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RESEARCH ARTICLE

ENVIRONMENTAL MONITORING PERFORMANCE ANALYSIS: A COMPARATIVE STUDY OF CLASS C AND CLASS D CONTROLLED ENVIRONMENTS

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Abstract

Monitoring and controlling of clean area environment is of paramount importance to ensure product safety and quality. This comprehensive analysis evaluates environmental monitoring (EM) data from Class C and Class D controlled environments in pharmaceutical manufacturing, utilizing Active Air (AA), Passive Air (PA), and Contact Plate (CP) or Replicate Organism Detection And Counting (RODAC) surface samples. The study aims to identify contamination trends, anomalies, and compliance with ISO 14644-1 and EU GMP Annex 1 standards. Results reveal unexpected findings: Class C Active Air (43 CFU/m³) and RODAC (3 CFU/plate) overall averages are higher than Class D Active Air (34 CFU/m³) and RODAC (2 CFU/plate), respectively, deviating from expected cleanroom classification. Class D Passive Air (22 CFU/plate) is higher than Class C (17 CFU/plate), aligning with expectations. Persistent hotspots were identified in Class C (e.g., location labelled "AA C 12 NG0"AA averages± Standard Deviation (SD): 67.33±17 CFU/m³), indicating localized control failures, while Class D showed extreme individual spikes (e.g., AA D 99 Ac: Max 171 CFU/m³). Sporadic contamination events in Class C suggest transient breaches, necessitating root-cause investigations. The study also highlights limitations of Class D monitoring, which obscures temporal trends and risks missing critical excursions due to long intervals between samples. Recommendations include targeted engineering assessments for high-load zones, enhanced Standard Operating Procedures (SOPs) for cleaning and gowning, adoption of real-time biofluorescent particle counters to replace manual sampling, and increased monitoring frequency in Class D hotspots.
Keywords: Active air sampling, contact plate sample, contamination control, environmental monitoring, ISO 14644, pharmaceutical manufacturing.

INTRODUCTION

Environmental monitoring (EM) is a foundational cornerstone of any robust pharmaceutical quality management system, serving as the primary method for providing objective, quantifiable evidence of contamination control across both sterile and non-sterile manufacturing operations[1]. The integrity of this process is critical, as it directly supports the release of safe and effective medicinal products by demonstrating that the production environment is maintained in a state of consistent microbial control[1-4]. Adherence to these principles is not merely a best practice but a strict regulatory requirement, governed by stringent international standards that form the basis for cGMP compliance.

EM programs are meticulously regulated by standards such as the European Union's Good Manufacturing Practice (EU GMP) Annex 1 and the International Organization for Standardization's ISO 14644-1. The fundamental purpose of these programs is to safeguard final product integrity by systematically quantifying the microbial burdens present in the manufacturing environment, including the air, critical surfaces, and the gowns of personnel involved in processing[2,3]. Based on the classifications of controlled environments, different levels of microbial contamination are deemed acceptable. Class C (ISO 7) cleanrooms, often designated for support tasks like preparing solutions for aseptic processes, are managed with moderate contamination limits, including an active air sampling threshold of no more than 100 CFU/m^3. Conversely, Class D (ISO 8) environments serve as vital transition areas and buffer zones, such as gowning rooms; consequently, they are allowed higher, though still strictly regulated, microbial counts, with a specified active air limit of ≤200 CFU/m³. In addition to active air testing, thorough

environmental monitoring protocols establish clear limits for other methods, such as passive air (PA) monitoring with settle plates and surface testing with contact plate (CP) samples[4-6]. This research provides an in-depth analysis of a large set of EM data from Class C and D zones. The primary goals are to assess the facility's compliance with these regulatory limits, uncover any systemic weaknesses or specific contamination points that signal a potential loss of control, and finally, to recommend focused, evidence-based actions to improve environmental management and maintain product safety.

MATERIALS AND METHODS

Data Source

The dataset comprised 12 months of Class C samples (n=65 locations) and Class D samples (n=98 locations), covering Active Air (AA), Passive Air (PA), and Contact Plate (CP) or Replicate Organism Detection And Counting (RODAC) surface tests.[6] Data originated from an Asian pharmaceutical facility (covering Bangladesh, Pakistan and India region) as an model for medicinal industry in developing countries, anonymized for analysis. Additional statistical outputs, including descriptive statistics, probability distribution, and goodness-of-fit tests for various sample categories (AA C, PA C, CP C, AA D, PA D, CP D), were derived from a supplementary analysis report[7].

