Training Program Development

Microorganism recovery in broad spectrum test samples and in neutralization broths with three different microbiologists (*accuracy and precision evaluation)*

Certified Laboratories
Cosmetic Microbiology Department, Melville, New York

R. A. Boehler, Jr., MSc.

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- 10:15 AM to 11:00 AM
- 12:30 PM to 2:00 PM
- 3:30 PM to 4:15 PM

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ACCURACY VS. PRECISION

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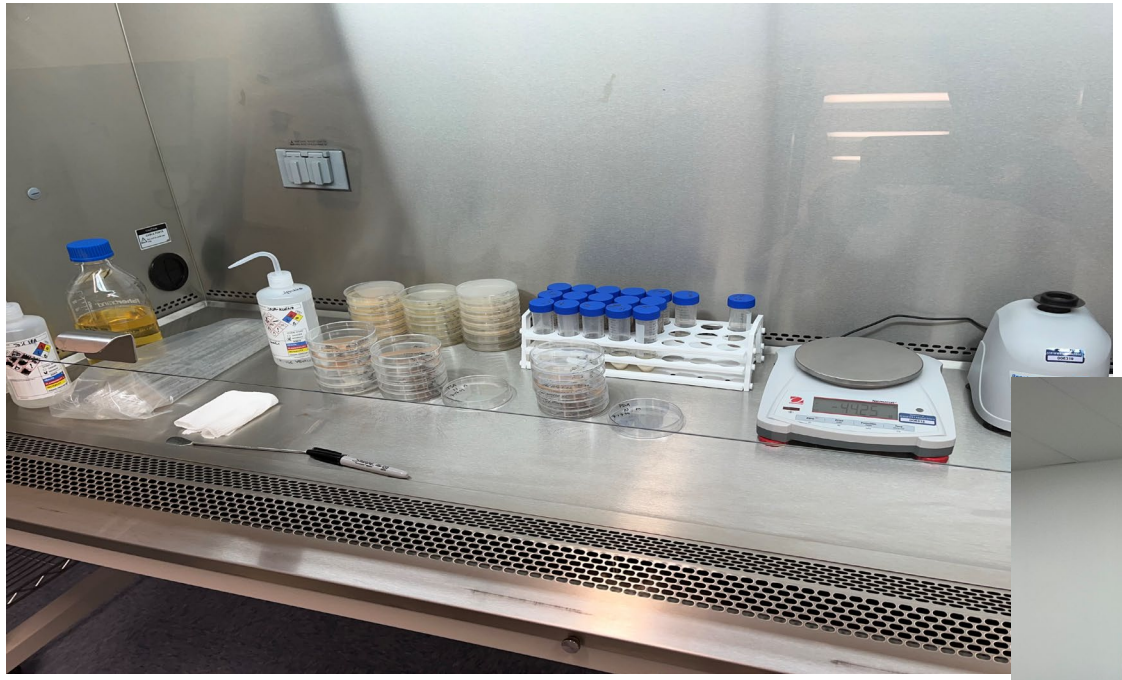
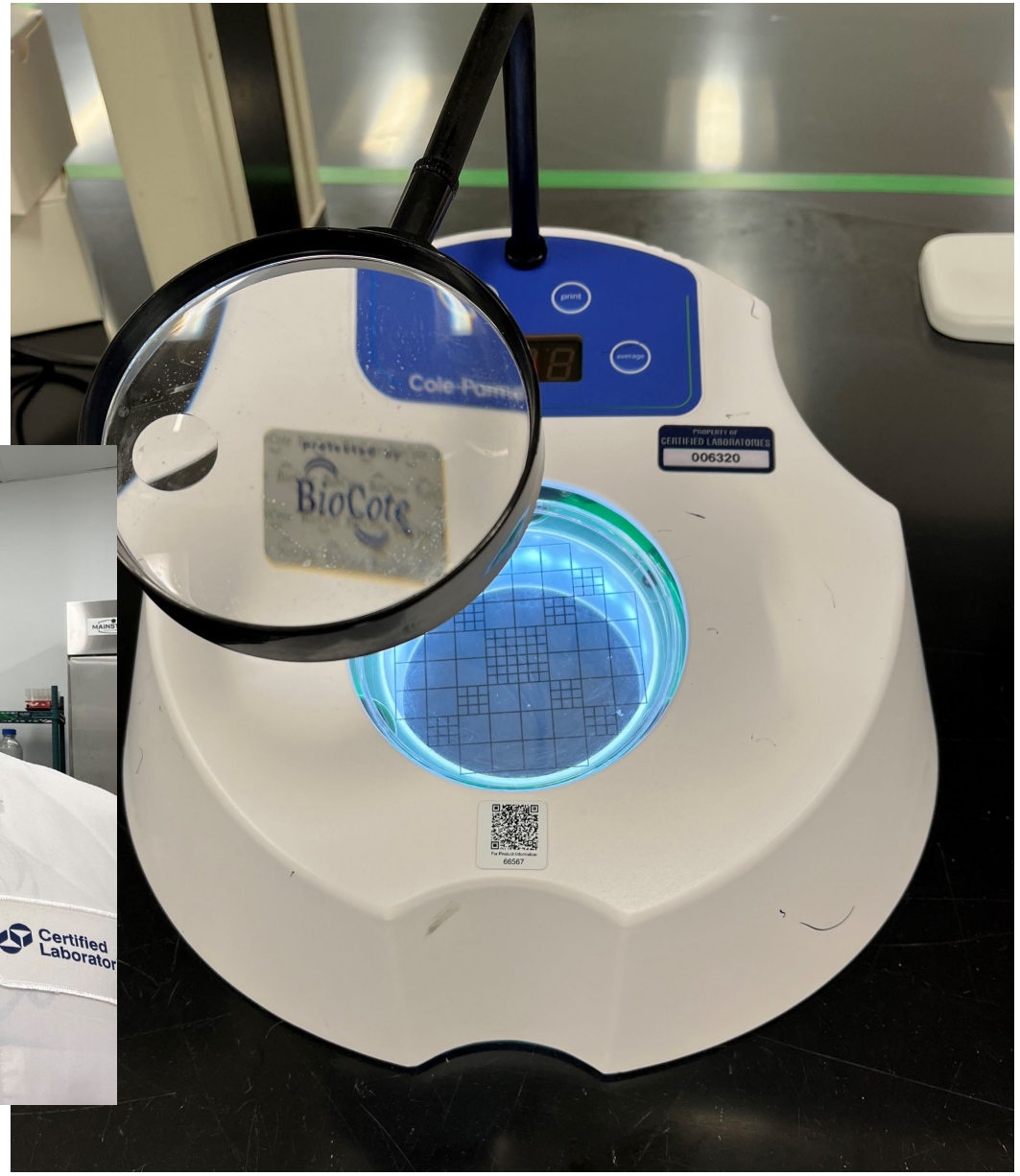
Laboratory Tools and Personnel





