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Environmental Monitoring and Risk Assessment of Cleanrooms within Pharmaceutical Industry

Azam Mehdi[1], Muhammad Danish Mehmood[2]*, Muhammad Usman Ghani[2], Muhammad Ismail[2], Faisal Ameen[3], Saud-ul-Hassan[3]

[1]Institute of Molecular Biology and Biotechnology, University of Lahore, Pakistan,
[2]Ottoman Pharma (Immuno-Division), Lahore, Pakistan
[3]Grand Parent Diagnostic Laboratory, Lahore, Pakistan

***Corresponding author e-mail: drdanishmehmood@gmail.com**

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ABSTRACT

Environmental monitoring is a scrutinizing program for microbial and particulate contamination of clean rooms and associated clean zones for aseptic processing. The current study was therefore, designed to evaluate the quality of clean rooms environment of local vaccine manufacturing unit in compliance with the international cleanroom standards proposed by the World Health Organization (WHO) for the production of quality biological products. The classified areas were divided into 60 gridlines where 30 sampling sites were randomly identified in which 20 critical and 10 non-critical sites were allocated and 700 liters/m^3 air was aspirated through air sampler. Furthermore, particles size of 0.5 um and 5.0 um were also detected for 1 minute from all critical and non-critical sites. It was revealed that viable count obtained from sampling sites designated as class A of Production Section I and II was less than ≤ 1 CFU/m^3. Whereas, the number of non-viable particles having size of ≤ 0.5 µm in class A at rest were ≤ 3,520 CFU/m^3 as compare to 20/m^3 of particle size ≥ 0.5 from each sampling sites in both the sections. Moreover, Finger DAB testing confirmed the hygienic status of personnel and proper use of antiseptics and sterile garments (<1 CFU/gloves). Manometer readings in three airlocks in production section were recorded as 0.05, 0.10 and 0.15 inch of water gauge respectively. Consequently, it is concluded that all sections of vaccine manufacturing unit are comply with WHO cleanroom standards. In each sampling site of all clean zones shows viable and non-viable counts within the limits set by local authorities and international cleanroom standards.

Keywords: Environmental monitoring, Air sampler, Finger DAB test and Viable count.

INTRODUTION

Environmental monitoring is a designed scrutiny system for microbial contamination of cleanrooms and other closed processing environments. It is a cascade of procedures which gives monitoring, testing and responses to the microbial concentration in aseptic processing environments. Environmental monitoring defines the procedures and actions that require occurring to characterize and scrutinize the quality of the environment. The prime goal of environmental monitoring of cleanrooms or isolators are to regulate the numbers of airborne viable and Non-viable particles within defined limits, predict the risk to the environment and regularly estimate the efficacy of cleaning and disinfecting processes in sterile areas such as filling, formulation, compounding, packing and other associated areas

[1]. Pharmaceutical manufacturing comprises a complicated, multi-phase processing system associated with major risks of microbial contamination through diverse sources. The product quality is greatly influenced by microbial contamination in several processing step [2]. There are two types of pharmaceutical products currently being manufactured are sterile and non-sterile products [3]. Parenteral (injectable) drugs are mainly included in sterile category as they enter directly into blood system. International standards have been set for the manufacturing of sterile products in which microbial limits are adjusted for the area to be used for their production to maintain the product quality [4].

Terminal sterilization and aseptic processing are two methods used for sterilization of drugs. Terminal sterilization typically includes filling and stoppering of product bottles under highly sterilized environmental states. The product at its finished stage including vials or bottles is then targeted to a sterilization procedure through heat or radiation. In some situations, the drug and bottle contain low biological load but still it is not deprived of it. In an aseptic processing, all the components are first aimed to sterilize individually through defined methods at each step of production, as recommended and then mixed together in sterilized areas. Both sterilization method requires highly controlled environment [5].

Environment plays key role in microbial access in Pharmaceutical and biological products. The quality of product depends on environmental controls in manufacturing area. 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 product. Environmental monitoring provides the systems and functions that require establishing and monitoring the quality of the environment. The frequency of monitoring ensures contamination free environment maintenance. Environmental monitoring is a programed scheme to explain the regulation of viable and non-viable particles in critical sites [2].

Clean room

Clean Area Separation is a segregation area which is required between classified area and external environment to prevent cross contamination. With the aim of air quality maintenance in classified areas, it is important to acquire appropriate airflow and a differential air positive pressure comparative to adjacent lesser clean areas. Upper cleanroom class should keep air positive pressure differential comparative to next lower classified areas of usually at least 10-15 Pascals (Pa) which is equal to 0.03 to 0.05 inches of water gauge (when door is shut). When doors are unlocked, outward air pressure must be sufficient to prevent entry of particles from external environment [6].

Microbiological risk

Microbial risk is defined in the ISO as, "A combined product of the probability of occurrence of harm and the severity of harm" [7]. As stated by the World Health Organization (WHO), a risk is, "An event in the production, control and supply of a drug which has potential an adverse health effect" [8]. Risks can be either intrinsic or extrinsic. Intrinsic risks are those which are integral part of manufacturing system having influence on quality of product. These are linked with mechanical problem or failure in sterility of manufacturing material i.e. contaminated water for injection (WFI), active pharmaceutical ingredient (API), or final product. Extrinsic risks are those come outside manufacturing process i.e. environment, personnel and surfaces. These are bigger challenge than intrinsic risks [9]. These risks, if not controlled, can endanger the quality of the product and can ultimately affect the patient. It is therefore no wonder that the practical approach of risk management has been formally recognized as actively supported by many pharmaceutical regulatory agencies [10]. Recognition of the nature of a hazard and its means of impacting (risk) product quality are the critical initial steps in performing a risk assessment. The results from these steps generally assist the choice of the most suitable risk assessment technique like FMEA, FMECA and HACCP [9]. The practice of risk assessment tools is an essential current Good Manufacturing Practice (cGMP) approaches in environmental monitoring. Although, every class of clean rooms will be considered slightly different [11]. The microbiological risk for drugs produced under high air quality conditions providing by pharmaceutical cleanrooms can be evaluated by the dispersion, transfer and accumulation of microbiological contamination onto drug. The risk assessment can be possible in two-steps, the first step will be used to evaluate the transfer of microorganism from all of the sources within the cleanroom premises and the second

step applied to find both air and surface contact contamination within critical production sites. These two steps can be applied to assess and minimize microbiological risk at the initial target step of the cleanroom and related production process [12]. The current study is therefore, designed to monitor the environment of commercial veterinary vaccine manufacturing unit in compliance with the international cleanroom standards of World Health Organization (WHO) for the production of quality biological products.

The objectives of the current study are below as;

1. Evaluate the environmental monitoring system cGMP standards for the production of veterinary vaccine manufacturing
2. Measuring the impact of air differential pressure on maintenance of controlled environment for biopharmaceutical product manufacturing
3. Designate specified grades to clean areas of local vaccine manufacturing unit on the basis of the world health organization (WHO) Cleanrooms standards

MATERIAL AND METHODOLOGY

The research was conducted in Ottoman Pharma Immuno division after getting written consent by management of the firm. The company has been manufacturing inactivated viral vaccines for poultry use and distributing its products throughout the Pakistan.

The research was designed on the basis of statistical sampling model proposed by revised ISO 14644-1 for airborne particles quantification in cleanroom. A randomized sampling plan was adopted in which a systematic grid was drawn across the room for getting the minimum number of sample locations.

Vaccine manufacturing unit layout

The vaccine manufacturing plant is categorized into three basic controlled areas where different activities have performed at different steps of vaccine manufacturing. Production section I, II and Microbiology Laboratory are classified areas of vaccine manufacturing plant. Production Section II is declared as highly sensitive and sterile area used for cultivation and emulsification of vaccine. Filling line machine is placed in Production Section II which used to fill the all components of vaccine in bottles at final stage of manufacturing under laminar air flow (LAF). Production section I is used for inoculation and cultivation of virus in embryonated eggs under the Aspiration assembly having unidirectional air flow (UDAF). Microbiology Laboratory is used for testing and feedback to the microbiological quality levels in all stages involved in biopharmaceutical production. Transportation of material is carried out through transfer bay from one section to other section which prevents cross contamination between two environments by the mean of Unidirectional Pathway of material.

Incubation room is site where substrate is used for candling and incubation of embryonated eggs at 37°C. Quality control laboratory is used for all of tests pertaining to quality of raw material, in process and finished products are being performed. Any material supposed to be used in manufacturing process is retained at Quarantine room till further clearance from Q.A. department. Packing Hall is used for labeling the bottles of finished product and assembling of bottles into transport packing. In-process intermediates and final product is refrigerated at 2°C and 8°C in Chiller Room till transportation. The route of entering for personnel is separated from the route of exiting personnel from vaccine manufacturing area.

Whereas, Non-classified areas include Incubation, Quarantine room, Packing Hall and Quality Control laboratory as shown in Figure 1.