Cleanroom Classification

Classification adhered to ISO 14644-1: Class C: ISO 7, for support zones (e.g., preparation areas).Class D: ISO 8, for transition zones (e.g., gowning rooms).[2,4]

2.3. Sampling Protocols

Active Air: Volumetric samplers (1 m³/sample) with Tryptic Soy Agar (TSA) plates; results in CFU/m³.[8]

Passive air: Settle plates (4-hour exposure); results in CFU/plate[9].

Contact plate surface: Contact plates (24 cm² agar surface); results in CFU/plate[10]. All methods followed USP <1116> and internal validated SOPs[11].

Statistical Analysis

Data underwent comprehensive statistical analysis, including descriptive statistics (mean, standard deviation (SD), median, minimum, maximum, range, skewness, and kurtosis) to characterize central tendency, dispersion, and shape of distributions. Coefficient of Variation (CV) was calculated to identify locations with high relative variability, indicating a less stable state of control. Probability plots and goodness-of-fit tests were utilized to assess the underlying distribution of the data for various sample categories. Control charts will be considered for evaluating process stability per PDA Technical Report 55 elsewhere in future study after gathering statistically significant number of samples.[12] Commercial statistical programs were used (Minitab® v17.1.0. GraphPad Prism v6.01 for windows) for datasets processing and analysis[11].

RESULTS AND DISCUSSION

In this study classes A and B were excluded from the analysis as all results of sampled locations returned zero CFU/sample. Thus, no further interpretation or examination is required. The remaining focus was centered on C and D areas. Most datasets exhibited normality with the exception of CP samples of Class C which failed to follow any other distribution type (p<0.05).

Class C Performance

Overall descriptive statistics for Class C sample categories were as follows:

AA (AA C): Mean=43.00 CFU/m³, SD=11.89, Median=44, Min=23, Max=66.

PA (PA C): Mean=17.00 CFU/plate, SD=6.39, Median=16, Min=10, Max=29.

CP Surface (CP C): Mean=3.00 CFU/plate, SD=1.71, Median=3, Min=2, Max=8.

AA Sample Analysis (Class C)

Active air monitoring in Class C environments showed varied performance. While most samples maintained moderate average counts (e.g., AA C 1 Ac: Avg 28.42; AA C 6 BM: Avg 14.83), several samples demonstrated notable variability and high maximum values, suggesting intermittent contamination events rather than consistently high baseline levels. For instance, AA C 10 Ac had an average of 32.42 CFU/m³ but recorded a maximum of 88 CFU/m³ and a high standard deviation (SD) of 31.50. Similarly, AA C 43 Rs showed an average of 28.29 CFU/m³ but reached a maximum of 89 CFU/m³ and an SD of 29.48. Conversely, some locations, such as AA C 12 NG0 (average 67.33 CFU/m³) and AA C 13 NG0 (average 69.67 CFU/m³) in particular spot, consistently presented higher average counts, which may indicate zones of higher activity or less effective air control within the Class C designation[6].

PA sample analysis (Class C)

PA monitoring in Class C environments generally showed lower counts compared to AA samples. However, some passive air samples exhibited higher averages and significant maximums, indicating localized settling issues. For example, PA C 7 Lsic recorded an average of 20.75 CFU/plate with a maximum of 70 CFU/plate, and PA C 41 Lsic showed an average of 36.60 CFU/plate with a maximum of 70 CFU/plate. Elevated counts in samples like PA C 7 Lsic (SD 22.19) and PA C 42 Ls (SD 24.05) suggest localized settling hotspots, particularly in areas such as sides and corners[6].

CP surface sample analysis (Class C)

CP surface samples in Class C environments generally showed very low counts, with many samples recording zero, which is the desired outcome for surface cleanliness. Nevertheless, a few samples exhibited notable spikes or higher averages, indicating specific surface contamination issues. CP C 11 W had an average of 12.17 CFU/plate but experienced a significant maximum of 72 CFU/plate in April. Similarly, CP C 30 NCu (Near CIP unit) showed an average of 7.33 CFU/plate with a maximum of 18 CFU/plate. These elevated readings for samples like

CP C 11 W (SD 19.59) and CP C 30 NCu (SD 5.94) directly imply a breakdown in surface cleanliness in specific locations such as Wall and Near CIP unit[6].

Class D Performance

Overall descriptive statistics for Class D sample categories were as follows:

AA (AA D): Mean=34.00 CFU/m³, SD=8.79, Median=29, Min=26, Max=44.

PA (PA D): Mean=22.00 CFU/plate, SD=6.79, Median=22, Min=14, Max=32.

