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Statistical Analyses of Second Indoor Bio-Release Field Evaluation Study at Idaho National Laboratory

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Executive Summary

In September 2008 a large-scale testing operation (referred to as the INL-2 test) was performed within a two-story building (PBF-632) at the Idaho National Laboratory (INL). The INL-2 test consisted of five tests (events) in which a floor (level) of the building was contaminated with the harmless biological warfare agent simulant *Bg* (*Bacillus globigii*, subsequently *Bacillus subtilis* var. *niger*, and recently renamed *Bacillus atrophaeus*). Samples were taken in most, if not all, of the rooms on the contaminated floor for each event. After the sampling, the building was decontaminated, and the next test performed. Judgmental samples and a predetermined number of probabilistic samples were determined and taken during each test. Vacuum, wipe, and swab samples were taken within each room. The report “Operational Observations on the INL-2 Experiment” (Grotte and Hebner) defines the seven objectives for this test and discusses the results and conclusions. This is further discussed in the introduction of this report.

It was determined that additional, in-depth statistical analysis of the data may provide some additional information that would help with sampling strategies and data evaluations in a real bio-terrorist event. Topics of interest included:

- *Statistical Distribution of the Data*: When determining the required number of samples to meet certain Data Quality Objectives (DQOs), if one can assume normality or log-normality, the required number of samples might significantly decrease.
- *Quantification of Sub-Elements of Sample Variations*: Having an estimate of the within-room and between room variations may provide a basis for determining the number of samples required for future bio-agent releases in similar office buildings.
- *Spatial Relationships between Sample Results*: If samples that are close together or within the same room are more similar than samples that are further apart, the number of samples required within each room might be decreased without much loss of information.
- *Surface Type and Sampling Methods Comparisons*: If results are consistent across various surface types and using different sampling methods, only certain surface types or sampling methods may be required in the future. Inconsistencies would suggest otherwise.

PNNL was tasked to perform these statistical analyses and report on the findings. This document reports the study results for the additional four topics that were not within the scope of the Grotte and Hebner report. The specific analyses performed are: 1) assess the quantitative assumptions about the data being normally or log-normally distributed; 2) evaluate differences and quantify the sample to sample variability within a room and across the rooms; 3) perform geostatistical types of analyses to study spatial correlations; and 4) quantify the differences observed between surface types and sampling methods for each scenario and study the consistency across the scenarios. The following four paragraphs summarize the results of each of the four additional analyses.

Sampling was performed before and after decontamination for each event. All samples after decontamination came back non-detect. Because of this, it was not appropriate to determine if these clearance samples were normally distributed. As Table 1 shows, the characterization data consists of values between and inclusive of 0 and 100 CFU/cm² (100 was the value assigned when the number is too numerous to count). The 100 values are generally much bigger than the rest of the data, causing the data to be right skewed. There are also a significant number of zeros. QQ plots show the characterization data has a lack of normality. Normality is improved when looking at log(CFU/cm²), but is still too non-normal to assume a normal distribution. This is discussed in depth in Section 2.1.

Variance component analysis (VCA) and analysis of variance (ANOVA) were used to estimate the amount of variance due to each source and to determine which sources of variability were statistically significant. In general, the across event variability and the across room variability were dependent on the sampling method used. For this reason, it was decided to do analyses for each sampling method, individually. The between event variability and between room variability were significant for each method, except for the between event variability for the swabs. For both the wipes and vacuums, the within room standard deviation was much larger (26.9 for wipes and 7.086 for vacuums) than the between event standard deviation (6.552 for wipes and 1.348 for vacuums) and the between room standard deviation (6.783 for wipes and 1.040 for vacuums). Swabs between room standard deviation was 0.151, while both the within room and between event standard deviations are less than 0.10 (all measurements in CFU/cm²).

The geostatistical analyses looked at the correlation between the amount of contamination and the distance the measurement was from other samples and the contamination point. It also used variogram models to help understand the spatial correlations. The amount of contamination was well dispersed across the floor and little to no correlation was found between the amount and the distance from the point of contamination. Spatial correlations were observed between samples during all but a few test cases. These spatial correlations were generally across short distances (< 150 inches), such that sample results that are over 150 inches apart were not correlated. The spatial contaminant distribution patterns observed for wipe samples and vacuum samples were not consistent, except in the case of Test Event 3.

Differences between surface types and consistency across scenarios (test events) were analyzed. Vacuums were not included in the analysis because nearly all samples were taken from carpet only. For swabs, there was no statistical difference in contaminant amounts when sampling from glass, smooth surfaces, or metal. There were also no differences across the scenarios. For wipes, there were significant differences in contaminant amounts when sampling from plastic, smooth surfaces, or metal. There were also significant differences across the scenarios and there was a significant interaction between sampling surface and scenario (test event). Figure 7 shows the contaminant amounts for each surface and test for wipes. This display shows that these amounts are not consistent across the test events. An example of this is that much higher contaminant amounts were wiped from metal surfaces during the first four events, but on the fifth event, the contaminant amount was highest from plastic surfaces.

Assuming that the sampling technologies provide fairly reproducible results, biological agents that behave similarly to that which was employed in this INL study appear to have fairly unique deposition patterns. Although the geostatistical concentration plots give the false impression that high contaminated areas (hotspots) may exist, the very high concentrations observed were quite isolated with adjacent samples often having very low concentrations. The high within room variations also confirm the patchiness and inconsistent spatial deposition behavior of this agent. Air flow, which was not studied in this analysis, may be a key factor in how the biological agent was spread throughout the building. Future sampling strategies developed and deployed should consider this unique deposition behavior, with a key emphasis on the effect of the air flow, if it is important to estimate concentrations over a spatial extent.

In summary, the overall findings of this study relative to the stated topics of interest are:

- *Statistical Distribution of the Data:* The data were not normally distributed and were highly skewed to the right with some values that were much larger (and truncated) than the bulk of the data. Thus, normality assumptions cannot be applied to reduce the number of samples required for future sampling strategies.
- *Quantification of Sub-Elements of Sample Variations:* The within-room standard deviation was the largest component of variation. This would suggest that information would be lost by reducing the number of samples in a room. Standard deviation estimates were obtained and may

be useful for determining the number of samples required for future, similar office building bio-agent releases.

- *Spatial Relationships between Sample Results:* For the characterization data, some minor spatial correlation was observed for some of the test events but generally only if samples were less than 150 inches apart. Spatial contamination patterns were not consistent across sample method. Observed spatial correlations were probably not sufficiently strong and are too inconsistent to justify taking fewer samples without losing important information.
- *Surface Type and Sampling Methods Comparisons:* Results were not consistent across various surface types and when using different sampling methods. Thus, no recommendation can be made on which surface types should be sampled or which sampling method employed.
- *Deposition Patterns:* The biological agent releases in this study did not appear to result in hotspot depositions. High concentration samples were isolated and often adjacent to very low concentration samples. The air flow within the building may be a key contributor to this. Future studies should focus on the effect of air flow during and after the release in order to better understand deposition patterns.

1.0 Introduction

In September 2007, a large-scale testing operation was conducted to focus on the evaluation of sample collection methods, within the context of building decontamination of biological agent. In September 2008, a second testing operation was performed. Both tests were performed within a two-story building (PBF-632) at the Idaho National Laboratory (INL). The objectives of this second evaluation (referred to as the INL-2 Test), as listed in the evaluation report “Operational Observations on the INL-2 Experiment” (Grotte and Hebner), were:

1. Operationally evaluate judgmental and probabilistic sampling for characterization, as well as evaluate and compare probabilistic and hybrid (judgment and probabilistic) sampling approaches for clearance and characterization in a building with localized contamination as well as gradient contamination (from low or moderate down to absent or not detectable) for different initial concentrations of the contaminant.
2. Explore judgment composite sampling approaches as a mechanism to reduce sample numbers but retain the robustness of coverage for characterization.
3. Identify operational factors that affect the minimum detectable concentration observed for agreed sampling methods in the field compared to laboratory validated performance data.
4. Operationally compare an alternative analytical method for assessing contamination [The Johns Hopkins University Applied Physics Laboratory (JHU/APL) modified Rapid Viability Polymerase Chain Reaction (RV-PCR) protocols²] and evaluate the utility of filter-plate and spiral-plate culturing methods.
5. Collect baseline data to serve as an indication of the actual levels of simulant contamination in the tests.
6. In an operational environment, gather baseline data on the ability of BROOM software and PDAs to track sample collection and processing activities; compile laboratory generated assay data and integrate information regarding location and *Bg* presence in a room/area that has been contaminated and treated.
7. In an operational environment, gather baseline data on the ability of procedural changes and risk mitigation strategies to minimize cross-contamination by the study agent of collected samples and controls in an environment of study agent prevalence.

Statistical analyses were performed in the report to help answer objectives (1), (2), and (4). With respect to these objectives, the analyses in the report concluded the following:

1. All 592 clearance samples came back negative. This supported the conclusion that the decontamination efforts were successful. Because all of the clearance samples were non-detects, comparing the different strategies during clearance was not meaningful.
2. During characterization there were no significant differences found between sampling strategies (probabilistic and judgmental) based on detection rate and overall recovery.
3. Only a small number of samples were collected as composite samples. For vacuums, there was not enough statistical evidence to indicate a difference in the response between the composite and non-composite samples. For wipes there was a moderately statistical significance ($p=0.0756$) that the composite sample values were in general higher than the non-composite sample values.

4. In comparing RV-PCR and plating methods, all clearance samples were non-detects, so all results were in agreement. When analyzing the 29 blanks and QC samples, the agreement between the two methods was 93%. For characterization the 93 samples which were analyzed using both methods showed an agreement rate of approximately 50%.

The purpose of this report is to study additional topics that were not within the scope of the original report. This was done to provide valuable information affecting future studies and sampling strategies. PNNL was tasked to focus on analyses related to the following four areas:

1. **Assess Assumptions** – Assess the quantitative results to determine whether normality or log-normality assumptions are applicable, thereby allowing for fewer samples to make the same confidence / % clean statements.
2. **Room Sample to Sample Differences** – Evaluate differences in sample to sample variability for within room versus across rooms and quantify the variation.
3. **Spatial Variation** – Perform geostatistical types of analyses to determine spatial correlation patterns.
4. **Scenario Consistency** – Evaluate the quantitative differences observed between surface types and sampling technologies for each scenario and determine the consistency across the scenarios.

The INL-2 test consisted of five tests (events) in which a floor (level) of the building was contaminated with the harmless biological warfare agent simulant *Bacillus atrophaeus* (*Bacillus globigii*, subsequently *Bacillus subtilis* var. *niger*, and recently renamed *Bacillus atrophaeus*) and samples were taken in most, if not all, of the rooms on the contaminated floor. After the sampling, the building was decontaminated, and the next test performed. Test 1, also called the ORI (Operational Readiness Inspection), test 2, and test 4 were performed on the first floor of the building, while tests 3 and 5 were performed on the second floor. Judgmental samples and probabilistic samples were determined and taken during each test. Each dissemination was performed at the same end of the building with the same amount for each floor. Vacuum, wipe, and swab samples were taken. Vacuum samples were mostly taken on carpet surfaces, wipe samples were usually taken on non-porous surfaces, and swab samples were usually taken on monitors and vents. A general summary of the sample results collected at the INL-2 test are found in Table 1. This data is also shown spatially in Figures A.1 – A.5 in Appendix A.

Table 1. Characterization Data Distribution (in CFU/cm²) for each Test and each Method (Each cell shows the number of samples for each method that fell within the CFU/cm² category levels. Percentages represent the percent of the samples that fell in each CFU category for a specific method).

Test	Method	0 CFU/cm ²	>0 - 0.01 CFU/cm ²	>0.01 - 0.10 CFU/cm ²	>0.10 - 1.0 CFU/cm ²	>1.0 - 10 CFU/cm ²	>10 - <100 CFU/cm ²	100 ^(a) CFU/cm ²
ORI	Vacuum	11 (17%)	17 (26%)	16 (25%)	10 (15%)	8 (12%)	2 (3%)	1 (2%)
	Swab	2 (20%)	0	4 (40%)	4 (40%)	0	0	0
	Wipe	13 (19%)	1 (1%)	9 (13%)	17 (25%)	18 (27%)	5 (7%)	4 (6%)
Test 2	Vacuum	11 (17%)	28 (43%)	18 (28%)	8 (12%)	0	0	0
	Swab	3 (30%)	0	6 (60%)	1 (10%)	0	0	0
	Wipe	6 (10%)	0	21 (33%)	20 (32%)	12 (19%)	0	4 (6%)
Test 3	Vacuum	36 (58%)	16 (26%)	4 (6%)	6 (10%)	0	0	0
	Swab	7 (58%)	0	2 (17%)	3 (25%)	0	0	0
	Wipe	11 (16%)	8 (12%)	16 (23%)	28 (41%)	5 (7%)	0	1 (1%)
Test 4	Vacuum	35 (45%)	31 (40%)	12 (15%)	0	0	0	0
	Swab	4 (44%)	0	3 (33%)	2 (22%)	0	0	0
	Wipe	4 (6%)	1 (1%)	5 (7%)	23 (32%)	21 (29%)	3 (4%)	15 (21%)
Test 5	Vacuum	33 (38%)	29 (34%)	18 (21%)	6 (7%)	0	0	0
	Swab	9 (75%)	0	3 (25%)	0	0	0	0
	Wipe	9 (17%)	2 (4%)	3 (6%)	16 (30%)	18 (34%)	0	5 (9%)

^(a) 100 CFU/cm² was assigned to samples that were too numerous to count (TNTC).

2.0 Statistical Analyses and Results

This section contains statistical analyses and results for the four areas of focus. Section 2.1 provides a discussion and assessment of the assumption of normality. Section 2.2 uses analysis of variance and variance component analysis to evaluate the causes of variability and estimate the amount of variability. Section 2.3 performs geostatistical analyses to determine spatial correlations. Section 2.4 investigates the differences between surface types and looks at the consistency in the results across the scenarios.

2.1 Assess Assumptions

The purpose of this investigation is to assess whether the normality assumptions are applicable in determining the numbers of samples needed for clearance. If a distribution like normality or log-normality can be established, then fewer samples could be taken to make the same level of confidence and percentage clean statement. For this study, all clearance samples came back negative. Because of this, it is not possible to determine the appropriateness of using a distribution for clearance sampling. Instead of looking at clearance data, it was decided to look at the distribution of the characterization data.

As Table 1 shows, the characterization data consists of values between 0 and 100 CFU/cm² (100 was the value assigned when the number is too numerous to count). The 100 values are generally much bigger than the rest of the data, causing the data to be right skewed. There are also a significant number of zeros. From the histograms in Figures A.6 of Appendix A these data characteristics can be seen, as well as a lack of normality in the data. Figure A.7 of Appendix A shows the data distributions if the log(CFU/cm²) is used. This does improve the normality of the data, except for the outliers at 0 and 100. The value of 0.001 was used in place of 0 when calculating the log. The value 0.001 was ½ the value of the smallest non-zero value.

Analysis of variance (ANOVA) is used in Section 2.2. It was performed using CFU/cm² and log(CFU/cm²). QQ plots of the residuals for each analysis can be found in Figure A.8. When the points on the QQ plot fall in a straight diagonal line, it indicates normality in the residuals. There is an improvement in the normality of the data when the log is used, however it still struggles with the extremes (the zeros and 100s). The literature is divided as to how robust ANOVA is in respect to the normality assumption. Some authors (Ferguson, 2005) believe it is very robust and normality is generally not an issue, while others disagree. In a compromise of these two beliefs, ANOVA's will be done in Section 2.2 that will summarize results for both CFU/cm² and log(CFU/cm²).

2.2 Room Sample to Sample Differences

Analyses were performed to evaluate the sources of variability in this study. These sources included: 1) differences in the three methods (wipes, swabs, and vacuums), 2) event to event variability, 3) room to room variability, 4) variability due to the interaction of method and event, 5) variability due to the interaction of method and room, and 6) general uncertainty (within room variability). Interactions measure the inconsistency of the different levels of one variable across the levels of the other variable, i.e. the interaction of method and event measures how differently the three methods change across the five events.

Analysis of variance (ANOVA) was used to determine which sources of variability were statistically significant. Different analyses were performed using CFU/cm² and log(CFU/cm²) as the response variable and three different subsets of the data – 1) all five events, with all judgmental and probabilistic samples, 2) the last four events (ORI removed), with all judgmental and probabilistic samples, and 3) all five events, with only probabilistic samples. These subsets were chosen so that the effect of the ORI can better be shown and differences between using all the samples and only the probabilistic samples can be expressed.

Variance component analysis (VCA) was used to estimate the amount of variance due to each source. VCA was performed using CFU/cm², in order to keep the variance and standard deviation estimates in the same scale and units as the original data. These estimated variances and standard deviations could be used in computing necessary sample sizes for future studies.

ANOVA and VCA used the following model for analyzing each subset of data:

$$Y = \mu + M + E + M \times E + R / E + M \times R / E + \varepsilon$$

where Y is the response variable of CFU/cm² or log(CFU/cm²), μ is the mean, M is the fixed factor Method, E is the random factor Event, $M \times E$ is the random term interaction of Method and Event, R / E is the random factor Room nested within Event, $M \times R / E$ is the random term interaction of Method and Room nested in Event, and ε is the residual.

Table 2 summarizes the ANOVA and VCA results for each of the three different subsets of the data. Each analysis concludes that there is a significant difference between the three methods. This is consistent with the conclusions in the INL-2 report. There was not a significant difference between events, and the variance component estimate for event was very small. Most of the analyses concluded that the interaction between method and event was significant. When analyzing the log(CFU/cm²) the room nested in event factor was significant, as well as the interaction between method and room nested in event.

The significant interaction between method and event and large variance component estimate for the interaction indicates that methods were not consistent across the events. Figure 1 plots the means for each method-event combination and it shows the lack of consistency over the events. Because of this interaction, it was decided to perform ANOVA and VCA for each method individually. Method was removed from the previous model, resulting in the use of the following model:

$$Y = \mu + E + R / E + \varepsilon$$

with the same definitions being used as the previous model.

