M02
Pathogen-Free
Microbiology Program

5-06-19 (Shipment Date)
6-06-19 (Report Issue Date)

Proficiency Testing Provider
Certificate 1782.01

AOAC INTERNATIONAL
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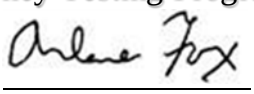
Report Authorization

This report has been authorized by Arlene Fox, Senior Director of the AOAC laboratory Proficiency Testing Program.



REPORT TO PARTICIPANTS IN
THE AOAC® LABORATORY PROFICIENCY TESTING PROGRAM

PATHOGEN FREE MICROBIOLOGY PROGRAM

1.0 Introduction

Test materials for the Pathogen Free Microbiology Program were shipped to participants on May 6, 2019. Each laboratory was given a site identification number in order to maintain confidentiality. Participants were encouraged to analyze the samples as soon as possible and urged to initiate analyses by Monday May 13, 2019. The participants were to submit an electronic response online to verify the condition of the test materials upon receipt. Participants were also instructed to report results electronically, if applicable to their laboratory. Participants were instructed to analyze the test materials according to procedures routinely used in their laboratories and to record their results on the report form provided by AOAC INTERNATIONAL. Instructions were provided for dilutions. The results were to be recorded and submitted to AOAC by May 21, 2019.

2.0 Preparation of Test Materials

Each set of test materials included four samples, each containing approximately 50 grams of frozen dehydrated mashed potato mixture. The samples were shipped in color coded vials to assist in maintaining proper sample identity. Samples number 1 and 2 were prepared as duplicates and samples 3 and 4 were prepared as duplicates. The matrix was screened prior to spiking. In addition these samples were analyzed on May 21, 2019. Ten quantitative samples were randomly selected and analyzed in duplicate prior to shipment to verify the homogeneity of the test material (Appendix II). The requirements of Section 4.4.2 of ISO/IEC Standard 17043:2010(E) Conformity Assessment - General requirements for proficiency testing schemes were met for all samples used for evaluation.
Samples were prepared by the following laboratory:

Silliker, Inc. Food Science Center
3600 Eagle Nest Drive, South
Bldg. Crete, IL 60417

3.0 Analyses Requested

Aerobic Plate Count MPN	Tube MPN Dilution 10^{-2} - 10^{-5}
Aerobic Plate Count	Dilution 10^{-2} - 10^{-5}
MPN Coli form MPN	Tube MPN Dilution 10^{-2} - 10^{-5}
Coli form Plate Count	Dilution 10^{-1} - 10^{-3}
MPN E. coli	Tube MPN Dilution 10^{-2} - 10^{-5}
E. coli Plate Count MPN	Tube MPN Dilution 10^{-2} - 10^{-5}
E. coli Plate Count	Dilution 10^{-1} - 10^{-3}
Yeast & Mold MPN	Tube MPN Dilution 10^{-1} - 10^{-4}
Yeast & Mold	Dilution 10^{-1} - 10^{-4}

The participants had the option of marking the analysis as “Not Tested” for any test. Information on the method used for each analysis was requested.

4.0 Calculation and Interpretation of z-scores:

For each individual result, a z-score was calculated as follows:

$$z = \frac{(x - X)}{s}$$

where:

z = the z score (standard score)

x = the reported value of analyte

X = the assigned value, the best estimate of the “ true” concentration

s = the estimate of variation (standard deviation)

The robust procedure from *ISO 13528:2005 (E), Statistical methods for use in proficiency testing by interlaboratory comparisons* is used in processing the result data. Robust statistics relies on medians rather than means, and uses more information from the central than from the outlying observations. This approach is being used to minimize the effect of extreme results on the calculation of z-scores.

There are two types of extreme results in microbial analyses: “Blunders” and “Outliers”. Blunder results were likely due to errors, such as transcription errors, incorrect sample identification, or a major problem performing the analysis. Blunders are defined as results that differ from the initial median of the participant’s results prior to $\log_{10}$ transformation, by a factor of 100. These results have been labeled as "pre-screened as an outlier."

The data was $\log_{10}$ transformed prior to the calculation of statistics. The assigned value was determined by the consensus of the majority after the removal of outliers by the robust procedure. Outlier results have been labeled “Outlier: Z beyond 3”.

The assigned value, standard deviation, and the z-scores were recalculated without the outliers. The blunder results and outlier results were still evaluated within the proficiency scheme and given the appropriate performance rating. Measurement uncertainty (standard uncertainty of the assigned value) has also been provided.

The following interpretation of z-scores for each individual test result is provided in of ISO/IEC Standard 17043:2010(E) Conformity Assessment - General requirements for proficiency testing as common examples of application of z-scores:

Results Obtained	Rating
$\lvert z \rvert \leq 2$	Satisfactory
$2 < \lvert z \rvert < 3$	Questionable
$\lvert z \rvert \geq 3$	Unsatisfactory

AOAC has calculated z-scores according to methodology for the Aerobic Plate Count (APC) and yeast and mold (Y&M) analyses. All statistics for the APC and Y&M analyses have been computed according to methodology when more than 10 participants specified the same method. When participants specified a method used by fewer than 10 participants (X for APC) (V for Y&M), z-scores for their results were based on all results for the specific analysis.

On the result page the Z-score calculation is now bolded and is listed in the second to last data column on the quantitative result page.

5.0 Results

5.1 General Discussion of Results

Confidentiality of results has been maintained by issuing site identification codes to the participants. Results in reports have only been identified by the site identification code. Results were submitted by as many as 63 participants per analysis. In response to customer feedback, APC MPN Technology and Yeast and Mold MPN Technology were added to the program. Some tests had fewer participants submitting results because some of the laboratories do not routinely perform all the analyses AOAC requires at least 12 data points, without blunders, for z-scores to be provided for an analyte. AOAC also requires at least 10 participating laboratories reporting, the use of the same method for an analyses for statistics to be computed based on that specific methodology. There was an insufficient number of usable data points to meet the criteria of issuing z-scores for MPN coliform, MPN *E.coli,* MPN APC*,* MPN *Enterobacteriaceae and* MPN *Yeast & Mold*. Samples 1 and 2 were negative for *E. coli* so no statistics were calculated for these samples. The results for yeast and mold were combined. The data was $\log_{10}$ transformed prior to the calculation of statistics.

AOAC is instituting an approach to provide information on the performance of the different methods used by participants. Results from equivalent methods were grouped together. This report provides this information for APC and Y&M. Future reports will provide additional information.

If a participant would like to appeal against the evaluation of their performance in this proficiency testing scheme please contact staff at LPTP@AOAC.org

Individual laboratory results are in Appendix A

APC

The table below indicates which methods were grouped together for statistical computation of APC.

APC Method Indicated in Individual Result Section	**APC** Methods Included
Method B	AOAC 990.12 and 3M Petrifilm
Method X	All Other Methods*

* When participants specified a method used by fewer than 10 participants, or when 2 or more methods have been used, z-scores for their results were based on all results for the specific analysis (X for APC).

