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

R. A. Boehler, Jr., MSc.

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Abstract

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.

Overview

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.

Acknowledgements

Certified Laboratories, Melville, New York

References

✓USP <61 62>

✓ USP <51>

✓ BAM Chapter 23

✓ BAM Chapter 3

Results

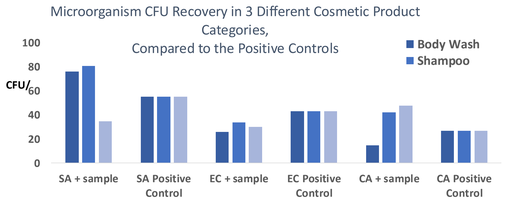
The percent recovery range for bacteria in cosmetic, compared to the positive controls was 96.8% to 100.4%. The yeast and mold recovery range exhibited 88.8% to 95.8%. The precision was determined by comparing the results between two different microbiologists using five different microorganisms, three 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.

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	200 to 229	N/A	30 to 48	N/A	125 to 150	N/A

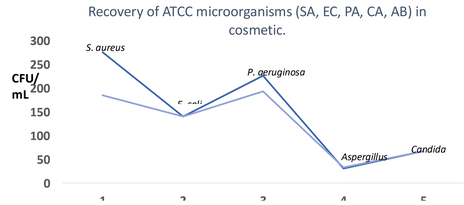
TABLE 1. Colony Forming Units recovered in samples, using ATCC microorganisms, Aerobic Plate Counts and Yeast/Mold Plate Counts. [USP <61>]

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

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



Graph 1. Accuracy



Graph 2. PRECISION PLATE COUNT DATA

Standard Deviation	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

Precision: 3 Microbiologists [neutralization broth toxicity / microorganism recovery]

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.

Method

Preservative Efficacy Testing (USP <51>)

Bacteriological Analytical Manual: Chapters 3 & 23: Aerobic Plate Count. Methods for Cosmetics

Aerobic Plate Count: (USP <61>)

Yeast & Mold Plate: Count (USP <61>)