Table 2. ANOVA using CFU/cm² and Log(CFU/cm²) and Variance Component Estimates using CFU/cm² for All Events, Events 2 through 5 (No ORI), and All Events Probabilistic Samples Only.

Source	DF	CFU/cm ²			log(CFU/cm ²)			Variance Component Estimates ^(a)	
		MS	F	p-value	MS	F	p-value	Variance	St. Dev
All Events, Judgmental & Probabilistic Samples									
Method ^(b)	2	6195.02	6.71	0.0195	935.16	20.62	0.0007	-	-
Event ^(c)	4	433.01	1.67	0.1704	37.68	2.13	0.0884	<0.001	0.001
Method x Event ^(d)	8	923.21	2.90	0.0065	45.22	6.86	<0.0001	17.557	4.190
Room / Event ^(e)	57	259.85	0.71	0.9453	17.66	4.44	<0.0001	<0.001	0.003
Method x Room / Event ^(f)	88	318.52	0.87	0.7862	6.60	1.66	0.0004	20.840	4.565
Residuals ^(g)	573	365.40			3.98			344.906	18.572
No ORI, Judgmental & Probabilistic Samples									
Method ^(b)	2	6513.27	6.53	0.0208	916.97	21.09	0.0019	-	-
Event ^(c)	3	570.26	2.32	0.0872	25.02	1.69	0.1822	0.038	0.195
Method x Event ^(d)	6	996.73	3.24	0.0072	43.49	6.94	<0.0001	22.895	4.785
Room / Event ^(e)	46	245.28	0.69	0.9408	14.80	4.35	<0.0001	0.169	0.411
Method x Room / Event ^(f)	71	307.94	0.86	0.7736	6.27	1.84	0.0001	20.936	4.576
Residuals ^(g)	462	356.38			3.41			336.128	18.334
All Events, Probabilistic Samples Only									
Method ^(b)	2	6354.44	9.29	0.0082	688.54	20.12	0.0008	-	-
Event ^(c)	4	181.62	0.37	0.8318	38.31	2.43	0.0589	0.002	0.042
Method x Event ^(d)	8	683.82	1.48	0.1807	34.22	5.59	<0.0001	15.133	3.890
Room / Event ^(e)	54	496.19	1.48	0.0196	15.78	3.83	<0.0001	<0.0001	0.006
Method x Room / Event ^(f)	70	462.42	1.38	0.0310	6.12	1.48	0.0109	49.877	7.062
Residuals ^(g)	399	334.95			4.13			320.321	17.898

- (a) Variance component estimates are recorded in terms of variance and standard deviations.
(b) Method represents the three methods. There are no variance component estimates because it is a fixed variable.
(c) Event represents event to event (between event) variability.
(d) Method x Event represents the interaction between method and event.
(e) Room is nested within event and it represents the room to room (between room) variability within events.
(f) Method x Room/Event represents the interaction between method and room.
(g) Residuals represent within room variability.

Tables 3 and 4 summarize the ANOVA and VCA results when analyzing each method separately. Table 3 summarizes all events with all samples (judgmental and probabilistic samples), while Table 4 summarizes the last four events (no ORI) with all samples. Analyzing only probabilistic samples was attempted; however, it was not much different from the results in Table 3 and 4, so it was not included here. The INL-2 report also concluded that there was not much difference between the probabilistic and judgmental samples. Figure 2 contains a plot summarizing the standard deviation estimates for each sampling method across the five events.

When analyzing the CFU/cm² for wipes only, the room nested in event effect (between room variability) was not significant. The event effect was significant (between event variability). The residual standard deviation was much larger than the others, indicating more variability is due to within room than between rooms or between events. The results plotted in Figure 2 confirm these findings. The results were the same whether analyzing all the events, or just the last four events (no ORI).

When analyzing the vacuums only, there was a significant difference between including all the events and including the last four events. The event effect was significant when including the ORI, but not

significant when removing the ORI. As Figure 1 and Table 1 show, the amount of contamination recovered using vacuums during the ORI was an order of magnitude larger than the other four events. When removing the ORI, the variance components were all very small. The within room variability (residual) was similar to the between room variability, with the between event variability being much smaller. The results plotted in Figure 2 confirm these findings.

The swab only analyses were similar whether including the ORI or removing it. The event effect was not significant, while the room nested in event effect was very significant. The variance component estimates verify this with the between room variability being larger (standard deviation = 0.151 with all events), while the within room variability (standard deviation = 0.091) and between event variability (standard deviation = 0.014) are smaller.

Figure 3 shows the %RSD (percent relative standard deviation), the ratio of the standard deviations and the means, for each of the sources of variability using all the events, and removing the ORI (last four events). Many of the %RSD values are over 100%, showing just how highly skewed the data really are. The vacuum within room %RSD was greatly reduced when removing the ORI. There were some too numerous to count ($100 \text{ CFU}/\text{cm}^2$) samples taken during the ORI with vacuums, but nothing above $1 \text{ CFU}/\text{cm}^2$ during the other events. It is very probable that the decontamination after the first ORI and then performed after each test event was responsible for the drastic decrease in the amount of contamination and that this effect mostly influenced the vacuum carpet samples.

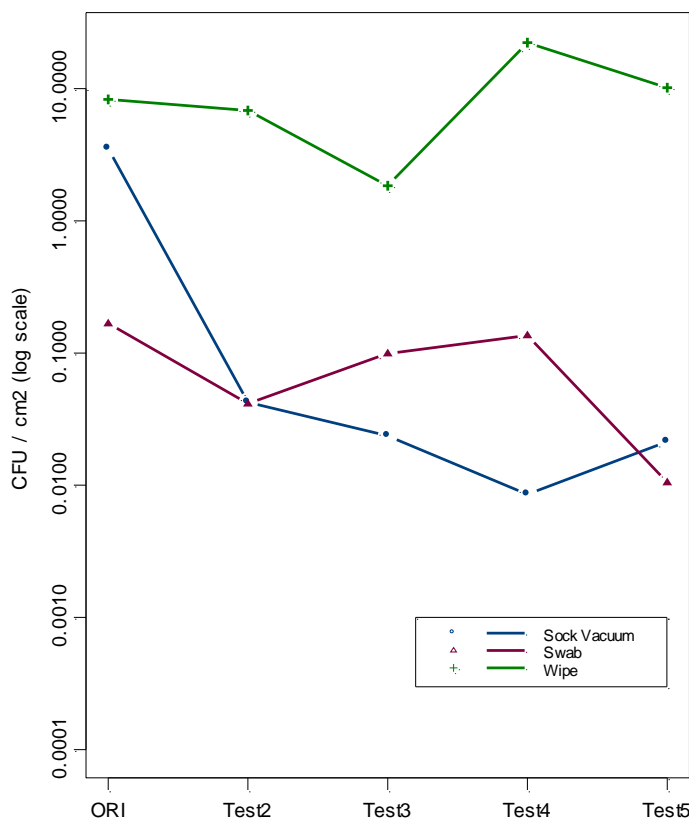


Figure 1. Interaction Plot of Means for Methods and Events (CFU/cm^2 in log scale)

Table 3. ANOVA and Variance Component Estimates (CFU/cm²) when Analyzing Each Method Individually Including All Events

Source	DF	CFU/cm ²			Log(CFU/cm ²)			Variance Component Estimates ^(a)	
		MS	F	P-value	MS	F	P-value	Variance	St. Dev
Wipes									
Event ^(b)	4	3037.62	3.32	0.0168	77.23	3.61	0.0111	42.932	6.552
Room / Event ^(c)	54	915.58	1.24	0.1369	21.39	3.29	<0.0001	46.005	6.783
Residuals ^(d)	265	737.35			6.50			723.362	26.900
Vacuums									
Event ^(b)	4	253.99	3.62	0.0106	73.28	6.01	0.0004	1.817	1.348
Room / Event ^(c)	57	70.08	1.47	0.0216	12.20	6.66	<0.0001	1.081	1.040
Residuals ^(d)	294	47.53			1.83			50.206	7.086
Swabs									
Event ^(b)	4	0.042	1.20	0.3306	12.11	1.79	0.1538	<0.001	0.014
Room / Event ^(c)	34	0.035	4.64	0.0018	6.77	4.56	0.0020	0.023	0.151
Residuals ^(d)	14	0.008			1.48			0.008	0.091

- (a) Variance component estimates are recorded in terms of variance and standard deviations.
- (b) Event represents event to event (between event) variability.
- (c) Room is nested within event and it represents the room to room (between room) variability within events.
- (d) Residuals represent within room variability.

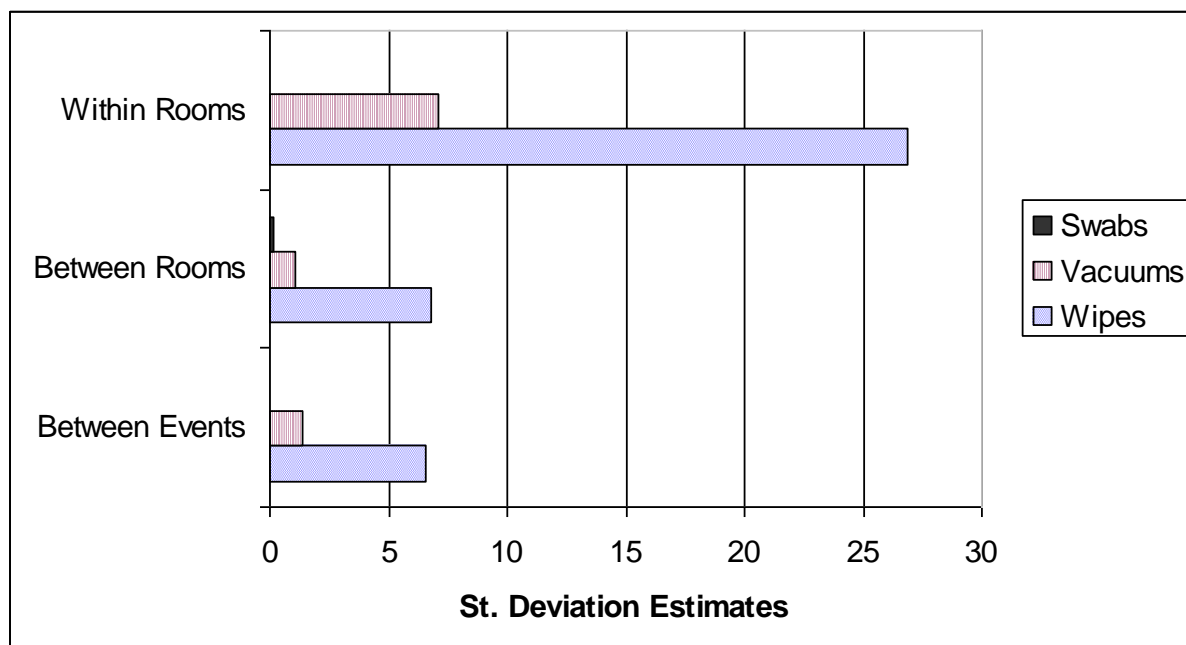


Figure 2. Standard Deviation Estimates from the Variance Component Analysis of All Events (results in Table 3) (Note that swab standard deviation estimates are too small to see on the plot for within rooms and between events estimates)

Table 4. ANOVA and Variance Component Estimates (CFU/cm²) when Analyzing Each Method Individually Including Events 2 through 5 (no ORI)

Source	DF	CFU/cm ²			Log(CFU/cm ²)			Variance Component Estimates ^(a)	
		MS	F	P-value	MS	F	P-value	Variance	St. Dev
Wipes									
Event ^(b)	3	3894.10	3.96	0.0141	102.51	5.58	0.0025	59.952	7.743
Room / Event ^(c)	43	983.77	1.25	0.1510	18.36	2.95	<0.0001	49.882	7.063
Residuals ^(d)	210	784.04			6.23			770.045	27.750
Vacuums									
Event ^(b)	3	0.020	1.45	0.2402	25.28	2.53	0.0685	<0.001	0.008
Room / Event ^(c)	46	0.014	5.85	<0.0001	9.98	9.74	<0.0001	0.002	0.049
Residuals ^(d)	241	0.002			1.02			0.002	0.049
Swabs									
Event ^(b)	3	0.045	1.30	0.2931	9.10	1.39	0.2671	<0.001	0.016
Room / Event ^(c)	28	0.035	43.80	<0.0001	6.56	3.89	0.0108	0.032	0.180
Residuals ^(d)	11	0.001			1.69			0.001	0.038

- (a) Variance component estimates are recorded in terms of variance and standard deviations.
- (b) Event represents event to event (between event) variability.
- (c) Room is nested within event and it represents the room to room (between room) variability within events.
- (d) Residuals represent within room variability.

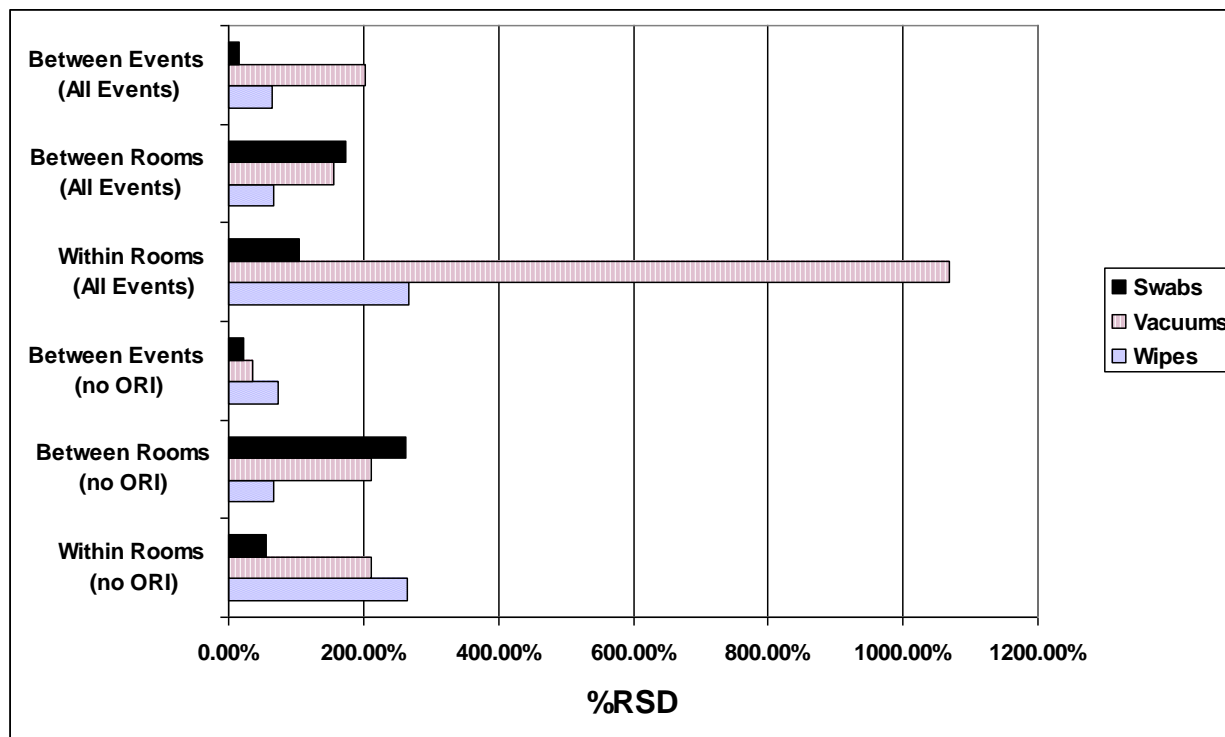


Figure 3. %RSD (Percent Relative Standard Deviation) Estimates from the Variance Component Analysis of All Events (first 3 rows) and Events 2 – 5 (last 3 rows)

2.3 Spatial Variation

The section investigates the spatial variation during the experiment. Section 2.3.1 investigates the contamination amounts with respect to the distance from the contamination point. Section 2.3.2 shows the results of geostatistical analyses.

2.3.1 Distance from Contamination Point Investigation

A series of analyses were performed to look at the amount of contamination and the distance the measurement was from the contamination point. The results can be found in Figures A.9 through A.11 in Appendix A. Each plot shows the results for an individual method. Each event is analyzed separately. The Spearman's correlation coefficient is calculated to determine the correlation between the distance from the contamination point and the amount of contamination for each test event / method combination. It was expected that this correlation would be negative and close to -1, indicating that the amount of contamination decreased as the distance from the contamination point increased.

Most of the correlations, as listed on the plot legends, were slightly negative or even positive. Only two test event / method combinations resulted in correlation coefficients < -0.5 . This indicates that there is not much correlation between distance from contamination point and amount of contamination.

2.3.2 Geostatistical Analyses

The sample results from the INL-2 tests provided one of the only data sets available for an in-depth evaluation of the spatial behavior of biological agents under an indoor aerosolized release scenario. Although the spatial dispersion and deposition of the simulant is affected by a multitude of factors, this INL-2 data set provided an opportunity to evaluate these patterns and spatial correlations for one of the many release and dispersion scenarios. Because the dispersion involved HVAC pathways as well as hallway/doorway pathways and samples were mainly taken from the floors and table-tops, a two-dimensional geostatistical evaluation of samples taken from the floors (or table tops projected upon the floors) was deemed sufficient. We did not attempt to adjust the calculated distances between samples accounting for doorway nor air duct pathways for these geostatistical analyses.

A geostatistical evaluation was performed using routines in Visual Sample Plan (VSP) on the characterization phase data from each of the 5 test events. Because the data values for the sock vacuum samples were significantly lower than those from the wipe samples, separate geostatistical models were derived for wipe and vacuum sample results. There were too few swab sample results to warrant a geostatistical evaluation. The analyses consisted of 1) selection of a variogram model, 2) fitting of the variogram, 3) kriging based on the variogram models, then 4) spatial display of the interpolated spatial maps. In all cases either the semivariogram of the logarithms or the semivariogram of the normalized values was used, primarily to deal with the highly skewed distributions. For example, Figure 4 shows the variogram and Figure 5 shows the kriged contaminant spatial distribution for the wipe samples on Test 1 (ORI).

The variogram and estimated Bg concentration maps for each test event are presented in Appendix B. The results of the geostatistical analyses for each of the test events are summarized in Table 5.