A table has been provided to compare the standard deviations and assigned values (log_{10} transformed) for the various methods. See section 4.0 for specific information on issuing results based on methodology.

APC	Sample 1	Sample 2	Sample 3	Sample 4
Method B Standard Deviation	0.230	0.198	0.206	0.171
Method X Standard Deviation	0.249	0.247	0.192	0.171
Method B Assigned Value	4.068	4.089	4.588	4.588
Method X Assigned Value	4.064	4.085	4.586	4.584

Y & M

The table below indicates which methods where grouped together for statistical computation of Yeast & Mold.

Y&M Method Indicated in Individual Result Section	**Y&M** Methods Included
Method S	AOAC 997.02, Petrifilm
Method V*	All Other Methods*

* When participants specified a method used by fewer than 10 participants, or when 2 or more methods have been used, z-scores for their results were based on all results for the specific analysis (V for Y&M).

A table has been provided to compare the standard deviations and assigned values (log_{10} transformed) for the various methods. See section 4.0 for specific information on issuing results based on methodology.

Y&M	Sample 1	Sample 2	Sample 3	Sample 4
Method S Standard Deviation	0.186	0.189	0.157	0.213
Method V Standard Deviation	0.185	0.184	0.173	0.209
Method S Assigned Value	4.565	4.558	4.212	4.206
Method V Assigned Value	4.558	4.555	4.221	4.206

5.2 Discussion of Data Plots

z-Score Plots

z-score plots allow a visual comparison of z-score results from each separate laboratory against the entire distribution of all data for a given test. These plots are made up of three components. For each sample, the leftmost “stripe" is the entire set of reported z-scores. Each separate value is a thin horizontal line. Tightly clustered values show as dense, dark areas. The entire distribution can be seen spread above and below the mean of zero. A box-whisker plot is included to focus attention on several well known descriptors, notably the median and the upper and lower quartiles (25th and 75th percentiles). The box in these plots itself represents the middle 50% of the data, while the whiskers give a sense of reasonable tails. Inside the box, the median is indicated by a horizontal line. The top whisker goes up to the largest data point which lies no further than 1.5 box-heights from the top of the box. The bottom whisker is analogous. The large black dot locates the individual z-score derived from the original value reported by the lab for each sample.

Distribution of Results Plots

The distribution of results plots provides information on the distribution of results converted to $\log_{10}$. For a given test, the minimum and maximum of all results (all samples combined) determine the range. For APC and Y&M, the minimum and maximum of results for specific methodology determine the range. The values are log10 transformed, and the range is divided into 10 intervals ("bins"). The number of reported results falling into each bin are counted. The bars depict these counts as percentages of the total for each sample.

Individual laboratory z-score Plots and Distribution of Results Plots are in Appendix B

AOAC® Laboratory Proficiency Testing Program
M02 Pathogen-Free Microbiology Instructions

Enclosed are four samples, each containing approximately 50 grams of frozen rehydrated mashed potato mixture for samples 1-4. The stability of potatoes stored frozen adds flexibility to the program by permitting participants to analyze samples at their convenience. Shelf-life studies performed on samples stored frozen demonstrated that the samples remained stable for a period of at least 7 days if stored properly.

Even with this added flexibility, please make every effort to analyze samples as soon as possible**.** Results are still due within 2 weeks of shipment, so your lab should **initiate analyses by no later than May 13, 2019. Results are due on May 21, 2019**.

Store samples frozen (-20 ^{O}C) until analysis date. Thaw quickly when ready to analyze. Place the samples into a plastic bag in a 45 ^{O}C water bath for 5 minutes, after 5 minutes in water bath, use sterile tongue depressor or stir rod to break up sample, put back in bath, and repeat after another 5 minutes. Do not place the samples in the 45 ^{O}C water bath for longer than 15 minutes. If the samples are still not adequately thawed, refrigerate the samples at 4 ^{O}C until thawed. Do not thaw the samples at room temperature.

Please verify that sample #1 is a red vial, #2 black, #3 purple, #4 green

Analyze samples numbered 1-4 for the following:

1. Aerobic Count (MPN Technology) - Dilution 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}
2. Aerobic Count (Plate Count Technology) - Dilution 10^{-2},10^{-3}, 10^{-4}, and 10^{-5}
3. Coliform (MPN Technology) - Dilution 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}
4. Coliform (Plate Count Technology) - Dilution 10^{-1}, 10^{-2}, and 10^{-3}
5. *E. coli* (MPN Technology) - Dilution 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}
6. E. coli (Plate Count Technology) - Dilution 10-1, 10-2, and 10-3
7. Yeast & Mold (MPN Technology) - Dilution 10^{-1}, 10^{-2}, $10^{-3,}$ and 10^{-4}
8. Yeast & Mold (Plate Count Technology) - Dilution 10^{-1}, 10^{-2}, $10^{-3,}$ and 10^{-4}

In response to participant feedback, these highlighted tests have just been added. Participating laboratories always have the right to report Not Tested for any tests that are not relevant to their analytical needs.

Complete additional dilutions if necessary.

Homogeneity Results -
M02 Proficiency Testing Program

Ship Date: May 6, 2019

Aerobic Plate Count (log cfu/g)

Sample #	APC 1,2 - Rep 1	APC 1,2 - Rep 2
1	4.08	4.23
2	4.18	4.43
3	4.56	4.53
4	4.08	4.43
5	4.28	4.15
6	4.43	4.41
7	4.26	4.23
8	4.28	4.48
9	4.23	4.18
10	4.28	4.40

Average ($\bar{x}$)	4.31
S_x	0.1180
S_w	0.1199
S_s	0.0820
	PASSED

Coliform Plate Count (log cfu/g)

Sample #	Coliform 1,2 - Rep 1	Coliform 1,2 - Rep 2
1	3.32	3.56
2	3.38	3.51
3	3.65	3.66
4	3.56	3.58
5	3.52	3.41
6	3.72	3.62
7	3.45	3.58
8	3.60	3.68
9	3.34	3.40
10	3.67	3.63

Average ($\bar{x}$)	3.54
S_x	0.1101
S_w	0.0771
S_s	0.0956
	PASSED

E. coli Plate Count (log cfu/g)

Sample #	E. coli 1,2 - Rep 1	E. coli 1,2 - Rep 2
1	<1.00	<1.00
2	<1.00	<1.00
3	<1.00	<1.00
4	<1.00	<1.00
5	<1.00	<1.00
6	<1.00	<1.00
7	<1.00	<1.00
8	<1.00	<1.00
9	<1.00	<1.00
10	<1.00	<1.00

Average ($\bar{x}$)	NA
S_x	NA
S_w	NA
S_s	NA
	PASSED

Aerobic Plate Count (log cfu/g)