CP (CP D): Mean=2.00 CFU/plate, SD=1.55, Median=2, Min=1, Max=5.

AA Sample Analysis (Class D)

Overall AA monitoring in Class D environments showed considerably lower average counts and variability compared to Class C. However, several individual samples exhibited extremely high maximum values, indicating significant contamination events, even for a Class D environment. Examples include AA D 26 Ac (Max 145 CFU/m³), AA D 71 Ac (Max 163 CFU/m³), AA D 91 Rs (Max 100 CFU/m³), and AA D 99 Ac (Max 171 CFU/m³)[5]. AA D 99 Ac, Average 97.75 CFU/m³ and AA D 95Ac, Average 71.25 CFU/m³ consistently showed particularly high average contamination, suggesting these are consistently high-load areas.

PA sample analysis (Class D)

PA monitoring in Class D environments generally showed higher counts than Class C. Several samples showed notable averages and maximums, indicating significant settling rates in specific locations. For instance, PA D 10 BM recorded a maximum of 60 CFU/plate, PA D 18 BM a maximum of 80 CFU/plate, and PA D 65 Btl a maximum of 100 CFU/plate[5]. Samples such as PA D 10 BM (Average 22.00), PA D 18 BM (Average 31.00), and PA D 65 Btl (Average 53.75) demonstrated significantly elevated passive air counts, particularly in locations behind machines and adjacent to the lines.

CP surface sample analysis (Class D)

CP surface samples in Class D generally showed low counts, with occasional higher maximums (e.g., CP D 66 Wne: Max 25 CFU/plate)[5]. Notably, 36 out of 37 Class D CP samples had averages of 6 CFU or less, with many at zero, indicating a predominance of very low counts despite the overall median of 2CFU/plate for the specific subset analyzed. For the specific subset of CP D samples analyzed in the supplementary report, the mean was 2.39 CFU/plate with a maximum of 5 CFU/plate[7]. Of particular note is CP D 76 NpA (close to changing area), which consistently showed 0.00 CFU across all available measurements, an anomalous finding for an active cleanroom environment.

Comparative overview of contamination levels and variability

Overall comparison

A direct comparison of the overall average contamination levels between Class C and Class D environments reveals unexpected patterns that deviate from the typical cleanrooms hierarchy. As shown in Table 1, AA: The overall average for Class C (43.00 CFU/m³) is higher than that for Class D (34.00 CFU/m³). This is contrary to the expected classification.PA: The overall average for Class D (22.00 CFU/plate) is higher than that for Class C (17.00 CFU/plate), which aligns with the expected cleanroom gradient. CP: The overall average for Class C (3.00 CFU/plate) is higher than that for Class D (2.00 CFU/plate), which is also contrary to expectations. This quantitative comparison highlights a critical deviation from the presumed cleanliness hierarchy, necessitating further investigation into the underlying causes.

Identified hotspots and high-variability samples

Specific samples within both classes exhibited notable deviations or consistent patterns that warrant focused attention (Table 2).

Class C Hotspots: Active air samples like AA C 13 NG0 (Max 88 CFU/m³) and AA C 43 Rs (Max 89 CFU/m³) showed sporadic spikes. Persistently high averages were observed in AA C 12 NG0 (Avg 67.33 CFU/m³) and AA C 13 NG0 (Avg 69.67 CFU/m³) for active air. For CP, CP C 11 W (Max 72 CFU/plate) and CP C 8 Wne (Max 27 CFU/plate) indicated surface contamination concerns.

Table 1: Summary of overall average contamination levels by class and sample type.

Class	Sample Type	Overall Average (CFU)	Overall Standard Deviation (SD)	Overall Maximum (CFU)
C	AA	43	11.89	66
	PA	17	6.39	29
	CP	3	1.71	8
D	AA	34	8.79	44
	PA	22	6.79	32
	CP	2	1.55	5

Table 2: Top 5 Samples with highest average contamination (across all classes and sample types).

Rank	Sample ID	Class	Sample Type	Average	Maximum
1	AA D 99 Ac	D*	Active Air	97.75	171
2	AA D 95 Ac	D*	Active Air	71.25	77
3	AA D 26 Ac	D*	Active Air	70.25	145
4	AA C 13 NG0	C	Active Air	69.67	88
5	AA C 12 NG0	C	Active Air	67.33	84

*In the center of the area

Table 3: Samples with highest variability (high coefficient of variation) through the whole samples.