For Test 1 vacuum and Test 5 Wipe sample evaluations, no spatial correlation appeared to be present. The variograms for these two cases are shown in Figure 6. We are not aware of any explanation as to why no spatial correlation was evident on these two Test/Sample-Type combinations but is evident on all others.

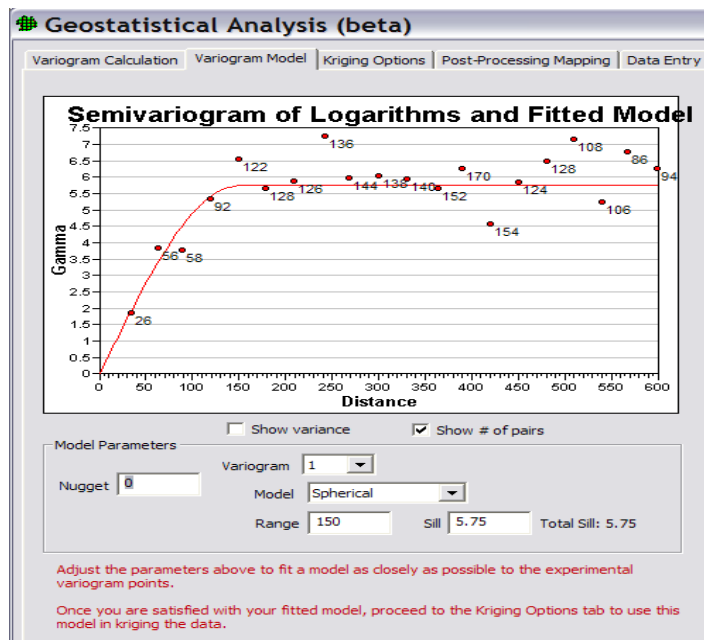


Figure 4. Variogram model for the wipe samples from Test 1 (ORI).

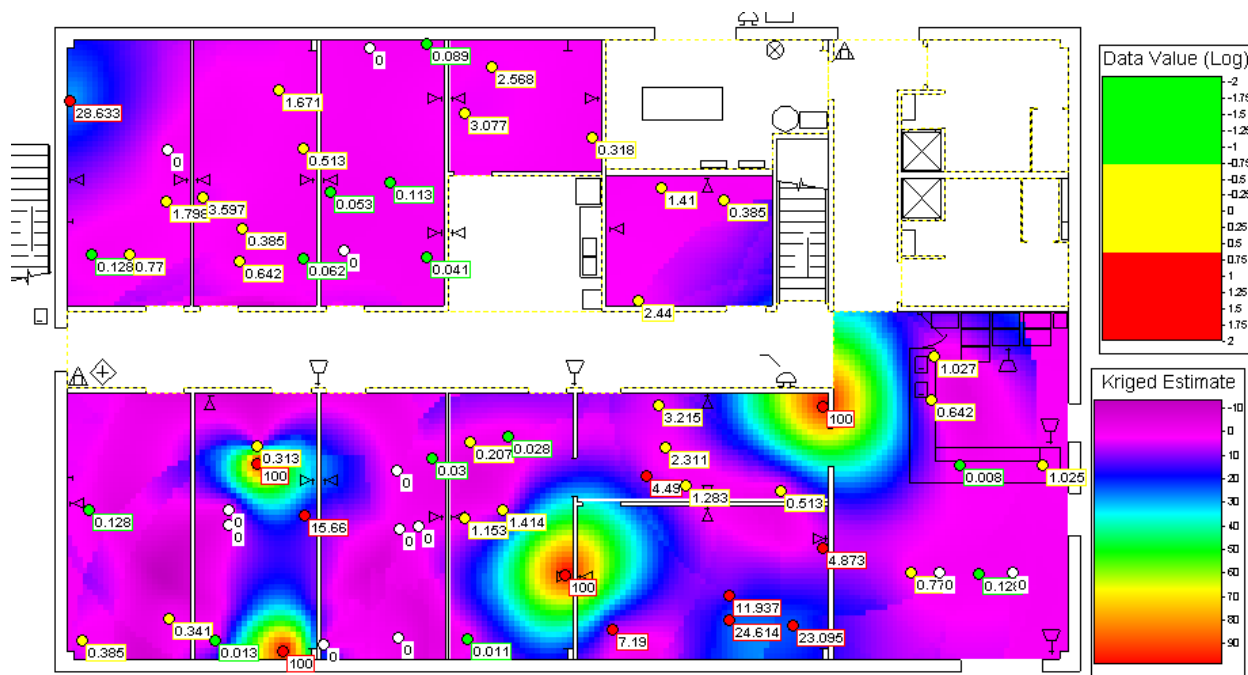


Figure 5. Kriged Map and Posting Plot for the wipe samples from Test 1 (ORI). Note dominance of samples where value is 100.

Table 5. Summary of Spatial Correlations Observed from the Geostatistical Evaluation.

Test Event	Floor	Sample Type	Semi-Variogram (SV) Model	Spatial Correlation	Wipe/Vac Consistency?
Test 1 (ORI)	1 st	Wipe	SV of Logs	<150 inches	No
Test 1 (ORI)	1 st	Vacuum	No Spatial Corr.	None	No
Test 2	1 st	Wipe	SV of Logs	<150 inches	No
Test 2	1 st	Vacuum	SV of Logs	<50 inches	No
Test 3	2 nd	Wipe	SV of Logs	<150 inches	Yes
Test 3	2 nd	Vacuum	SV of Normalized	<150 inches	Yes
Test 4	1 st	Wipe	SV of Normalized	<80 inches	No
Test 4	1 st	Vacuum	SV of Logs	<150 inches	No
Test 5	2 nd	Wipe	No Spatial Corr.	None	Unclear
Test 5	2 nd	Vacuum	SV of Normalized	<400 inches	Unclear

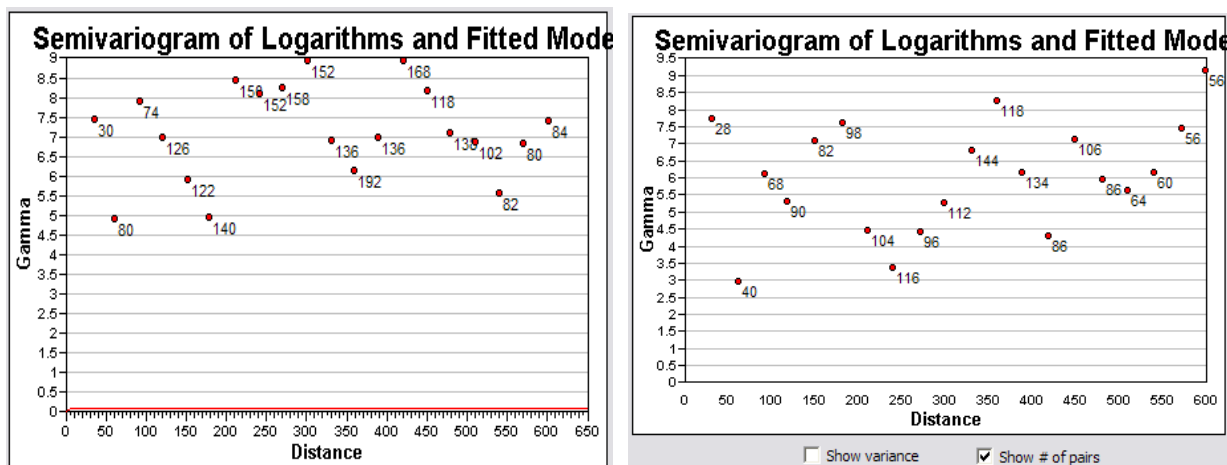


Figure 6. Semi-variograms for Test Event 1 vacuum samples (on left) and Test Event 5 wipe samples (on right) showing no spatial correlation.

Two general conclusions can be made from the geostatistical analyses and the summarized results in Table 5.

- If any spatial correlation is observed, it is generally across short distances (<150 inches) such that sample results that are over 150 inches apart are not correlated.
- Spatial contaminant distribution patterns observed for wipe samples are not consistent with vacuum sample patterns with the exception of Test Event 3.

If there would have been strong, consistent spatial correlation patterns observed over larger distances, this may have provided justification for significantly reducing the number of samples required (no need for samples taken in close proximity to each other because they are somewhat duplicative). However, given the inconsistent spatial correlations (or lack thereof) observed and the relatively short distances over which some of those observed spatial correlations are present, **there is little support for significant reductions in the number of samples required for characterization.** It is also troubling that the Bg concentration maps for wipe samples are not generally consistent with those for vacuum samples. The

scaling and color visualizations on the maps may contribute to this conclusion but it appears that the inconsistencies are real and perhaps should be studied further.

2.4 Scenario Consistency

This section investigates the differences between surface types and examines the consistency in the results across the scenarios. There is really just one scenario, either played out on the first floor or the second floor. All disseminations were performed with the same level of contaminant and from the same end of the building. For this reason, the five events will be used to represent the scenarios.

Table 6 summarizes the contamination for each method on each surface, for each event. Each cell contains three numbers – the top number is the mean amount of contamination (measured in CFU/cm²), the number in parentheses is the number of samples, and the bottom number is the standard deviation. Cells containing NA mean that no samples were taken for that combination.

Table 6. Means (top number), Number of Samples (in parentheses) and Standard Deviations (bottom number) for Each Method, Surface, and Event (measured in CFU/cm²).

Method	Event	Carpet	Glass	Metal	Plastic	Smooth
Sock Vacuum	ORI	3.584 (64) 16.882	NA	NA	NA	0.004 (1) NA
Swab		NA	0.234 (2) 0.255	0.189 (2) 0.165	NA	0.138 (6) 0.208
Wipe		NA	NA	NA	16.115 (2) 17.703	8.440 (62) 24.716
Sock Vacuum	Test 2	0.0042 (65) 0.099	NA	NA	NA	NA
Swab			0.030 (3) 0.038	0.046 (7) 0.043	NA	NA
Wipe		0.235 (1) NA	NA	50.440 (6) 54.298	0.436 (6) 0.797	2.675 (46) 14.692
Sock Vacuum	Test 3	0.024 (62) 0.078	NA	NA	NA	NA
Swab		NA	0.1442 (5) 0.266	0.067 (7) 0.112	NA	NA
Wipe		0.185 (2) 0.002	NA	12.639 (8) 35.299	0.077 (12) 0.118	0.539 (46) 1.358
Sock Vacuum	Test 4	0.009 (78) 0.019	NA	NA	NA	NA
Swab		NA	0.009 (2) 0.013	0.230 (5) 0.348	0.054 (1) NA	0.0 (1) NA
Wipe		NA	0.070 (1) NA	61.825 (10) 49.543	25.651 (16) 44.339	11.016 (43) 29.093
Sock Vacuum	Test 5	0.021 (86) 0.045	NA	NA	NA	NA
Swab		NA	0.005 (4) 0.009	0.018 (4) 0.036	NA	0.009 (4) 0.018
Wipe		NA	NA	10.323 (11) 29.758	13.034 (16) 33.954	8.351 (26) 26.994

Table 7. ANOVA's for Swabs and Wipes Testing for Differences in Surfaces.

Source	DF	CFU/cm ²			log(CFU/cm ²)		
		MS	F	p-value	MS	F	p-value
Swabs							
Surface (Glass, Smooth, Metal)	2	0.013	0.73	0.5199	12.28	3.79	0.0863
Event	4	0.043	1.42	0.2467	30.69	2.46	0.0616
Surface x Event	6	0.018	0.60	0.7296	3.24	0.26	0.9524
Residuals	39	0.030			12.50		
Wipes							
Surface (Plastic, Smooth, Metal)	2	10207.37	4.88	0.0471	115.78	14.20	0.0034
Event	4	5103.35	7.47	<0.0001	76.00	5.96	0.0001
Surface x Event	7	2092.79	3.06	0.0040	8.15	0.64	0.7235
Residuals	296	683.56			12.76		

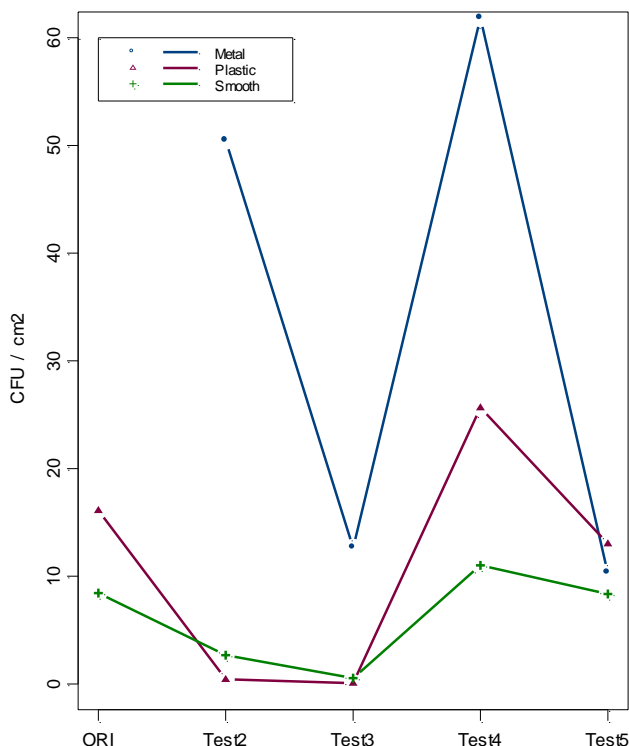


Figure 7. Interaction Plot of Means for Surface and Event for All Wipe Samples (CFU/cm²)

The vacuum samples in Table 6 show that all but one sample taken were from carpet. Because this analysis is interested in comparing across surfaces, vacuum samples were removed from this analysis. Most of the swab samples were taken from glass, smooth, or metal surfaces, with only one sample taken from a plastic surface. The plastic surface sample was removed from the subsequent analysis for swabs. Most of the wipe samples were taken from plastic, smooth, or metal surfaces, with three samples taken from carpet and one sample taken from glass. The carpet and glass samples were removed from the subsequent analysis for wipes.

Analysis of variance was used to analyze swabs and wipes, each individually, to determine if there were differences in the surfaces, and if there was consistency in the surface measurements across the events. The analysis of variance results are found in Table 7.

The analysis of the swabs in Table 7 shows no differences between the surfaces ($\alpha = 0.05$). There was also no interaction between surface and event. The analysis of the wipes in Table 7 indicates a significant difference between the surfaces (p-value = 0.0471). There was also a significant difference between the events (p-value < 0.0001) and a significant interaction between surface and event (p-value = 0.0040). Figure 7 displays the interaction by graphing each surface type across all five events. From this plot it can be seen that there is no consistency across the scenarios (events), for any of the surfaces. The metal surface sample values were higher than the others for all but the last event.

It is important to note that this experiment was not designed to look at differences between sampling surface for each method. Location of the sample, and the resulting amount of contamination, were not controlled, so that may be having an effect on the differences between surfaces. Further tests, controlling these extraneous variables, would be able to better show true differences between sampling surfaces.

3.0 Summary and Conclusions

The first analysis looked at the normality of the data. With many zeros and some 100 CFU/cm² measurements when the amount was too numerous to count, the characterization data did not look normally distributed. Taking the log of this data did help, but spikes at 0 and 100 were still present. Thus, normality assumptions cannot be applied to reduce the number of samples required for future sampling strategies.

The other analyses all focused on different aspects of the variability in the data. Vacuum and wipe samples each found the largest variability between samples to be within room variability. This variability within rooms was spatially correlated in most cases when samples were taken within 150 inches of each other. Observed spatial correlations were probably not sufficiently strong and are too inconsistent to justify taking fewer samples without losing important information. Vacuum and wipe contaminant amounts were also not consistent across events, although when the ORI (Test Event 1) was removed, the vacuum contaminant amounts were consistent across events. Wipe contaminant amounts also varied significantly for the types of surfaces sampled from and these amounts were not consistent across the events. The swab contaminant amounts were consistent across different types of surfaces, as well as consistent across events.

In general, as Figure 3 shows, the variability between samples is very large. It was not uncommon to see %RSD values greater than 100%. The %RSD for within room for vacuums was actually larger than 1000%, although when the ORI was removed from the analysis, the %RSD dramatically dropped to about 200%. With the large amounts of variability observed in this experiment, it shows just how difficult it is to control where contaminant will be spread throughout a building, possibly due to air flow factors, and just how different the sampled amounts can be when taken from within the same room. This would suggest that information would be lost by reducing the number of samples in a room. Standard deviation estimates were obtained and may be useful for determining the number of samples required for future, similar office building bio-agent releases.

4.0 References

Ferguson, George A. and Yoshio Takane. 2005. *Statistical Analysis in Psychology and Education, Sixth Edition*. Montréal, Quebec: McGraw-Hill Ryerson Limited.

Grotte, Jeffrey H. and Margaret Hebner. 2009. *Operational Observations on the INL-2 Experiment*. IDA Paper P-4449.

Kuehl, RO. 1994. *Statistical Principles of Research Design and Analysis*. Duxbury Press, Belmont, CA.

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Appendix A

Appendix A contains sets of figures which look at different aspects of the data. Because each set contains multiple figures it was decided to place them into the Appendix instead of interrupting the flow of the report. The following discussion describes what each set of plots are and lists any observations that were noticed about them.

