Research Article

Evaluation of Environmental Microbial Contamination and Effectiveness of Cleaning Protocols in Controlled Manufacturing Environments

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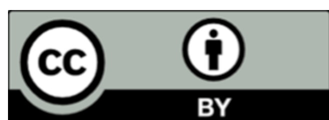

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Abstract : Environmental microbial monitoring program is an essential protocol to ensuring the safety, and quality of food-grade and cosmetic-grade products derived from plant-based extracts. This study uses air and surface sampling techniques to evaluate the microbial contamination risks in the product contact surface areas. The data collected through settle plates, manual swabs, and ATP tester/Luminometer, highlights the microbial risk assessment and implementation of hygiene protocols in critical areas. This study highlights best practices and improvement measures for contamination control by classifying clean room zones and utilizing quantitative microbial risk assessment models.

Keywords: Environmental monitoring, Risk assessment, Microbial contamination, Clean room, Hygiene monitoring.

Introduction

Microbial contamination is a persistent challenge in industries such as pharmaceuticals, food processing, and cosmetics [1,2]. Ensuring product quality and safety necessitates stringent control of microbial presence, as even minor contamination can lead to serious repercussions, including product recalls, regulatory violations, financial losses, and compromised consumer trust. The primary sources of microbial contamination include personnel, raw materials, equipment, and environmental factors such as air and surfaces [3]. Environmental contamination indicator microorganisms like *Salmonella sp., Escherichia coli, Staphylococcus aureus, P. aeruginosa*, and *Listeria monocytogenes* pose significant health risks, emphasizing the importance of effective monitoring and mitigation strategies [4, 5].

The global cost of product recalls due to microbial contamination has been estimated at billions annually, with significant impacts on brand reputation and consumer confidence. For example, a study by Hameed et al., 2020 reported that over 50% of contamination incidents in cleanroom environments stemmed from inadequate hygiene protocols and ineffective environmental controls.

Such findings underscore the need for robust microbial monitoring systems.

To address these risks, risk management frameworks like Hazard Analysis and Critical Control Points (HACCP) and Failure Mode and Effect Analysis (FMEA) have been widely adopted. HACCP systematically identifies potential hazards and implements control measures at critical points in the production process, while FMEA evaluates potential failure modes in equipment or processes, prioritizing actions based on their impact and likelihood. These frameworks are indispensable in cleanroom settings, where maintaining sterility is paramount [6,7].

Cleanrooms are specialized environments designed to minimize airborne and surface contamination. They are classified into zones based on contamination risk, ranging from high-risk critical areas to low-risk non-critical zones. Advanced technologies, such as high-efficiency particulate air (HEPA) filtration systems and rapid hygiene detection tools, are increasingly being integrated into these environments to enhance contamination control. However, the success of these technologies depends on comprehensive monitoring protocols and a thorough understanding of microbial dynamics within the facility [8].

Mathematical models play a crucial role in quantifying and managing microbial risks. For example, the equation for microbial risk assessment in Airborne contamination: $R = A \times B \times C \times D$

where:

A: Microbial concentration at the source.

B: Dispersion potential.

C: Proximity to critical areas.

D: Effectiveness of control measures.

Similarly, for surface contamination, the Equation: $S = C \times Tr \times A \times F$

Where:

C: Surface microbial load,

Tr: Transfer coefficient,

A : Contact area, and

F: Frequency of contact.

This study investigates microbial contamination in the packing areas of Indfrag Biosciences, which specializes in food-grade and cosmetic-grade plant extracts. By combining traditional sampling techniques with advanced risk assessment models, the research evaluates critical contamination sources, assesses cleaning protocols, and provides actionable recommendations for improvement. The integration of statistical tools, visualized through graphs and tables, further enhances the understanding of microbial dynamics, paving the way for optimized contamination control.

Materials and Methods

The methodology employed in this study integrates advanced microbial monitoring techniques with comprehensive data analysis to assess

contamination risks in cleanroom packing areas. A combination of traditional sampling methods and quantitative models was utilized to evaluate microbial load and determine the effectiveness of cleaning protocols.

