ASIDE Health Sciences

Original Article

Environmental Monitoring in a Class D Pharmaceutical Facility: Microbial Load and Hygiene Practices, a Risk-Based Cross-Sectional Study

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ARTICLE INFO

Article history:
Received 13 Jul. 2025
Received in revised form 24 Sep. 2025
Accepted 26 Sep. 2025
Published 7 Oct. 2025

Keywords:
Environment Monitoring Test
Near Return Air Loop
Clean rooms
Finger Dab Test

ABSTRACT

Background: Environmental monitoring is a crucial current Good Manufacturing Practice (cGMP) tool for assessing the status of the working environment in a Pharmaceutical Manufacturing Facility.
Methods: The test was conducted between May 20 and 25, 2025. 90 mm Diameter Settle Plates methods, 4 hours exposure under dynamic conditions, were used as a test method to study the microbial load in controlled and classified areas. The finger dab test was used to assess hygiene and sanitization practices in the plant. The non-viable count was excluded from the study due to limitations, including a lack of facilities for conducting the tests.
Result: The result of the environmental monitoring test was below 100 colony-forming units (CFU) in the rooms. The mean value of Total Aerobic Microbial Count and Total Yeast and Mold Count show a higher microbial count at the Near Return air loop. The p-value of Total aerobic microbial count and Total Yeast and Mold Count was found to be 0.8685 and 0.8716 respectively. The result is not significant at $p<0.05$. The result of the Finger dab test was below 100 CFU/5 fingerprints in both hands and complies with the internal action limit.
Conclusion: The result of the study suggests that the higher load of organisms was found at the "near return loop area". The result of the Finger dab test was satisfactory according to the in-house limit (100 CFU). The result can serve as a basis for selecting a sample spot for regular Environmental Monitoring in a Manufacturing Facility.

1. Introduction

The purpose of microbiological environmental monitoring is to assess the cleanliness of pharmaceutical (sterile and non-sterile) and medical device manufacturing environments. Environmental monitoring involves the collection of data relating to the numbers or incidents of microorganisms present on surfaces, in the air, and from people. [1]. The primary goal of environmental monitoring in cleanrooms is to regulate the numbers of airborne viable and non-viable particles within defined limits, predict the risk to the environment, and regularly assess the efficacy of cleaning and disinfecting processes [2].

Pharmaceutical manufacturing involves a complex, multi-phase processing system that is associated with significant risks of microbial contamination from various sources. The quality of the product is significantly influenced by microbial contamination in several processing steps [3]. To obtain a pharmaceutical product free of contamination, you need an adequate environmental monitoring system. The system includes identification, testing, and removal of bioburden to ensure the quality of the product [4].

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Citation:Singh S. Assessment of Microbial Load and practices, during Environment Monitoring Test in Pharmaceutical Industry. ASIDE Health Sci. 2025;2(1):8-14, doi:10.71079/ASIDE.HS.100725170

Risk assessment approaches are used to determine the location of environmental monitoring [5]. Risk-based approaches include Failure Mode and Effects Analysis (FMEA), Fault Tree Analysis (FTA), Hazard Analysis and Critical Control Points (HACCP), and Quantitative Microbiological Risk Assessment (QMRA) [6, 7]. The scope of this study is to fix the sampling spot based on risk assessment for performing environmental monitoring and to correlate the results of the Finger dab test and the non-viable count report with the sanitization of the area and the cleanliness of Personnel.

2. Method

2.1. Study Design

The study was designed as a cross-sectional pilot study and conducted between May 20 and 22, 2023. Test conditions were Dynamic and Temperature less ($\leq$ 25 °C), and Humidity ($\leq$ 60%) was maintained during study periods. Statistical analysis of the obtained data was done using the chi-square test, and the p-value was calculated. The test was performed by exposing 90 mm presterilized Petri plates containing Soyabean Casein Digest Agar and Sabouraud Dextrose Agar at a height of 40 cm on the Petri plate stands for 4 hours at each location. After 4 hours of exposure time, the Petri plates were covered with a lid and transported to the Microbiology Lab aseptically in a closed container. Soybean Casein Digest Agar plates were incubated at 35 °C for 72 hours, and Sabouraud Dextrose Agar Petri plates were incubated at 25 °C for 5 days. The Equipment used during the study was well-calibrated, and a sterility check of the used media was done prior to conducting the test.



Figure 1: Petri plate Exposed Near the Return Air Loop on the Stand.