- **Figures A.1 to A.5.** These figures consist of five figures, each one representing an event. Each figure shows the spatial location of each sample and the sampling method (plotted using the letters W (wipe), V (vacuum), and S (swab)). The measured amount of contamination (CFU/cm²) is represented by the color, where blue represents 0, red represents 100 (highest), with shades of light blue, green, yellow, and orange representing values between 0 and 100.
 - These plots show how large the variability is during an individual event, on each individual floor.
 - It also shows that event 3 has heavier contamination near the deposition site, with light contamination elsewhere, while the other events also tended to have heavier contamination in areas away from the deposition.
- **Figures A.6 to A.7.** These figures display histograms of the contamination amount for each event and each method. The histograms in Figure A.6 are produced using CFU/cm², while the histograms in Figure A.7 are produced using Log (CFU/cm²). On each histogram the x-axis was allowed to change according to the minimum and maximum values.
 - These plots show the large number of samples that are at or near zero.
 - Swab samples all range between 0 and 1 CFU/cm².
 - Wipes samples tended to be either at or near zero, or at 100 CFU/cm². There are not many samples that are between near 0 and 100 CFU/cm².
 - During the ORI there were a few vacuum samples at 100 CFU/cm². For all other events the vacuum samples all ranged between 0 and 1 CFU/cm².
- **Figure A.8.** This figure contains QQ (normal quantile) plots of the residuals from the analysis of variance performed in Section 2.2. The plot on the left corresponds to the analysis of CFU/cm², while the plot on the right corresponds to the analysis of Log (CFU/cm²).
 - These plots show outliers on either end of the scale, which is to be expected with so many zero and 100 CFU/cm² values. Taking the Log of the data did seem to help the normality of the data, although there is still some skewness on either end.
- **Figures A.9 to A.11.** These figures show the amount of contamination versus the distance from the contamination deposition, with each test event defined by a different color and symbol. Figure A.9 plots only the wipe samples; Figure A.10 plots only the swab samples; and Figure A.11 plots only the vacuum samples. The legend of each plot lists the Spearman's correlation coefficient (r) for each test event. The Spearman's correlation coefficient was used because it is a non-parametric measure of correlation.
 - If there was a strong gradient of contamination across the floor, then it would be expected that these plots would show strong contamination near the deposition of the contamination, with contamination getting weaker further from the deposition. This would result in negative correlation values, close to -1. The correlation values for each event for wipes showed very weak correlations ranging from -0.22 to 0.11, except for event 2, which had a correlation of -0.59.
 - For swab samples, the correlation values were actually positive for three events, with event 5 having a correlation value of -0.42.
 - All of the vacuum sample correlations were negative, although small. The exception was event 3 with a correlation of -0.67.

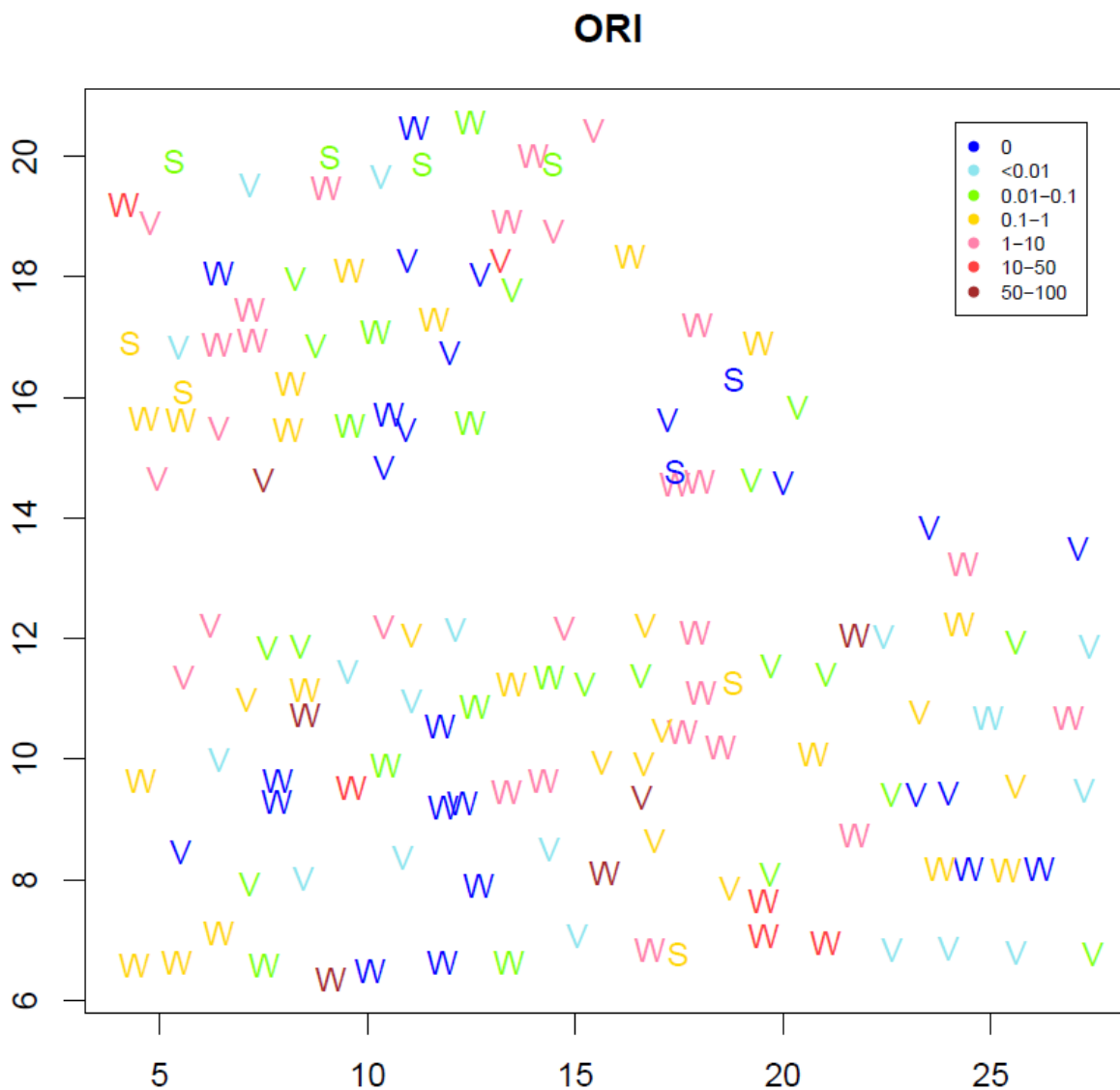


Figure A.1. ORI (Test Event 1) Judgmental & Probabilistic Sample Locations (measured in feet from corner of the first floor) and Data Values (measured in CFU/cm²). (W=wipe, V=vacuum, S=swab) (Contamination occurred in the bottom right of the plot.)

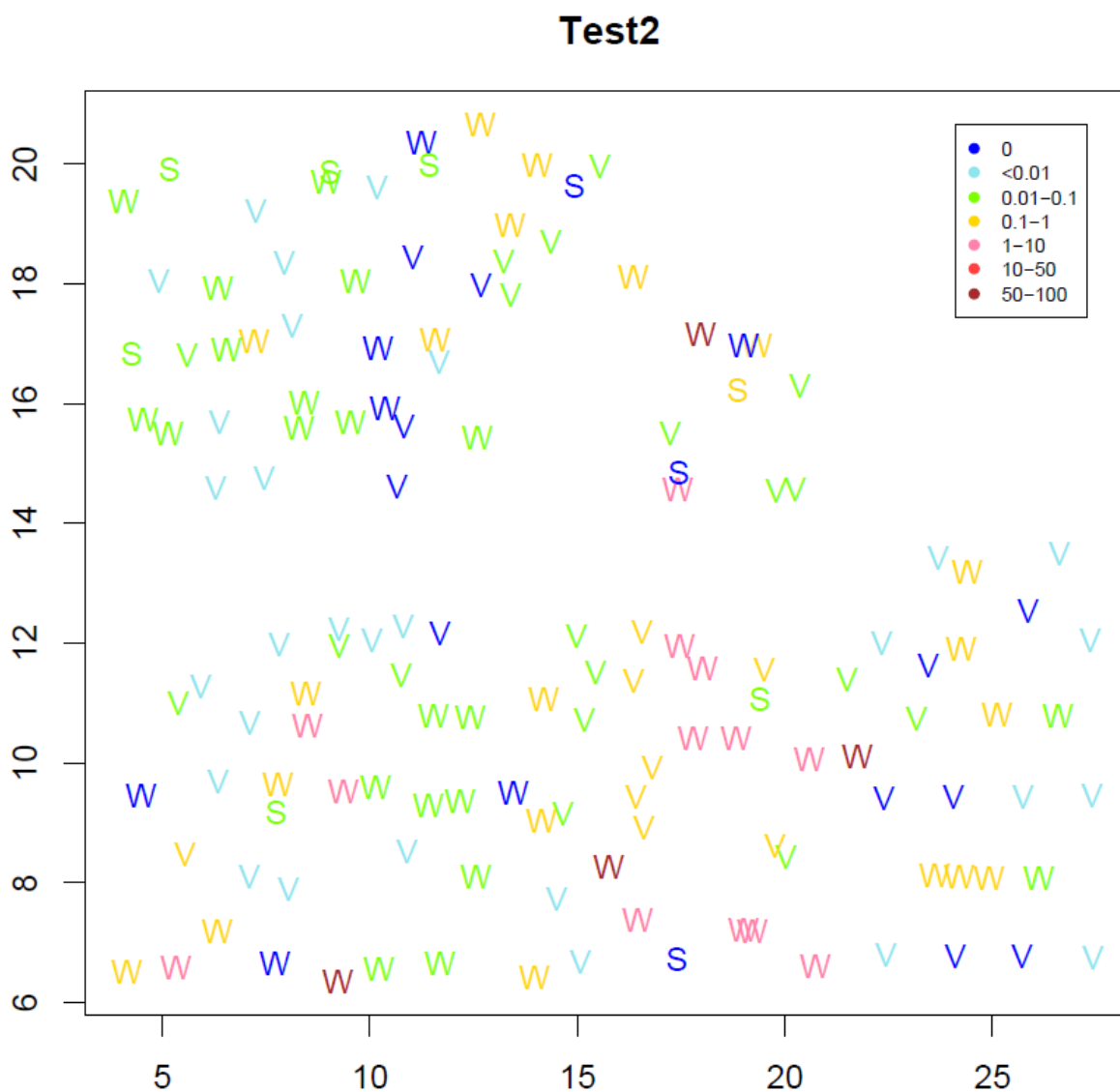


Figure A.2. Test Event 2 Judgmental & Probabilistic Sample Locations (measured in feet from corner of the first floor) and Data Values (measured in CFU/cm²). (W=wipe, V=vacuum, S=swab) (Contamination occurred in the bottom right of the plot.)

Test3

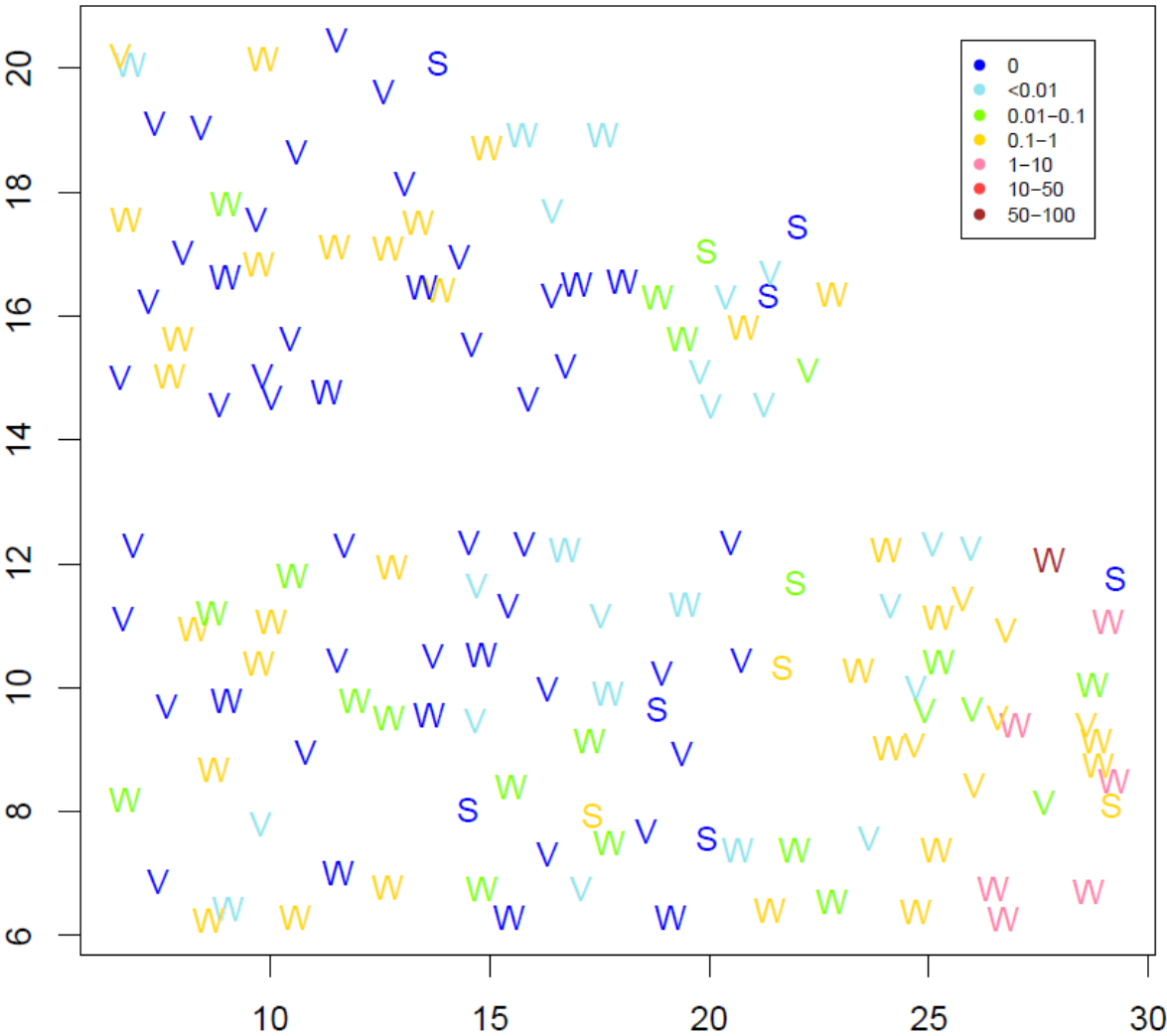


Figure A.3. Test Event 3 Judgmental & Probabilistic Sample Locations (measured in feet from corner of the second floor) and Data Values (measured in CFU/cm²). (W=wipe, V=vacuum, S=swab) (Contamination occurred in the bottom right of the plot.)

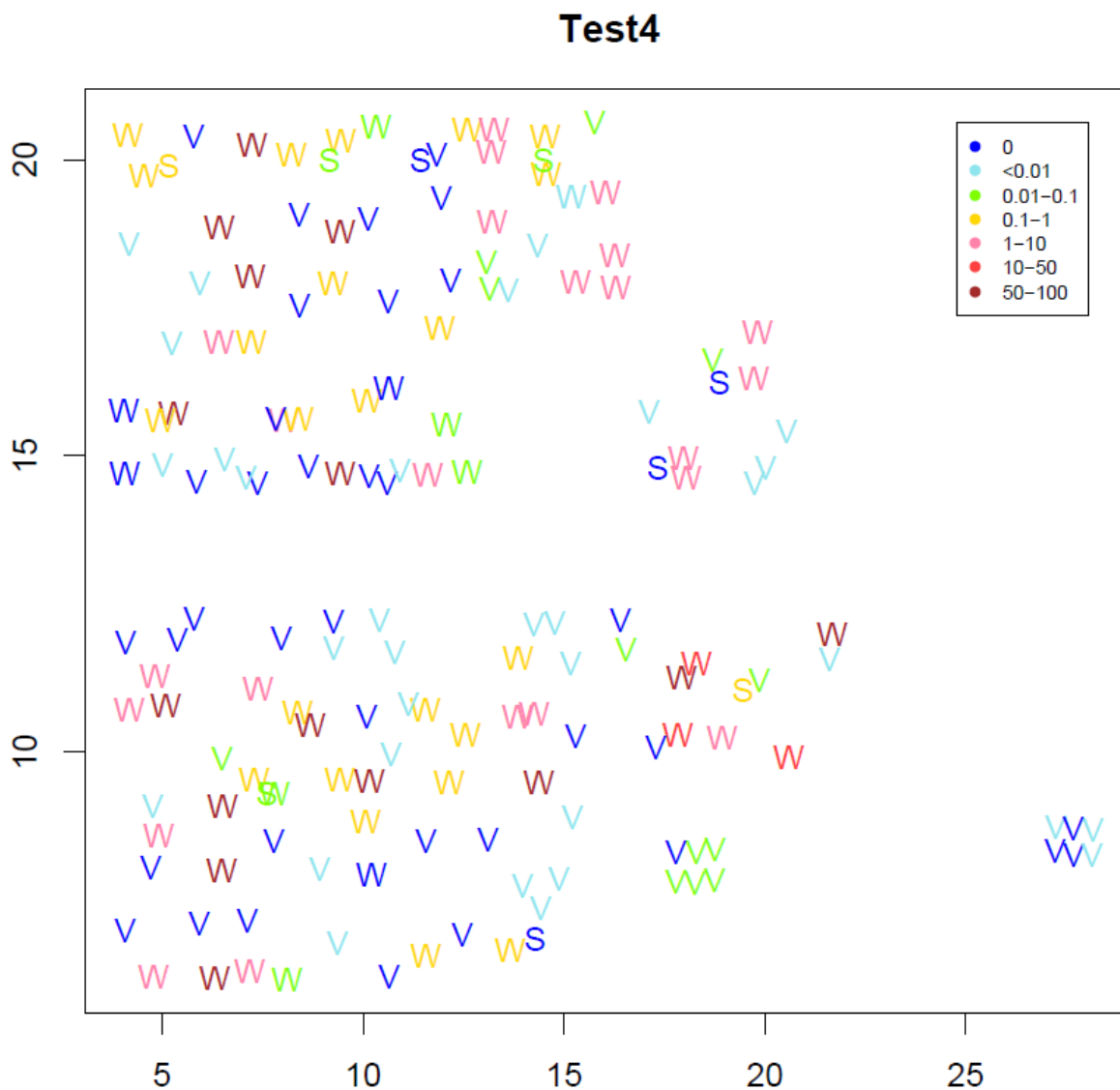


Figure A.4. Test Event 4 Judgmental & Probabilistic Sample Locations (measured in feet from corner of the first floor) and Data Values (measured in CFU/cm²). (W=wipe, V=vacuum, S=swab) (Contamination occurred in the bottom right of the plot.)

