Jana Nexus: Journal of Health and Medicine

RESEARCH ARTICLE

Article DOI: 10.21474/JNHM01/162
DOI URL: http://dx.doi.org/10.21474/JNHM01/162

OPTIMIZING SANITIZATION AND ACCESS CONTROL: LESSONS FROM A CLEANING VALIDATION STUDY

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Manuscript Info

Manuscript History
Received: 08 May 2026
Final Accepted:10 June 2026
Published: July 2026

Key words:-
Cleaning Validation, Contamination Variables, Findings, Swab technique, Root cause analysis

Abstract

Background: Cleaning Validation studies is an important aspect in pharmaceutical studies to identify and correct potential problems previously unsuspected, which could compromise the safety, or quality of subsequent batches of product produced within the equipment.
Methods The study was designed as experimental study and conducted between January-June 2025.The study was conducted on Clobetasol 0.05% cream manufacturing equipments. Total 36 samples from 3 consecutive batches were included in the study. Swabbing technique was used to find microbial load on equipment under the study. Total aerobic Microbial Count (TAMC) 100 cfu/ 100 cm ^{2} and Total Yeast and Mould Count 10cfu/ 100 cm ^{2} was set as pass limit during cleaning validation study. Different contamination variables were studied during cleaning validation study.Statical analysis was done using Standard deviation on obtained data.
Result: The mean value of cleaning validation study for Total aerobic Microbial count and Total yeast and mould count was found to be 8.38 and 0.55 for all three batches of used equipments respectively. Standard deviation was found to be 6.08 and 0.511.
Conclusion: Several contamination variables like sanitizationtechnique, frequency of fumigation, access control in area, Microbial load over lint free clot used for mopping was coined during study and after mitigating such contamination variables the study result was found satisfactory.Changed technique like change from chemical method of sterilization to moist heat sterilization of templates , use of lint-free cloths soaked in 70% IPA provided better control, Increased fumigation frequency and limited access in area acts as major preventive measures for contamination Control.

Introduction:-

Cleaning validation is study done to find the effectiveness and reliability of cleaning pharmaceutical production equipment. People in the pharmaceutical industry use equipment validation and cleaning procedures mainly to prevent cross-contamination that makes these practices crucial.[1] In general, cross-contamination usually happens when an active ingredient from one product is transferred to other product through the instruments improper cleaning that can pose real risks to consumers and second type is contamination by foreign materials, which could be

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bacteria or fungi .Poor maintenance or storage conditions may let microbes flourish in processing equipment and becomes a serious issue[2]

The most important benefit of conducting cleaning validation work is to identify and correct potential problems previously unsuspected, which could compromise the safety, efficacy or quality of subsequent batches of drug product produced within the equipment.[3]Cleaning validation guarantees the safety, identity, purity, and strength of products, which are fundamental to cGMP (Current Good Manufacturing Practice) [4]

Significance of Cleaning Validation study:-

The primary purpose of cleaning validation is to prevent cross-contamination between different pharmaceutical products, thereby ensuring product integrity and patient safety. By verifying the effectiveness of cleaning procedures, it provides assurance that active ingredient or cleaning agent from a previous batch remains under acceptable level on equipment that could not adulterate subsequent products[5].The process typically includes the development of cleaning methods, assessment of worst-case scenarios, establishment of acceptance criteria, and verification using appropriate analytical techniques to quantify residuals and ensure reproducibility [6].

Cleaning Method Development [8,9]

Cleaning method development is a critical component of the pharmaceutical validation lifecycle and is carried out alongside drug development to ensure that cleaning processes are scientifically sound, efficient, and compliant with regulatory expectations. Stages of Cleaning Method Development Cleaning method development typically progresses through three main stages:

1. **Feasibility**: This stage evaluates whether the proposed method is suitable for the specific sample, equipment, and contaminants in question.
2. **Development**: Optimization of cleaning parameters such as time, temperature, cleaning agents, and equipment to achieve maximum residue removal.
3. **Validation**: Demonstration that the optimized cleaning method consistently meets acceptance criteria across multiple runs and conditions

