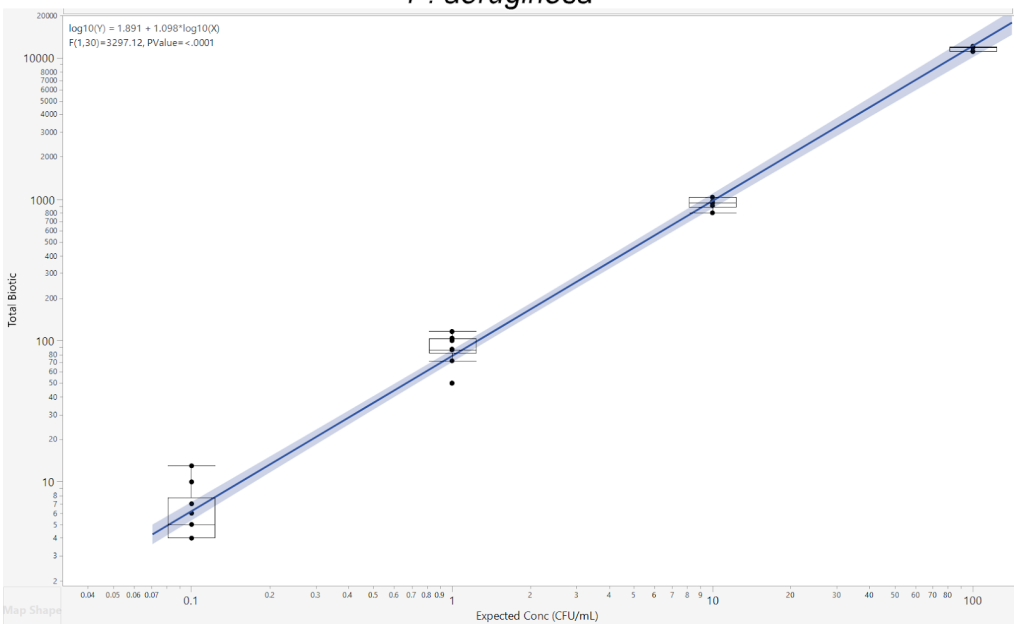
C. albicans



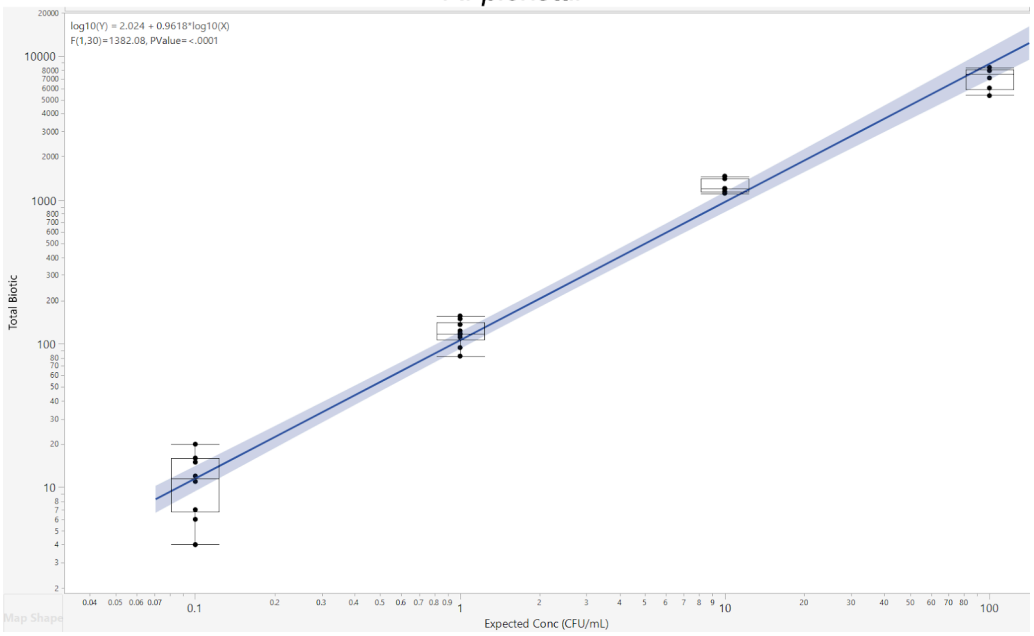
E. coli