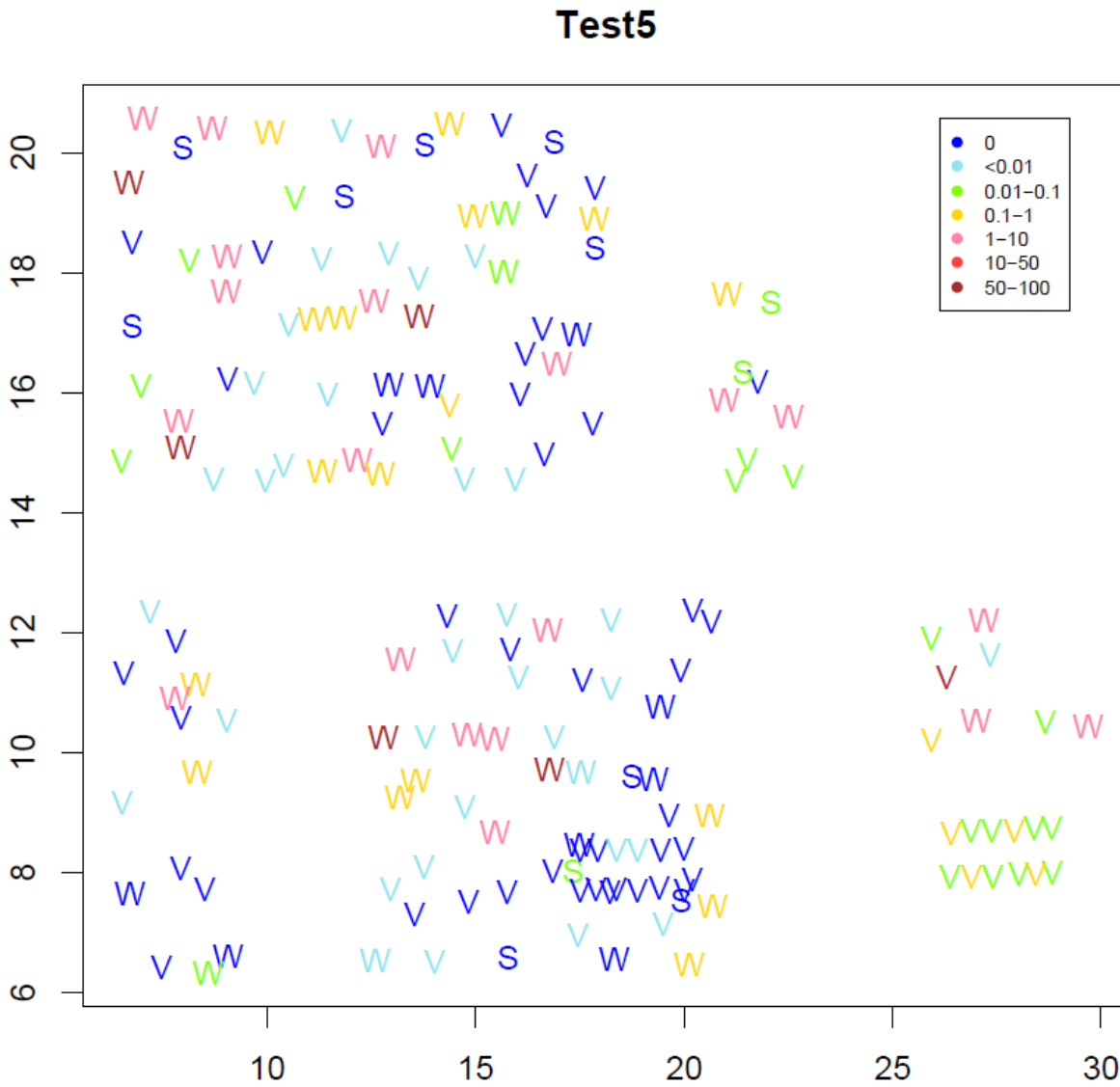


Figure A.5. Test Event5 Judgmental & Probabilistic Sample Locations (measured in feet from corner of the second floor) and Data Values (measured in CFU/cm²). (W=wipe, V=vacuum, S=swab) (Contamination occurred in the bottom right of the plot.)

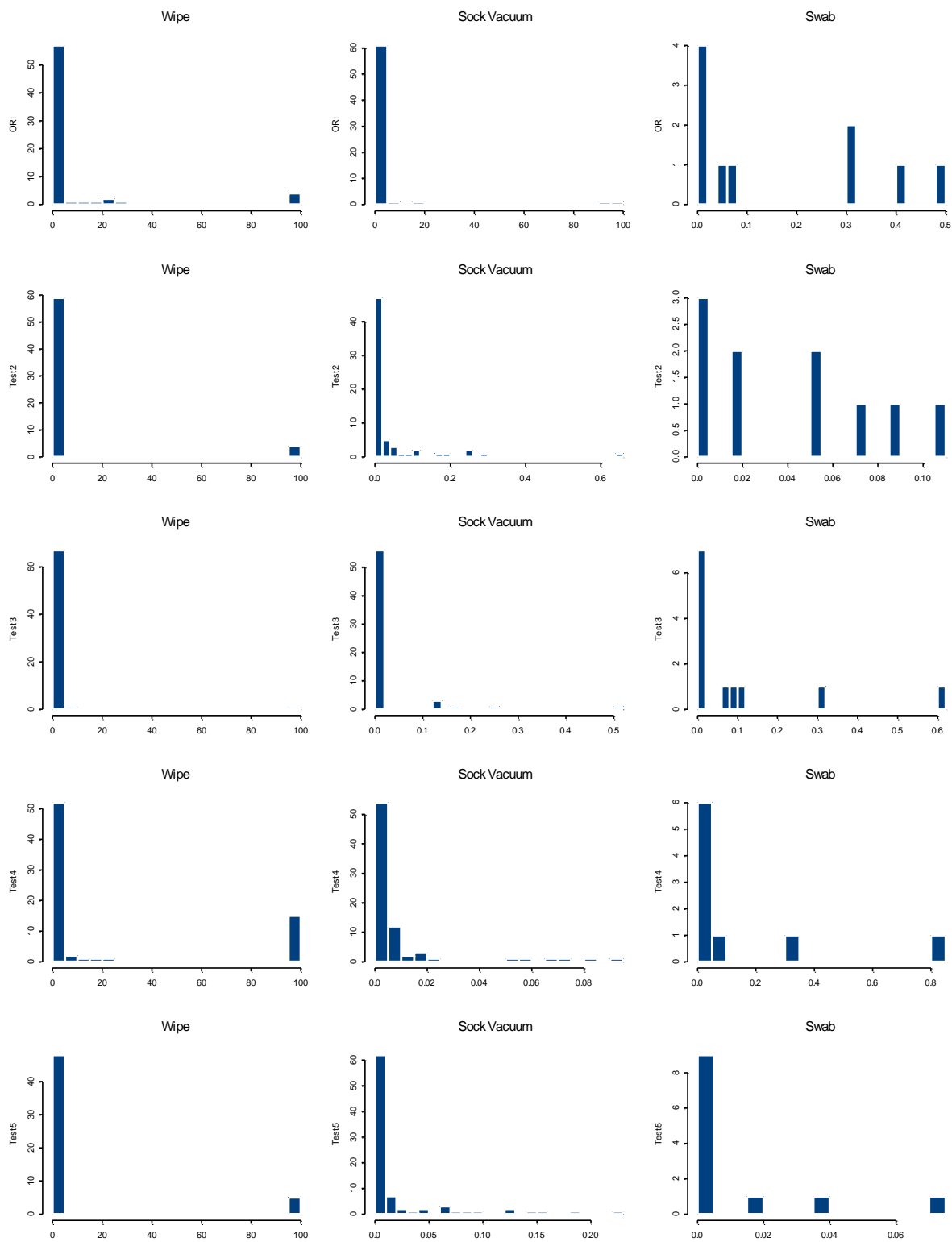


Figure A.6. Histograms of CFU/cm² for Each Event (listed by row) and Each Method (listed by column)

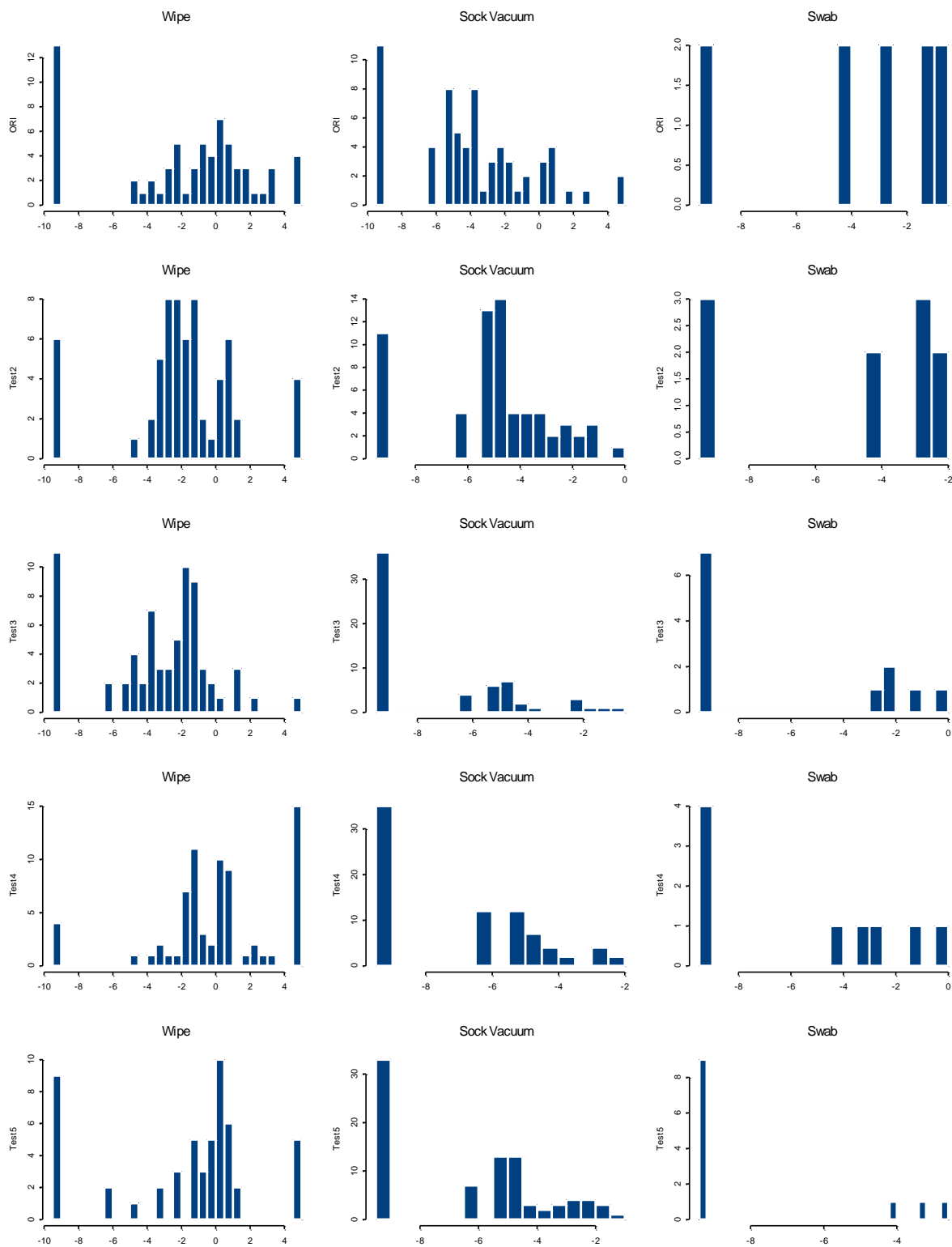


Figure A.7. Histograms of $\log(\text{CFU}/\text{cm}^2)$ for Each Event (listed by row) and Each Method (listed by column)

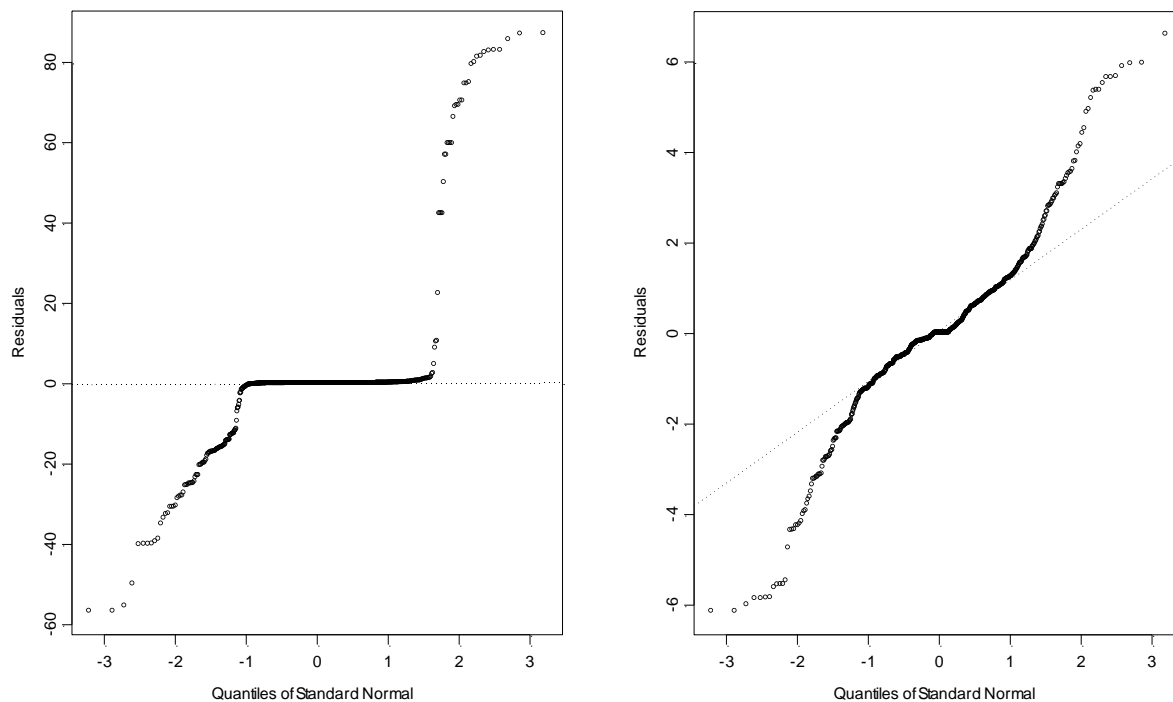


Figure A.8. QQ Plots of the Residuals from the Analysis of Variance Performed in Section 2.2. The left plot corresponds to the analysis of CFU/cm² summarized in Table 2, and the right plot corresponds to the analysis of log(CFU/cm²) summarized in Table 3.

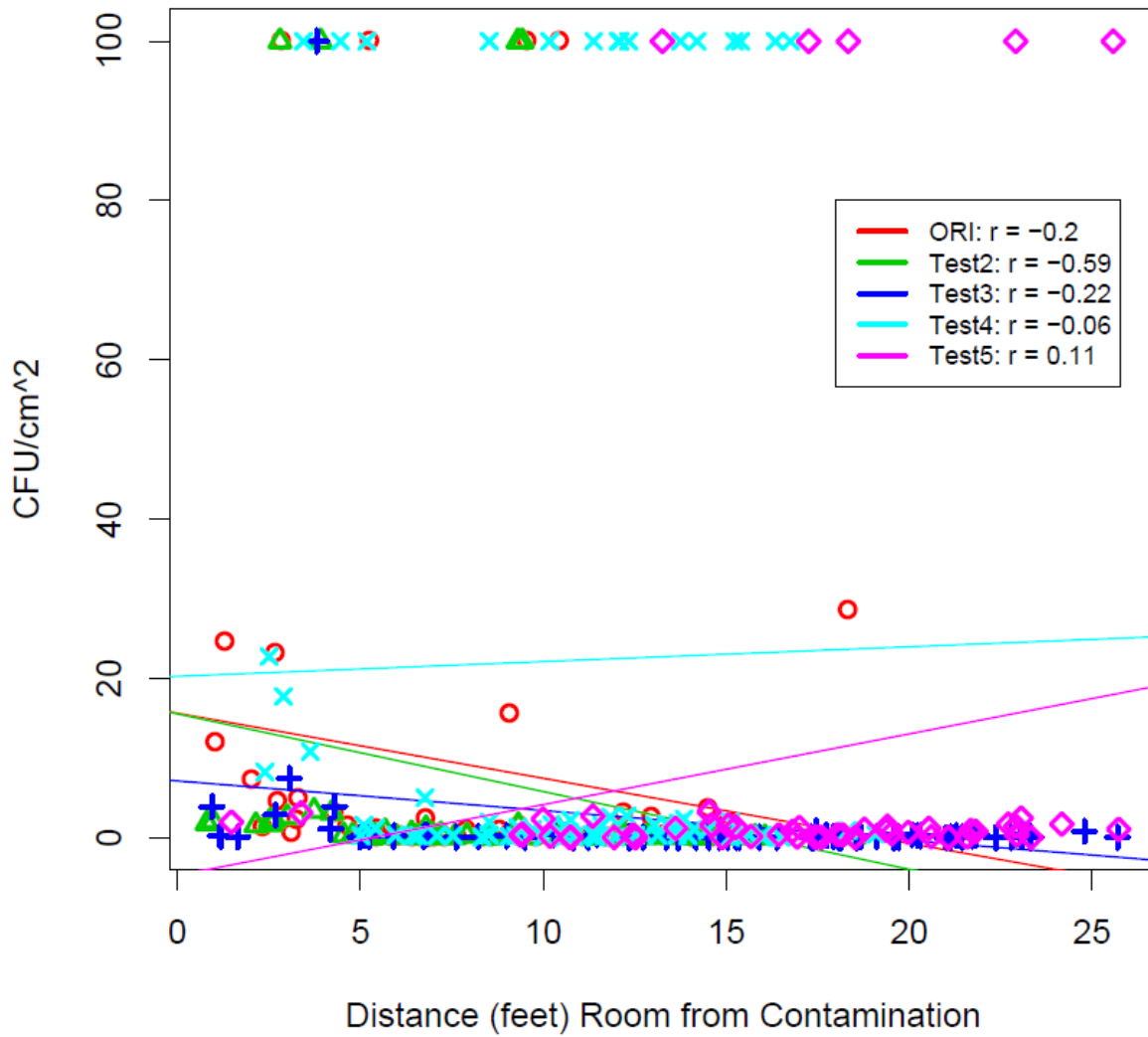


Figure A.9. Distance (feet) from the Contamination Point and CFU/cm² for Wipe Samples for Each Test Event. (Spearman correlation (r) coefficients in the legend)