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Accuracy and Precision

Two key test parameters for the traditional aerobic plate count test and the traditional yeast & mold plate count test.

Training Program Execution

- During a training program, data was collected for test area microbiologists
- Spike three different cosmetic categories
  - baby sunscreen lotion
  - biotin collagen shampoo
  - body wash
- Cosmetics first neutralized with tryptone azolectin broth/tween 20 and lecithin
- Recovery of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus brasiliensis* was determined with sterile petri dishes
  - 1:10 test dilutions in neutralization broth, vortex mixing, pipetting and addition of tryptic soy agar for bacteria enumeration and potato dextrose agar for yeast/mold enumeration.
  - The test plates were incubated at 32.5℃ and 22.5℃, respectively.
  - Duration was 3 days for bacteria and 5 days for fungi.
  - Colonies were counted using a magnifying glass and colony counter with light source.

Accuracy and Precision

Two key test parameters for the traditional aerobic plate count test and the traditional yeast & mold plate count test.

Training Program Results

- Comparing each microorganism "positive control" to the average colony forming units recovered in each cosmetic, the plate count test method proved to be very accurate.
  - The percent recovery range for bacteria in cosmetic, compared to the positive controls was 96.8% to 100.4%.
  - The yeast and mold recovery range exhibited 88.8% to 95.8%.
- The precision was determined by comparing the results between two different microbiologists using five different microorganisms, three different product categories and 15 dilutions per test run.
- Good precision was observed with little variability with the recovery of *Escherichia coli*, *Candida albicans* and *Aspergillus brasiliensis*.
- More variability was observed with the recovery of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.
  - Standard deviations of 64 and 23, respectively were noted.
- Both microbiologists 1 and 2 recovered mold in cosmetic samples at a 109.8 % rate
- **The training program emphasized the importance of aseptic technique for testing preparation and during testing processes. Pipetting techniques and vortex mixing were also an important part of the overall training program for newly hired microbiologists.**

Accuracy and Precision are two key test parameters for the traditional aerobic plate count test and the traditional yeast & mold plate count test. During a training program that was implemented for a newly launched cosmetic testing microbiology laboratory, data was collected for test area microbiologists. The American Type Culture Collection microorganisms were utilized to spike three different cosmetic categories (baby sunscreen lotion, biotin collagen shampoo and body wash). Positive control data was also collected and compared to determine test accuracy and test precision. The cosmetics were first neutralized with tryptone azolectin broth/tween 20 and lecithin. After neutralization a small population of microorganisms were used as separate inoculations into each cosmetic sample. The recovery of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus brasiliensis* was determined with sterile petri dishes, 1:10 test dilutions in neutralization broth, vortex mixing, pipetting and addition of tryptic soy agar for bacteria enumeration and potato dextrose agar for yeast/mold enumeration. The test plates were incubated at 32.5℃ and 22.5℃, respectively. Incubation duration was 3 days for bacteria and 5 days for fungi. Colonies were counted using a magnifying glass and colony counter with light source. Comparing each microorganism "positive control" to the average colony forming units recovered in each cosmetic, the plate count test method proved to be very accurate. The percent recovery range for bacteria in cosmetic, compared to the positive controls was 96.8% to 100.4%. The yeast and mold recovery range exhibited 88.8% to 95.8%. The precision was determined by comparing the results between two different microbiologists using five different microorganisms, three different product categories and fifteen dilutions per test run. Good precision was observed with little variability with the recovery of *Escherichia coli*, *Candida albicans* and *Aspergillus brasiliensis*. More variability was observed with the recovery of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Standard deviations of 64 and 23, respectively were noted. Both microbiologists 1 and 2 recovered mold in cosmetic samples at a 109.8 % rate. The training program emphasized the importance of aseptic technique for testing preparation and during testing processes. Pipetting techniques and vortex mixing were also an important part of the overall training program for newly hired microbiologists.

INTRODUCTION

Accuracy:

- ✓ The correctness of a result, relative to an expected outcome.
- ✓ The closeness of agreement between a test result or a measurement result and the true value.
- ✓ Essentially the “absence of error”; the more accurate a result the lower the associated error of the test.

Precision:

- ✓ Depends only on the distribution of random errors.
- ✓ Expressed usually in terms of imprecision and computed as a standard deviation of the test results.
- ✓ Better levels of precision are reflected by smaller standard deviations (e.g., < 10 %).
- ✓ Quantitative measures of precision:

  Repeatability and Reproducibility.

Error:

- ❑ Training Error.
- ❑ Testing Error.

Test Variability: technique, experience, equipment calibrations, tolerance, drift, test environment (EM program), ATCC culture maintenance, inherent cellular variability, test limitations, test limit of detection.

Causes of Variability:

- ❑ Non-standard test conditions (area/test station).
- ❑ Human error in weighing, dispensing, pipetting.
- ❑ Skill levels: beginning, intermediate, expert (SME).