Sample #	APC 3,4 - Rep 1	APC 3,4 - Rep 2
1	4.78	4.91
2	4.75	4.90
3	4.96	4.80
4	4.88	4.76
5	4.83	4.74
6	4.83	4.74
7	4.92	5.00
8	4.76	4.91
9	4.79	4.79
10	4.95	4.81

Average ($\bar{x}$)	4.84
S_x	0.0552
S_w	0.0855
S_s	0.0000
	PASSED

Coliform Plate Count (log cfu/g)

Sample #	Coliform 3,4 - Rep 1	Coliform 3,4 - Rep 2
1	3.76	3.85
2	3.83	3.67
3	3.71	3.72
4	3.76	3.59
5	3.64	3.66
6	3.69	3.85
7	3.99	3.54
8	3.67	3.69
9	3.62	3.58
10	3.66	3.62

Average ($\bar{x}$)	3.71
S_x	0.0657
S_w	0.1199
S_s	0.0000
	PASSED

E. coli Plate Count (log cfu/g)

Sample #	E. coli 3,4 - Rep 1	E. coli 3,4 - Rep 2
1	3.76	3.85
2	3.83	3.67
3	3.71	3.72
4	3.76	3.59
5	3.64	3.66
6	3.69	3.85
7	3.99	3.54
8	3.67	3.69
9	3.62	3.58
10	3.66	3.62

Average ($\bar{x}$)	3.71
S_x	0.0657
S_w	0.1199
S_s	0.0000
	PASSED

Homogeneity Results -
M02 Proficiency Testing Program

Ship Date: May 6, 2019

Yeast (log cfu/g)

Sample #	Yeast 1,2 - Rep 1	Yeast 1,2 - Rep 2
1	4.40	4.26
2	4.52	4.49
3	4.62	4.65
4	4.58	4.52
5	4.66	4.66
6	4.72	4.78
7	4.46	4.63
8	4.61	4.60
9	4.51	4.69
10	4.71	4.62

Average ($\bar{x}$)	4.58
S_x	0.1146
S_w	0.0708
S_s	0.1030
	PASSED

Mold (log cfu/g)

Sample #	Mold 1,2 - Rep 1	Mold 1,2 - Rep 2
1	<1.00	<1.00
2	<1.00	<1.00
3	<1.00	<1.00
4	<1.00	<1.00
5	<1.00	<1.00
6	<1.00	<1.00
7	<1.00	<1.00
8	<1.00	<1.00
9	<1.00	<1.00
10	<1.00	<1.00

Average ($\bar{x}$)	NA
S_x	NA
S_w	NA
S_s	NA
	PASSED

Yeast (log cfu/g)

Sample #	Yeast 3,4 - Rep 1	Yeast 3,4 - Rep 2
1	<1.00	<1.00
2	<1.00	<1.00
3	<1.00	<1.00
4	<1.00	<1.00
5	<1.00	<1.00
6	<1.00	<1.00
7	<1.00	<1.00
8	<1.00	<1.00
9	<1.00	<1.00
10	<1.00	<1.00

Average ($\bar{x}$)	NA
S_x	NA
S_w	NA
S_s	NA
	PASSED

Mold (log cfu/g)

Sample #	Mold 3,4 - Rep 1	Mold 3,4 - Rep 2
1	4.34	4.46
2	4.30	4.26
3	4.28	4.41
4	4.18	4.18
5	4.28	4.18
6	4.36	4.38
7	4.11	4.41
8	4.32	4.40
9	4.26	4.20
10	4.26	4.36

Average ($\bar{x}$)	4.30
S_x	0.0735
S_w	0.0884
S_s	0.0386
	PASSED

Quantitative Results: M02 05/06/2019 Site ID: xxxxx - Set A

Test	Sample	Method	Number of Reported Results	Reported result	log10 of reported result	Min result	Lower quartile	Upper quartile	Max result	Median	Geometric mean	Assigned value (Mean log10)	Std dev	Z-score	Standard uncertainty of the assigned value	Notes
MPN APC	1		7	Not tested	.	3.004	3.279	4.167	4.254	.	.	.	.	.	.	Z not computed
	2		6	Not tested	.	3.041	3.748	4.230	4.234	.	.	.	.	.	.	Z not computed
	3		7	Not tested	.	3.398	4.473	4.802	4.845	.	.	.	.	.	.	Z not computed
	4		7	Not tested	.	3.544	4.238	4.806	4.826	.	.	.	.	.	.	Z not computed
APC	1	B	46	17300	4.238	0.000	3.875	4.217	4.672	4.079	11702	4.068	0.230	***0.74***	0.042	
	2	B	46	16000	4.204	0.000	3.903	4.204	4.643	4.093	12265	4.089	0.198	***0.58***	0.037	
	3	B	47	39413	4.596	0.000	4.431	4.708	5.077	4.596	38698	4.588	0.206	***0.04***	0.038	
	4	B	46	37600	4.575	0.000	4.477	4.699	4.839	4.591	38683	4.588	0.171	***-0.07***	0.032	
MPN Coliform	1		10	Not tested	.	0.000	2.362	2.748	3.643	.	.	.	.	.	.	Z not computed
	2		9	Not tested	.	0.000	2.467	3.086	3.663	.	.	.	.	.	.	Z not computed
	3		10	Not tested	.	0.000	2.851	3.255	3.633	.	.	.	.	.	.	Z not computed
	4		10	Not tested	.	0.000	2.851	3.176	3.380	.	.	.	.	.	.	Z not computed
Coliform plate count	1		51	1016	3.007	0.000	2.431	2.944	3.398	2.708	507	2.705	0.349	***0.87***	0.061	
	2		51	1080	3.033	0.000	2.415	2.954	3.591	2.826	609	2.785	0.294	***0.85***	0.052	
	3		52	2142	3.331	0.000	2.803	3.205	3.708	3.042	1177	3.071	0.258	***1.01***	0.045	
	4		51	2148	3.332	0.000	2.748	3.190	3.623	2.973	1055	3.023	0.276	***1.12***	0.048	
MPN E. coli	1		11	Not tested	.	0.000	0.000	0.000	0.000	.	.	.	.	.	.	Z not computed
	2		10	Not tested	.	0.000	0.000	0.000	0.000	.	.	.	.	.	.	Z not computed
	3		11	Not tested	.	0.000	2.968	3.398	3.633	.	.	.	.	.	.	Z not computed
	4		11	Not tested	.	0.000	2.908	3.362	3.591	.	.	.	.	.	.	Z not computed
E. coli plate count	1		33	0	.	0.000	0.000	0.000	2.279	.	.	.	.	.	.	Negative sample
	2		33	0	.	0.000	0.000	0.000	2.243	.	.	.	.	.	.	Negative sample
	3		33	2142	3.331	0.000	2.839	3.255	3.505	3.058	1093	3.039	0.320	***0.91***	0.070	
	4		33	2148	3.332	0.000	2.740	3.223	3.398	2.973	973	2.988	0.316	***1.09***	0.069	
MPN Enterobacteriaceae	1		3	Not tested	.	2.190	2.190	2.778	2.778	.	.	.	.	.	.	Z not computed
	2		3	Not tested	.	2.243	2.243	2.544	2.544	.	.	.	.	.	.	Z not computed
	3		3	Not tested	.	2.000	2.000	3.079	3.079	.	.	.	.	.	.	Z not computed
	4		3	Not tested	.	2.000	2.000	3.176	3.176	.	.	.	.	.	.	Z not computed
Enterobacteriaceae plate count	1		6	Not tested	.	2.505	2.568	3.041	3.204	.	.	.	.	.	.	Z not computed
	2		6	Not tested	.	2.477	2.602	2.964	3.053	.	.	.	.	.	.	Z not computed
	3		6	Not tested	.	3.176	3.204	3.265	3.556	.	.	.	.	.	.	Z not computed
	4		5	Not tested	.	2.954	3.000	3.233	3.301	.	.	.	.	.	.	Z not computed
MPN Yeast+Mold	1		4	Not tested	.	2.398	3.521	4.866	4.869	.	.	.	.	.	.	Z not computed
	2		3	Not tested	.	4.380	4.380	5.491	5.491	.	.	.	.	.	.	Z not computed
	3		4	Not tested	.	4.204	4.263	4.667	4.954	.	.	.	.	.	.	Z not computed
	4		4	Not tested	.	4.204	4.318	4.680	4.869	.	.	.	.	.	.	Z not computed
Yeast+Mold	1	S	34	45492	4.658	3.301	4.435	4.672	5.000	4.591	36690	4.565	0.186	***0.50***	0.040	
	2	S	34	43475	4.638	3.000	4.414	4.695	4.895	4.562	36180	4.558	0.189	***0.42***	0.041	