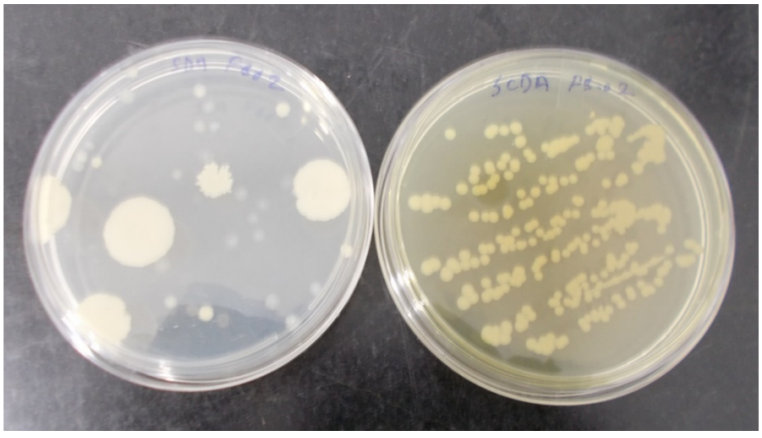


Figure 2: Petri plates after incubation (cfu per 4-hr settle plate).

Table 1: Materials used during the study

S. No	Materials and Equipment	Manufacturer
1	Pre-sterilized Petri plates	Tarsons
2	Soyabean Casein Digest Agar (SCDA)	Hi Media
3	Sabouraud Dextrose Agar (SDA)	Hi Media
4	Hot Plate	Lab Quest
5	Autoclave	Equitron
6	Bio-safety Cabinet	Thermolab
7	Incubators	Allyone
8	Colony Counter	Lapiz
9	Stainless Steel Petri Plate Stand	Sanitt
10	70% IPA	Qualigens

SCDA, Soyabean Casein Digest Agar; SDA, Sabouraud Dextrose Agar; IPA, Isopropyl Alcohol.

Table 2: Study area

S. No	Sampling rooms	Sampling location in the Room
1	Dispensing Room	Near Machine
2	Granulation Room	Area with maximum man movement
3	Punching Room	Difficult to clean area
4	Coating Room	Near return air loop
5	Blister Packing	Near the Drainage area

The Finger Dab test was performed in the Dispensing Room, Granulation Room, punching room, Coating room, and Blister Packing room by the personnel working in the respective areas. The in-house limit of the Finger Dab test was set at 100 CFU/5-finger print, as the production area was classified as a Class D area and non-sterile solid dosages were formulated in the area. Personnel working in areas were selected randomly, and the finger DAB test was evaluated for each of them. All the fingers, including the thumb of personnel's gloves, were gently imprinted, and impressions of all these workers were obtained on labeled Petri plates containing Soyabean casein digest agar. All the plates were incubated at 35°C for 72 hours, and the results were recorded. The finger dab test

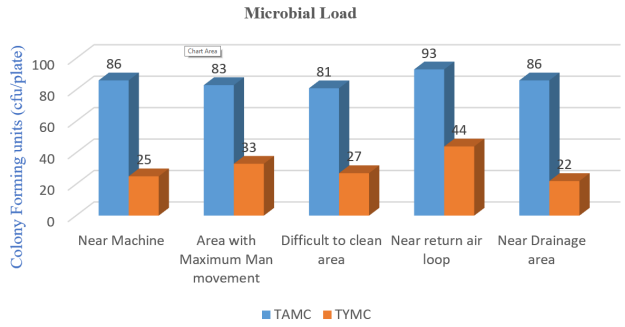


Figure 3: Bar Diagram of Dispensing Room (CFU per 4-hrs settle plate).

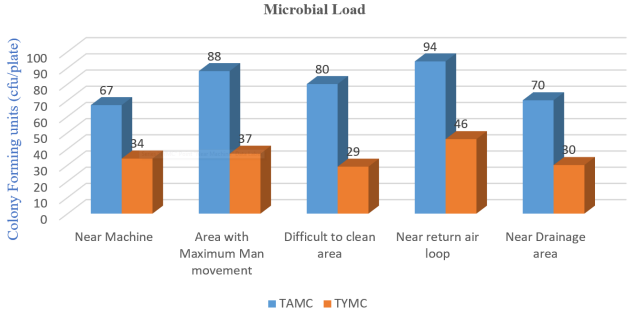


Figure 4: Bar Diagram of Granulation Room (CFU per 4-hrs settle plate)