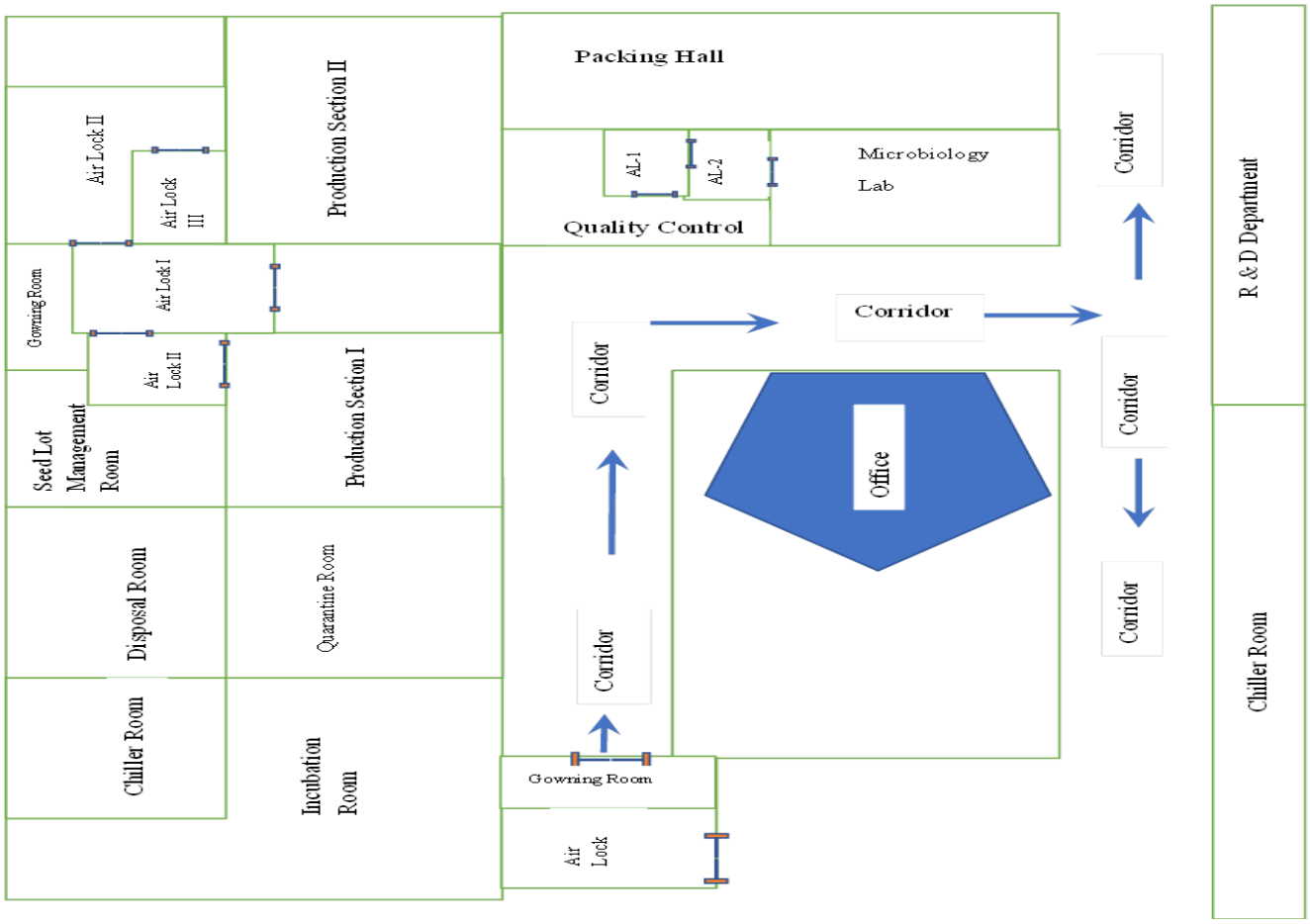


Figure 1: The layout of vaccine manufacturing unit.

Statistical sampling model

A statistical approach was adopted in this study to gain 95% statistical confidence level for representative sampling model. An imaginary boundary (grid) is drawn which divides the total area in square meter into regions called area units those were selected as sample location randomly. The minimum sample locations are retrieved by square root of total area (√A) of respective room. The particle count was measured in each randomly selected location within each section. The minimum numbers of sample locations (NL) in square meter are shown as in Tables 1-2.

Table 1: The minimum number of sample locations according to total area of cleanroom proposed by revised ISO 14644-1.

Area (m^2) less than or equal to	Minimum number of sample locations (N_L)
2	1
4	2
6	3
8	4
10	5
24	6
28	7
32	8
36	9
52	10
56	11
64	12
68	13
72	14
76	15
104	16
108	17
116	18
148	19
156	20
192	21
232	22
276	23
352	24
436	25
500	26

To gain minimum number of sample locations, the total area of each section of vaccine manufacturing unit was separated by imaginary boundary (grid) into area units as ISO 14644-1 recommendations. The minimum number of sample locations and Area Unit size and of each site in the vaccine manufacturing unit is given in Table 3.

Table 2: The minimum number of sample locations for total area of each classified areas of vaccine manufacturing unit.

Sites	**Total area (m^2)**	**N_L**	**Gridline Division**	**Unit size (m^2)**
Production Area I	19	5	10	2
Air Lock-I (P-1)	6	3	6	1
Air Lock-II (P-1)	5	2	4	1.3
Production Area II	29	7	14	2
Air Lock-I (P-2)	6	3	6	1
Air Lock-II (P-2)	6	3	6	1
Air Lock- III (P-2)	3	1	2	1.5

Culture media preparation

The media was prepared by adding 40 grams of Tryptone Soy Agar (Thermo Scientific™ Oxoid™, England) to 1000 milliliters of distilled water for bacteria. Water was boiled to dissolve completely. Final mixture was sterilized by autoclaving through (China manufacturer™) at 121°C on 15 psi atmospheric pressure for 15 minutes. Sterilized media was poured into Petri plates and incubated at 37°C for 24 hours to verify the sterility of biochemical media. For fungi and molds, 65 grams of Sabouraud Dextrose Agar (Thermo Scientific™ Oxoid™, England) dissolved into 1 liter of distilled water. Mixture was autoclaved at 121°C on 15 psi for 15 minutes. Finally, media was poured and incubated at 37°C for 24 hours to check the sterility of biochemical media. Both cultures were used in air sampler and settle plate but only TSA media was also used in finger DAB test. Different quantities of plates were used in different sections of vaccine manufacturing plant.

Microbiological techniques for sample collection

Methodology was strategized to achieve the assessment of environmental monitoring of cleanroom. The environmental monitoring was assessed by the monitoring of air, surfaces and personnel.

Non-viable particles counting

Non-viable particles of two sizes such as 0.5 µm and 5.0 µm were monitored by laser particle counter, brand Name CEM (Model Number DT-9881) and results were recorded from the digital screen.

Viable particles sampling

There are two methods used for viable particles count sampling.

Active air sampling

Thousand liters per cubic meter of air was collected by air sampler MAS® air sampler (model 007) from Cleanroom Class A at both rest and operation states and seven hundreds per cubic meter of air was taken from Class B at rest state. Ten liters at operational state and same quantity of air was taken from Cleanroom Class C at rest and Only Two liters of air aspirated at operational state from Cleanroom Class C.

Table 3: Recommended volume of air by ISO for active air sample of cleanroom.

Cleanroom Class	Volume of Active Air Sample	
	At Rest	At Operation
Class A	Thousand liters	Thousand liters
Class B	695 liters	Ten liters
Class C	Ten liters	Two liters
Class D	Two liters	Two liters

Passive air sampling (settle plate method)

Open pre-incubated media plates were exposed for 4 hours at rest and whole manufacturing time at operation state. The technique was used for both sterile and non-sterile area.

Surfaces

Sterile swab was used for the collection of surface samples from corners, floors, walls, machinery and equipment etc.

Personnel

Finger DAB test was used for evaluate microbial contamination of personnel involved in production of biological products. All the workers involved in production were included in the study.

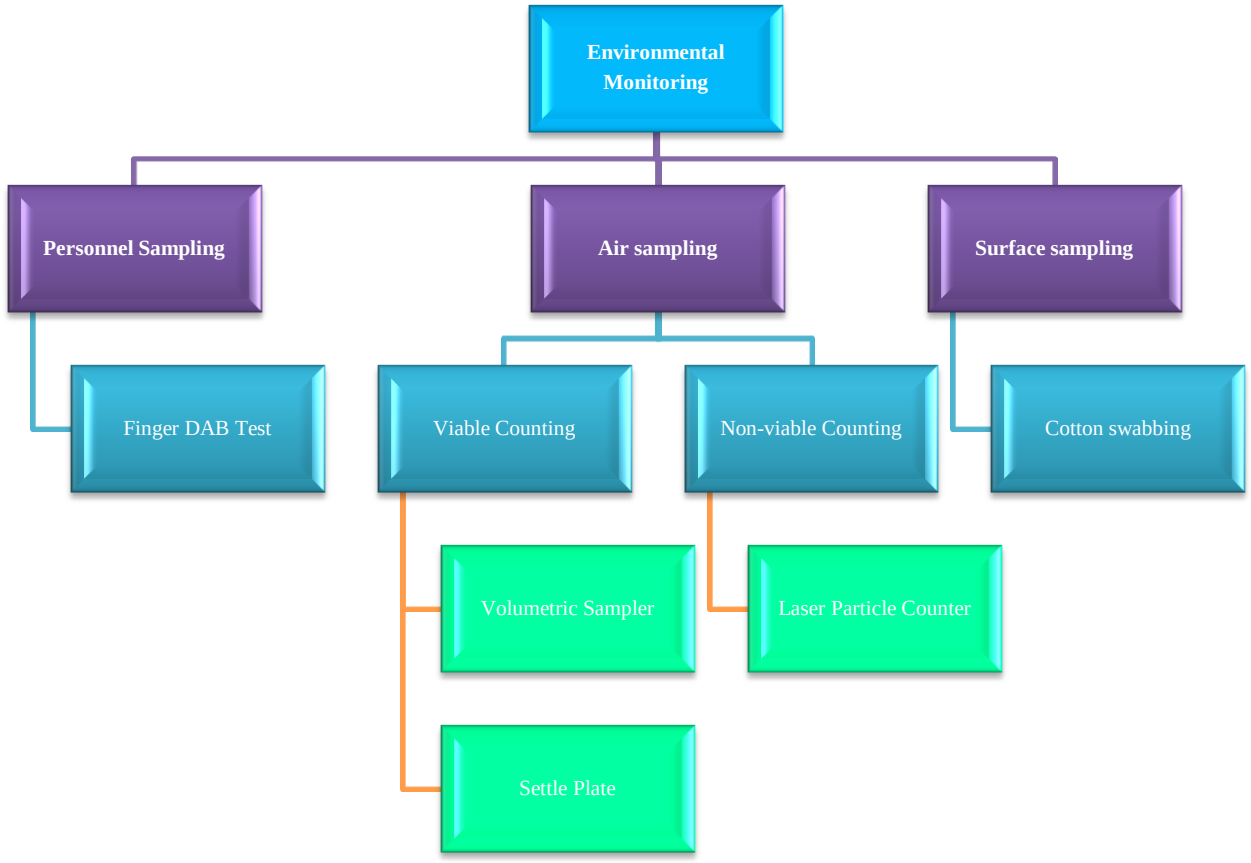


Figure 2: Flow chart of microbiological sampling methods for environment monitoring.

Classified areas

Production section I and II are two classified areas in the vaccine manufacturing unit those included in this study.

Preparation of classified area

Classified area was cleaned and disinfected using existing SOPs of the Biopharmaceutical manufacturing plant. The line clearance certificate was released by the Q.A. Officer (Attached here with as ISO 14644).

Environmental monitoring

The environmental monitoring including tops, walls and floors for viable counts in each sample locations in every section was done through conventional and allied techniques.

Production section I

The total area of the room was 19 m^2 which was divided into 10 gridlines having air pressure of 0.15 inch of water gauge inside the room and seemed with two Air locks (I and II) having positive air pressure as shown in Figure 4.

Selected critical and non-critical sites

Total of 5 sample sites were chosen in Production Section I in which 3 critical sites were identified including each single site along with HVAC inlet and outlet, door and 2 sites along with aspiration assembly. Air lock I and II were also considered as two additional locations including into the critical locations for Production Section I. These sites were declared as critical sites after visit of researcher and management. It was unanimously decided as critical sites within Production Section I premises as shown in Figures 2 and 3.

Two Non-critical sites were marked including each site along with Transfer bay and worker benches in Production Section I.

Air monitoring

Environmental air was monitored by the estimating viable and non-viable particle counts in Production Section I.

