ISSN(O)-3107-7889 Volume:1 Issue 8, August,2025 01(08), 18-21
JANA NEXUS: JOURNAL OF HEALTH AND MEDICINE

Article DOI: 10.21474/JNHM01/108
DOI URL: http://dx.doi.org/10.21474/JNHM01/108

RESEARCH ARTICLE

MICROBIAL LOAD ASSESSMENT DURING DIRTY EQUIPMENT HOLD TIME STUDY

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Manuscript Info

Manuscript History
Received: 08 June 2025
Final Accepted:10 July 2025
Published: August 2025

Key words:-
Clean equipment, Hold time study, Bioburden, TAMC, TYMC

Abstract

The objective of this Dirty Equipment Hold Time Study (DEHT) to establish a validated maximum dirty hold time for equipment before cleaning. DEHT study helps us in determining the maximum time equipment can remain uncleaned after production without compromisin g cleaning effectiveness. The dirty equipment hold time study was carried out for Amlodipine. The study was carried at 96 hours of hold time of dirty Equipments and microbial sampling was done at the end of 96 hours. The Result of visual inspection was found satisfactory and no visible signs of microbial growth was observed. The results of Microbial Sample were found below 100 cfu/25cm2.

Introduction:-

Dirty Hold Time refers to the maximum duration that equipment can remain idle after the completion of a manufacturing process and before the start of the cleaning procedure. During this period, equipment surfaces are exposed to risks such as: Drying and hardening of product residues, which can reduce cleaning effectiveness, Microbial growth, particularly when residues retain moisture or contain nutrient-rich material and Chemical degradation, potentially altering residue characteristics and complicating removal[1]. Qualification of the cleaning process is carried out using methods of a visual control of cleanliness, a wet-swab sampling and analysis of controlled substances [2].Typically washing process in the pharmaceutical plant begins immediately after the production batch. The postponement of several days can make more efficient use of working time and lead to cost reductions, but the residuals of the produced material, however, may become more difficult removable after the delay [3]. Dirty-hold time is part of the cleaning validation process from the beginning, but often overlooked [4]. The API (active pharmaceutical ingredient), an excipient or the detergent are the controlled substances. For a single production line or the production device one product representing all products manufactured on this line can be determined based on a method of "worst case" [5].

The criteria for selecting the worst case are a complexity of the cleaning process, a solubility in a cleaning media, the greatest toxicity, the lowest therapeutic dose and the lowest limit (eg from the therapeutic dose). Sampling is usually performed after a maximum number of produced batches. Process qualifications of the washing procedure are carried out for newly manufactured products, a new equipment, new systems CIP (Clean in Place) and recently used detergents [6]. The work was aimed on the cleaning process efficiency and the cleanliness of the pharmaceutical apparatuses after the end of the production and subsequent cleaning process. Influence of the dirty-hold time, e.g. the time interval between the end of the production period and beginning of the cleaning process on the cleanliness of the equipment was studied for Microbial Load.

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Material and Method

Table 1: Materials and Equipment Used for Dirty Equipment Hold Time Study

S.No.	Material and Equipment	Manufacturer
1	Sterile Swab	Hi Media
2	Soyabean casein Digest Agar	Hi Media
3	Sabouraud chloramphenicol agar	Hi Media
4	Peptone Water	Hi Media
5	Autoclave	Equitron
6	Incubator	Allyone
7	Biosafety Cabinet	Thermolab
8	70% iso Propyl alcohol	Qualigens
9	Hot Plate	Lab Quest
10	Colony Counter	Lapiz
11	Membrane filter	Merk
12	Stensils	

Material:-

The materials and equipment utilized in this study are detailed in (Table 1).

Media preparation

All media used in the study were prepared strictly per the manufacturer's recommendation (Hi Media).