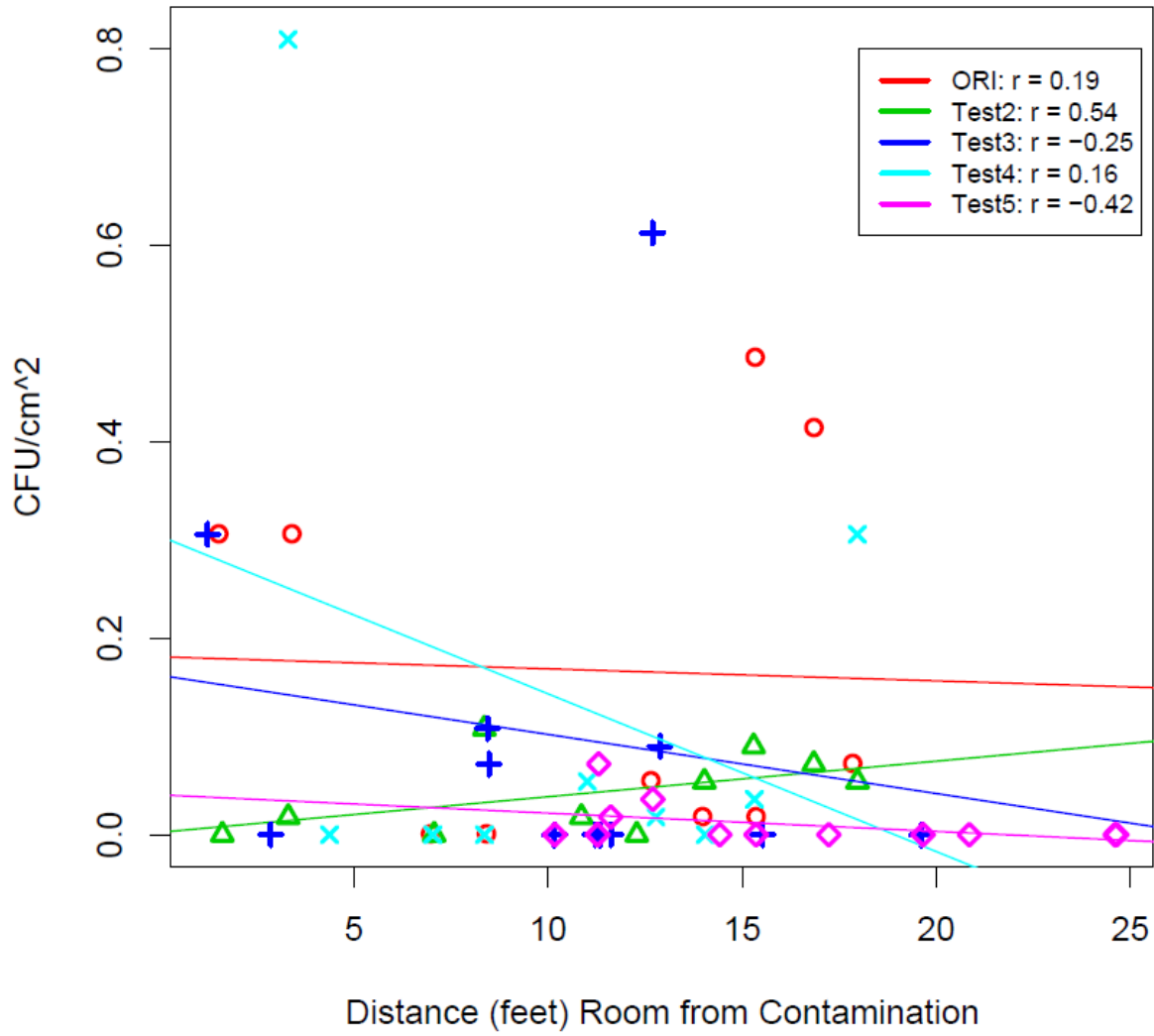


Figure A.10. Distance (feet) from the Contamination Point and CFU/cm² for Swab Samples for Each Test Event. (Spearman correlation (r) coefficients in the legend)

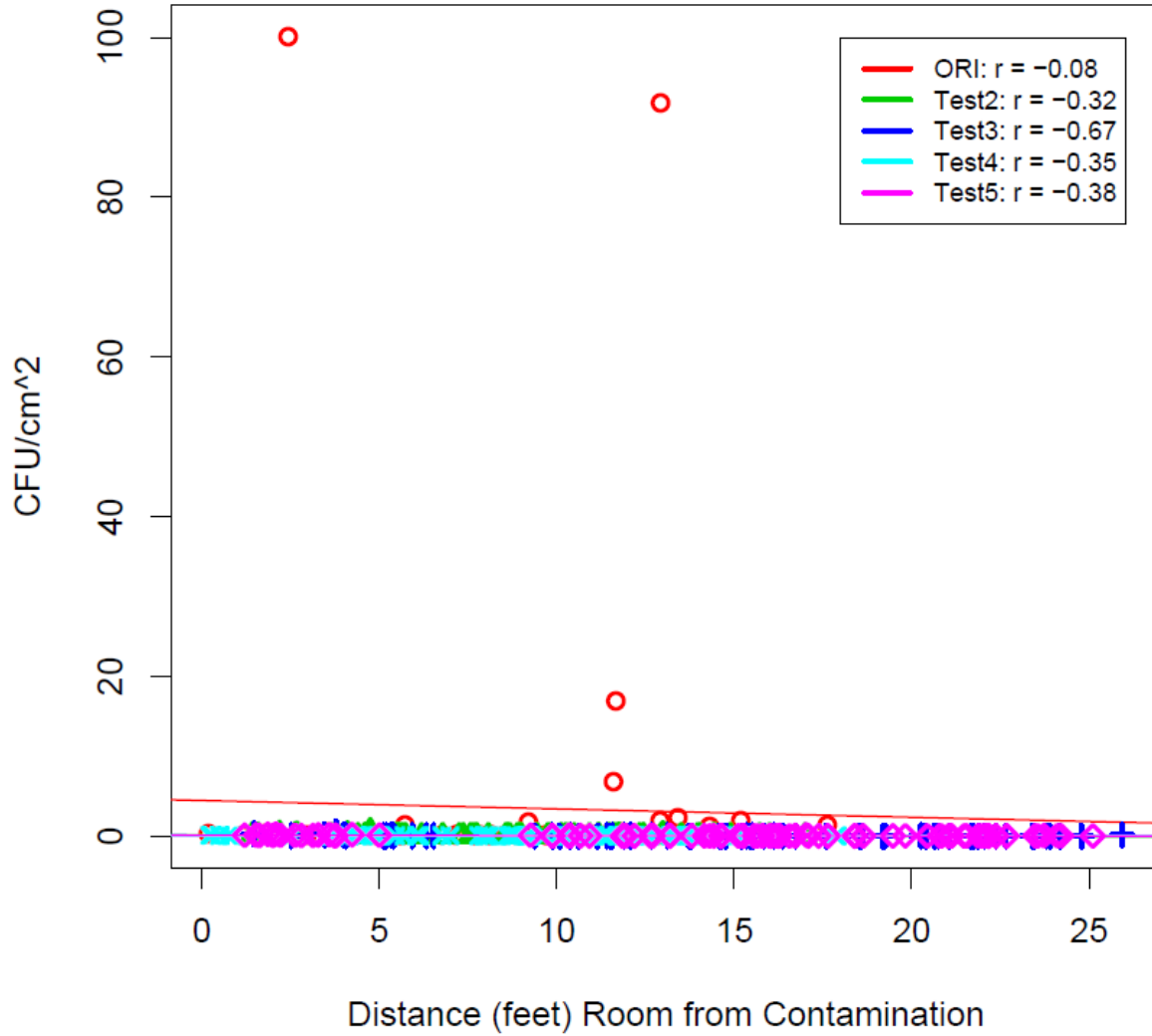


Figure A.11. Distance (feet) from the Contamination Point and CFU/cm² for Vacuum Samples for Each Test Event. (Spearman correlation (r) coefficients in the legend)

Appendix B

Appendix B contains sets of figures related to the Geostatistical analyses found in Section 2.3.2. Each set consists of a figure for the semivariogram models and a kriged *Bg* concentration map for each of five test events and two sample types (wipe or vacuum) combinations, resulting in 20 (2x5x2) figures. A summary of the spatial correlations displayed in the semivariogram model figures can be found in Table 5. The following observations are made for each of the kriged concentration maps:

- **Figures B.2 and B.6** show a few hotspots where 100 CFU/cm² values were found using wipes during the ORI and Test 2.
- **Figure B.4** shows that there were no spatial correlations from the vacuum samples during the ORI.
- **Figure B.8** shows slight spatial correlations from the vacuum samples during Test 2.
- **Figures B.10 and B.12** show that the higher values are in the far right lower rooms for both the wipe and vacuum samples, indicating some spatial correlation there, but only slight spatial correlations elsewhere.
- **Figure B.14** shows some spatial correlations, especially around the many hotspots where 100 CFU/cm² values were found using wipes during Test 4.
- **Figure B.16** shows that most of the higher values are in the far right lower rooms for vacuum samples during Test 4, indicating spatial correlations in that area. Not much spatial correlation elsewhere on the floor.
- **Figure B.18** shows that there were no spatial correlations from the wipe samples during Test 5.
- **Figure B.20** shows very slight spatial correlations from the vacuum samples during Test 5.

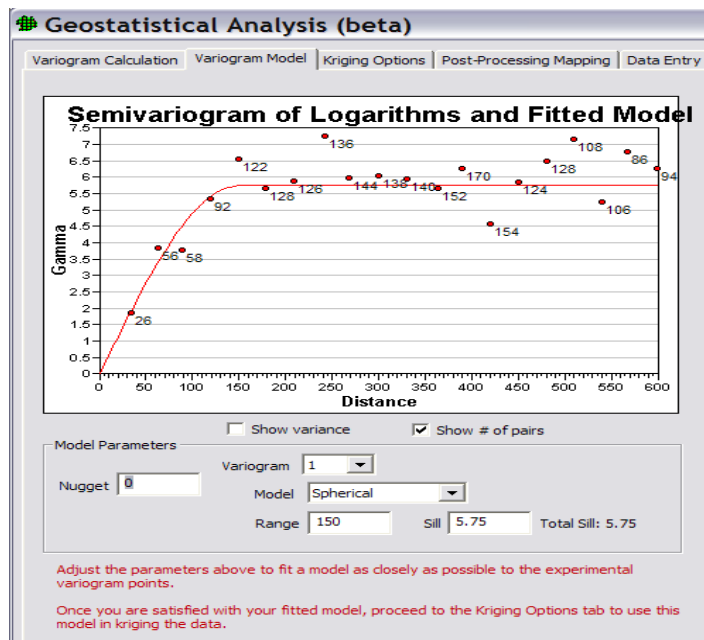


Figure B.1. Variogram Model for the Wipe Samples from Test Event 1 (ORI).

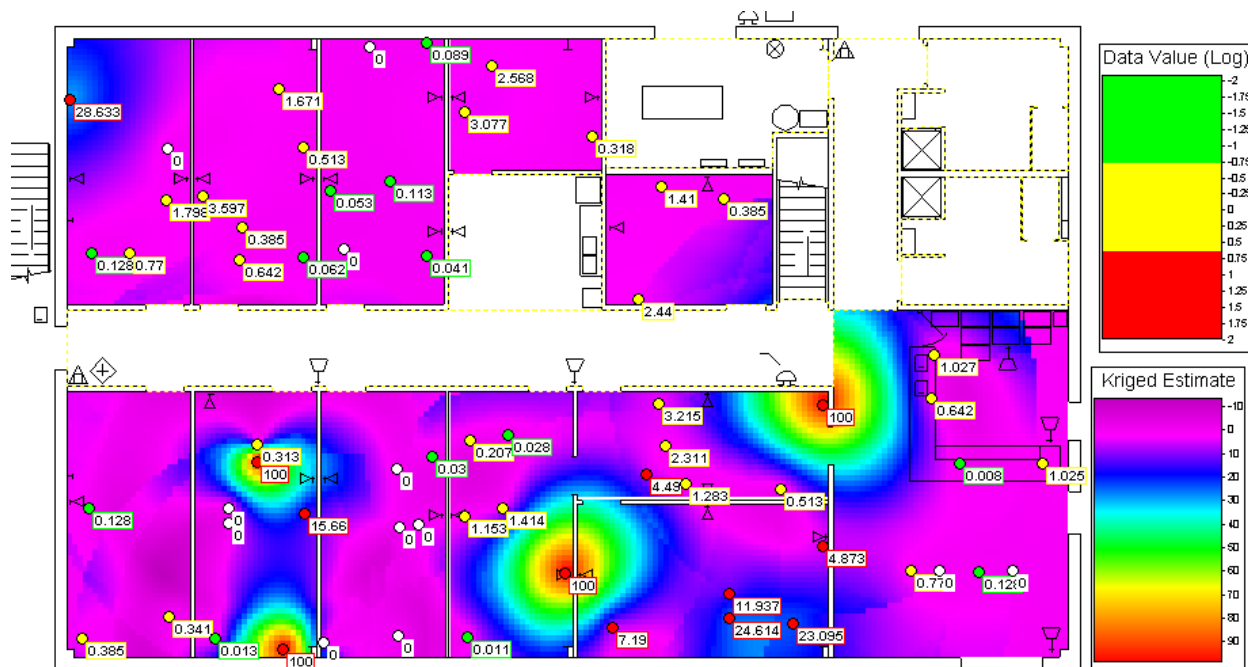


Figure B.2. Kriged Map and Posting Plot for the Wipe Samples from Test Event 1 (ORI).

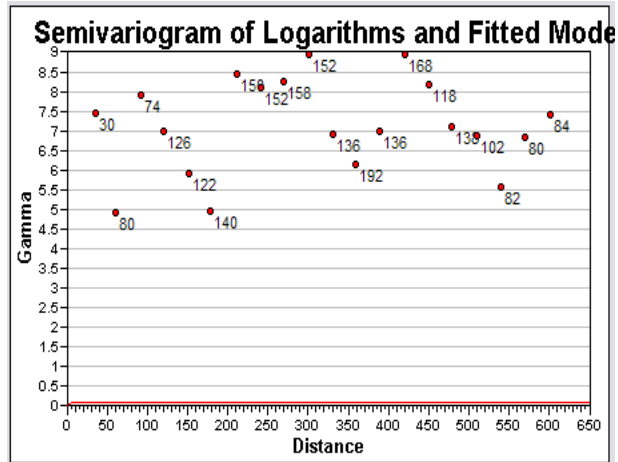


Figure B.3. Variogram Model for the Vacuum Samples from Test Event 1 (ORI).

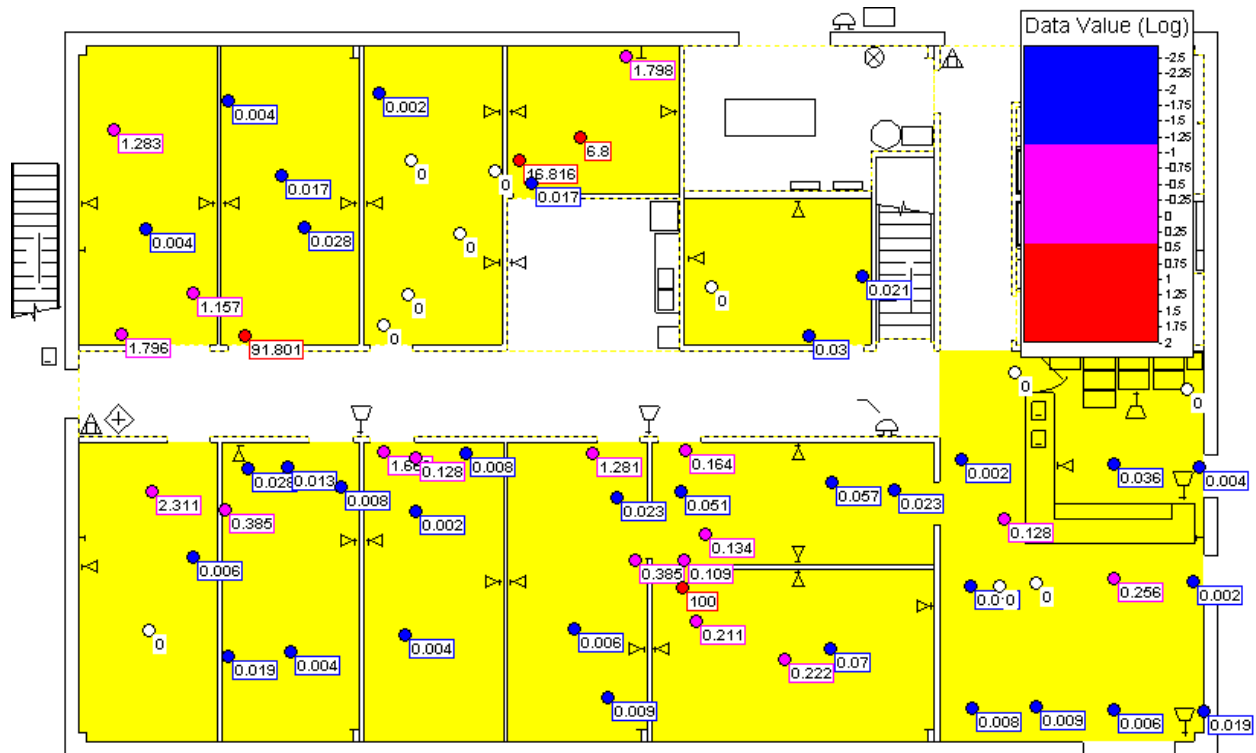


Figure B.4. Posting Plot for the Vacuum Samples from Test Event 1 (ORI).