Criteria for selecting a sampling point:

High risk	- Daily basis
Medium risk	- Weekly basis
Low risk	- Monthly basis

Simplified Zone Classification in Environmental Monitoring

To maintain cleanliness, control contamination risk, and ensure product safety, industrial facilities such as pharmaceutical and food processing units classify areas into four distinct zones based on risk levels.

Zone 1: Critical Area (High-Risk)

This zone presents the highest contamination risk as the product is directly exposed. Common examples include aseptic processing, packaging lines, blending, and sieving. To maintain strict hygiene, critical areas require advanced air quality control using HEPA filters, total plate count (TPC), and total yeast and mold count (TYMC). Continuous microbial monitoring is essential, including airborne contamination levels measured in CFU/m^3 and surface contamination levels in CFU/cm^2. Environmental conditions must be tightly regulated, with temperature maintained below 24°C (±2°C) and humidity under 45% (±5%). Frequent cleaning protocols, sterile equipment usage, and intensive microbiological testing ensure contamination control.

Zone 2: Controlled Area (Moderate-Risk)

This area has a moderate contamination risk and supports critical operations. It includes switches, airlocks, and maintenance tools that interact with high-risk zones. Air quality is maintained at controlled levels, with periodic microbial sampling. Temperature and humidity are monitored within broader acceptable ranges. Cleaning is conducted regularly, though gowning requirements are less stringent compared to critical areas. Routine environmental monitoring ensures compliance with safety standards.

Zone 3: Low-Risk Area

This zone does not directly participate in product processing but plays a crucial role in maintaining hygiene. Hallways, intermediate storage areas, floors, and walls fall under this classification. Routine microbial monitoring is performed with higher contamination thresholds than in controlled areas. Temperature and humidity are monitored, though strict controls are not required. Cleaning occurs periodically, and monitoring is conducted infrequently compared to higher-risk zones.

Zone 4: Non-Critical Area

With minimal contamination risk, non-critical areas require only basic environmental controls. Locker rooms, hallways, and finished goods storage

are typical examples. Air quality monitoring is minimal, with occasional microbial checks to ensure cleanliness. Basic temperature and humidity controls are in place, while general housekeeping practices maintain hygiene. Since product exposure is absent, monitoring frequency and cleaning intensity are significantly reduced.

Sampling

Air Monitoring

Airborne microbial contamination was evaluated using the settle plate method, a widely recognized passive sampling technique. SCDA agar plates were strategically positioned at critical locations within the packing area to capture airborne microorganisms. These plates were exposed for 2-3 hours, allowing microorganisms carried by airborne particles to settle naturally onto the agar surface.

The microbial deposition was calculated using the equation: $N=D \times A \times T$

N = D \times A \times T where:

N: Number of microorganisms deposited (CFU).

D: Deposition rate (CFU/cm²/s).

A: Surface area of the plate (cm²).

T: Duration of exposure (s).

Following exposure, the plates were incubated at 37°C for bacterial growth and at 25-30°C for fungal growth. The Colony Forming Units (CFU) were subsequently counted providing a quantitative measure of microbial concentration in the air. This data was crucial for identifying high-risk areas and assessing compliance with cleanliness standards.

Surface Monitoring

Surface microbial contamination was assessed through swabbing and contact plate methods, focusing on critical surfaces such as equipment and workstations. Sterile swabs were used to collect samples from irregular surfaces, while contact plates were directly applied to flat surfaces to detect microbial residues. To safeguard consumers various regulatory bodies including EU cGMP, U.S. FDA Aseptic Guidance, and USP <1116> & USP <61> have established methods to regulate industries such as pharmaceuticals, cosmetics, food and beverages.

The microbial transfer from surfaces to products was modelled using:

$Ms=C \times Tr \times A \times F$

Ms = C \times T_r \times A \times F where:

Ms: Microbial load transferred to the product (CFU).

C: Microbial count on the surface (CFU/cm²).

Tr: Transfer coefficient (fraction of microorganisms transferred).

A: Contact area (cm²).