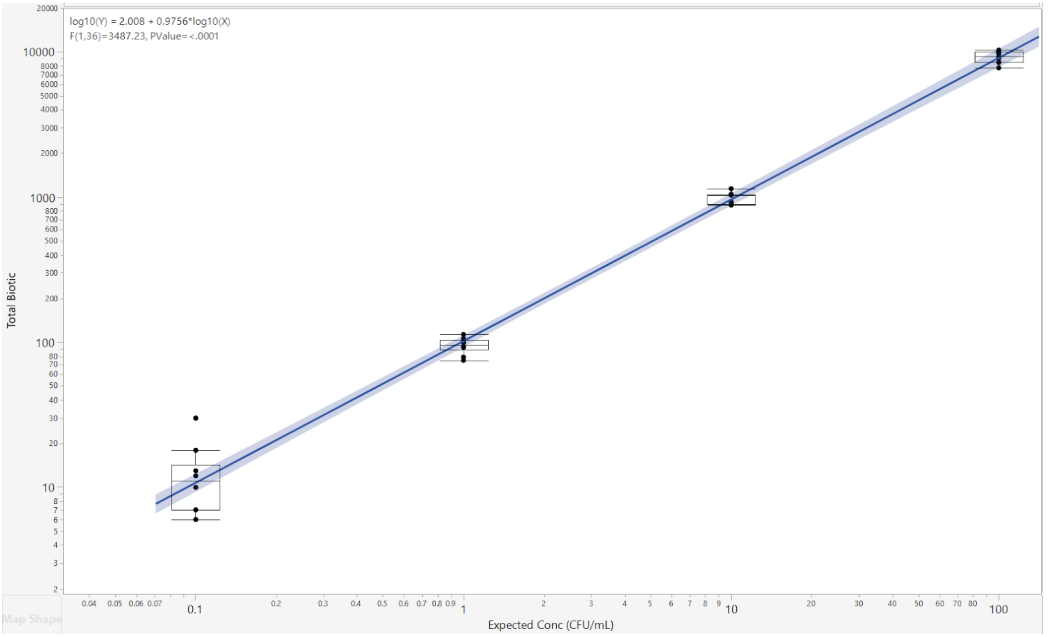
P. aeruginosa



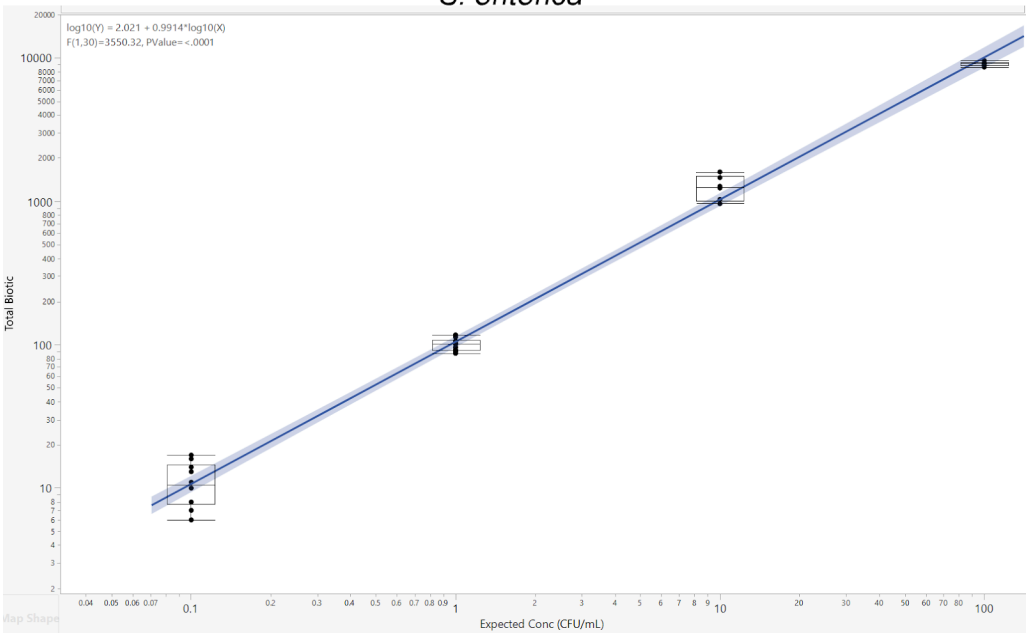
R. pickettii