Test Method:-

The test was conducted in Class D Pharmaceutical Manufacturing Facility. The test areas of 5×5 cm^2 were measured with sterile Stensils. The sterile swabs were moistened with sterile water and sample was collected from two different area of 25cm^2 from dirty equipments. Altogether 2

samples were collected, in unidirectional movements, first with 10 horizontal strokes followed by 10 vertical strokes from each equipments for test of Total aerobic microbial count and Total yeast and mould count. The swabs were dipped in a test tube containing 10 ml peptone water and transported to microbiology lab for further analysis.

Sample Analysis

Each tube containing the swabs sample was shaken for 1-2 minutes. 1 ml of the sample solution was pipetted individually in 50 ml peptone water, mixed well by shaking and the whole content were filtered through membrane filter having pore size of 0.45 µm. The filter paper was
transferred on Soyabean casein digest agar (SCDA) with sterile forceps and incubated at 35 ◦C for 72 hours, for test of Total aerobic microbial count and the SDA chloramphenicol agar plates at 25 ◦C for 5 days for test of Total yeast and mould count.

Calculation of test Result:-

TAMC or TYMC (cfu/ml No. of colonies per ml × Dilution factor Volume of Sampl

Result:-

Table 2: Observation of microbial count and Visual Inspection

Equipment	Sampling Location	Test Parameter	Observations Cfu/25cm$^{2.}$	Visual Observation
Saizoner Mixer Granulator (SMG)-I	Base of SMG	Total Aerobic Microbial Count	30 cfu/swab	No visual signs of microbial proliferations and no any foul odours were observed
		Total Yeast and Mould Count	No Growth	
Fluidized Bed Dryer (FBD)-II	Inner wall of FBD dome	Total Aerobic Microbial Count	10 Cfu/swab	
	Inner wall of FBD bowl	Total Yeast and Mould Count	No Growth	
Binder Preparation Vessel-II	Base of Vessel	Total Aerobic Microbial Count	20 Cfu/swab	
	Wall of Vessel	Total Yeast and Mould Count	No Growth	
		Total Aerobic Microbial Count	50 fu/swab	
Square Bin-II	Inner wall near lid	Total Yeast and Mould Count	No Growth	
Mesh	Surface of mesh	Total Aerobic Microbial Count	10 Cfu/Swab	
		Total Yeast and Mould Count	2 Cfu/swab	
Tablet Compression Machine-I	Surface of Turret	Total Aerobic Microbial Count	50 Cfu/swab	
		Total Yeast and Mould Count	No Growth	
Colloid Mill	Inner wall of hopper	Total Aerobic Microbial Count	50 Cfu/swab	

		Total Yeast and Mould Count	No Growth
Coating Suspension Vessel	Inner wall of vessel	Total Aerobic Microbial Count	70 Cfu/swab
		Total Yeast and Mould Count	No Growth
Blister Packing Machine-III	Inner surface of hopper	Total Aerobic Microbial Count	40 Cfu/swab
		Total Yeast and Mould Count	1 Cfu/swab

Result and Discussion:-

Dirty equipment hold time study was done at Class D Pharmaceutical Manufacturing facility.

The equipments were hold for 96 hours and sampling was done at single point of time from all equipments.Higher bacterial load was found at coating suspension vessels (Table 2). Fungal load was very low in comparison to bacterial load on dirty equipments at 96 hours hold time. Higher load on coating suspension vessels might be due to use of purified water to make suspension and excepients used in preparation of coating suspension which itself have bioburden .The bioburden present present in excipients and purified water contribute to have higher bioburden in comparison of other equipments.

Conclusion:-

The dirty equipment hold time study was carried out for Amlodipine. Visual inspection and microbial sampling was done at the end of 96 hours.

The Result of visual inspection was found satisfactory and no visible signs of microbial growth was observed. The results of Microbial Sample were found below 100 cfu/25cm^2 forTotalaerobic microbial count and Total yeast and mould count in dirty equipments at 96 hours hold time. Based on the outcomes of study, the cleaning methods is effective to clean the dirtyequipments up to 96 hours hold time. Limitation1The study was done at single point of time and single product (Amlodipine), so further studies should be done to on other products also to point out the real figure

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