F: Frequency of contact.

After incubation, microbial counts were compared pre- and post-cleaning to evaluate the effectiveness of cleaning protocols. Rapid detection techniques, such as ATP luminometry, were employed alongside traditional methods to provide real-time feedback on contamination levels.

Rapid Surface Monitoring

Manufacturers seeking quick results can use rapid methods to quantify microbial residues after sanitation and before operation. These methods include colorimetric assays that do not require instruments, as well as sampling swabs that require an instrument reader such as a luminometer. The testing procedure for using a luminometer involves several key steps to ensure accurate measurement of ATP or microbial activity. First, prepare the area by ensuring it is dry, free of visible debris, and handled with aseptic techniques while wearing gloves. Power on the luminometer and allow it to complete its self-check process. Swab the surface using a sterile test swab following the manufacturer's instructions typically using a zigzag or circular motion. Insert the swab into the luminometer's testing chamber ensuring it is securely seated, then initiate the test by pressing the appropriate button. The luminometer will display results in Relative Light Units (RLU), which should be recorded for documentation. Compare the results to acceptable thresholds to determine the cleanliness or contamination levels. After testing, clean the luminometer's exterior with a disinfectant and store it in a safe, dry place as per the manufacturer's guidelines. If contamination levels are high, clean the area thoroughly and repeat testing until it reaches the acceptable standards.

Risk = (No. of Critical Transfers + Production Time) / (No. of Cleanings × Cleaning Time)

No. of Critical Transfers: Refers to the number of times materials or products are transferred between equipment or areas, which can introduce contamination risks.

Production (Work Time) ÷ Cleaning Time: Represents the ratio of the total production time to the cleaning time. Longer production periods relative to cleaning frequency increase contamination risk.

No. of Cleanings: Indicates how many times the equipment is cleaned. More frequent cleaning reduces the overall risk of contamination.

Personnel Monitoring:

Personnel monitoring is conducted to maintain the sterile environment. Most contamination within a facility can be attributed to individuals working in cleanrooms. Therefore, personnel must adhere to specific gowning and sterilization procedures before entering and exiting the aseptic cleanroom condition.

Airborne risk = [amount of personnel's body within area] × [effectiveness of clothing] × [proportion of time personnel are within critical zone during production]

Swab testing and contact plate sampling from personnel are crucial for monitoring hygiene in controlled environments. For swab testing, sterile swabs pre-moistened with a neutralizing buffer or saline are used to sample high-contact areas such as fingertips, palms or specific garment sections like sleeve cuffs and chest areas. The swab is collected using a zigzag motion, stored in sterile containers and labelled before laboratory analysis for microbial or ATP contamination. Contact plate sampling involves pressing pre-filled growth

media plates (e.g., SCDA-RODAC) onto hands, gloves, or garment surfaces without smearing. Plates are covered immediately, labelled, and incubated in the lab to count colony-forming units (CFU). Both methods are performed routinely or randomly, with results recorded and corrective actions taken if necessary to ensure compliance with hygiene standards.

Environmental indicator microbes' detection:

In indicator microbes' detection, the streak plate method is commonly employed to isolate individual colonies of microorganisms from a sample. A loopful of the sample is first streaked across the surface of an agar plate in a zigzag pattern (primary streak), followed by sterilization of the loop and a second streak to further isolate individual colonies (secondary streak). This process is repeated until well-separated colonies appear. Incubation is typically conducted at 35-37°C for 24-48 hours, depending on the microorganism being cultured. Various selective media are used for specific Indicator organisms: Escherichia coli is grown on MacConkey Agar or Eosin Methylene Blue (EMB) Agar, Salmonella spp. on XLD Agar, Staphylococcus aureus on Mannitol Salt Agar (MSA), Pseudomonas aeruginosa on Cetrimide Agar, and Listeria monocytogenes on PALCAM Agar. According to USP <62> guidelines for microbiological testing of nonsterile products, microbial limits must be defined, with specific incubation conditions and times for each pathogen. The methods used for pathogen detection must be validated for their sensitivity, specificity, and reproducibility, and both negative and positive controls should be included to verify the testing process. Finally, confirmatory testing, such as biochemical tests (e.g., Oxidase, catalase and coagulase tests) is performed to identify the isolated environmental indicator organisms.