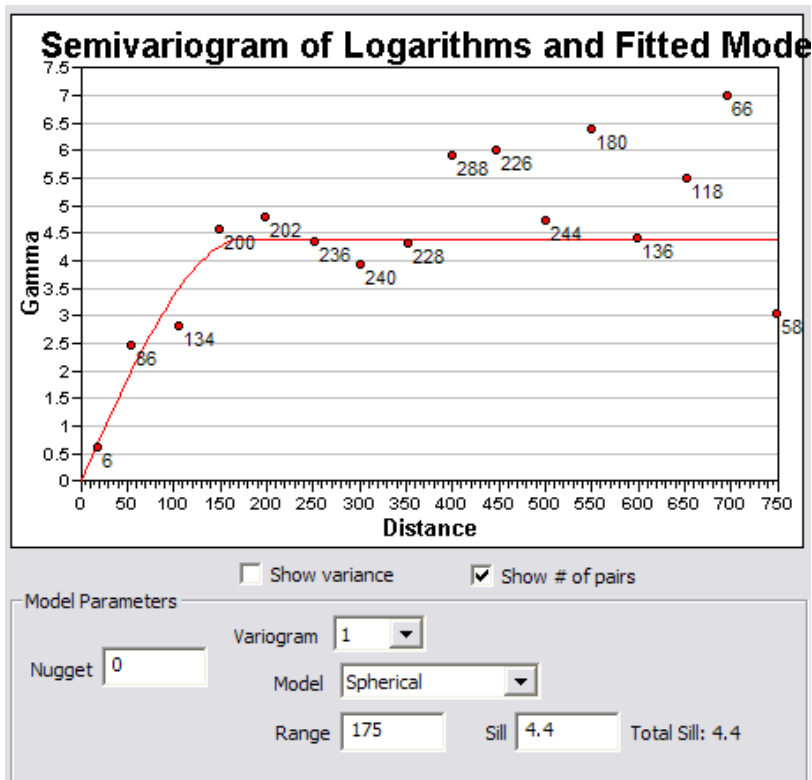


Figure B.5. Variogram Model for the Wipe Samples from Test Event 2 (Variogram with 15 lags of 50 distance).

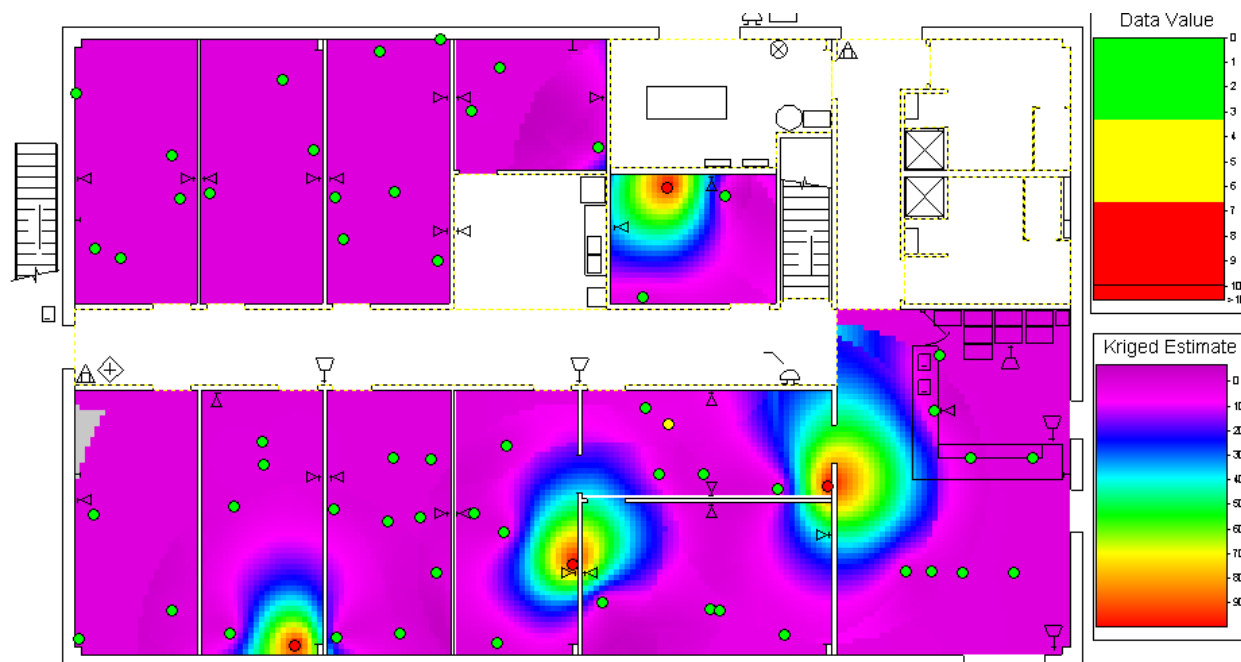


Figure B.6. Kriged Map and Sample Results for the Wipe Samples from Test Event 2.

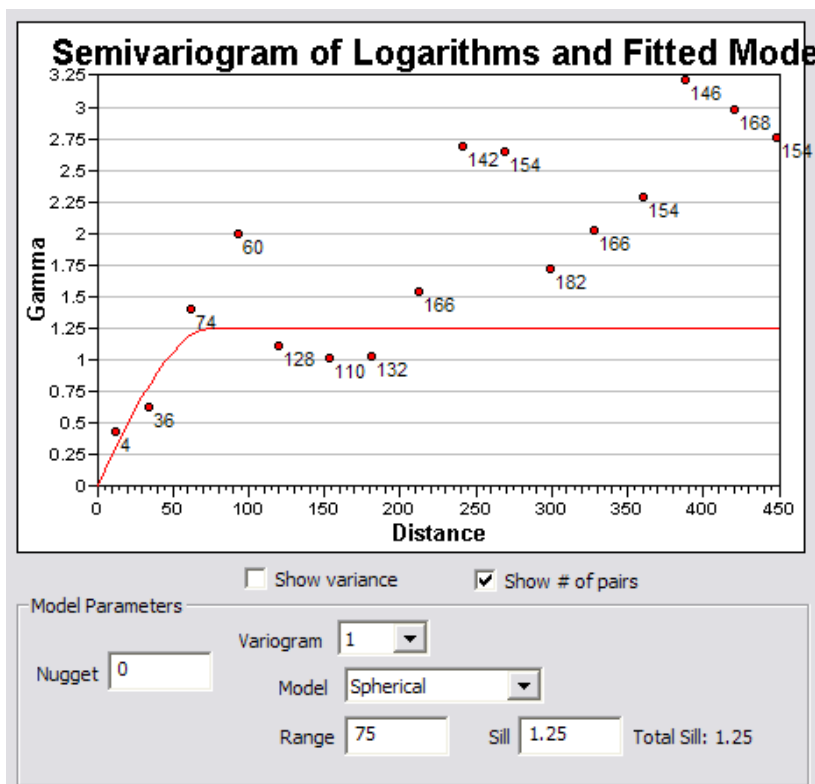


Figure B.7. Variogram Model for the Vacuum Samples from Test Event 2. (Variogram with 15 lags of 30 distance)

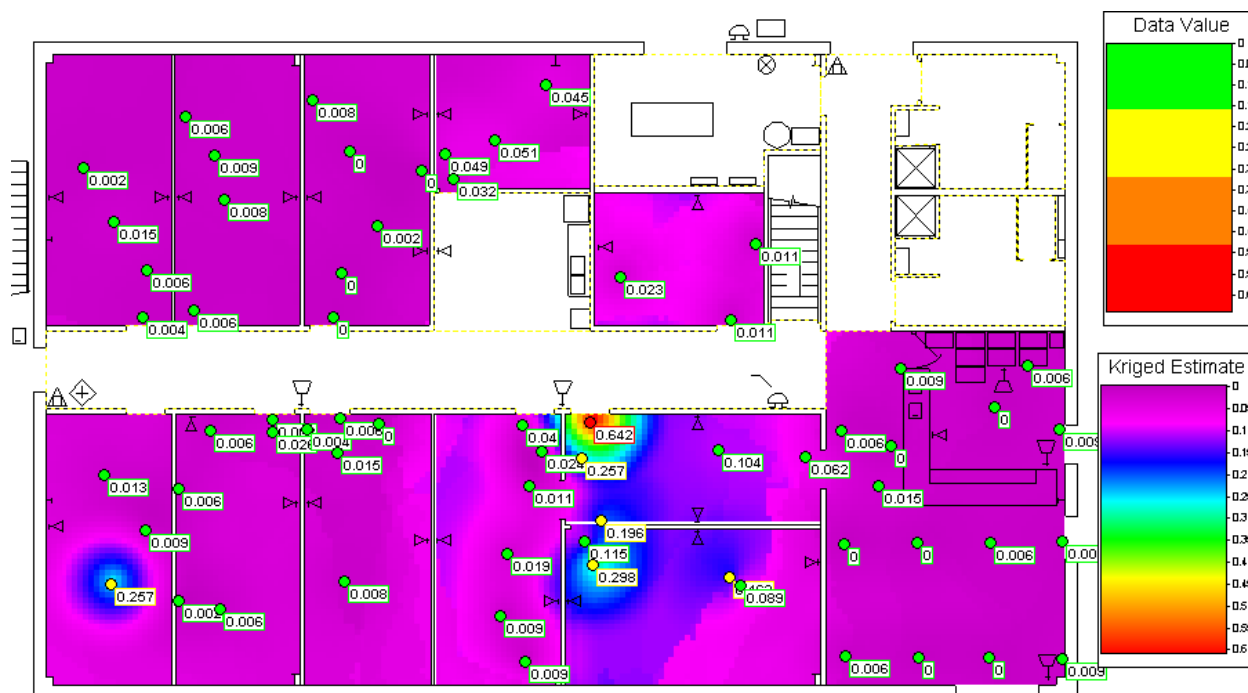


Figure B.8. Kriged Map and Posting Plot for the Vacuum Samples from Test Event 2.

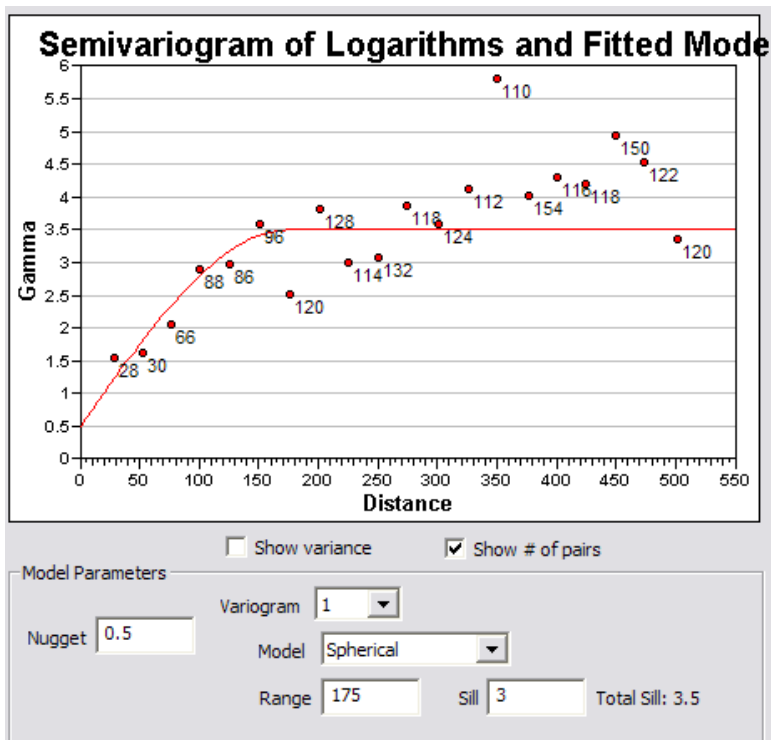


Figure B.9. Variogram Model for the Wipe Samples from Test Event 3. (Variogram with 20 lags of 25 distance)

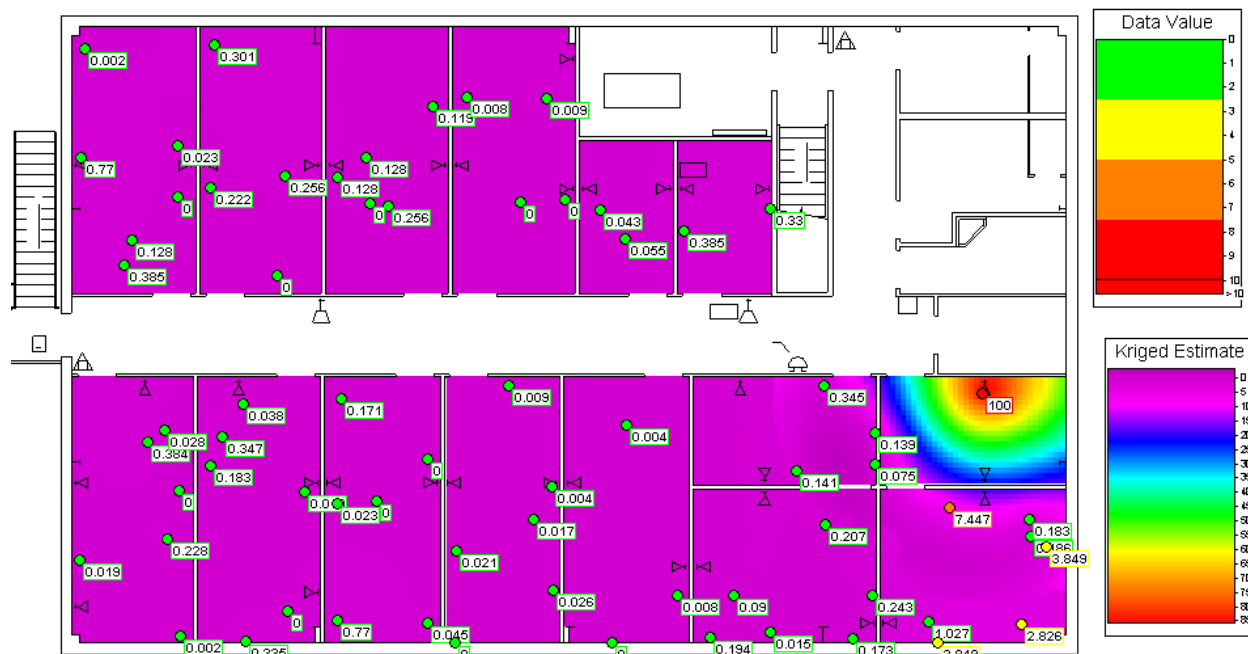


Figure B.10. Kriged Map and Posting Plot for the Wipe Samples from Test Event 3.

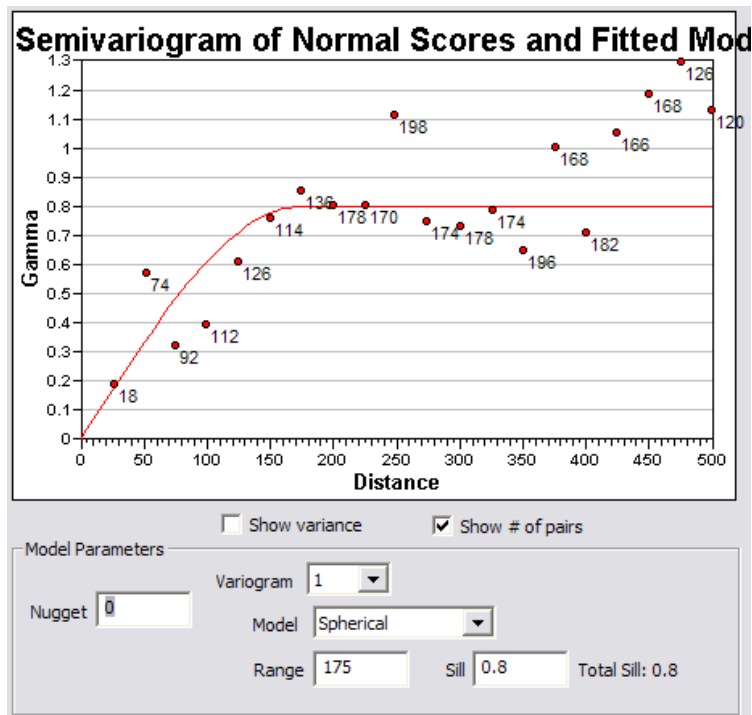


Figure B.11. Variogram Model for the Vacuum Samples from Test Event 3. (Variogram with 20 lags of 25 distance)

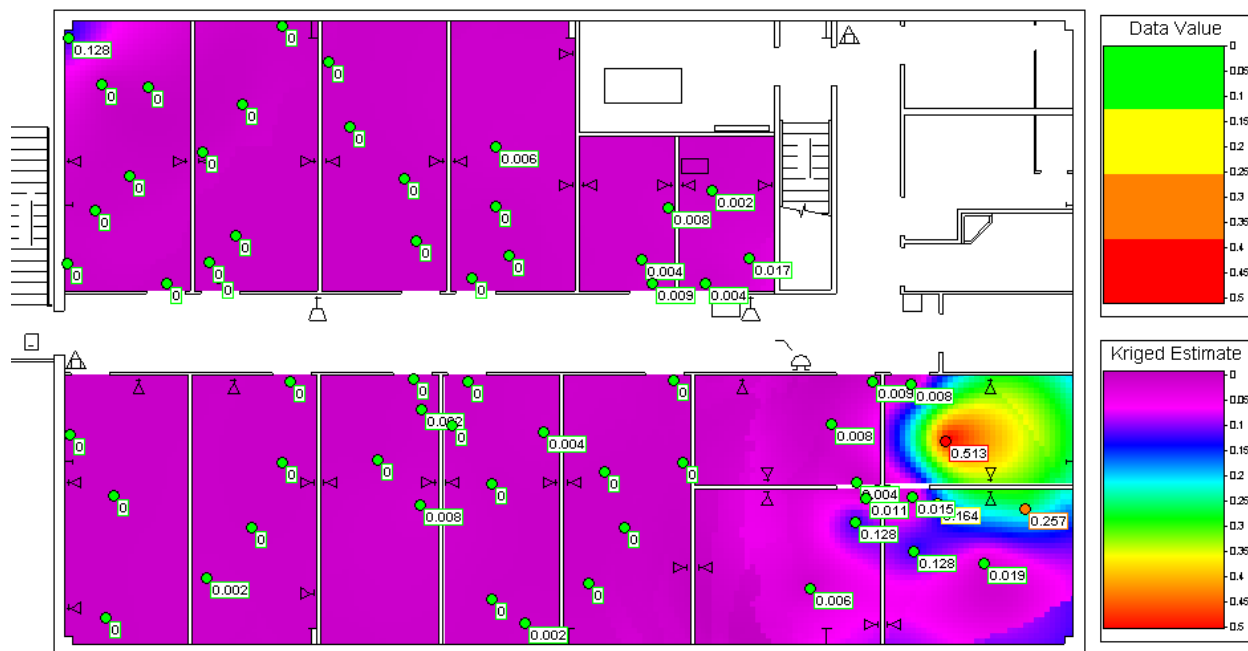


Figure B.12. Kriged Map and Posting Plot for the Vacuum Samples from Test Event 3.