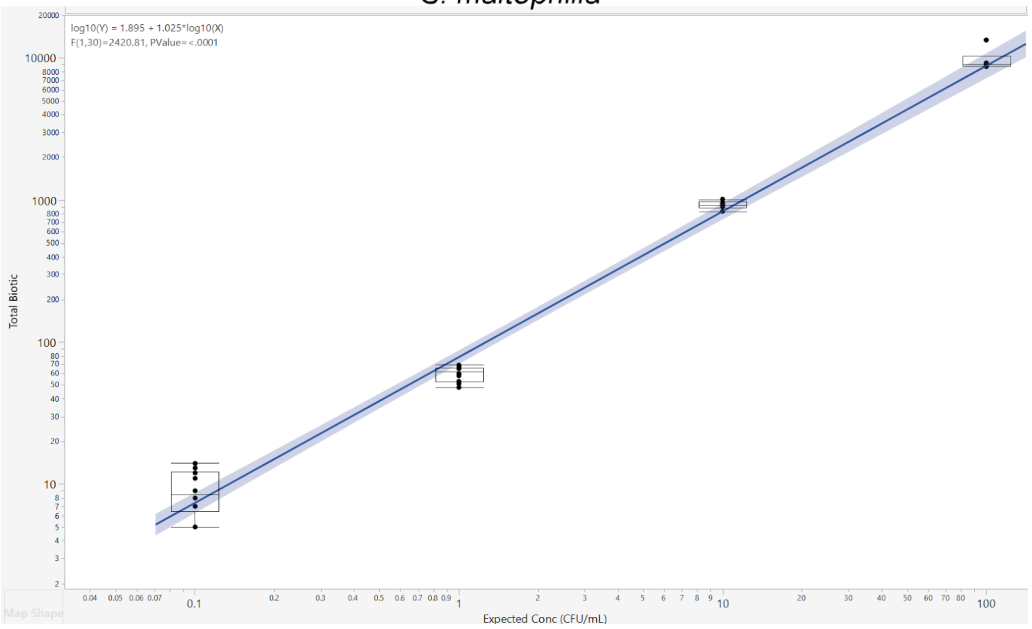
S. aureus



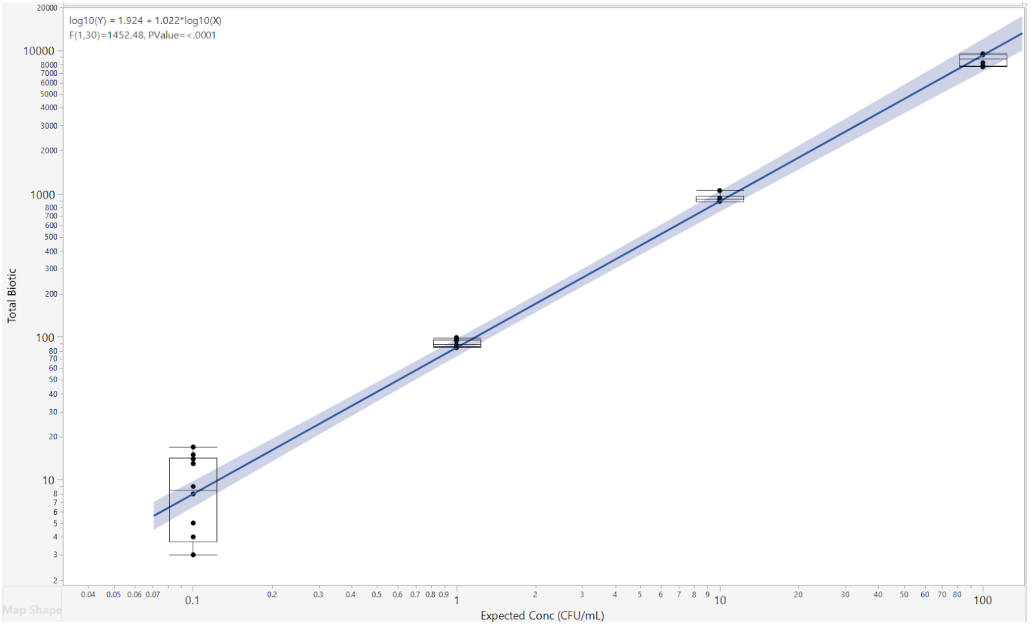
S. enterica



S. maltophilia