Results

Air Monitoring

The air monitoring results indicated satisfactory environmental conditions with respect to both microbial and particulate contamination.

Equipment Room Before and After Cleaning (Dryer)

The dryer area air monitoring results, both before and after cleaning, indicated a significant improvement in cleanliness, similar to the air quality results. Before cleaning, the equipment surfaces exhibited microbial contamination, with a Total Plate Count (TPC) of 12 CFU (colony-forming units) per surface area, which was higher than the acceptable limits for the monitored area. However, after cleaning, the TPC was reduced to <1 CFU per surface area, it was demonstrating the effectiveness of the cleaning procedures in removing the microbial contaminants. No indicator organisms such as Escherichia coli, Salmonella spp., Staphylococcus aureus, Pseudomonas aeruginosa and Listeria monocytogenes. on any equipment surfaces before or after cleaning. It is confirming that the cleaning process effectively eliminated the environmental indicator microorganisms. The rate at which these particles deposit onto or into a product can be determined by using settle plates placed near the exposed product [9] . This study analysed microbial counts in various sampling points pre- and post-cleaning. Data from

settle plates and swabs are summarized below:

Surface Hygiene Monitoring:

Swab Test Results:

Swab tests are typically used to sample surfaces in order to detect microbial contamination (bacteria, fungi, etc). The swab is rubbed on a surface and then cultured in a lab to see if any microorganisms is present.

The 30-day period indicates the length of time over which the tests were conducted. This suggests the data tracks microbial contamination over a month, which allows for trend analysis (e.g., changes in contamination levels over time). The comparison could involve showing the results from different surfaces, areas, or time points within the 30 days. It may display how microbial contamination levels fluctuate or remain consistent throughout the month. The figure could include a bar graph, line chart, or another visual format to represent the data, helping to identify trends, peaks in contamination, or days with unusually high contamination.

Table 1. Microbial Counts Zone-wise (CFU)

Grade	Frequency	Average		Min TPC	Max TPC	Alert level		Action level		Limits	
		TPC	TYMC			TPC	TYMC	TPC	TYMC	TPC	TYMC
A	1 to 30 days	<1	<1	<1	<1	1	1	>1	1	<1	<1
B	1 to 30 days	18	<1	<1	21	22	1	>22	1	<25	<1
C	1 to 30 days	20	<1	10	42	45	1	>45	1	<50	1
D	1 to 30 days	65	<1	40	53	90	1	>90	1	<100	1

Total Plate Count (TPC), Total Yeast and Mold Count (TYMC)

Personal Hygiene (Before Sterilization & After Sterilization)

The results from the personal hygiene monitoring table before and after sterilization and sanitation procedures demonstrate a significant reduction in microbial contamination on both aprons and gloves. Before sterilization, the manual swab results for aprons ranged from 160 to 190 CFU, with corresponding ATP (Adenosine Triphosphate) readings ranging from 610 to 920 RLU (Relative Light Units). After sterilization, these values decreased substantially, with all results showing less than 10 CFU on the manual swabs and ATP readings between 16 and 19 RLU, indicating effective sterilization and reduction of microbial contamination. Similarly, the glove hygiene results showed a notable improvement. Before sanitation, the manual swab results for

Table 2. Microbial Counts from Settle Plates (CFU) on Dryer Room

Sample ID	TPC Before Cleaning	TYMC Before Cleaning	TPC After Cleaning	TYMC After Cleaning
VTD-01	12	5	<1	<1
VTD-02	30	3	<1	<1
STD-01	22	6	<1	<1