PRODUCT CATEGORIES

- Body Wash
- Baby Sunscreen Lotion
- Biotin Collagen Shampoo
- Neutralization Broths:
  - TAT with lecithin and tween 20
  - Deys Engley Broth
- Lotions / Creams
- Bath Gels
- Lip Gloss
- Powders

METHODS

- Preservative Efficacy Testing (USP <51>)
- Aerobic Plate Count (USP <61>)
- Bacteriological Analytical Manual:

  Chapters 3 & 23:
  Aerobic Plate Count
  Methods for Cosmetics
- Yeast & Mold Plate Count (USP <61>)

SUITABILITY TESTING OF COSMETICS

▪ Sample preparation

Cosmetic + TAT with lecithin and tween 20

1:10

1:100

1:1000

▪ Sample Inoculation, Incubation, Plate Reading

- ❖ ATCC: SA, EC, PA, CA, AB
- ❖ Positive Controls
- ❖ Negative Controls
- ❖ < 300 CFU/g Bacteria, Yeast, Mold
- ❖ 30℃ to 35℃ (3 days),
  20℃ to 25℃ (5 days)
- ❖ Colony Counter, Light Source,
  Magnifying Glass

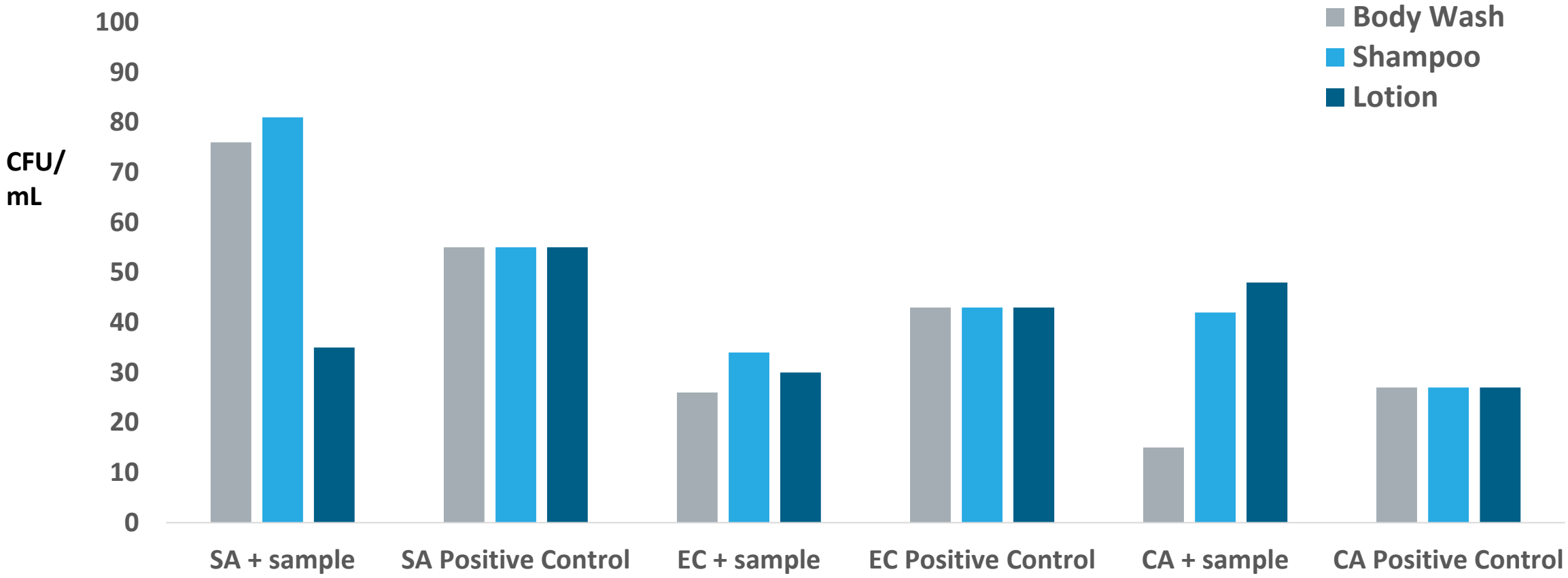
ACCURACY TEST DATA

TABLE 1. Colony Forming Units recovered in samples, using ATCC microorganisms, Aerobic Plate Counts and Yeast/Mold Plate Counts. [USP <61>]