Viable counts

Viable counts were sampled by two methods:

Active air sampling

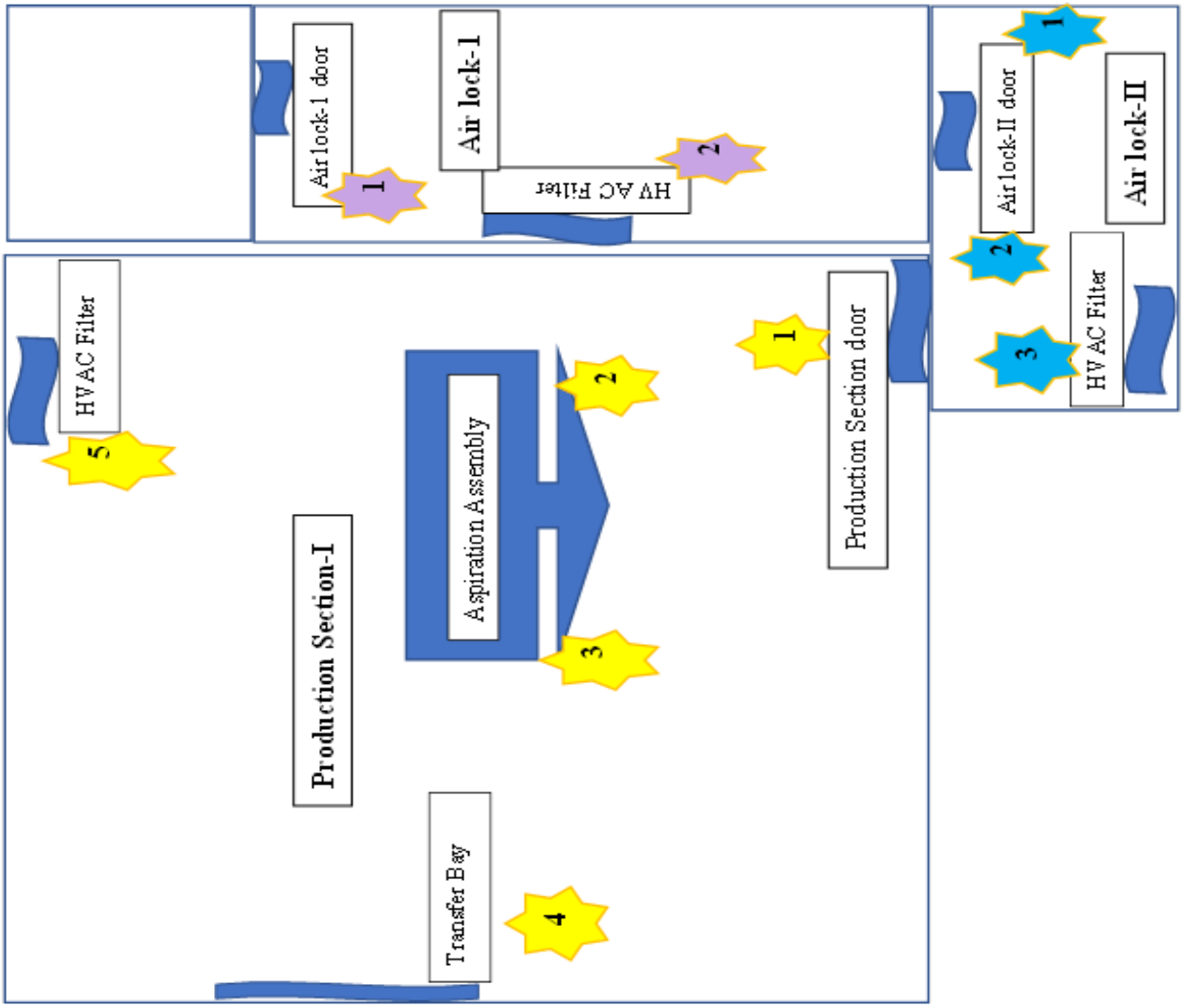
Thousand liters per cubic meter of air was aspirated by MAS® air sampler (model 007) from aspiration assembly used for inoculation of virus under LFH (laminar air flow hood) which

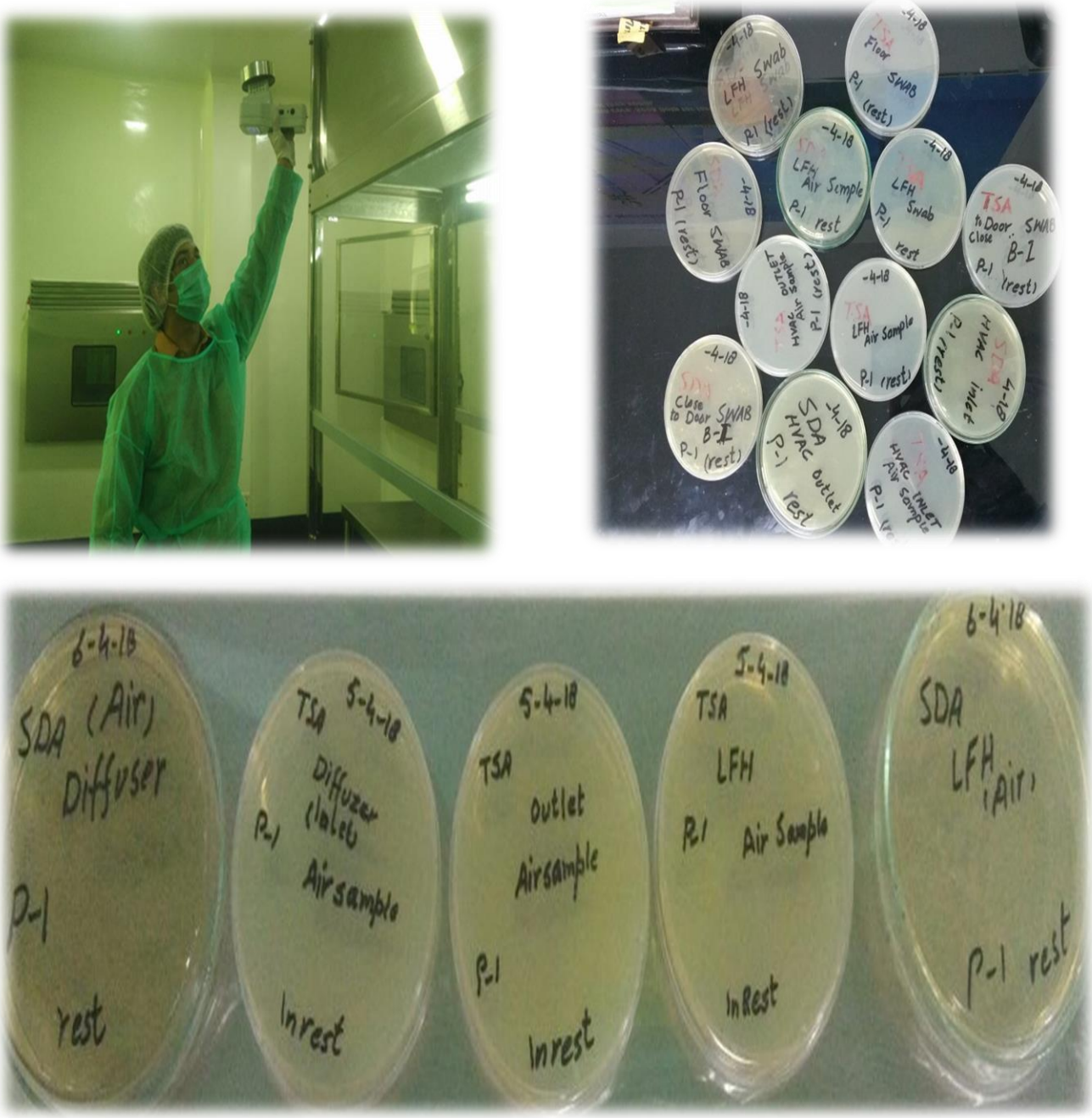
deemed class A of WHO cleanroom standards. Seven hundreds liters per cubic meter of air was collected from closer to the door, HVAC inlet and outlets those were considered as cleanroom class B. Four samples were taken from each sampling locations. Plates were incubated at 30 to 35˚C for 2 days followed by at 20 to 25˚C for 7 days and results were recorded.

Passive air sampling

4 pre-incubated media plates were placed in each corner and one plate was exposed in the center of each sampling site for 4 hours at rest state and exposed for full working time at operation state. TSA containing plates were incubated at 30-35˚C for 2-5 days and SDA plates were incubated at 20-25˚C for 3-7 days and results were recorded (Figures 3-6).

Figure 3: Passive air sampling.





Figures 4-6: Samples collection from production section I.

Non-viable counts

Same sample location plan includes critical and non-critical sites were adopted for Non-viable particle counting. Non-viable particle counts were measured by CEM ® particle counter, (Model DT-9881M) with regarding to 0.5 µm and 5.0 µm size of particles from 5 sample sites at rest and operation states and results were recorded from its digital screen.

Surface sampling (production section I)

Total area of the room was 19 square meters which was divided into 10 area units. 5 sample sites were randomly selected in each unit of 1 m^2 area for sample collection as shown in Table 4 Four surface swab samples were collected from each sample site and results are recorded (Figure 7).