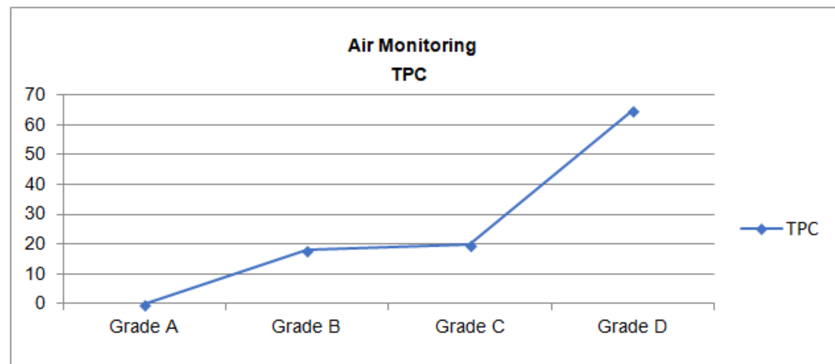

Figure 1: Graphical comparison of microbial reduction post-cleaning

gloves ranged from 60 to 90 CFU, with ATP readings between 117 and 156 RLU. After sanitation, all samples showed less than 10 CFU on manual swabs, with ATP readings significantly lower, ranging from 5 to 9 RLU, confirming the effectiveness of sanitation procedures in reducing microbial presence. These results highlight the success of the sterilization and sanitation protocols in ensuring personnel hygiene standards are met and maintaining a safe environment.