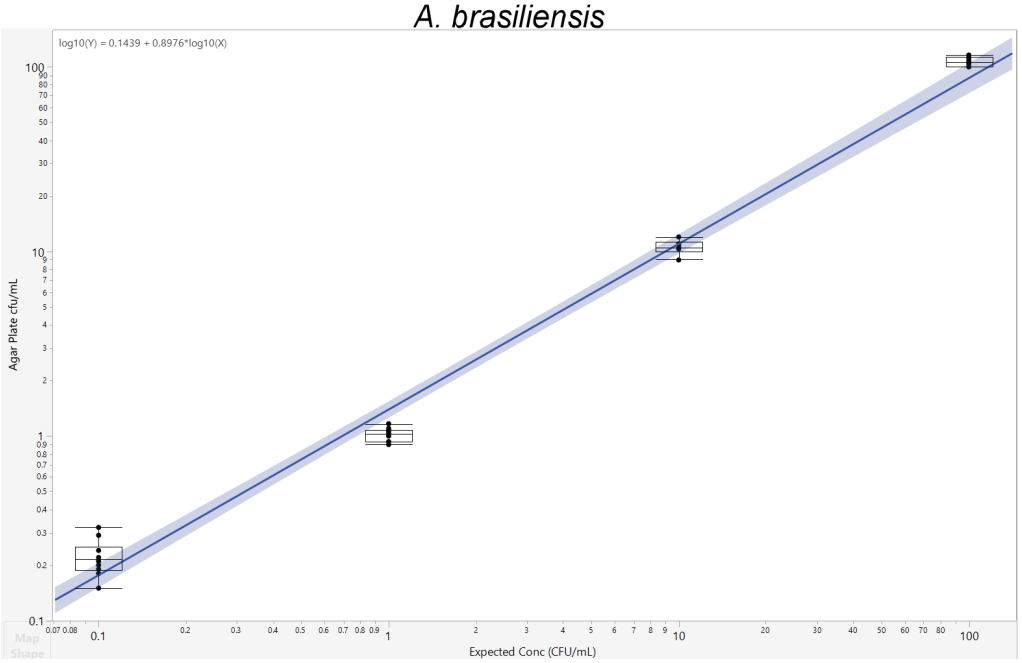


Mixture

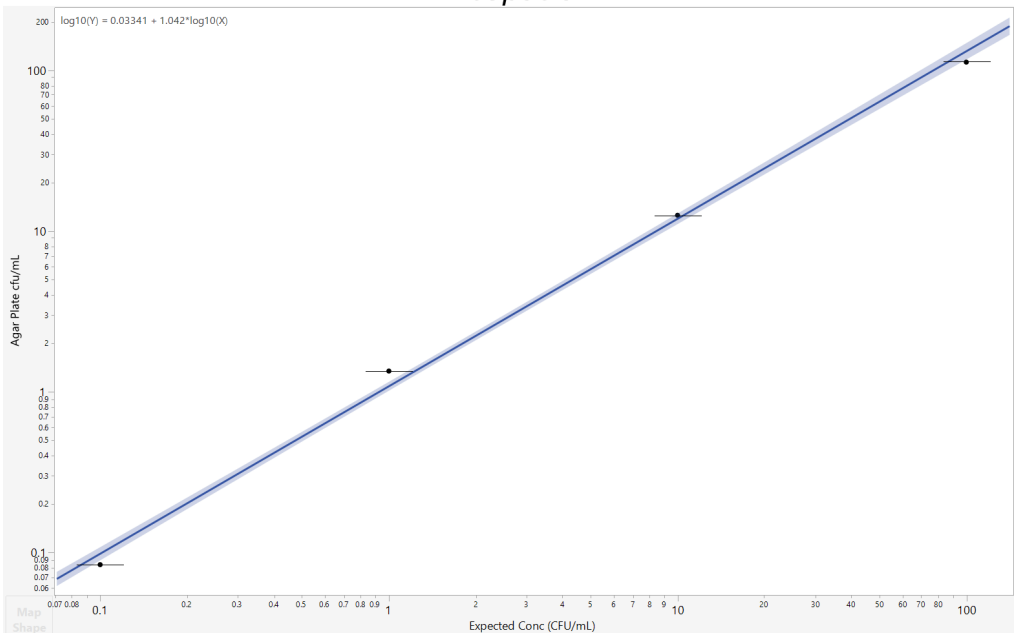


Agar Plates:

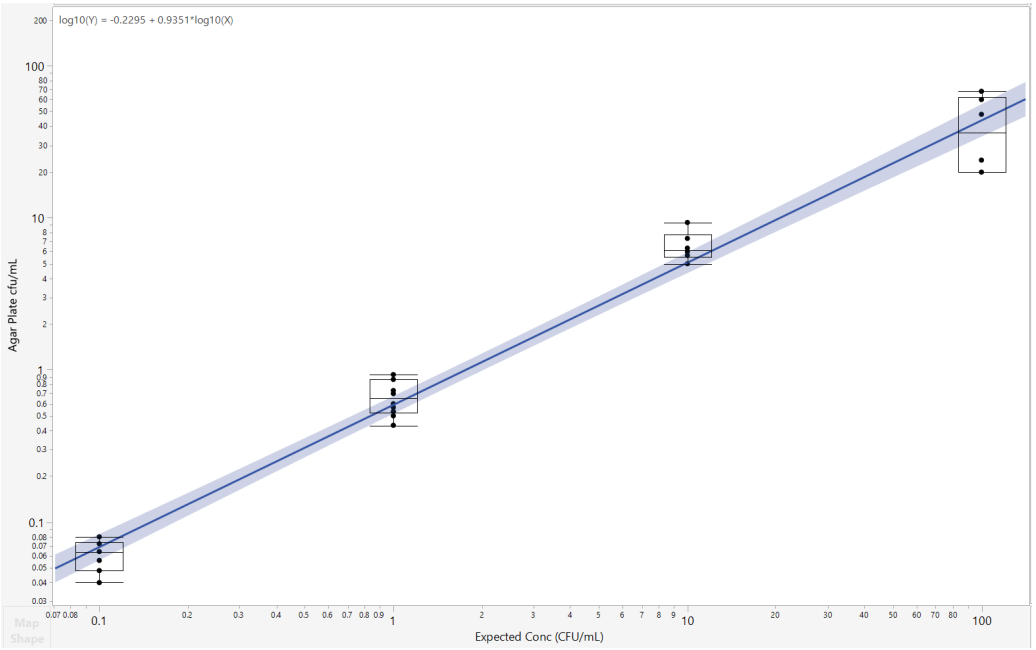
A. brasiliensis



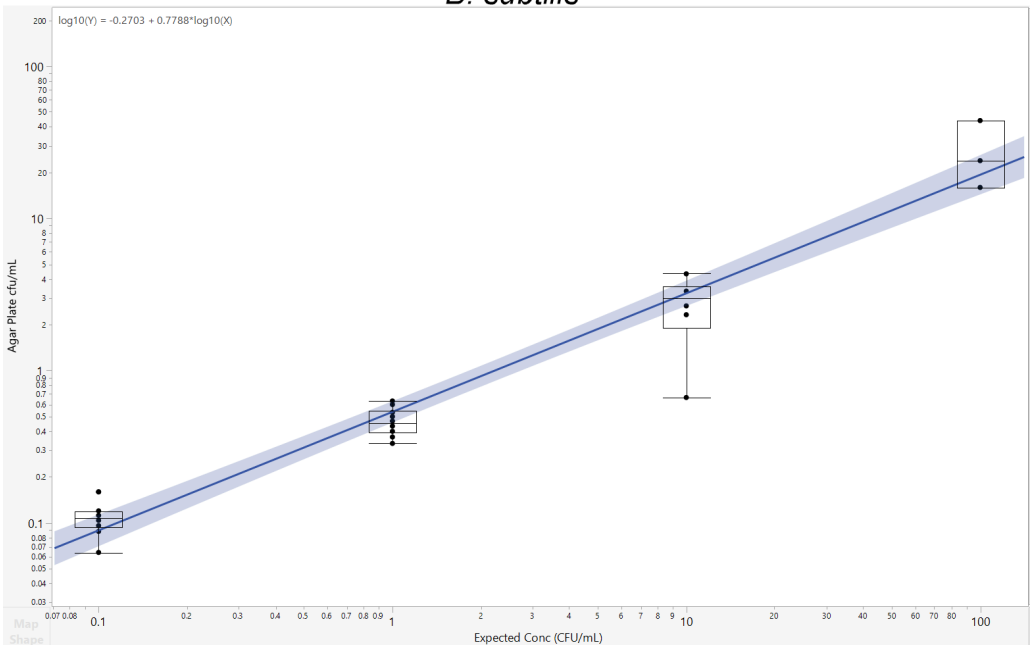
B. cepacia



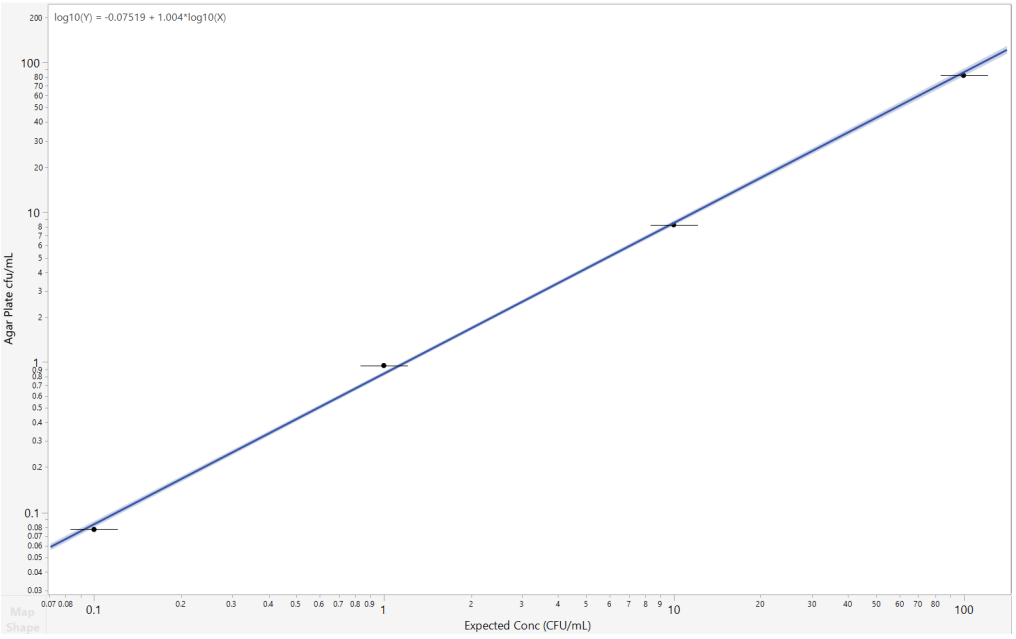