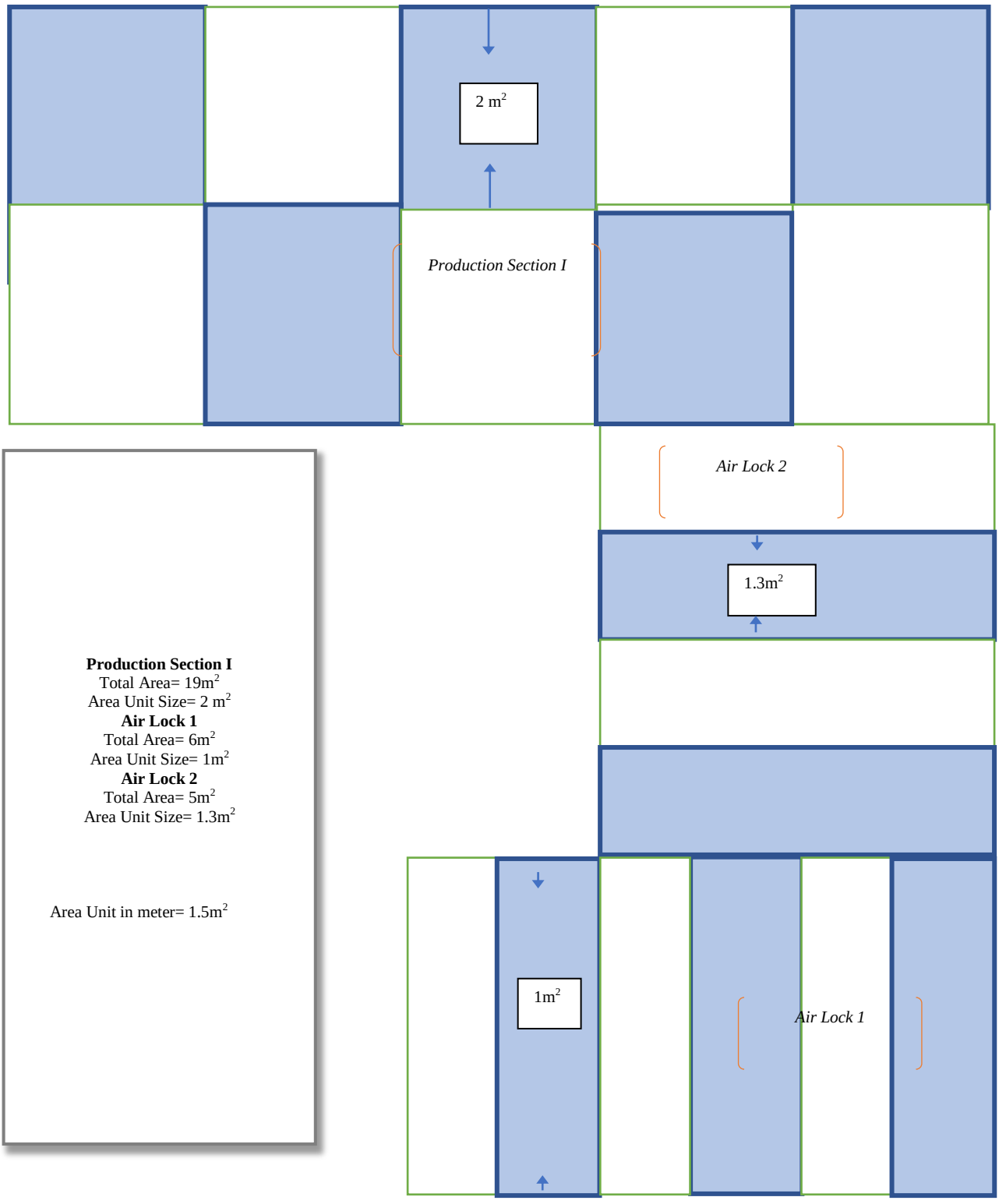

2 m²
Production Section I
Air Lock 2
1.3m²
1m²
Air Lock 1
Production Section I
Total Area= 19m²
Area Unit Size= 2 m²
Air Lock 1
Total Area= 6m²
Area Unit Size= 1m²
Air Lock 2
Total Area= 5m²
Area Unit Size= 1.3m²
Area Unit in meter= 1.5m²

Figure 7: Gridline mapping of production section II and adjacent air lock I and air lock II.

Air lock I (P-1)

Air lock-I was assessed by environmental air and surface monitoring.

Air monitoring

Air was monitored by counting the viable and non-viable counts inside the air lock.

Viable counts

Total of 3 sample sites were selected in which 2 critical sites were identified including each single site along with HVAC inlet and outlet. Area closed to the door was marked as non-critical site. Seven hundreds liters air per cubic was collected from each sample site and results were recorded. Five pre-incubated plates were placed in each sampling sites for 4 hours at rest state and exposed for full working time at operation state for passive air sampling and results were recorded.

Non-viable counts

Non-viable particle counts was measured by particle counter from 3 sample sites including critical and non-critical sites in rest and operation states and results were recorded.

Surface monitoring

Total area of air lock I was 6 square meters which was divided into 6 area units. 3 sample locations were marked randomly having each unit was 1 square meters area as shown in Table 3. Four samples were collected from each sample site and results are recorded.

Air locks II (P-1)

It was assessed by environmental air and surface monitoring.

Air monitoring

Air was monitored by counting the viable and non-viable counts inside the air lock.

Viable counts

Total of 2 sample sites were selected including each single site along with HVAC inlet and outlet. Seven hundreds liters air per cubic was collected from each sample site and results were recorded.

Five pre-incubated plates were placed in each sampling sites for 4 hours at rest state and exposed for full working time at operation state for passive air sampling and results were recorded.

Non-viable counts

Non-viable particle counts was measured by particle counter from 2 sample sites including critical and non-critical sites in rest and operation states and results were recorded.

Surface monitoring

Total of 2 sample location were obtained by separating the total area of 5 square meters into 4 gridlines having each unit was 1.3 square meters area as shown in Table 3. Four samples were collected from each sample site and results were recorded.

Production section II

The total area of the room was 29 m^2 which was divided into 14 gridlines having air pressure of 0.20 inches of water column inside the room and seemed with three buffers I, II and III having positive air pressure as shown in Figures 1-3.

Selected critical and non-critical sites

Total of 7 sample sites were chosen in Production Section I in which 5 critical sites were identified including each single site along with HVAC inlet and outlet, Vial Transportation Trolley, Homogenizer and 2 sites along with filling line. These sites were declared as critical sites after visit of researcher and management. 2 Non-critical sites were marked including each site along with Transfer bay and filling area door in Production Section II. Air lock I, II and III were also considered as two additional sites including into the critical sites for Production Section II as shown in Figure 8.

Air monitoring

Environmental air was monitored by the estimating viable and non-viable particle counts in Production Section II.

Viable counts

Viable particle counts were sampled by two methods:

Active air sampling

Thousand liters per cubic meter of air was aspirated by MAS® air sampler (model 007) from each sample site as recommended volume for class A of WHO cleanroom standards. Four samples were taken from each sampling locations. The bacterial plates were incubated at 30-35˚C for 2-5 days as compared to mold and fungal plates were incubated at 20-25˚C for 3-7 days.

Passive air sampling

4 pre-incubated media plates were placed in each corner and one plate was exposed in the center of each sampling site for 4 hours at rest state and exposed for full working time at operation state. TSA containing plates were incubated at 30-35˚C for 2-5 days and SDA plates were incubated at 20-25˚C for 3-7 days and results were recorded (Figures 9-12).

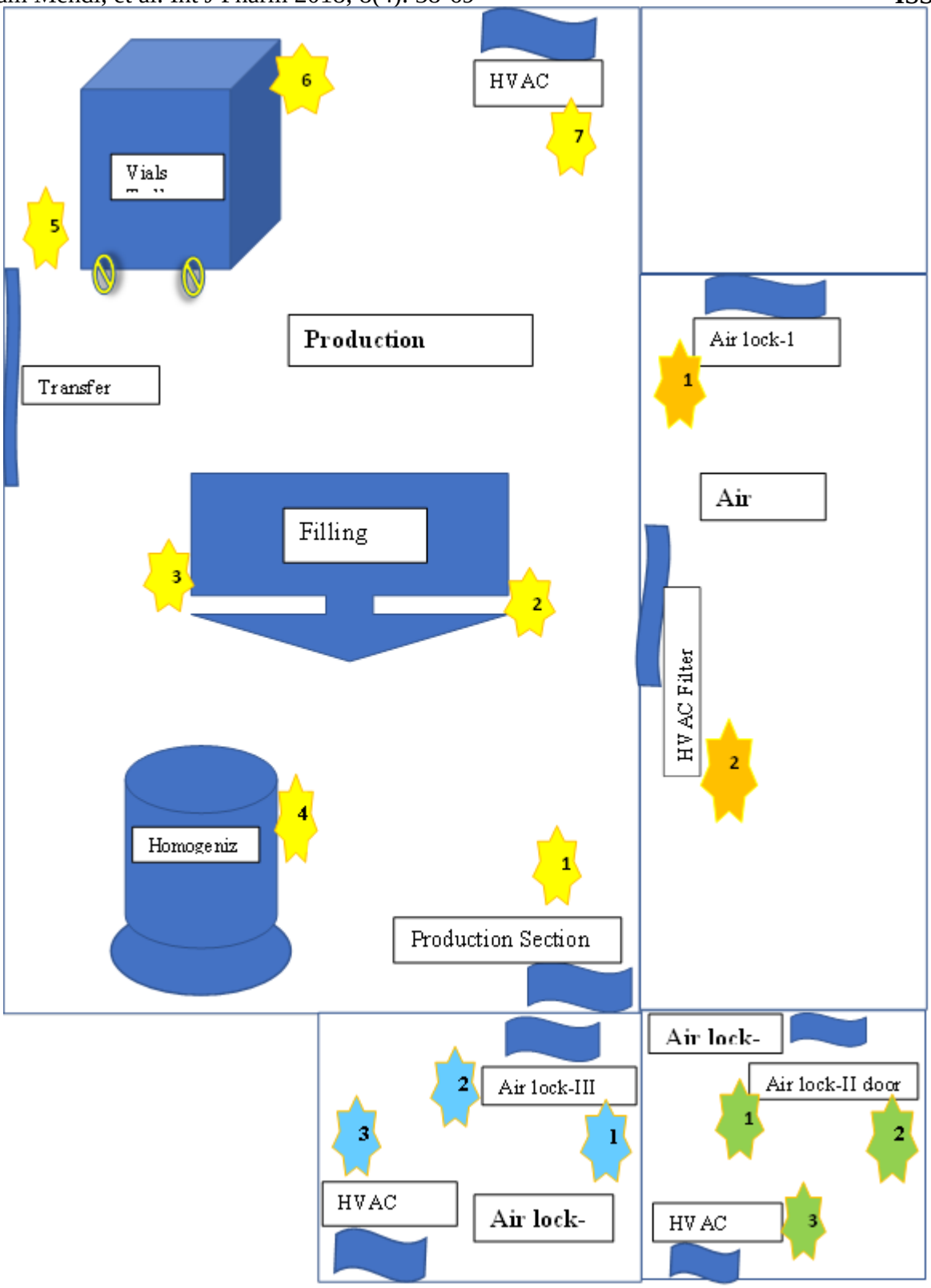


Figure 8: Selected critical and non-critical areas for sampling in production section II.

Figures 9-12: Sample collection from production section II.

Non-viable counts

Same sample location plan includes critical and non-critical sites were used for Non-viable particle counting the size of 0.5 um and 5.0 um. Non-viable particle counts were measured by CEM ® particle counter, (Model DT-9881M) from 7 sample sites at rest and in operation states and results were recorded from its digital screen.

Surface sampling (Production section II)

Total area of the room was 29 square meters which was divided into 14 area units. 7 sample sites were randomly selected in each unit of 2 m^2 area for sample collection as shown in Table 3. Four surface samples were collected from each sample site and results are recorded (Figure 13).