COSMETIC TEST SAMPLE	SA + sample	SA Positive Control	EC + sample	EC Positive Control	PA + sample	PA Positive Control	CA + sample	CA Positive Control	AB + sample	AB Positive Control
Body Wash "A"	48	48	31	32	229	218	41	45	125	144
Baby Sunscreen Lotion "B"	48	48	31	32	229	218	48	45	150	144
Biotin Collagen Shampoo "C"	45	48	32	32	200	218	30	45	140	144
CFU AVERAGE	47	48	31	32	219	218	40	45	138	144
STANDARD DEVIATION	1.73	N/A	0.58	N/A	16.74	N/A	9.07	N/A	12.58	N/A
CFU RANGE	45 to 48	N/A	31 to 32	N/A	219 to 229	N/A	30 to 48	N/A	125 to 150	N/A

Graph 1. Accuracy

Microorganism CFU Recovery in 3 Different Cosmetic Product Categories, Compared to the Positive Controls



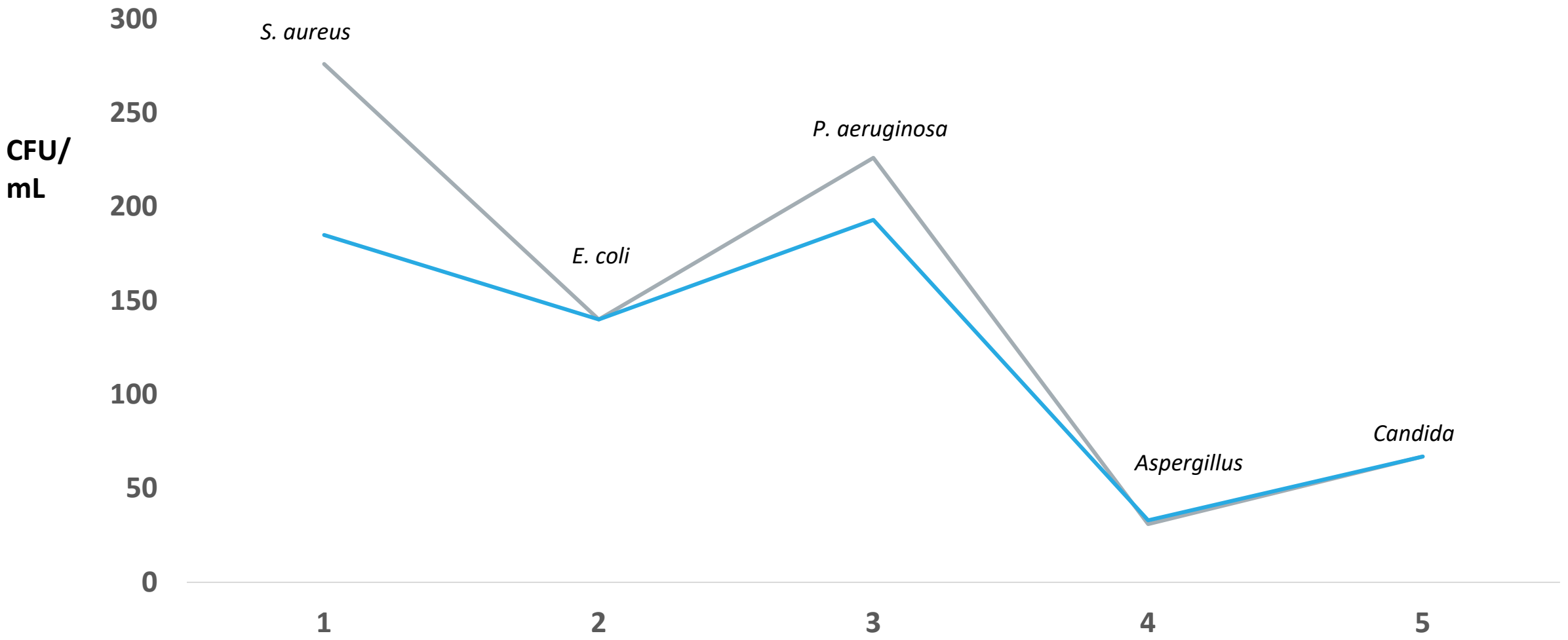
PRECISION TEST DATA

TABLE 2. Precision evaluation for microbiologists, performing suitability testing on a cosmetic sample using ATCC microorganisms.

Microbiologist	SA + sample	SA Positive Control	EC + sample	EC Positive Control	PA + sample	PA Positive Control	CA + sample	CA Positive Control	AB + sample	AB Positive Control
1	276	230	140	136	226	253	31	25	67	61
2	185	230	140	136	193	253	33	25	67	61
CFU AVG	**231**	**230**	**140**	**136**	**210**	**253**	**32**	**25**	**67**	**61**
STDEV	**64.35**	**N/A**	**0.00**	**N/A**	**23.33**	**N/A**	**1.41**	**N/A**	**0.00**	**N/A**

Graph 2. PRECISION PLATE COUNT DATA:

Recovery of ATCC microorganisms (SA, EC, PA, CA, AB) in cosmetic.



PRECISION: 3 microbiologists [neutralization broth toxicity / microorganism recovery]

- Standard Deviations

	Microbiologist		
Microbe	1	2	3
SA	67.6		
EC	15.6		
PA	54.2		
CA	7.0		
AB	6.0		
SDV Range	**6.0 to 67.6**		

- Microorganism Recovery (CFU/mL) in Neutralization Broths

	Microbiologist		
Microbe	1	2	3
SA	185	276	317
EC	140	113	140
PA	193	120	226
CA	33	31	20
AB	73	67	79