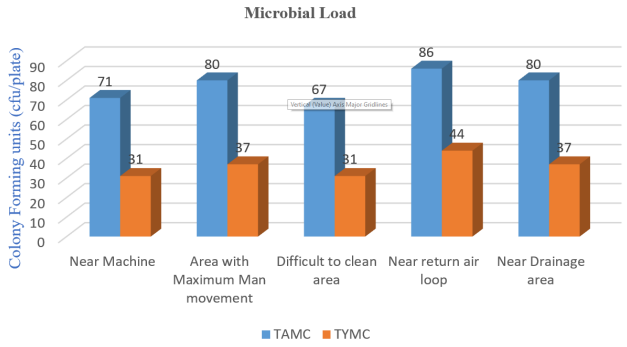


Figure 5: Bar Diagram of Punching Room (CFUper 4-hrs settle plate).

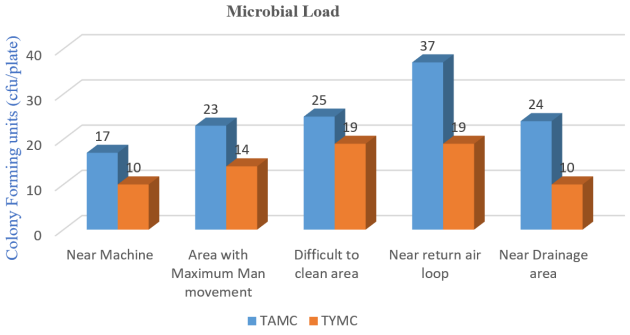


Figure 6: Bar Diagram of Coating Room (CFUper 4-hrs settle plate)

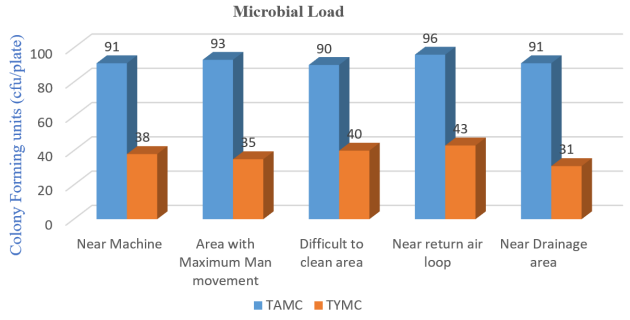


Figure 7: Bar Diagram of Blister Packing Room (CFU per 4-hrs settle plate).

in class D was proposed in a study to screen operator hygiene in non-sterile areas, and a 100 CFU in-house limit was selected as the internal action limit by analogy to the Class D Settle plate method. In the Class D area, the limit for the finger dab test is not defined in the WHO Technical Report Series No. 961. 2011, 100 CFU is an internal action limit, not a regulatory limit.

2.2. Procedure

25 plates of Soyabean Casein Digest Agar (SCDA) and 25 plates of Sabouraud Dextrose Agar (SDA) were exposed for 4 hours in all sampling points. The study was conducted for three successive days. Sterile Culture media plates of Soybean Casein Digest Agar and Sabouraud Dextrose Agar were exposed on Petri plates, placed at their respective sampling sites in each room, for four hours. After the completion of the exposure time, the Petri plates were aseptically transported to the Microbiology laboratory. Soybean Casein Digest Agar plates were incubated in an incubator for 72 hours, and Sabouraud Dextrose Agar Petri plates were incubated in a Biological Oxygen Demand (BOD) Incubator for 5-7 days. After the completion of the incubation period, colonies on Petri plates were counted using a Colony Counter, and the results were interpreted.

3. Result

3.1. Microbial count in different areas

The results obtained for the different locations of five rooms were counted, and the mean CFU was calculated for each sample location and sampling point within the room. The obtained data suggest that a higher Microbial load was observed in the "Near Return Air Loop" sample spot in each room. The higher count near the return loop warrants a study of grill cleanliness, personal proximity, and equipment, as well as heat plumes around the return filters. The p-value of Total aerobic microbial count was 0.8685, and the p-value of Total Yeast and Mold Count was 0.8716. The result is not significant at $p<0.05$.