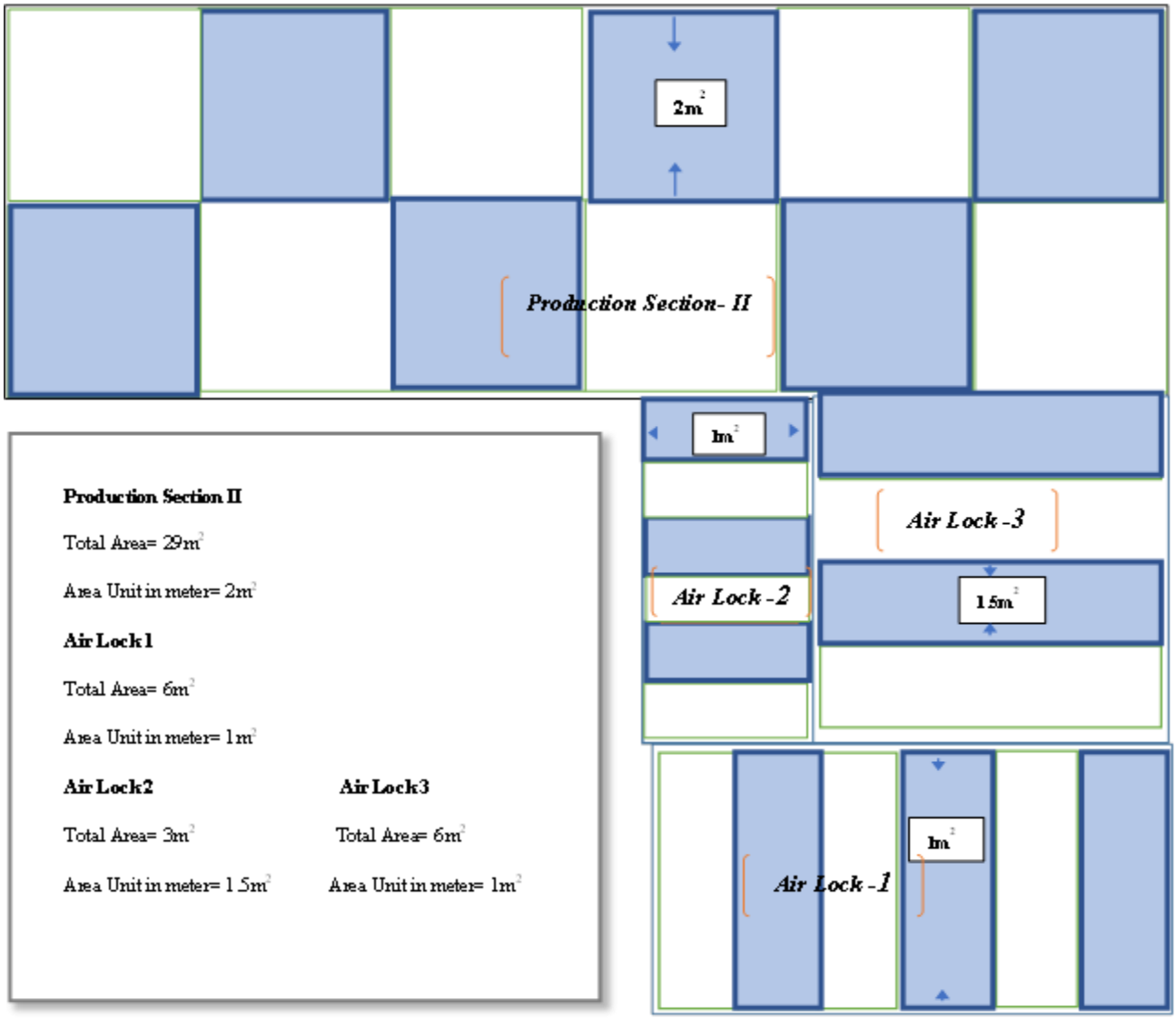


Figure 13: Gridline map of productions II and its air lock I and air lock II.

Air lock-I (P-II)

Air lock-I was assessed by environmental air and surface monitoring.

Air monitoring

Air was monitored by counting the viable and non-viable counts inside the air lock.

Viable counts

Total of 3 sample sites were selected in which 2 critical sites were identified including each single site along with HVAC inlet and outlet. Area closed to the door was marked as non-critical site. Four samples were collected from each sample site and results were recorded. Five pre-incubated plates were

placed in each sampling sites for 4 hours at rest state and exposed for full working time at operation state for passive air sampling and results were recorded.

Non-viable counts

Non-viable particle counts was measured by particle counter from 3 sample sites including critical and non-critical sites in rest and operation states and results were recorded as shown in Table 4.

Surface monitoring

Total area of air lock I was 6 square meters which was divided into 6 area units. 3 sample locations were marked randomly having each unit was 1 square meters area as shown in Table 3. Four samples were collected from each sample site and results are recorded.

Air lock-II (p-II)

Air lock-I was assessed by environmental air and surface monitoring.

Air monitoring

Air was monitored by counting the viable and non-viable counts inside the air lock.

Viable counts

Total of 3 sample sites were selected in which 2 critical sites were identified including each single site along with HVAC inlet and outlet. Area closed to the door was marked as non-critical site. Four samples were collected from each sample site and results were recorded. Five pre-incubated plates were placed in each sampling sites for 4 hours at rest state and exposed for full working time at operation state for passive air sampling and results were recorded.

Non-viable counts

Non-viable particle counts was measured by particle counter from 3 sample sites including critical and non-critical sites in rest and operation states and results were recorded.

Surface monitoring

Total area of air lock I was 6 square meters which was divided into 6 area units. 3 sample locations were marked randomly having each unit was 1 square meters area as shown in Table 3. Four samples were collected from each sample site and results were recorded.

Air lock-III (P-II)

Air lock-II was assessed by environmental air and surface monitoring.

Air monitoring

Air was monitored by counting the viable and non-viable counts inside the Air Lock.

Viable counts

Total of 2 sample sites were selected including each single site along with HVAC inlet and outlet. Four samples were collected from each sample site and results were recorded. Five pre-incubated plates were placed in each sampling sites for 4 hours at rest state and exposed for full working time at operation state for passive air sampling and results were recorded.

Non-viable counts

Non-viable particle counts was measured by particle counter from 2 sample sites including critical and non-critical sites in rest and operation states and results were recorded.

Surface monitoring

Total of 1 sample location was obtained by separating the total area of 3 square meters into 2 gridlines having each unit was 1.5 square meters area as shown in Table 3. Four samples were collected from each sample site and results are recorded.

2.7.

The research was strategized to assess the microbiological risk for environmental monitoring of cleanroom to pertain the product quality. It was assessed by two parameters which are at highly risks as below;

1. Personnel
2. Differential positive pressure

Personnel

Personnel (operators) worked in all selected sections of vaccine manufacturing unit were screened and each of them was evaluated by finger DAB test. All the fingers including thumb of personnel's gloves were gently imprinted and impression of all these workers were obtained on labeled perti plates containing Tryptic Soya Agar (TSA). All the plates were incubated for at 37°C for 48 hours and results were recorded.

Differential positive pressure

The differential positive pressure in air lock I, II and III in comparison with Production Section II (Aseptic area) was monitored through manometer, Brand Name Dwyer™ (Model, Inclined-Vertical Scale Mark II-25) in inches of water and readings were noted. Similarly, air lock I and air lock II of Production Section I were also assessed positive pressure comparative to adjacent area with the help of manometer and

readings were recorded.

RESULTS

Non-viable count

In classified area, airborne particles the size of 0.5 µm per cubic meter dispersed from sampling sites including Filling line, Homogenizer, Vial Trolley, Aspiration Assembly and Production Section II Room which are considered as cleanroom class A were ranged from 14 to 40 at rest state and from 24 to 92 at operation state. Similarly, the number of airborne particles 5.0 µm at rest state of all sites which deemed as class A ranged from 1 to 7 and from 9 to 20 at operation state during environmental monitoring assessment as shown in Figures 14-16.

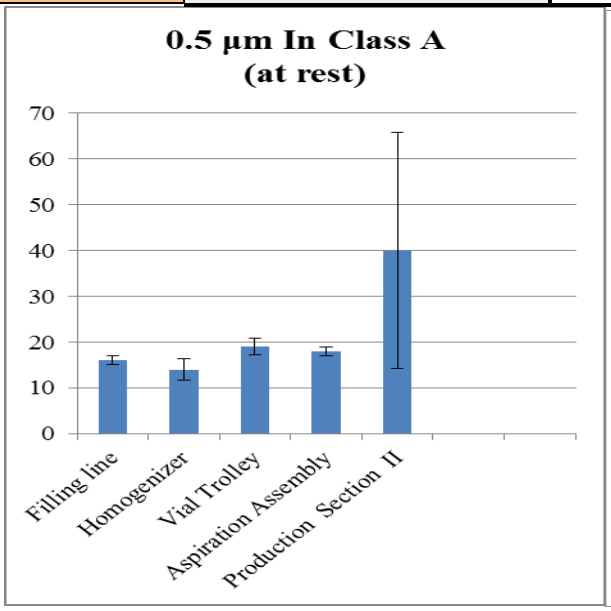
Particles count the size of 0.5 µm collected from sampling sites including Air Lock-III of Production Section II and Production Section I Room which considered as cleanrooms class B were ranged from 45 to 46 at rest state and from 110 to 119 at operation state. Similarly, the number of airborne particles 5.0 µm at rest state of sites proposed as class B ranged from 16 to 17 and from 20 to 21 at operation state as displayed in Figures 17-20.

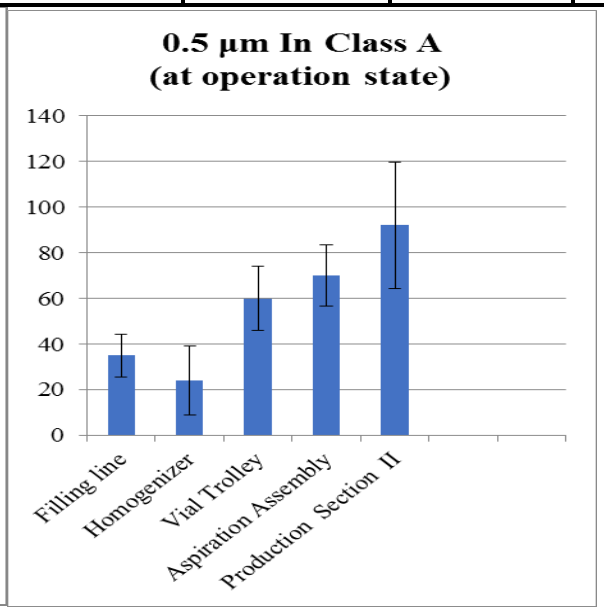
Figures 14-16: Results particles count from sampling sites of classified areas.

Environmental particles quantification of 0.5 µm sized particles in all sampling sites including each Second (II) Air Lock of classified areas deemed as class C of cleanrooms distributed at rest state ranged from 110 to 121 and from 234 to 267 at operation state. Similarly, the number of airborne particles 5.0 µm at rest state of all sites considered as class C ranged from 31 to 34 and from 67 to 78 at operation state during environmental monitoring assessment. Particles the size of 0.5 µm dispersed at rest state of all sampling sites including first (I) Air Locks of cleanrooms considered class D ranged from 198 to 219 and from 124 to 434 at operation state. Similarly, the number of airborne particles 5.0 µm at rest state of all classes D ranged from 123 to 194 and from 298 to 354 at operation state as shown in Table 4 (Figures 21 and 22).