B. diminuta



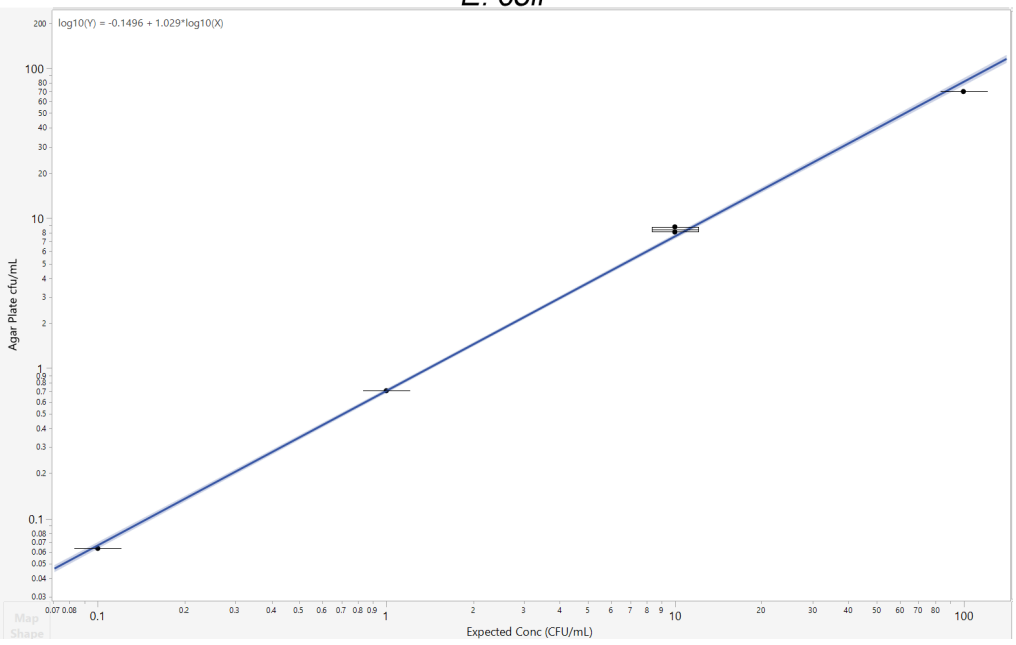
B. subtilis



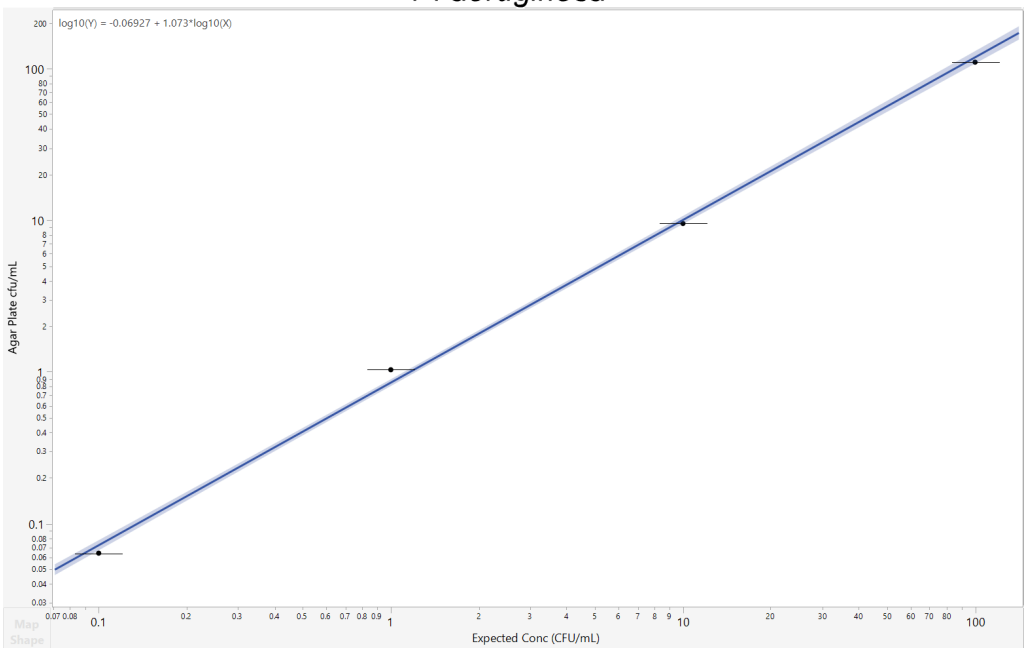
C. albicans