Table 3: Observation of the Dispensing Room

S. No	Sampling Location	Total Aerobic Microbial Count (CFU/Plate)				Total Yeast and Mold Count (CFU/Plate)			
		Day-I	Day-II	Day-III	Mean	Day-I	Day-II	Day-III	Mean
1	Near Machine	84	90	84	86	21	28	26	25
2	Area with maximum man movement	92	75	82	83	30	31	38	33
3	Difficult to clean area	84	81	78	81	24	26	31	27
4	Near return air loop	96	93	90	93	43	47	42	44
5	Near the Drainage area	91	77	90	86	16	26	24	22

CFU, Colony Forming Units.

Table 4: Observation of the Granulation Room

S. No	Sampling Location	Total Aerobic Microbial Count (CFU/Plate)				Total Yeast and Mold Count (CFU/Plate)			
		Day-I	Day-II	Day-III	Mean	Day-I	Day-II	Day-III	Mean
1	Near Machine	65	67	69	67	32	36	34	34
2	Area with maximum man movement	85	89	90	88	33	39	39	37
3	Difficult to clean area	80	76	84	80	26	31	30	29
4	Near return air loop	90	94	98	94	45	45	48	46
5	Near the Drainage area	75	65	70	70	32	25	33	30

CFU, Colony Forming Units.

Table 5: Observation of the Punching Room

S. No	Sampling Location	Total Aerobic Microbial Count (CFU/Plate)				Total Yeast and Mold Count (CFU/Plate)			
		Day-I	Day-II	Day-III	Mean	Day-I	Day-II	Day-III	Mean
1	Near Machine	74	71	68	71	25	32	36	31
2	Area with maximum man movement	82	78	80	80	38	33	40	37
3	Difficult to clean area	65	67	69	67	26	35	32	31
4	Near return air loop	84	86	88	86	46	42	44	44
5	Near Drainage area	80	78	82	80	37	40	34	37

CFU, Colony Forming Units.

Table 6: Observation of the Coating Room

S. No	Sampling Location	Total Aerobic Microbial Count (CFU/Plate)				Total Yeast and Mold Count (CFU/Plate)			
		Day-I	Day-II	Day-III	Mean	Day-I	Day-II	Day-III	Mean
1	Near Machine	17	12	22	17	11	10	9	10
2	Area with maximum man movement	23	26	20	23	12	14	16	14
3	Difficult to clean area	31	24	20	25	17	17	23	19
4	Near return air loop	34	37	40	37	14	18	28	20
5	Near the Drainage area	21	29	22	24	7	10	13	10

CFU, Colony Forming Units.

Table 7: Observation of the Blister Packing Room

S. No	Sampling Location	Total Aerobic Microbial Count (CFU/Plate)				Total Yeast and Mold Count (CFU/Plate)			
		Day-I	Day-II	Day-III	Mean	Day-I	Day-II	Day-III	Mean
1	Near Machine	90	95	88	91	36	37	41	38
2	Area with maximum man movement	95	94	90	93	40	35	30	35
3	Difficult to clean area	95	96	97	96	42	40	38	40
4	Near return air loop	90	88	92	90	42	45	42	43
5	Near Drainage area	90	93	90	91	38	40	22	31

CFU, Colony Forming Units.

Table 8: Statistical Analysis for Total Aerobic Microbial Count (Chi-Square Test)

Room	Near Machine	Area with maximum Mean value	Difficult to clean area	Near return air loop	Near the Drainage area	Row Total
Dispensing room	86 (79.17) (0.59)	83 (87.53) (0.23)	81 (81.79) (0.01)*	93 (96.82) (0.15)	86 (83.70) (0.06)	429
Granulation room	67 (73.63) (0.60)	88 (81.40) (0.54)	80 (90.05) (0.17)	94 (90.05) (0.17)	70 (77.85) (0.79)	399
Punching room	71 (70.87) (0.00)*	80 (78.38) (0.04)*	67 (86.66) (0.01)*	86 (86.66) (0.01)*	80 (74.92) (0.34)	384
Coating room	17 (23.25) (1.68)	23 (25.70) (0.28)	25 (24.02) (0.04)*	37 (28.44) (2.58)	24 (24.58) (0.01)*	126
Blister packing room	91 (85.08) (0.41)	93 (94.05) (0.01)*	90 (87.89) (0.05)	96 (104.04) (0.62)	91 (89.94) (0.01)*	461
Column total	332	367	343	406	351	1779

(*) Statistically Significant. P-value: 0.8685. The result is not significant at $p < 0.05$

Table 9: Statistical Analysis for Total Yeast and Mold Count (Chi-Square Test)