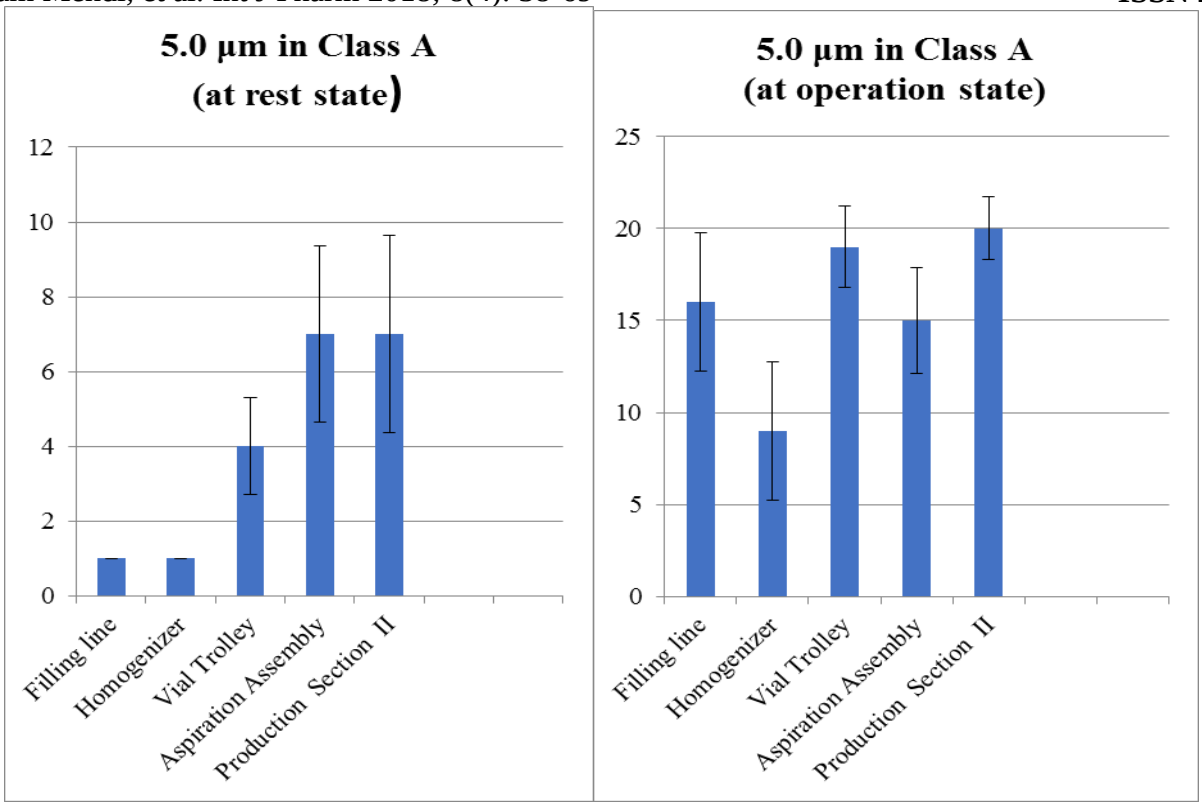
Table 4: Non-viable counts of environmental monitoring in each sampling sites.

Classification of Area	Sample locations	Non-viable counts (particles /m^3)			
Class	Sites	0.5 µm		5.0 µm	
		At Rest	At Operation	At Rest	At Operation
A (under the LFH)	Filling line	16	35	1	16
	Homogenizer	14	24	1	9
	Vial Trolley	19	60	4	19
	Aspiration Assembly	18	70	7	15
	Production Section II	40	92	7	20
B	Production Section I	46	119	17	21
	Air Lock-III (P-2)	45	110	16	20
C	Air Lock-II (P-1)	110	234	31	67
	Air Lock-II (P-2)	121	265	34	78
D	Air Lock-I (P-1)	219	434	161	354
	Air Lock-I (P-2)	198	124	123	298

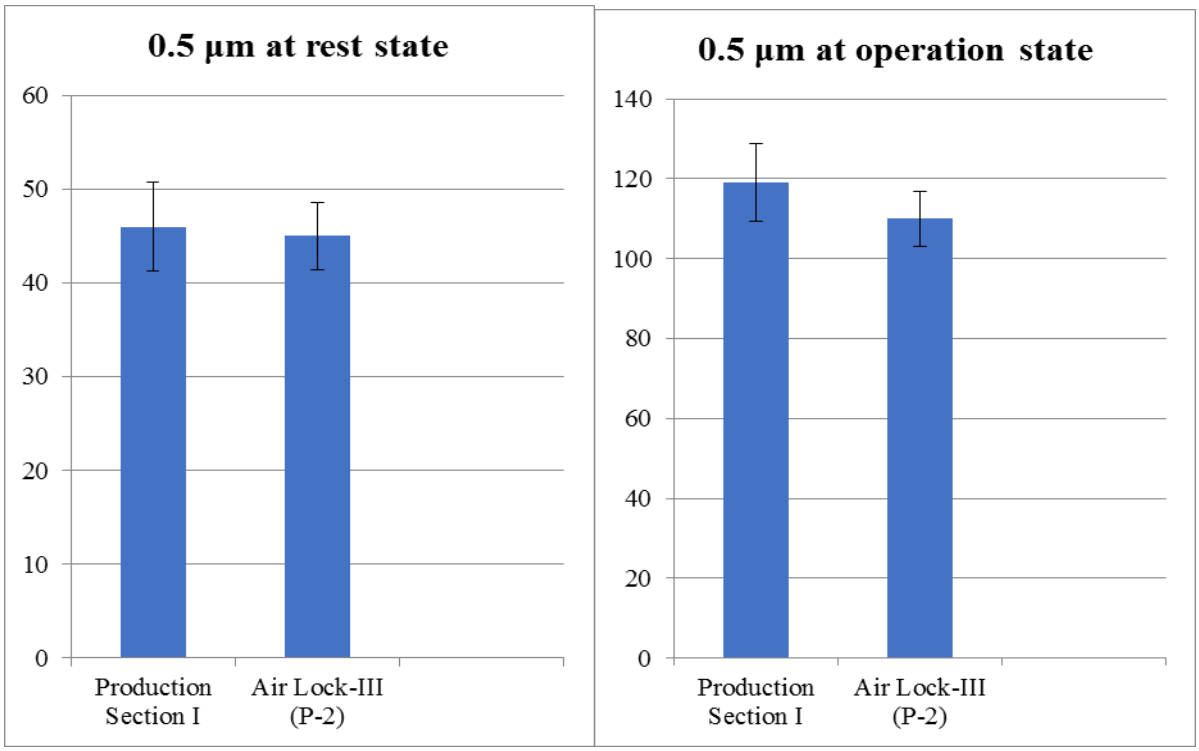




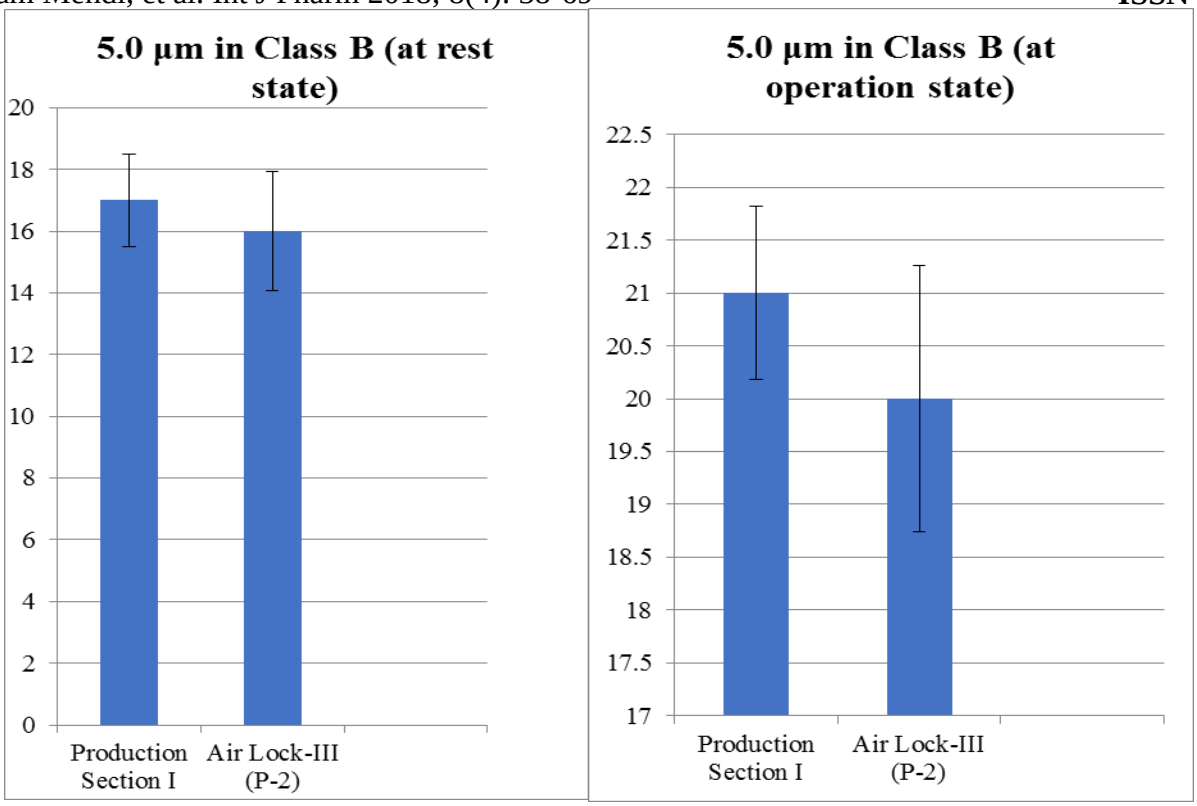
Figures 17 and 18: 0.5 particles quantification in class A at rest and operation state.