Risk-Based Approach:-

A risk-based strategy is fundamental in cleaning validation studies. It involves identifying and prioritizing equipment, surfaces, or products that pose a higher risk of contamination or cross-contamination. This approach allows the validation team to focus resources and attention on critical areas where product safety or quality may be most vulnerable. It involves in choosing appropriate cleaning method, test method and justify the result based on regulatory guidelines. 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) [10]

Scope of study:-

- To study different variables for contamination of clean equipments
- To provide scientific rationale and documentation for cleaning effectiveness.
- To perform cleaning validation study on equipments used for formulation of products

Methods:-

Study Design:-

The study was designed as experimental study and conducted between January-June 2025.The study was conducted on Clobetasol 0.05% cream manufacturing equipments in class D manufacturing facility in pharmaceutical Industry.Sampling method was selected as Swabbing technique, to study the microbial load on equipments included the study.Total aerobic Microbial Count (TAMC) 100 cfu/ 100 cm^2 and Total Yeast and Mould Count 10cfu/ 100 cm^2 was set as pass limit during cleaning Validation study [12].The worst case product was selected on basis of cleaning validation master plan with a RPN score of 27 points and sampling point was selected based on areas with higher potential for microbial growth (E.g. areas with stagnant water, areas that are open to environment etc). 1 sample from each site were studied during validation studies from 3 consecutive batches of product. Validation of swabbing recovery technique was done prior study using USP<61> (United States Pharmacopoeial Convention, 2025 and limit of validation study was taken asreference from Indian Pharmaceutical Alliance [12].

Study were conducted in 3 phases:-
a.To study the actual status of microbial load on clean equipments under study
b.To find the different variables for contamination of clean equipments
c.To perform cleaning validation study on equipments used for formulation of products
Test conditions of cleaning validation was designed under static condition.Temperature less (≤ 25°C), and Humidity (≤ 60%) was maintained in sampling areas during study periods.Swabbing technique was used for sampling and membrane filter test method was used for detection of microbial load on equipments included in the study.

Materials used under study:-
Materials and Manufacturer:-
1 Buffered peptone water: Hi Media
2 Soyabean Casein Digest Agar (SCDA): Hi Media
3 Sabouraud Dextrose Agar (SDA): Hi Media
4 Sterile swab: Hi Media
5 Autoclave: Equitron
6 Bio-safety Cabinet:Thermolab
7 Incubators: Allyone
8 Colony Counter: Lapiz
9.70% IPA: Qualigens

Sampling Procedure:-
Test areas of 10 × 10 cm² were measured using sterile stencils. The sterile swabs were moistened with sterile water, and samples were collected from two different 100cm² areas of each piece of clean equipment. A total of two swab samples from each equipment were collected using unidirectional movements-first 10 horizontal Strokesfollowed by 10 vertical strokes-for the determination of total aerobic microbial count and total yeast and mould count. The swabs were placed into separate test tubes containing 10 mL ofBuffered peptone water (Fig.1) and transported to themicrobiology laboratory.

Fig 1: Swab Sample in Buffered peptone water

Sample analysis and Quality Control:-
The equipment used during the study was well calibrated. Stencils used for measuring surface area were sterilized in an autoclave at 121 °C and 15 psi for 15 minutes. Soybean Casein Digest Agar was used for the isolation of bacteria,

and Sabouraud dextrose agar with chloramphenicol was used for the isolation of fungi.Growth promotion testand Sterility checks of the swab sticks was performed as per USP<61> (United States Pharmacopoeial Convention, 2025[7].Each tube containing the swab sample was shaken for 2-3 minutes.10 ml of the sample solution was individually pipetted into 50 mL of peptone water, mixed thoroughly, and the entire contents were filtered through a membrane filter with a pore size of 0.45 μm (Fig 2).