Room	Near Machine	Area with maximum Mean value	Difficult to clean area	Near return air loop	Near the Drainage area	Row Total
Dispensing room	23 (26.49) (0.46)	33 (30.38) (0.23)	27 (28.44) (0.07)	44 (38.37) (0.83)	22 (25.32) (0.44)	149
Granulation room	34 (31.29) (0.23)	37 (35.89) (0.03)*	29 (33.59) (0.63)	46 (45.32) (0.01)*	30 (29.91) (0.00)*	176
Punching room	31 (32.00) (0.03)*	37 (36.71) (0.00)*	31 (34.35) (0.33)	44 (46.35) (0.12)	37 (30.59) (1.34)	180
Coating room	10 (12.98) (0.68)	14 (14.89) (0.05)	19 (13.93) (1.84)	20 (18.80) (0.08)	10 (12.41) (0.47)	73
Blister packing room	38 (33.24) (0.68)	35 (38.13) (0.26)	40 (35.69) (0.52)	43 (48.16) (0.55)	31 (31.78) (0.02)*	187
Column total	136	156	146	197	130	765

(*) Statistically Significant. P-value: 0.8716. The result is not significant at $p < 0.05$.

Table 10: Limit of 90 mm Diameter Settle Plate [2].

Grade	Air Sample (CFU/m^3)	90 mm Diameter Settle Plates (CFU/4 hours)	55 mm Diameter Contact Plates (CFU/plate)	Gloves Print (5 fingers) CFU/Glove
Class A	<1	<1	<1	<1
Class B	10	5	5	5
Class C	100	50	25	-
Class D	200	100	50	-

CFU, Colony Forming Units.

Table 11: Observation of Finger Dab Test

S. No.	Sample Code No.	Sampling Location	TAMC (Right Hand, CFU/Plate)	TAMC (Left Hand, CFU/Plate)
1	09	Dispensing Room	30	22
2	19	Dispensing Room	47	29
3	24	Granulation Room	24	48
4	51	Granulation Room	30	41
5	62	Punching Room	58	48
6	28	Punching Room	59	48
7	42	Coating Room	46	47
8	11	Coating Room	58	45
9	36	Blister Packing Room	32	30

TAMC, Total Aerobic Microbial Count; CFU, Colony Forming Units.

The result of a higher count near the return air loop may be due to the airflow path. The result aligns with a similar study by Shrawan et al. (2025), who reported a higher load at the return loop [8]. The Finger Dab test was conducted in the Class D area to screen operator hygiene in a non-sterile environment, with a limit of 100 CFU/5 fingerprints, as the internal action level was selected as a limit in analogy to the Class D Settle plate method, but not a regulatory limit.

The result was satisfactory, but the study warrants consideration of whether it can be done or not, as no guidelines suggest the finger dab test in a Class D area.

4. Discussion and Conclusion

The result of the study suggests that the pharmaceutical industry is operating in compliance with the standard for viable particles. Although the study is a single-site pilot study, all viable counts were within expectation for Class D. Seasonal variability and inter-site comparability study remain the major aspects to be concluded in further studies. So far, the results of the viable count of microorganisms indicate that higher counts are found near the Return loop areas. This result can serve as a basis for selecting the sample spot for the regular environmental monitoring Program in Class D areas of the pharmaceutical industry. Operator hygiene should be considered at higher Personnel Proximity in a Pharmaceutical Manufacturing Facility. Although 100 CFU/5 fingerprint is not a regulatory limit, it can be considered an internal action limit to maintain hygiene within a Class D pharmaceutical manufacturing facility.

Conflicts of Interest

The authors declare that they have no competing interests that could have influenced the objectivity or outcome of this research.

Funding Source

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Acknowledgment

Thank you, Dr. Kamlesh Dutta, for your everlasting support during the study period.

Institutional Review Board (IRB)

This study did not involve human participants, human data, or human tissue. Therefore, approval from an IRB or ethics committee was not required. All procedures were conducted in accordance with the relevant guidelines and regulations for laboratory-based research.

Large Language Model

No large language model was used in the preparation of this manuscript.

Authors Contribution

SKS contributed to conceptualization, methodology, investigation, data collection, analysis, and writing of the original draft and final manuscript.

Data Availability

No datasets were generated or analyzed for this study beyond the summary results presented in the article; therefore, no additional data are available. Data sharing does not apply to this research. For reasonable queries about the summarized results or methods, please contact the corresponding author listed in the manuscript.

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