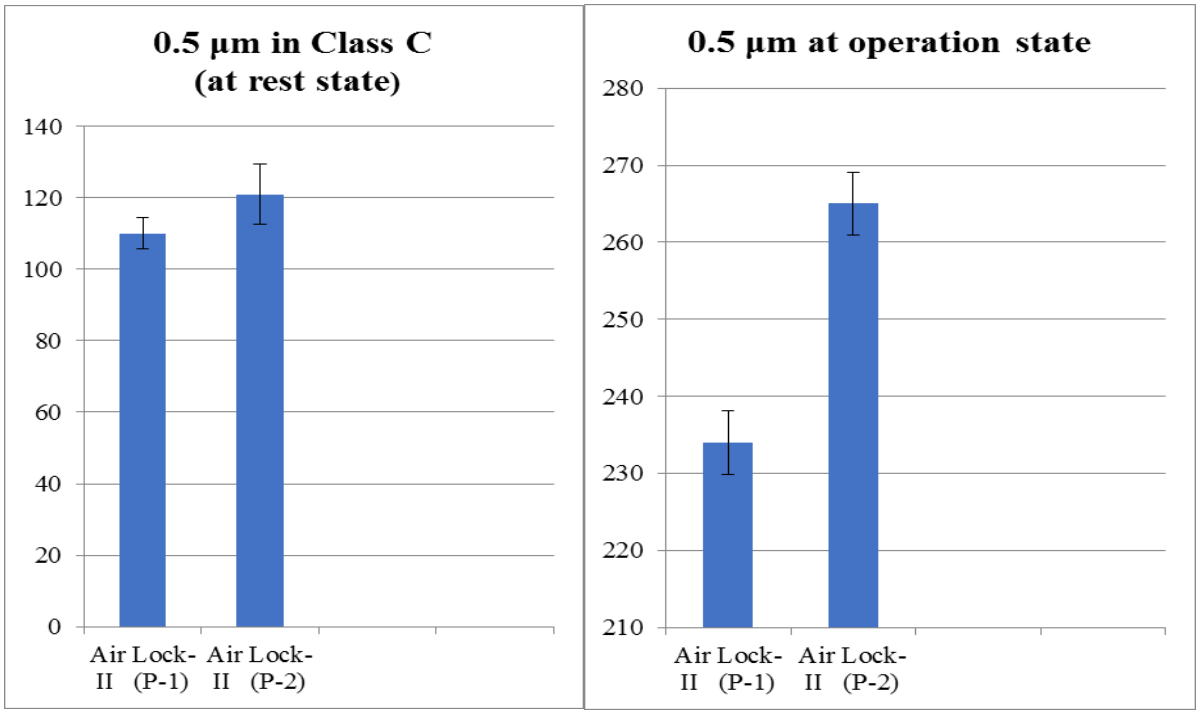
Figures 19 and 20: 5.0 particles quantification in class A at rest and operation state.



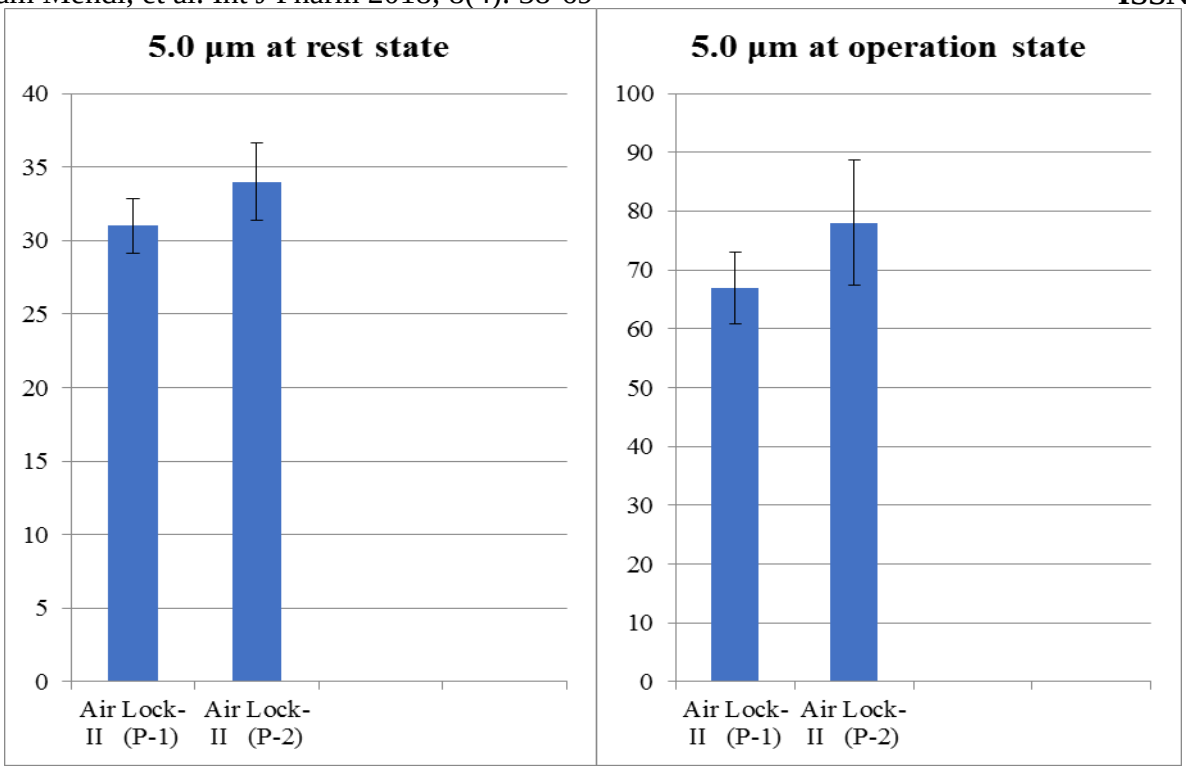
Figures 21 and 22: 0.5 particles quantification in class B at rest and operation state.



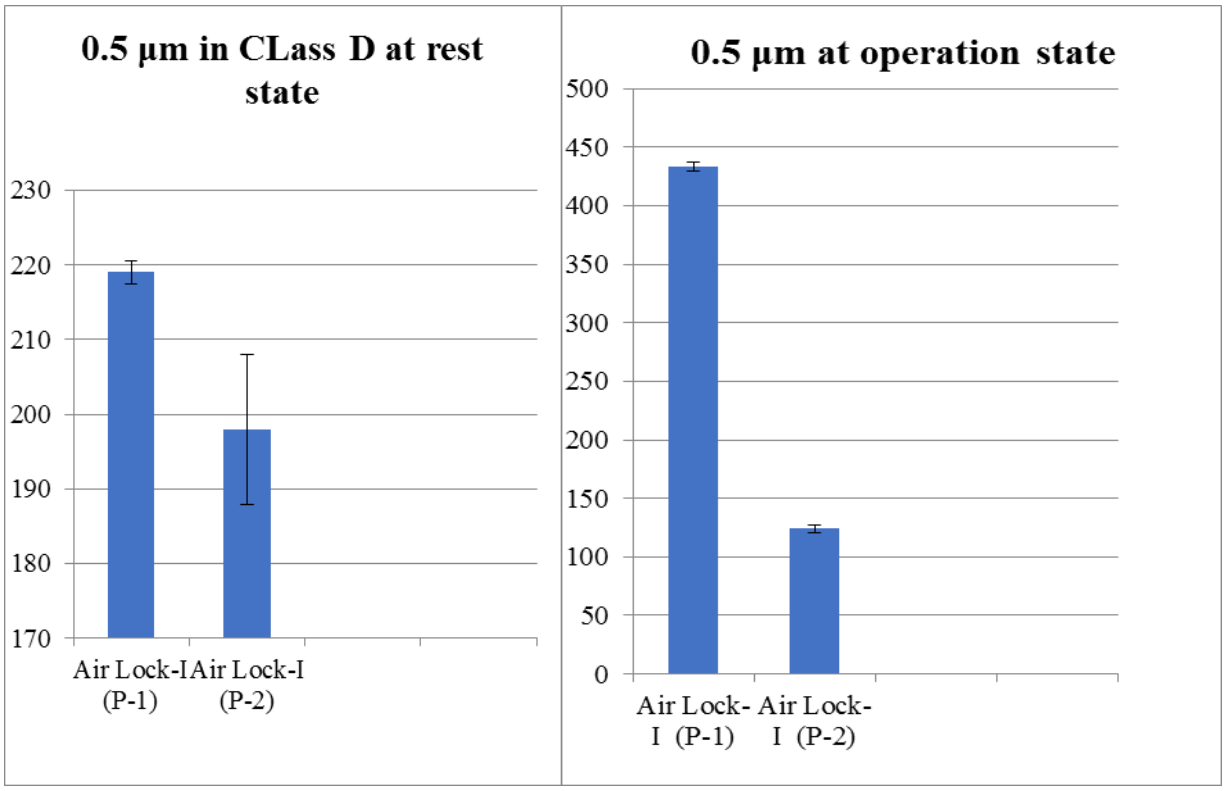
Figures 23 and 24: 5.0 particles quantification in class B at rest and operation state.



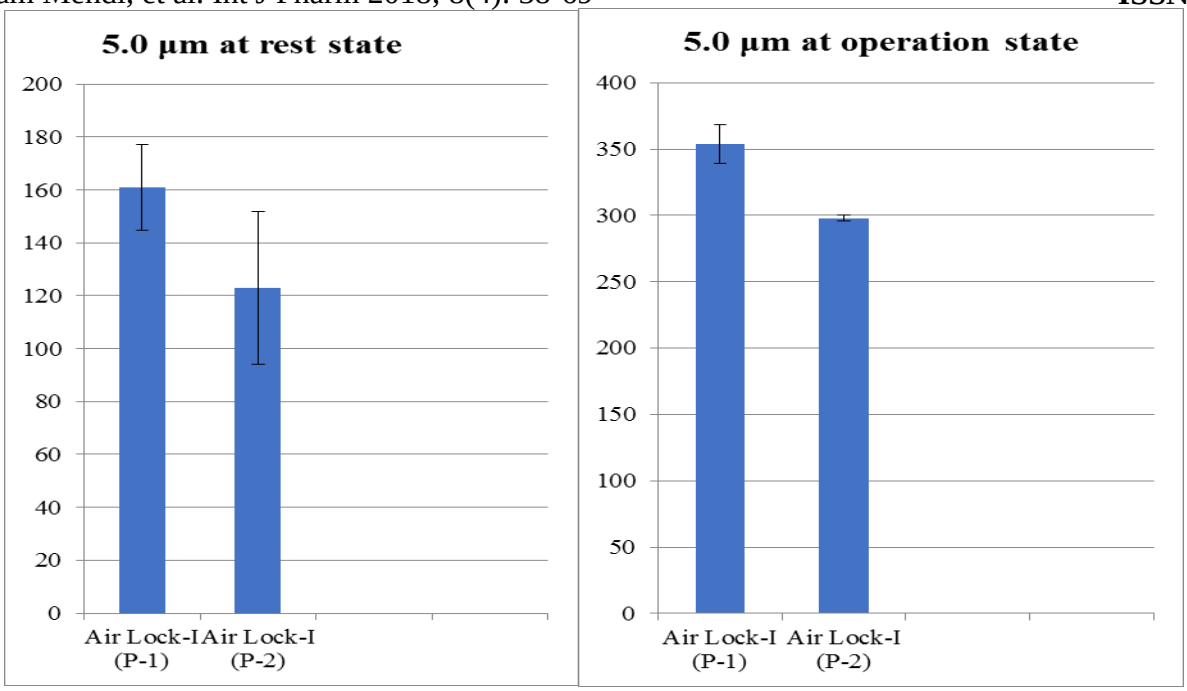
Figures 25 and 26: 0.5 particles quantification in class C at rest and operation state.