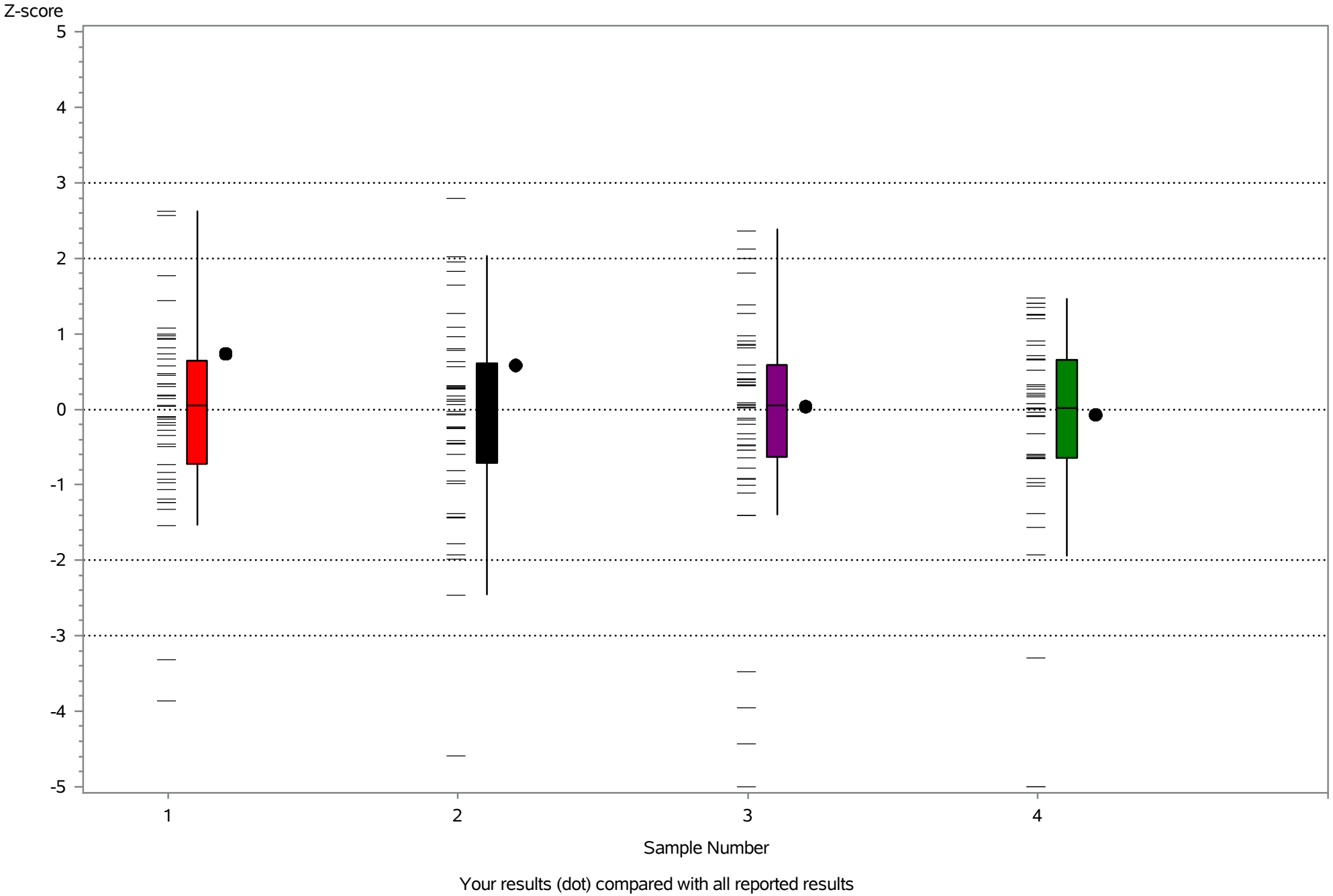
Quantitative Results: M02 05/06/2019 Site I xxxxx - Set A

Test	Sample	Method	Number of Reported Results	Reported result	log10 of reported result	Min result	Lower quartile	Upper quartile	Max result	Median	Geometric mean	Assigned value (Mean log10)	Std dev	Z-score	Standard uncertainty of the assigned value	Notes
	3	S	35	20158	4.304	3.000	4.114	4.301	4.845	4.217	16298	4.212	0.157	***0.59***	0.033	
	4	S	35	23283	4.367	3.000	4.079	4.322	4.949	4.242	16067	4.206	0.213	***0.76***	0.045	