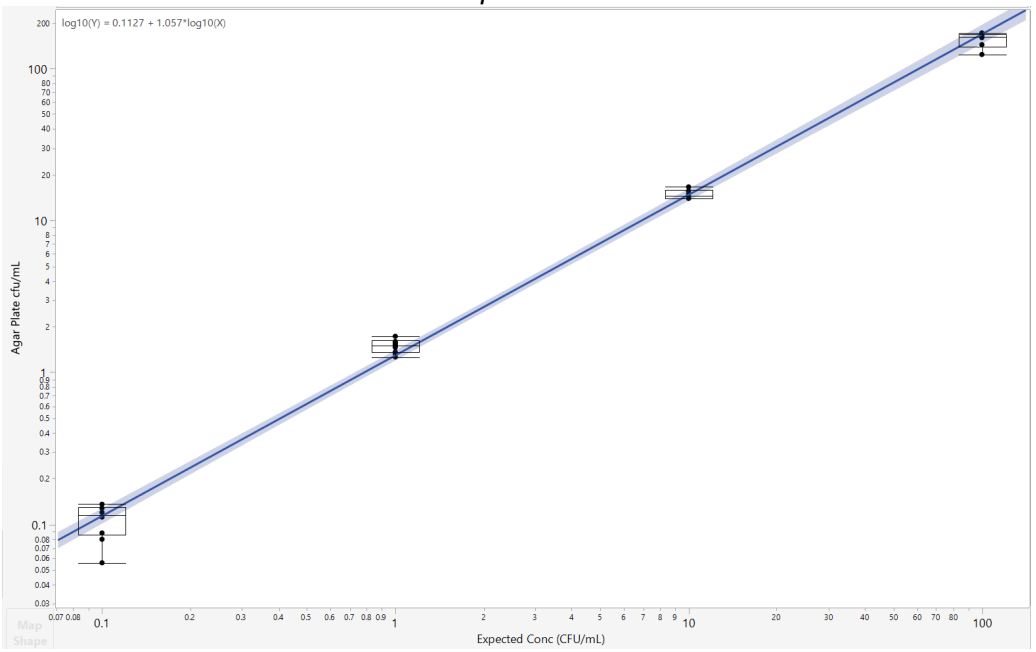
E. coli



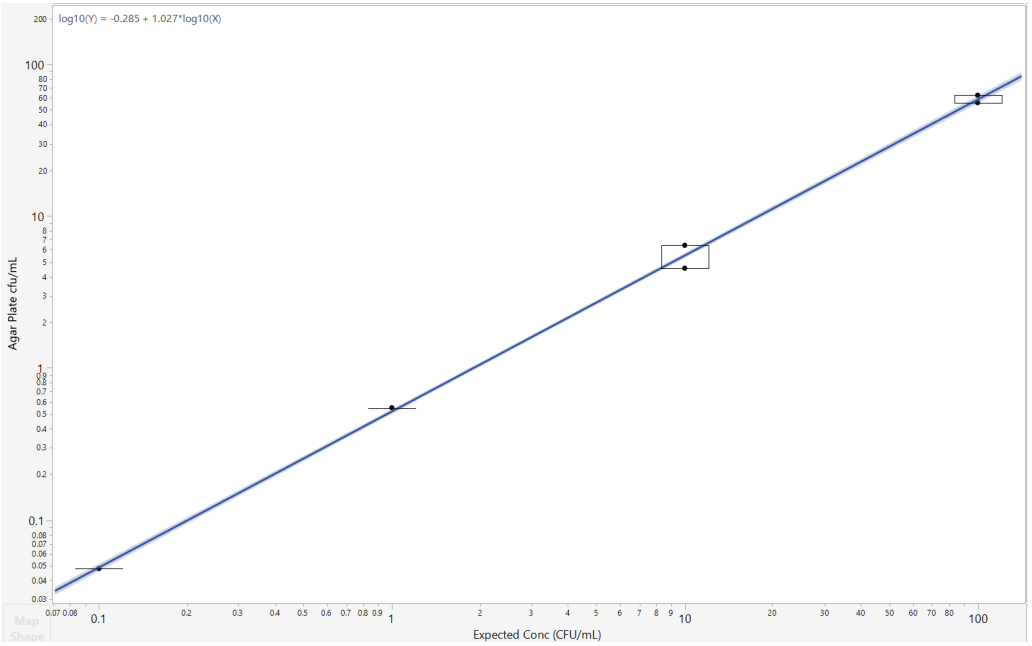
P. aeruginosa



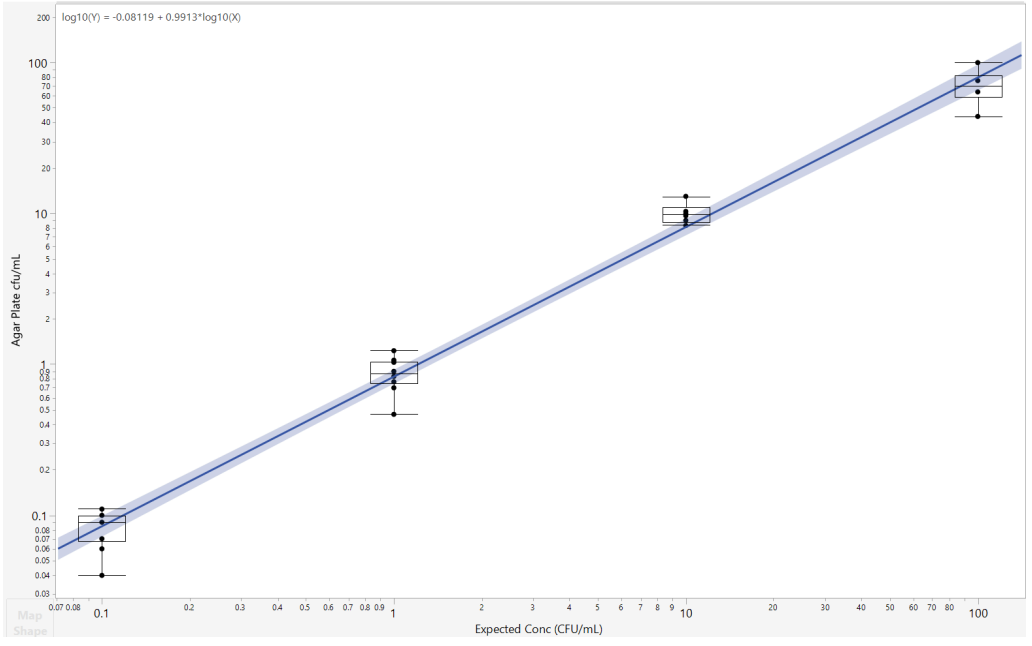
R. pickettii