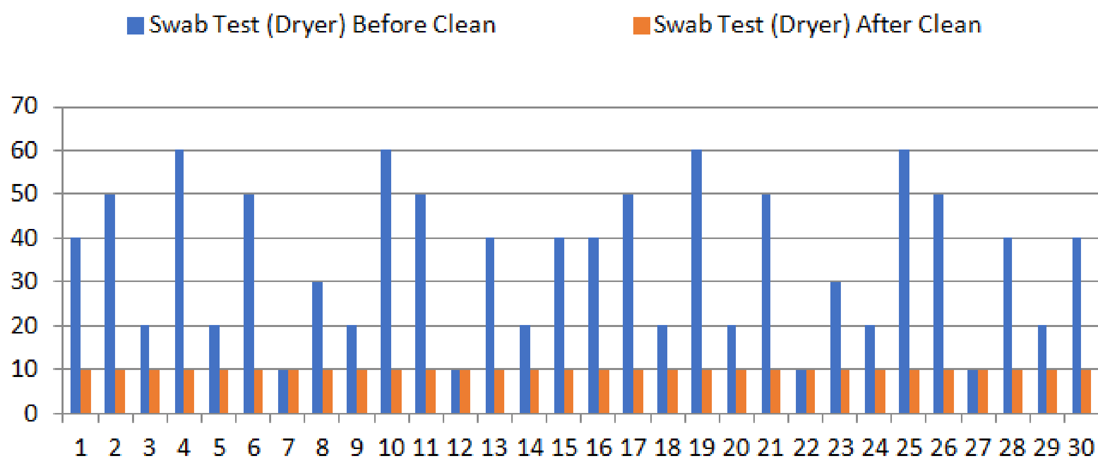

Figure 2: Comparison of swab test results for a period of 30days

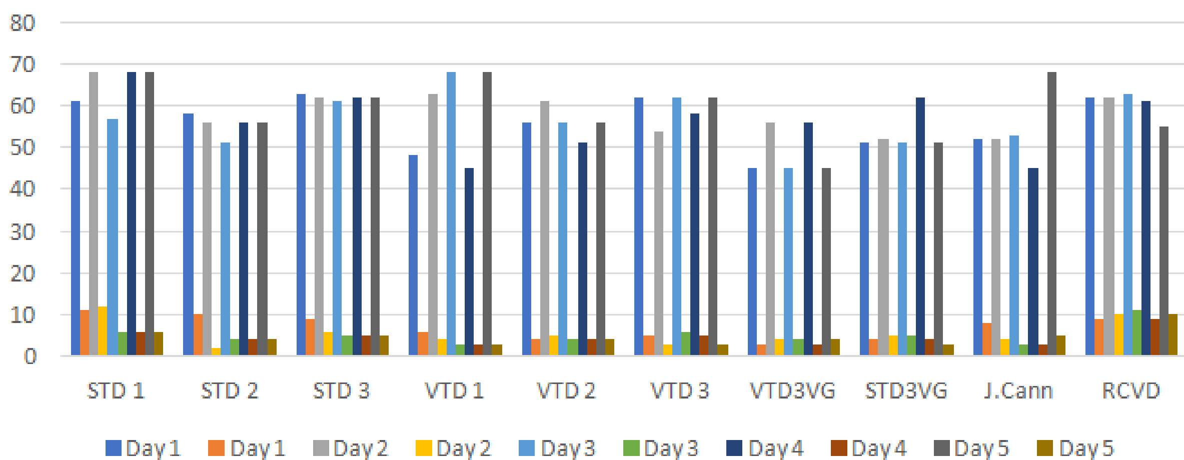

Figure 3: ATP luminometer results comparing pre- and post-cleaning

Table 3. Personal Hygiene (before sterilization & after Sterilization)

	Before Sterilization		After Sterilization	
	Manual (Swab)	**ATP (Swab)**	**Manual (Swab)**	**ATP (Swab)**
Person A	170	750 RLU	<10	16 RLU
Person B	180	690 RLU	<10	17 RLU
Person C	190	785 RLU	<10	19 RLU
Person D	160	610 RLU	<10	16 RLU
Person E	170	920 RLU	<10	17 RLU
	Before sanitation		**After sanitation**	
Person A	70	117 RLU	<10	06 RLU
Person B	60	125 RLU	<10	05 RLU
Person C	80	145 RLU	<10	07 RLU
Person D	90	141 RLU	<10	07 RLU
Person E	80	156 RLU	<10	09 RLU

Discussion:

Environmental microbial monitoring is essential for ensuring product safety and quality, particularly in industries where contamination can result in significant health risks and economic losses [9]. In this study, the integration of conventional sampling techniques with advanced approaches, such as quantitative microbial risk assessment (QMRA), enabled a comprehensive evaluation of contamination dynamics within cleanroom environments [10]. The analysis of microbial loads across different zones demonstrated clear spatial variability, highlighting the importance of effective environmental control strategies. Critical areas (Zone I) consistently exhibited near-zero microbial counts following cleaning procedures, indicating the effectiveness of established sanitation practices in high-risk zones. However, increased contamination levels observed in non-critical areas (Zone IV) suggest inconsistencies in hygiene practices and potential gaps in routine monitoring [11,12]. These findings emphasize the need for uniform contamination control measures across all operational zones, regardless of their classification.

The application of ATP luminometry further demonstrated its value as a rapid and reliable tool for real-time microbial monitoring. This approach facilitates the identification of contamination hotspots and allows for immediate assessment of cleaning effectiveness, thereby improving response efficiency [13]. Moreover, adherence to stringent Good Manufacturing Practices (GMP), along with the implementation of HEPA filtration systems and automated microbial detection technologies, plays a critical role in maintaining controlled environments [14]. Personnel training and awareness programs were also identified as key contributors to ensuring compliance with hygiene protocols and maintaining operational consistency [15]. Together, these measures form a robust framework for minimizing microbial contamination and ensuring product integrity.

Conclusion

This study highlights the critical importance of environmental microbial monitoring in mitigating contamination risks and ensuring product safety across cleanroom environments. While effective cleaning protocols maintained near-zero microbial loads in critical zones (Zone I), higher contamination levels in non-critical areas (Zone IV) indicate the need for more consistent and standardized hygiene practices across all operational zones. The integration of advanced tools, such as ATP luminometers and automated microbial detection systems, significantly enhances real-time monitoring and supports rapid identification of contamination hotspots. Coupled with strict adherence to Good Manufacturing Practices (GMP), HEPA filtration systems, and continuous personnel training, these strategies contribute to a more robust and proactive contamination control framework. Future efforts should focus on incorporating predictive analytics and machine learning approaches to optimize cleaning schedules, improve contamination risk prediction, and strengthen real-time detection capabilities, ultimately reducing product recalls and ensuring long-term consumer safety and industrial sustainability.

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