Rank	Sample ID*	Average	Standard Deviation (SD)	Maximum	Coefficient of Variation (CV%)
1	CP D 20 Wif	1.50	3.00	6	200.00
2	CP D 88 Wne	0.75	1.50	3	200.00
3	CP D 8 Wif	0.50	1.00	2	200.00
4	CP D 81 Wif	0.25	0.50	1	200.00
5	CP C 51 Wbpb	1.42	2.78	10	196.12

* Wall RODAC samples

Class D Hotspots: AA samples, including AA D 71 Ac (Max 163 CFU/m³) and AA D 99 Ac (Max 171 CFU/m³), exhibited extremely high individual maximums or averages. PA sample PA D 65 Btl (Max 100 CFU/plate) indicated high settling rates. The anomalous CP D 76 NpA for CP, with consistently zero readings, requires data integrity verification. Table 3 showed that the highest variation from the overall samples comes from CP wall specimens from which 80% pertaining to Class D in selected top highest 5 CV%.

Distributional characteristics

The supplementary statistical analysis revealed important insights into the distributional characteristics of the data. For Class D RODAC (CP D) samples, despite a low mean (~2.4 CFU/plate) and maximum (5 CFU/plate) for the subset analyzed, attempts to fit various distributions (e.g., 3-Parameter Lognormal, 2-Parameter Exponential, 3-Parameter Weibull, 3-Parameter Gamma, 3-Parameter Loglogistic) encountered warnings regarding non-convergence of algorithms or non-existence of variance/covariance matrices. This indicates that the data may not adhere to these standard distributions, potentially due to the high frequency of zero counts or a limited number of distinct data values. Similar warnings were observed for other Class D and Class C sample categories (PA D, AA D, CP C, PA C, AA C) when attempting to fit certain distributions. These warnings highlight the non-normal nature of microbial data, which is common in environmental monitoring, and underscore the need for careful interpretation of parametric statistical methods.

Environmental monitoring performance analysis: A comparative study of class C and class D controlled environments

Environmental monitoring data reveals unexpected cleanliness trends: Class C AA (43 CFU/m³) and CP (3 CFU/plate) averages are higher than Class D (34 CFU/m³ and 2 CFU/plate respectively), challenging the expected hierarchy. Conversely, Class D PA (22 CFU/plate) is higher than Class C (17 CFU/plate), aligning with expectations. This deviation necessitates re-evaluation of local conditions, sampling, or activity profiles. Class C contamination shows sporadic spikes from transient breaches (human factors, equipment issues), requiring event-driven root-cause investigations. Persistent high counts in Class C AA suggest continuous sources or systemic airflow/traffic issues, demanding engineering assessments[12]. Class D faces pervasive high contamination and extreme individual spikes, indicating breakdowns in basic controls (gowning, material transfer, HVAC). Elevated passive air in high-activity Class D areas points to localized particle generation. An anomalous zero RODAC reading requires data integrity verification.

Differing Class C and Class D monitoring frequencies impact trend analysis, with Class D's limited data potentially missing critical excursions. Uncontrolled contamination poses risks to product quality and compliance[13]. While Class D maximums generally meet ISO 14644-1 limits (e.g., AA D 99 Ac: 171 CFU/m³ vs. 200 CFU/m³ limit; PA D 65 Btl: 100 CFU/4 hours vs. 100 CFU/4 hours limit; CP D 66 Wne: 25 CFU/plate vs. 50 CFU/plate limit), proximity to action limits and high Class C variability (e.g., AA C 6 BM CV 1.43) demand vigilance. Recommendations include targeted investigations, procedural enhancements, monitoring optimization, and proactive trend analysis.

CONCLUSIONS

The environmental monitoring data for Class C and D controlled environments reveals both expected and unexpected contamination patterns. While the Class D PA averages align with the expected hierarchy (higher than Class C), the overall averages for Class C AA and RODAC samples are unexpectedly higher than their Class D counterparts. A detailed analysis has revealed critical insights into specific areas and sample types that warrant focused attention. These include sporadic contamination events in Class C, indicative of transient excursions that require event-specific investigations. Conversely, persistently elevated contamination in certain Class D locations highlights areas for more systemic process or engineering optimization. The findings underscore that effective environmental control necessitates a nuanced, data-driven approach that extends beyond routine compliance checks. Continuous vigilance, robust data integrity, and a commitment to thorough root cause analysis are paramount for maintaining a consistent state of control in these critical environments.

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None to declare.

AUTHOR'S CONTRIBUTIONS

Eissa ME: conceived the idea, writing the manuscript, literature survey, formal analysis, critical review.

DATA AVAILABILITY

The accompanying author can provide the empirical data that were utilized to support the study's conclusions upon request.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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