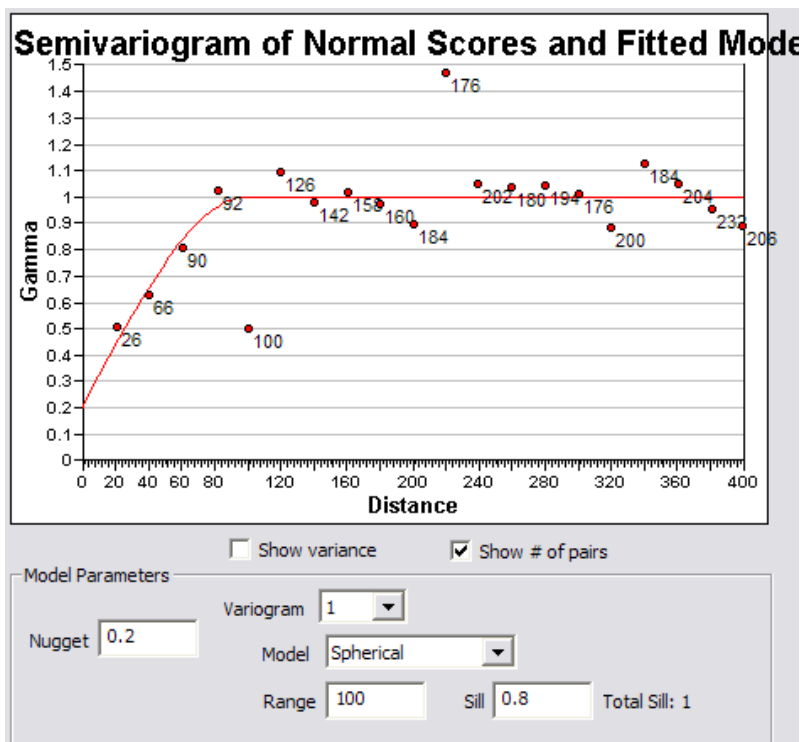


Figure B.13. Variogram Model for the Wipe Samples from Test Event 4. (Normalized Variogram with 20 lags of 20 distance)

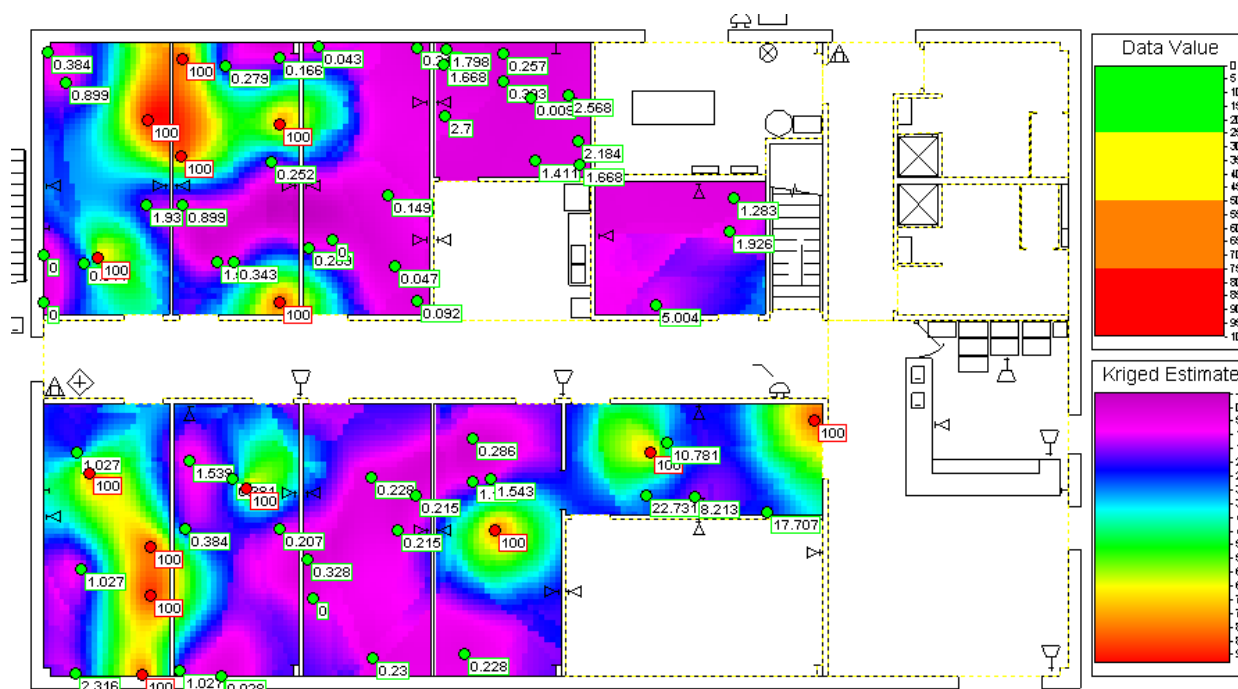


Figure B.14. Kriged Map and Posting Plot for the Wipe Samples from Test Event 4.

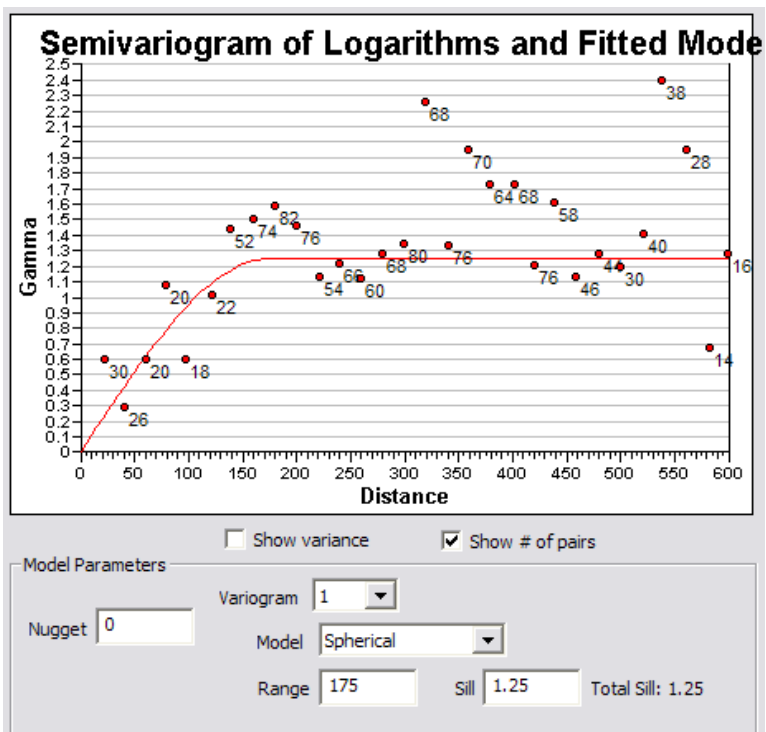


Figure B.15. Variogram Model for the Vacuum Samples from Test Event 4. (Variogram with 30 lags of 20 distance)

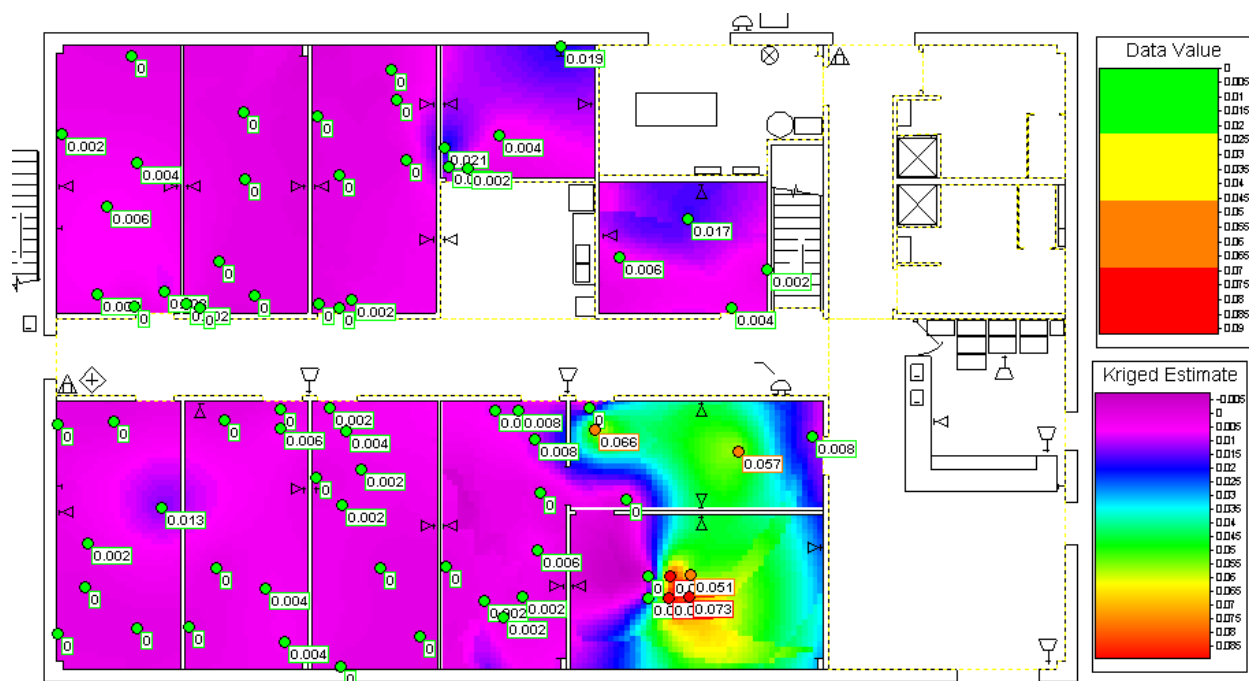


Figure B.16. Kriged Map and Posting Plot for the Vacuum Samples from Test Event 4.

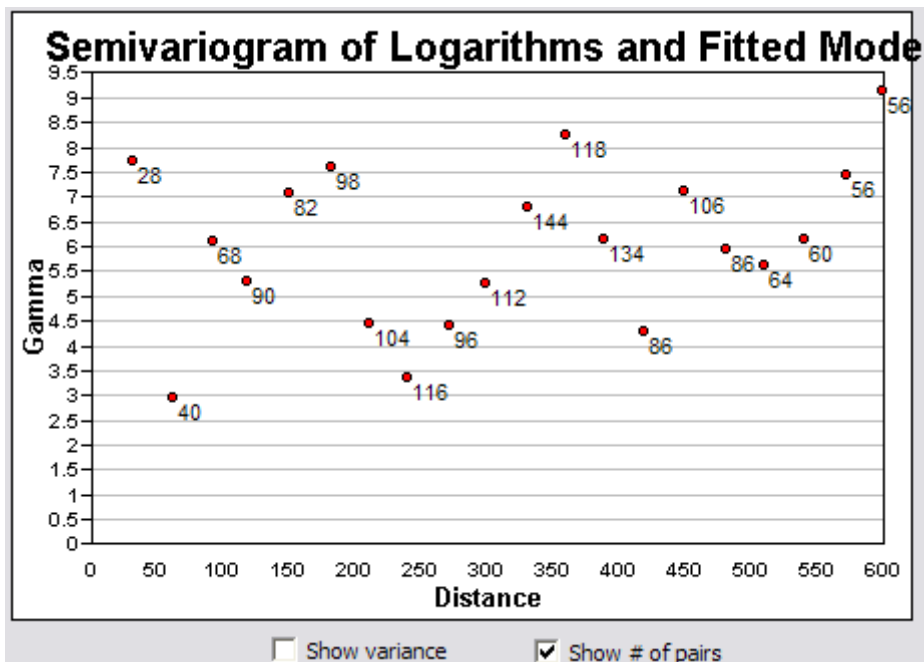


Figure B.17. Variogram Model for the Wipe Samples from Test Event 5.



Figure B.18. Posting Plot for the Wipe Samples from Test Event 5.

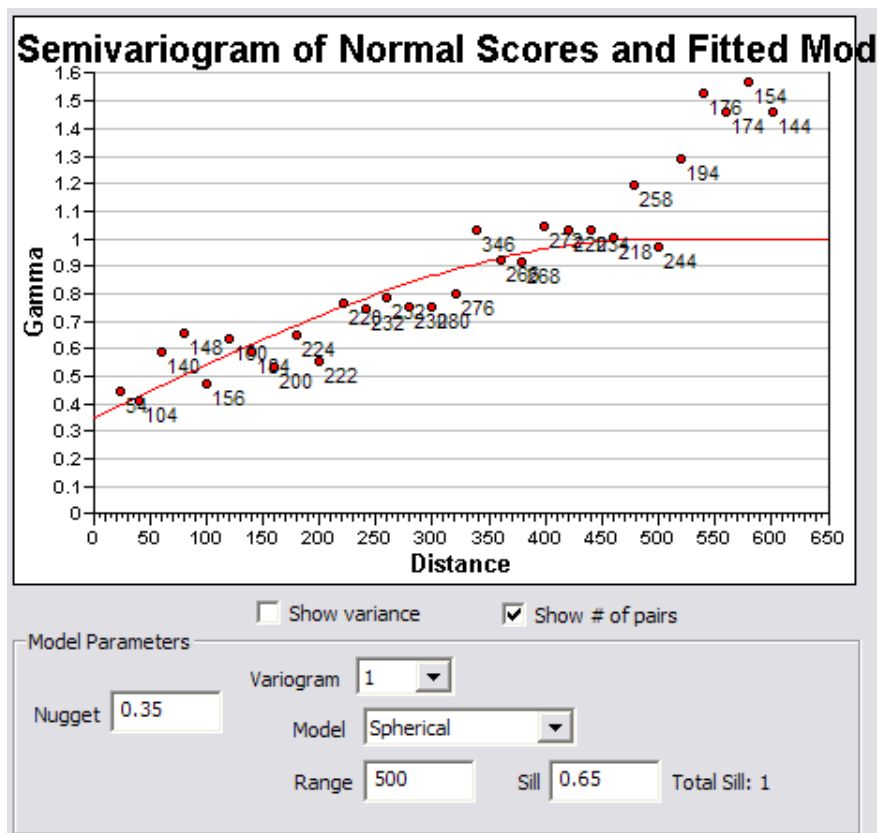


Figure B.19. Variogram Model for the Vacuum Samples from Test Event 5. (Variogram with 30 lags of 30 distance)

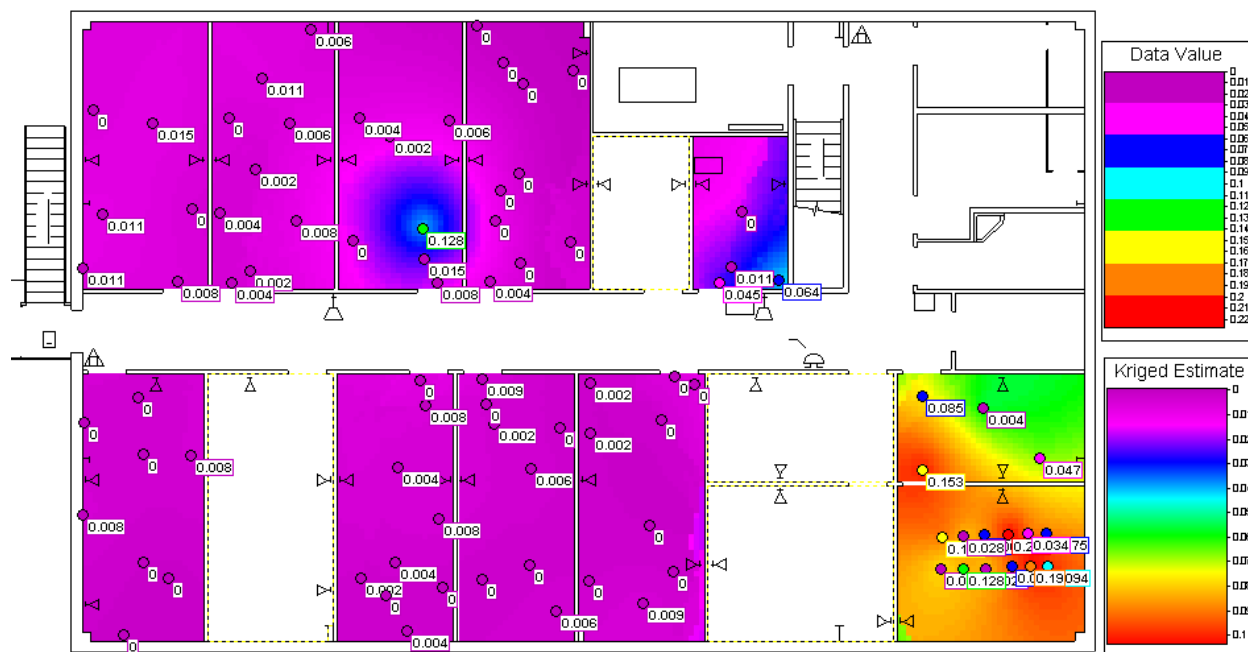


Figure B.20. Kriged Map and Posting Plot for the Vacuum Samples from Test Event 5.