05/31/2019 22:33:21

Z-score distribution analysis M02 May 2019 - Set A

Site ID= xxxxxTest=APC Method=B



Your results (dot) compared with all reported results

05/31/2019 22:33:21

Z-score distribution analysis M02 May 2019 - Set

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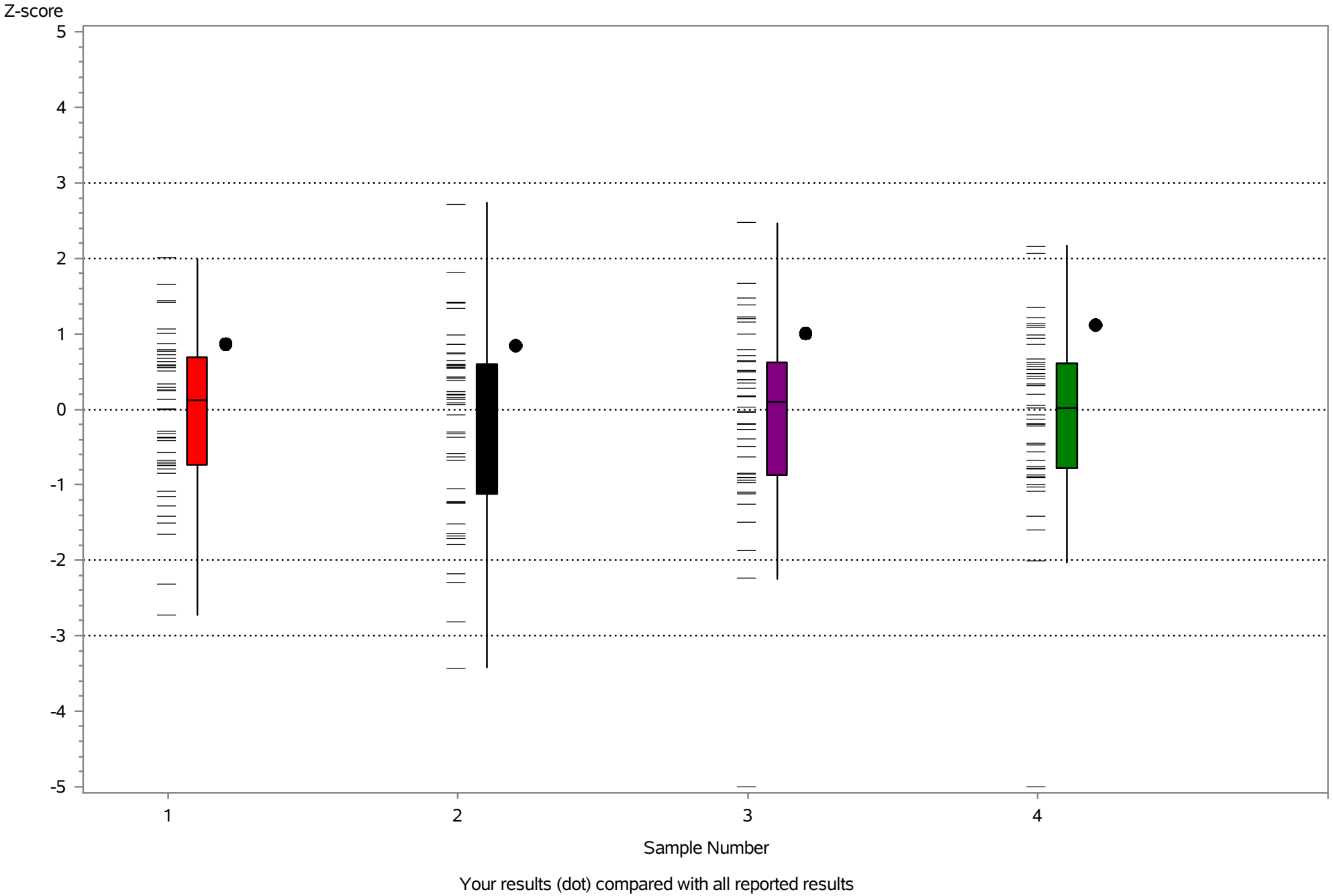