DISCUSSION/CONCLUSIONS

- 100.4% PA recovery in the cosmetic, compared to the control.
- 88.8% CA recovery in the cosmetic, compared to the control.
- 95.8% AB recovery in the cosmetic, compared to the control.

Comparing each microorganism "positive control" to the average CFU recovered in each cosmetic category, the plate count test method exhibited very good accuracy.

- 97.9% SA recovery in the cosmetic, compared to the control.
- 96.8% EC recovery in the cosmetic, compared to the control.

- ✓Although SA and PA bacteria were both recoverable in cosmetics, more variability (standard deviations of 64 and 23, respectively) in the level of recovery was observed between different microbiologists.

- ✓**Training, technique and experience will close the gap to improve precision.**

  - ✓Additional data sets will strengthen the statistical evaluations.

- ✓**The precision** in the recovery of bacteria, yeast and mold was evaluated during the training process.

- ✓**There was little variability (good precision)** with the recovery of *E. coli, Candida albicans* yeast and *Aspergillus* mold between different microbiologists that spiked and plated cosmetic samples.

- ✓**Toxicity / Microorganism Recovery in neutralization broths:** EC, CA and AB data supported better precision among three microbiologist. More variability was observed with the recovery of SA and PA in neutralization broths.

ACKNOWLEDGEMENTS

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REFERENCES

- ✓ USP <61 62>
- ✓ USP <51>
- ✓ BAM Chapter 23
- ✓ BAM Chapter 3

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T H A N K Y O U

Richard A. Boehler, Jr., MSc.
Cosmetic Microbiology Laboratory Manager, Melville New York

(631) 742 – 0784

richard.boehler@certifiedgroup.com