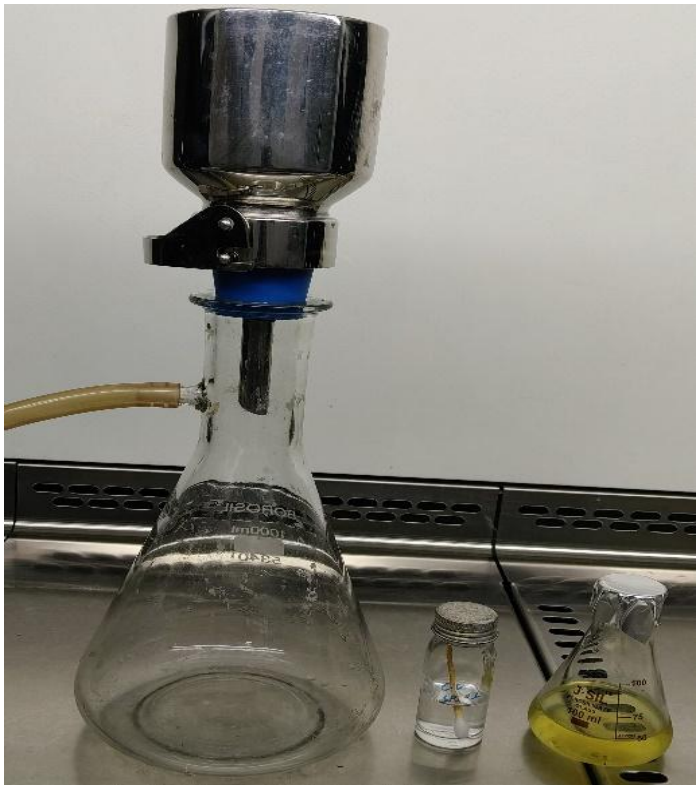

Fig.2: Filter Assembly, Swab sample and Buffered Peptone water,

The membrane filter was aseptically transferred onto Soybean Casein Digest Agar (SCDA) using sterile forceps (Fig.3) and incubated at 35 °C for 72 hours. For total yeast and mould count, the filter was placed on Sabouraud Dextrose Agar with chloramphenicol plates and incubated at 25 °C for 5 days [7].The microbial growth was counted using colony counter and result were interpreted. (Fig 4)

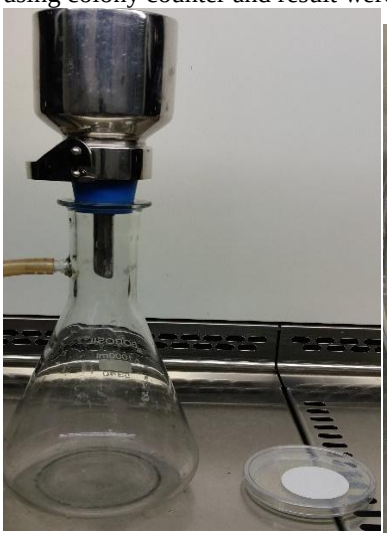

Fig 3: Filtered sample on agar plate

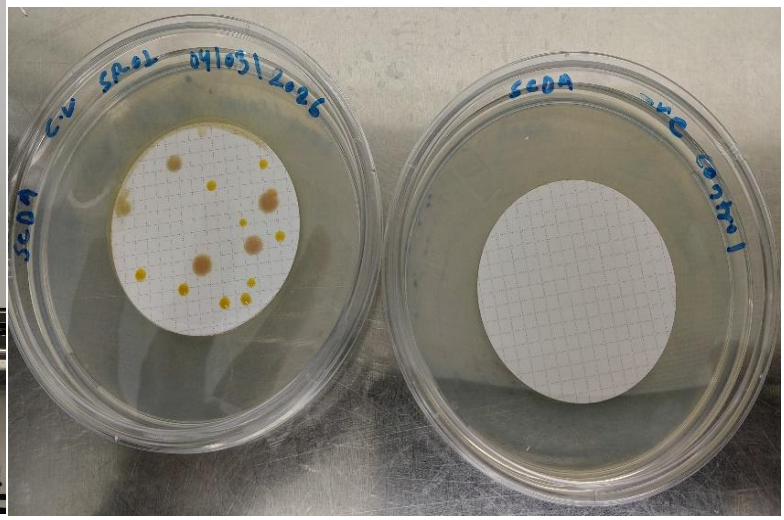

Fig 4: Microbial growth on Sample after Incubation

Result:-

Initial Result of cleaning validation study:-

The load of Microorganism (Total aerobic microbial count) was found out of limit (i.e.> 100 Cfu/100cm$^{2)}$ and the total yeast and mould count was found with in pass limit;<10 Cfu/100cm^2. During the disinfection process of equipments, final rinse was done with purified water. Then 70% IPA was sprayed and mopped with cotton cloth. The result showed that multiple factor may have contributed to the increase bacterial load of on clean equipments under study (Table 1)