Your results (dot) compared with all reported results

05/31/2019 22:33:21

Z-score distribution analysis M02 May 2019 - Set

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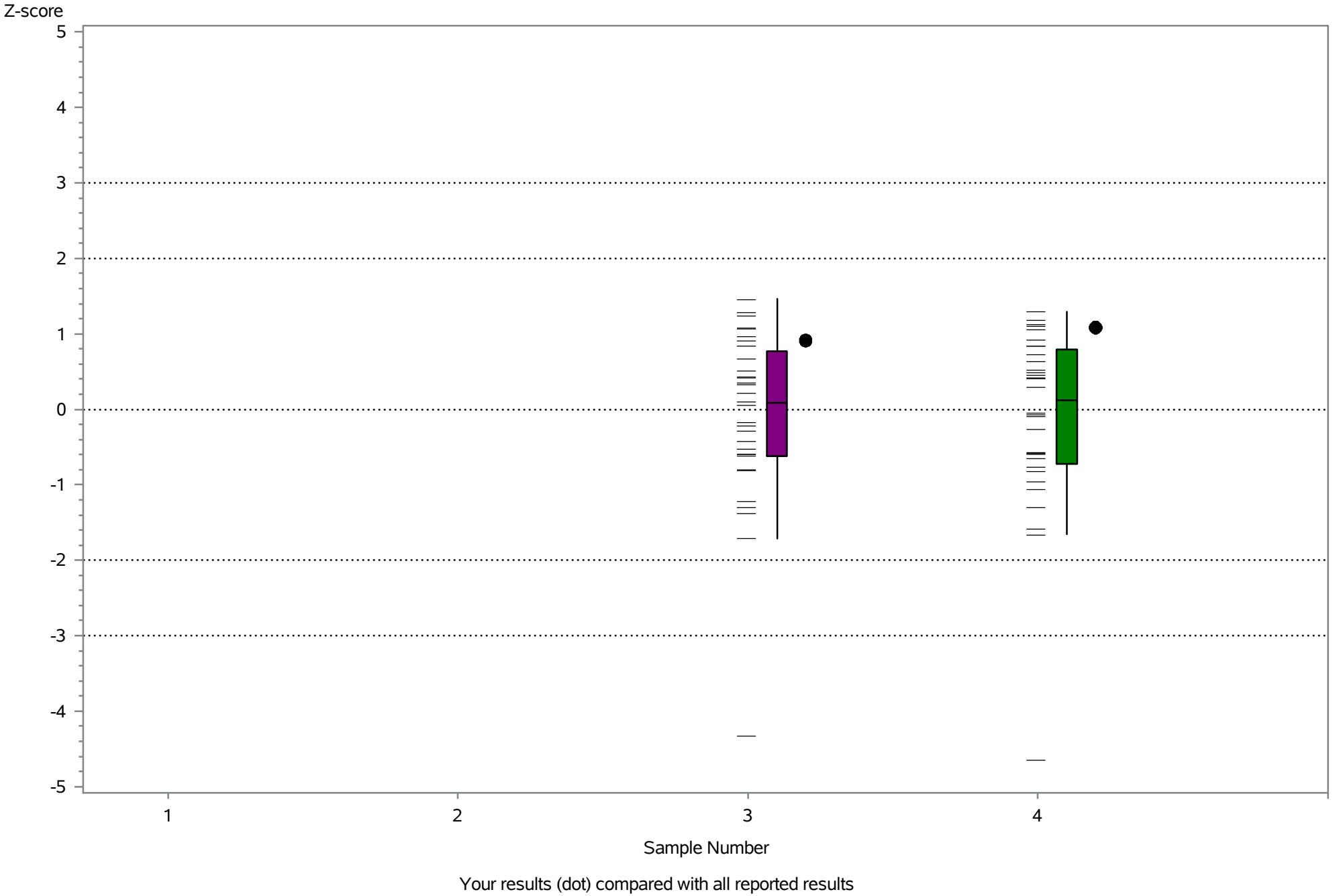
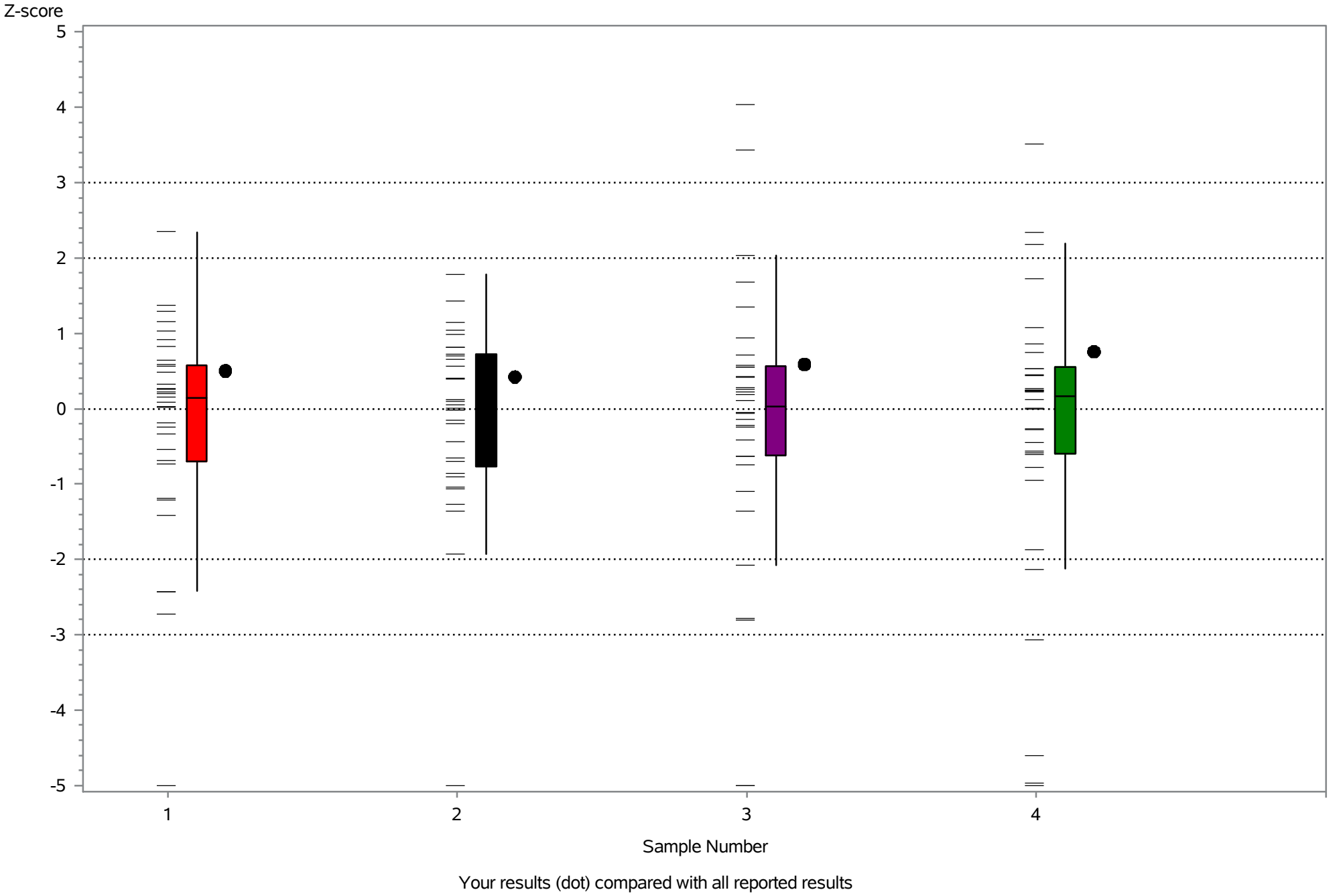