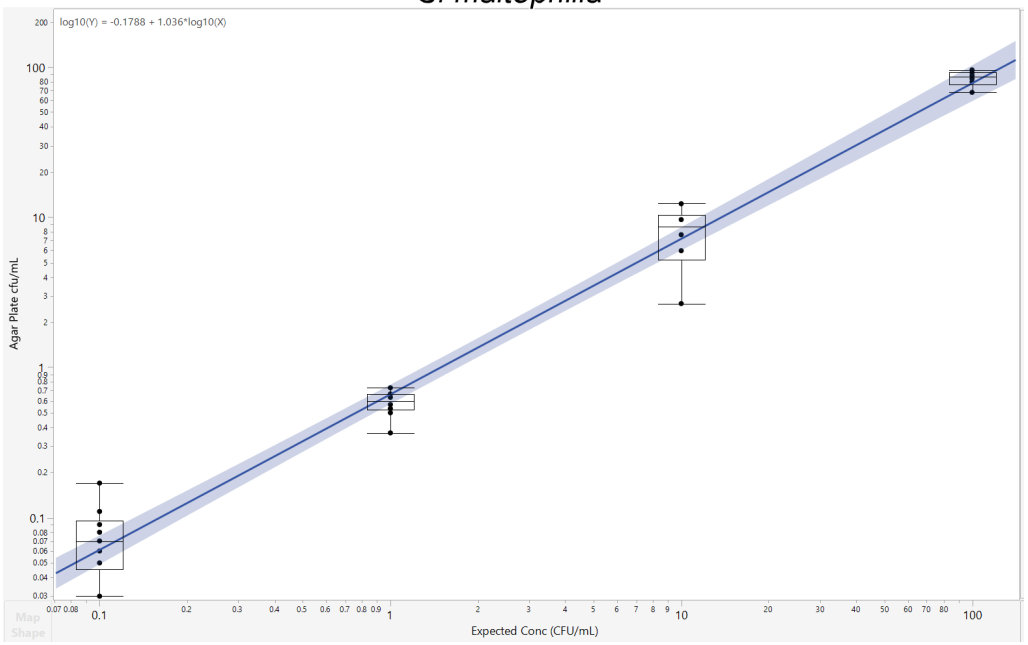
S. aureus



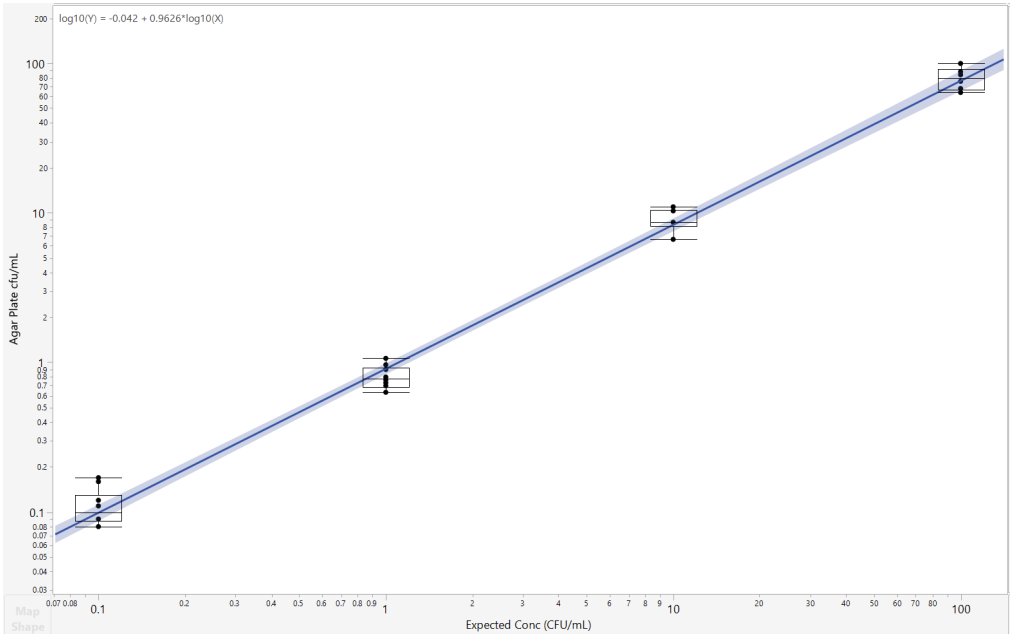
S. enterica



S. maltophilia



Mixture



Accuracy