Table1: Initial Microbial Load on equipments

S.No.	Equipment	TAMC (Cfu/100cm$^{2)}$ (Limit:<100 cfu)		TYMC (Cfu/100cm^2) (Limit:<10 cfu)	
		Sampling Location	Result	Sampling Location	Result
1	Wax vessel	From the both side of the baffles (MC-01)	300	From the base of the vessel near discharge(Mc-02)	1
2	Manufacturing Vessel	A sample from the Teflon flanges attached to the homogenizer. (MC-03)	250	A sample from the base of the equipment near the drain pipe(MC-04)	3
3	Storage Vessel/Paste preparation vessel	Sample from the Base on the other (MC-05)	150	Sample from the wall of the vessel (MC-06)	1
4	SS Containers	Wall of the container(MC-07)	180	Base of the container(MC-08)	2
5	SS Jugs	Base of the jugs(MC-09)	200	Base of the jugs(MC-10)	3
6	Semi- Automatic Tube Filling Machine	Inner surface of hopper(MC-11)	240	Surface of the stirrer(MC-12)	4

Root cause Analysis to find source of contamination:-

Different variables was selected to find the source of increase microbial load on clean.Test of the total yeast and mould count was excluded in the study as the result was found satisfactory (Table 1)

1. Sample taken without any disinfection process
2. Spray with 70% IPA directly on clean equipment, Air Dry
3. Spray 70% IPA on Lint free cloth and mop the clean equipment
4. Completely dip Lint free Cloth in 70% IPA and Mop the clean equipment
5. Mop the template with 70% IPA and swab taken
6. Lint free cloth soaked in sterile water and mopped the clean equipment
7. Limiting personnel access in the sampling area
8. Decreasing frequency of fumigation in the area

Table 2: Observation of different variables of Study for Microbial load

S.No.	Variables	TAMC (Cfu/100cm^2) (Limit:<100 cfu)
1	Sample taken without any disinfection process	190
2	Spray with 70% IPA directly on clean equipment, Air Dry	46
3	Spray 70% IPA on Lint free cloth and mop the clean equipment	330
4	Completely dip Lint free Cloth in 70% IPA and Mop the clean equipment	21
5	Mop the template with 70% IPA and swab taken	10
6	Lint free cloth soaked in sterile water and mopped the clean equipment	1300

The result showed that lint free cloth as main carrier of organism to the clean equipments during clean validation study and also the chemical sanitizing technique of template was not effective. (Table 2)

Remediation of Microbial Contamination on Clean Equipments:-

Changed Technique:-

1. Template sterilization technique was changed from chemical sterilization to moist heat sterilization (Autoclaving)
2. Cloth used for moping was dipped in 70% IPA and Dried before use of final moping on clean equipments.
3. Fumigation frequency in the area was decreased to 3 month to 1 month.
4. Limited access was done in area during sampling in the area.

Table 3:Result after correction of the contamination variables

S.No.	Equipment	Sampling Location	TAMC (Cfu/100cm$^{2)}$ (Limit:<100 cfu)
1	Wax vessel	From the both side of the baffles (Mc-01)	7
2	Manufacturing Vessel	A sample from the Teflon flanges attached to the homogenizer (MC-03)	9
3	Storage Vessel/Paste preparation vessel	Sample from the Base on the other (MC-05)	23
4	SS Containers	Wall of the container (MC-07)	30
5	SS Jugs	Base of the jugs (MC-09)	20
6	Semi- Automatic Tube Filling Machine	Inner surface of hopper (MC-11)	17

After correction of the contamination Variables, the result of Total Aerobic microbial count was found in pass limit < 100 Cfu/100cm^2.(Table 3)