Your results (dot) compared with all reported results

05/31/2019 22:33:21

Z-score distribution analysis M02 May 2019 - Set A

Site ID= xxxxx Test=Yeast+Mold Method=S

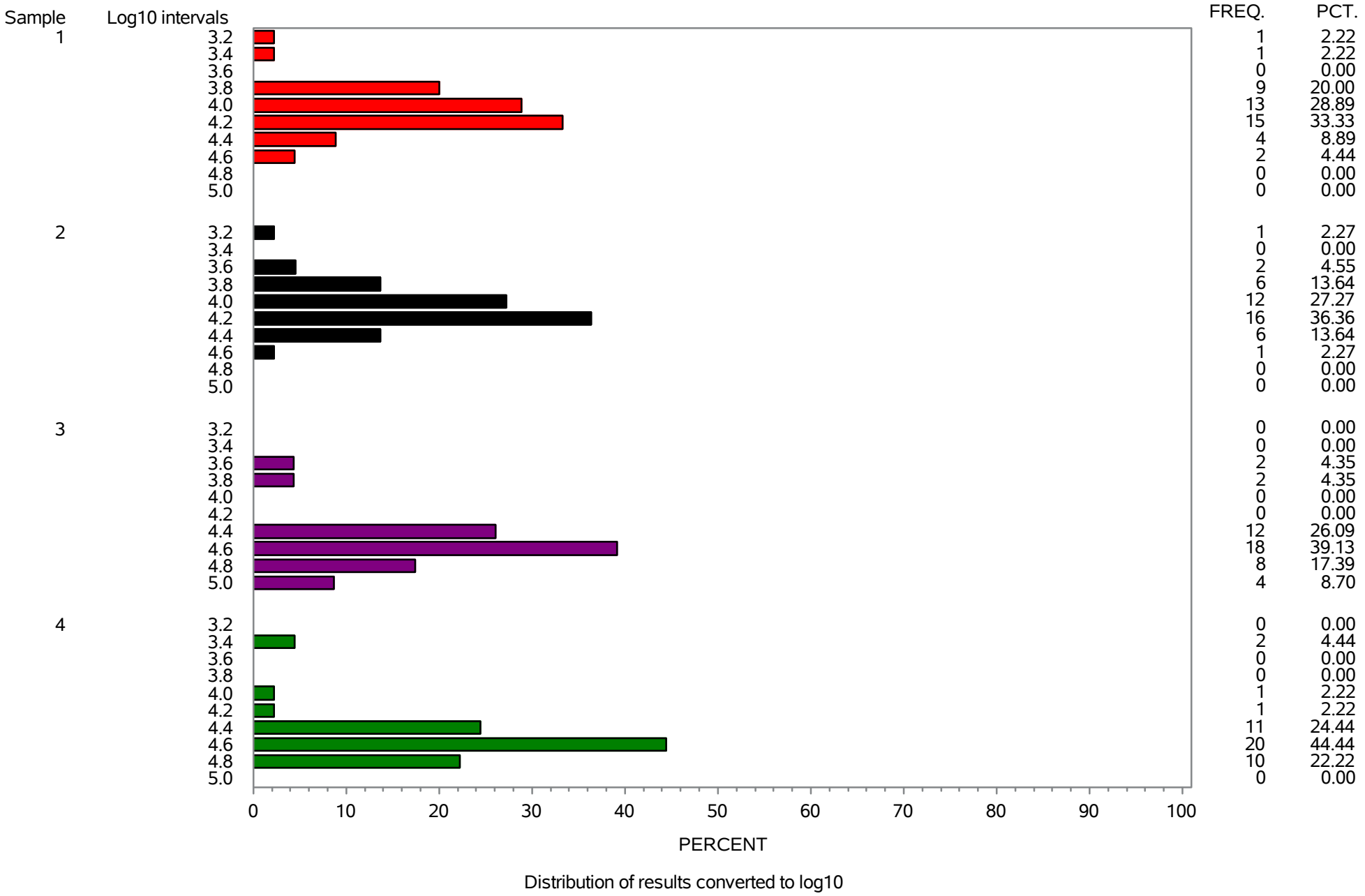


Your results (dot) compared with all reported results

05/31/2019 22:33:21

Distribution of Results Converted to log10 - Set A

Site ID= xxxxx Test=APC Method=B

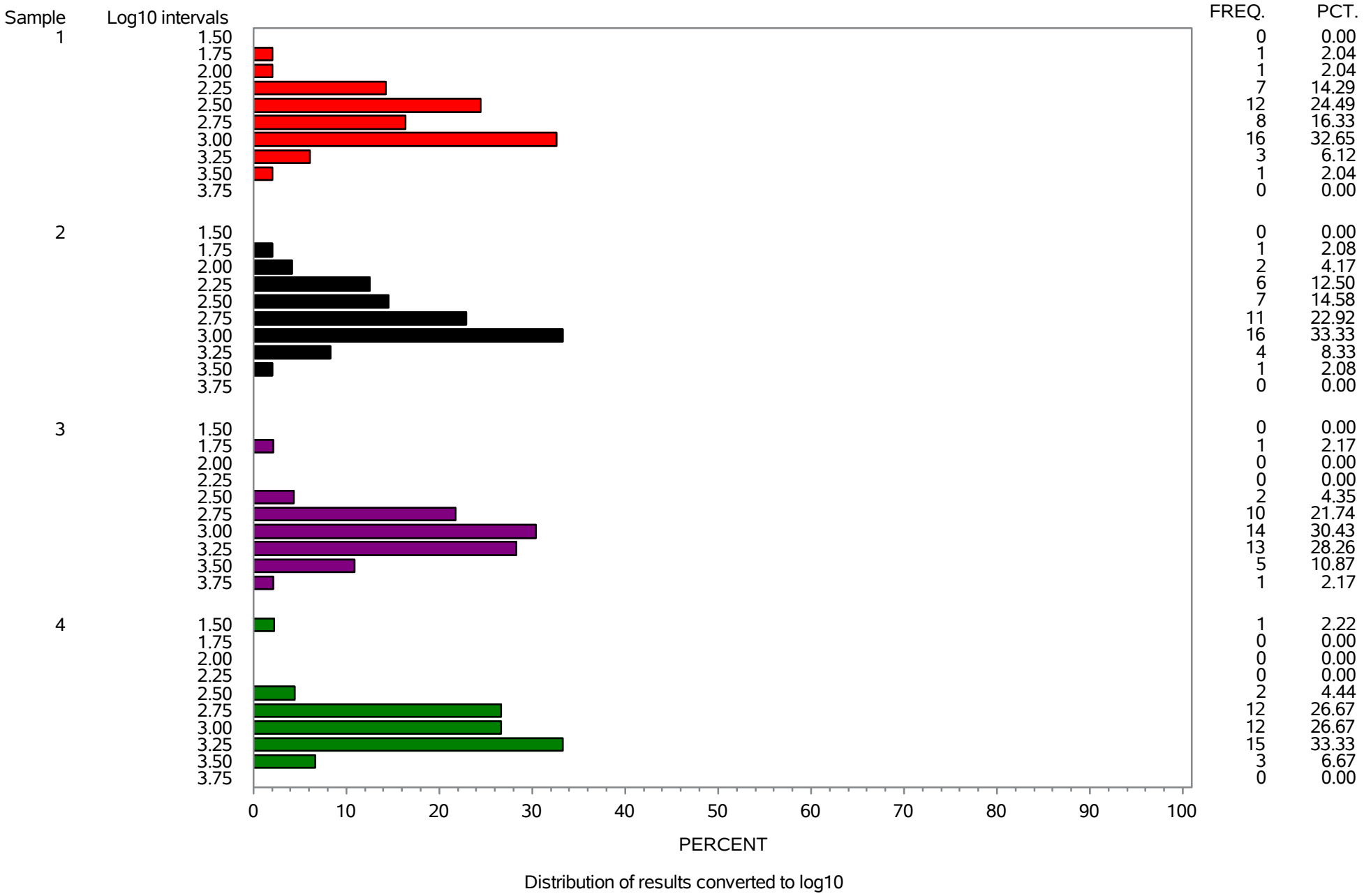


Distribution of results converted to log10

05/31/2019 22:33:21

Distribution of Results Converted to log10 - Set A