Table 4:Cleaning Validation Study Result

S.No.	Equipment	TAMC(cfu/100cm^2) (Limit:<100 cfu)				TYMC(cfu/100cm^2) (Limit:<10cfu)			
		Sampling Location	Result			Sampling Location	Result		
			Batch1	Batch2	Batch3		Batch1	Batch2	Batch3
1	Wax vessel	From the both side of the baffles(Mc-01)	7	1	9	From the base of the vessel near discharge (Mc-02)	0	1	0
2	Manufacturing Vessel	A sample from the Teflon flanges attached to the homogenizer. (MC-03)	10	3	2	A sample from the base of the equipment near the drain pipe(MC-04)	1	0	1
3	Storage Vessel/Paste preparation vessel	Sample from the Base on the other(MC-05)	6	3	2	Sample from the wall of the vessel (MC-06)	0	1	1
4	SS Containers	Wall of the container(MC-07)	20	23	14	Base of the container (MC-08)	1	0	1
5	SS Jugs	Base of the jugs(MC-09)	10	12	8	Base of the jugs(MC-10)	0	1	0

6	Semi-Automatic Tube Filling Machine	Inner surface of hopper(MC-11)	5	11	5	Surface of the stirrer(MC-12)	1	0	1
Mean value			8.38			Mean value	0.55		
Standard Deviation			6.08			Standard Deviation	0.511		

The result of cleaning validation on Clobetasol 0.05% cream manufacturing equipments in class D manufacturing facility in pharmaceutical Industry shows within the acceptance limit below 100 cfu and 10 cfu respectively for Total aerobic microbial count and Total Yeast and Mould count, after correcting the contamination variable (Table 4).The mean value of Total aerobic Microbial count and Total yeast and mould count was found to be 8.38 and 0.55 for all three batches of used equipments respectively.Standard deviation was found to be 6.08 and 0.511.

Discussion:-

Cleaning Validation studies is an important aspect in pharmaceutical studies to identify and correct potential problems previously unsuspected, which could compromise the safety, efficacy or quality of subsequent batches of drug product produced within the equipment. During the study we studied the potential contaminating variables and mitigate the causes to guarantees the safety, identity, purity, and strength of products, which are fundamental to cGMP during formulation of products. Several contamination variables like inappropriate disinfection techniques, use of lint free cloth with higher microbial load, fumigation time interval, and unauthorized personnel assess in the area were found as major variable which increased microbial load on clean equipments. (Table2)

After change in techniques like, Template sterilization technique was changed from chemical sterilization to moist heat sterilization (Autoclaving), Cloth used for moping was dipped in 70% IPA and Dried before use of final moping on clean equipments, Fumigation frequency in the area was decreased to 3 month to 1 month and Limited access was done in area during sampling in the area the result of cleaning validation was found satisfactory (Table 3) Cleaning validation study on Clobetasol 0.05% cream manufacturing equipments was performed after correcting the different contamination variables. The mean valueof obtained microbial count was found to be 8.38 and 0.55 for all three batches of used equipments respectively (Table 4).The result is in agreement with similar study done Patel et.al.2013[13] and Kathiresan et.al 2010[14]who reported more than 6 cfu from equipment surface during the study. The lower microbial load on equipments might be due to correction in contamination variables found during study (Table 3).Major Root cause of contamination found during study, include technique of sterilization, personnel access in area, duration of Fumigation in area and the carryover of microbial load from lint free cloth to clean equipments during sanitization of equipments.

Conclusion:-

1. The high Total aerobic microbial count was likely caused by poor sterilization of the sampling tools (Template/Cloth) rather than the equipment cleanliness itself.
2. The change from chemical method of sterilization to moist heat sterilization of templates was critical.
3. The use of lint-free cloths soaked in 70% IPA provided better control.
4. Increased fumigation frequency and limited access improved the environmental control.

Limitations of Study:-

1. Single Manufacturing Sites
2. Single Product evaluated
3. Relatively small sample size
4. Limited Duration of monitoring

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.

Acknowledgement:-

Thanks, Dr. Kamlesh Dutta and GobindaKumar for everlasting support during the study period.

Informed consent:-
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 article

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.

Ethics Approval and Consent:-
No human or animals samples were involved in the study, so no ethical approval and consent is required in this study.

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