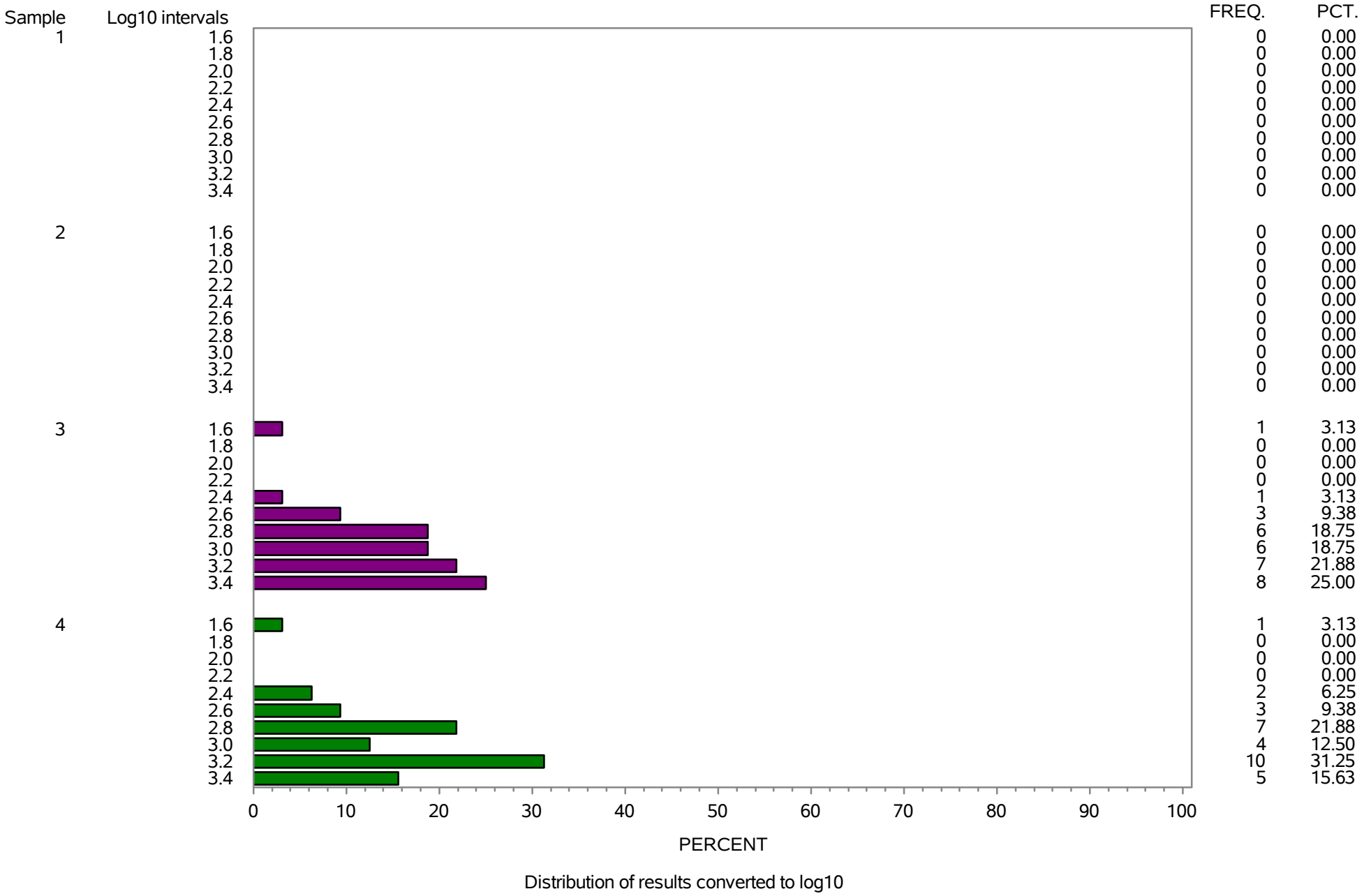
Site ID= xxxxx Test=Coliform plate count Method=' '



Distribution of results converted to log10

05/31/2019 22:33:21

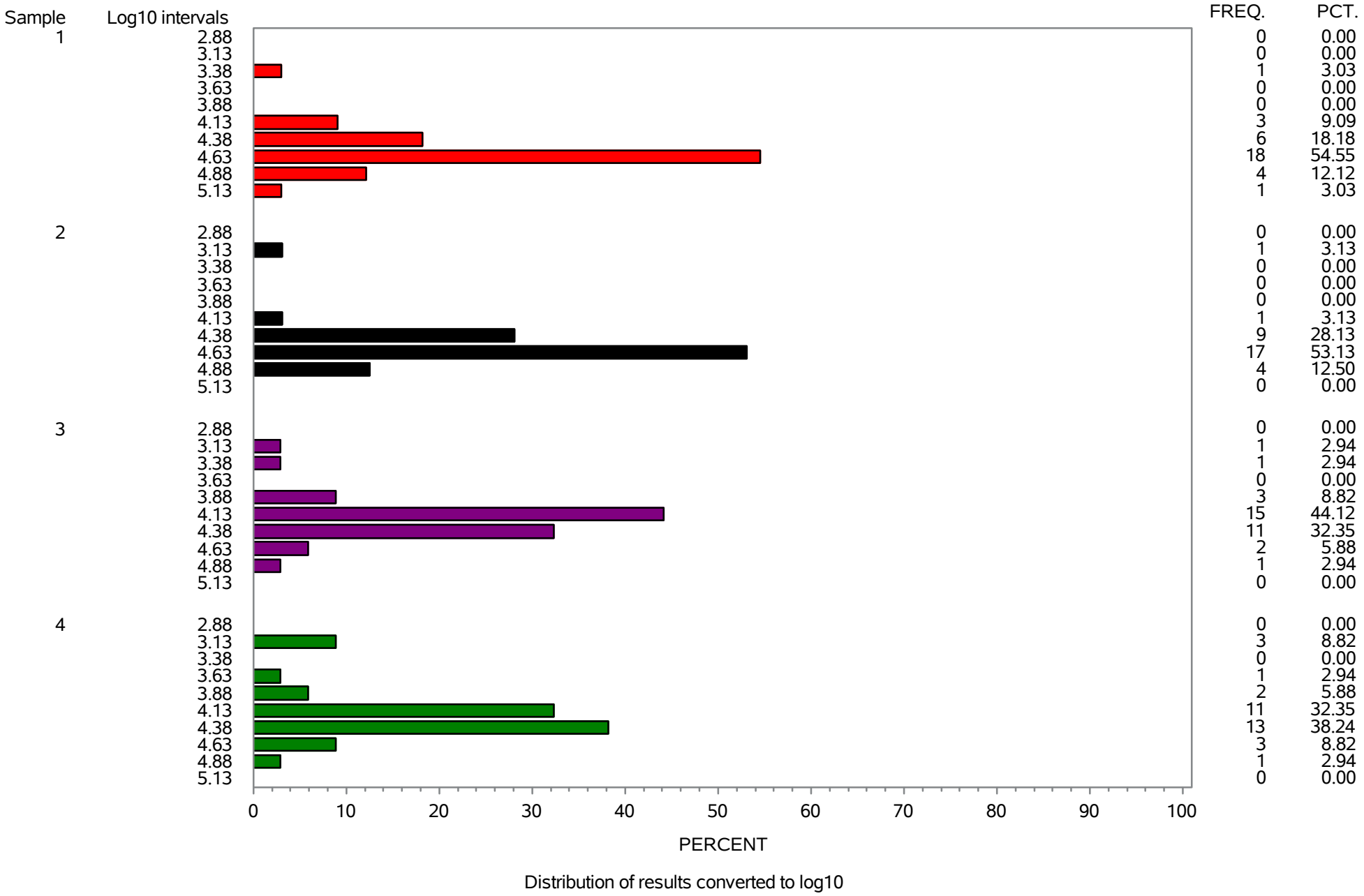
Distribution of Results Converted to log10 - Set
A Site ID= xxxxx Test=E. coli plate count Method=' '



Distribution of results converted to log10

05/31/2019 22:33:21

Distribution of Results Converted to log10 - Set
A Site ID= xxxxx Test=Yeast+Mold Method=S



Distribution of results converted to log10