Figures 27 and 28: 5.0 particles quantification in class C at rest and operation state.



Figures 29 and 30: 0.5 particles quantification in class D at rest and operation state.



Figures 31 and 32: 5.0 particles quantification in class D at rest and operation state.

Viable count

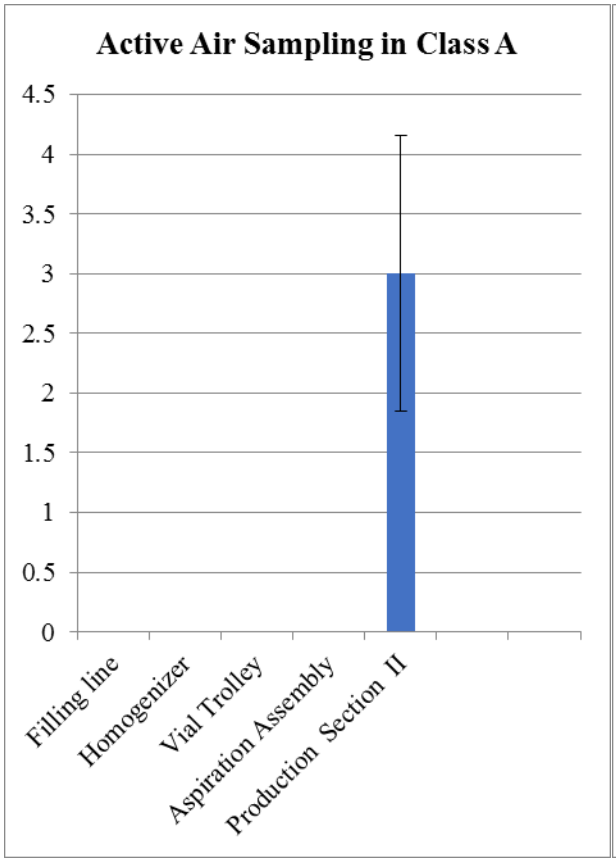
Sampling sites including Filling line, Homogenizer, Vial Trolley, Aspiration Assembly and Production Section II Room which are considered as Cleanroom Class A expressed less than one mean colony forming unit per cubic meter (CFU /m^3) in both air sampler technique and through settle plate method. In surface sampling by swabbing method, all sampling sites declared as class A showed zero colonies forming unit per cubic meter (CFU /m^3) as shown in Figures 23-42.

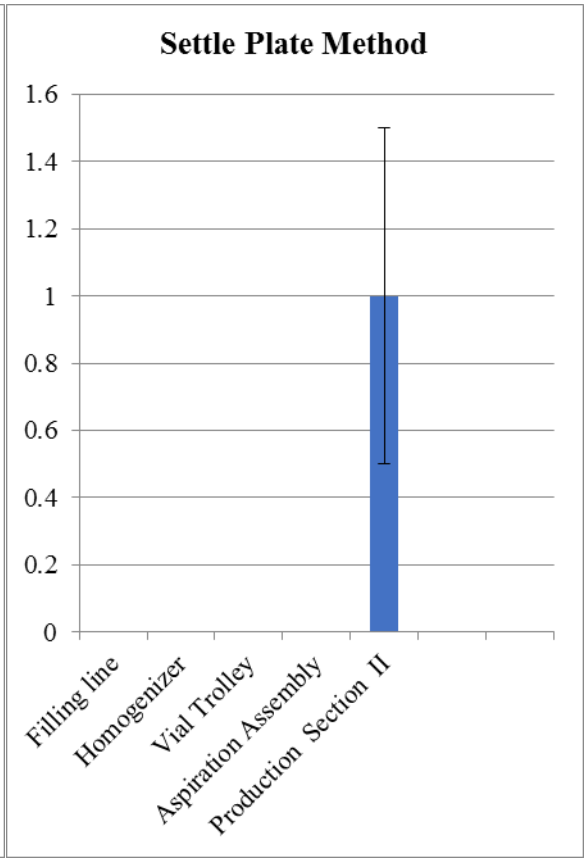
All sampling sites which deemed as Cleanroom Class B showed 5 to 9 mean colony forming unit per cubic meter (CFU /m^3) through air sampler technique and 2 to 5 (CFU /m^3) by settle plate method. The mean colony forming units per cubic meter (CFU /m^3) of all classes B of cleanrooms were obtained ranged from 6 to 11 in surface sampling as displayed in Figure 43. All sampling sites proposed as Cleanrooms Class C expressed ranging from 9 to 13 colony forming unit per cubic meter (CFU /m^3) through air sampler technique and 5 to 7 (CFU /m^3) through settle plate method as shown in Figure 44-48. The mean colony forming units per cubic meter (CFU /m^3) of all classes C of cleanrooms were obtained ranged from 11 to 15 in surface monitoring. All classes D showed 16 to 21 colony forming unit per cubic meter (CFU /m^3) through air sampling technique and 11 to 14 (CFU /m^3) by settle plate method. The mean colony forming units per cubic meter (CFU /m^3) of all classes D of cleanrooms were obtained ranged from 21 to 25 in surface monitoring as shown in Table 5.

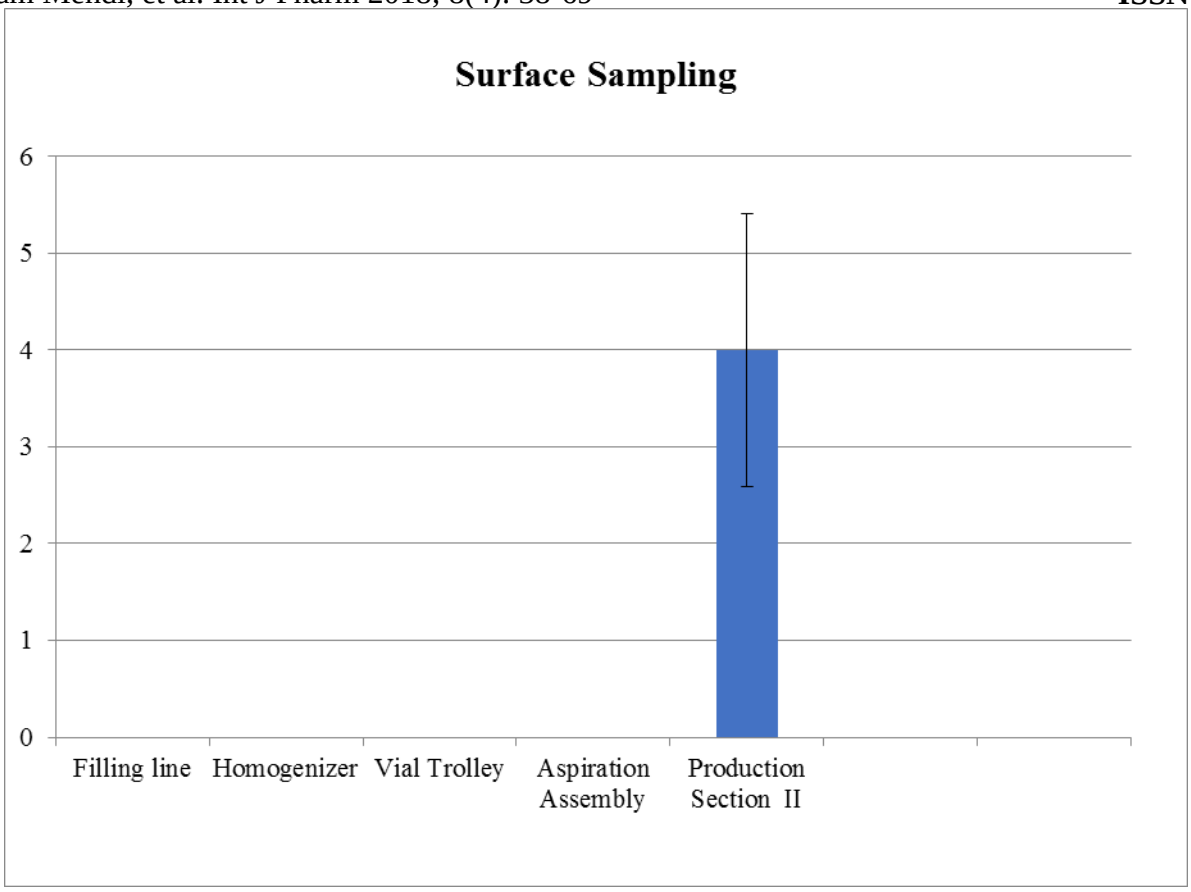
Figures 33-35: Viable results from sampling sites in classified areas.

Table 5: Viable counts of environmental monitoring in each sampling sites.

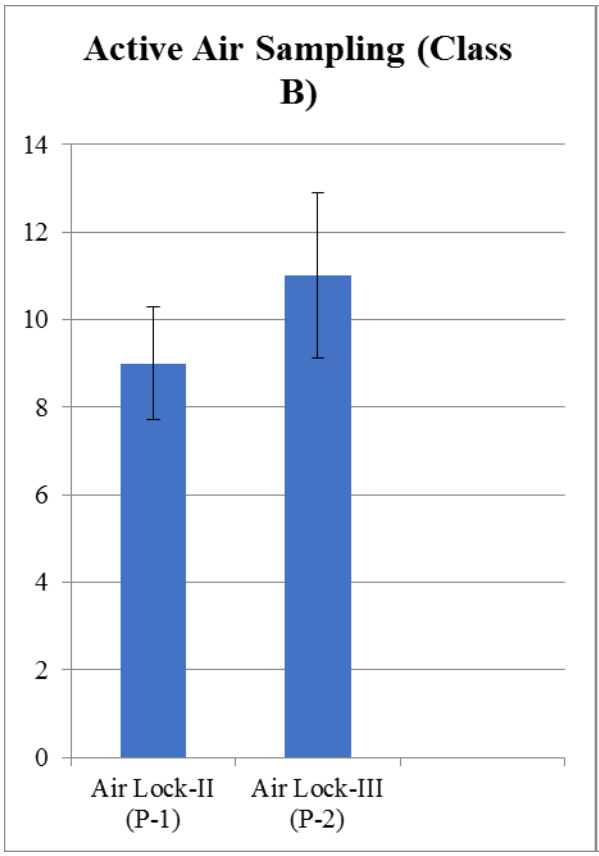
Area Classification	**Sample Locations**	**Viable Counts** (CFU /m^3)		
Class	Sites	Air Sampler	Settle Plate	Surface Sampling
A (under the LFH)	Filling line	0	0	0
	Homogenizer	0	0	0
	Vial Trolley	0	0	0
	Aspiration Assembly	0	0	0
	Production Section II	3	1	4
B	Production Section I	9	5	6
	Air Lock-III (P-1)	11	6	12
C	Air Lock-II (P-1)	11	6	12
	Air Lock-II (P-2)	13	7	15
D	Air Lock-I (P-1)	21	14	21
	Air Lock-I (P-2)	17	13	22

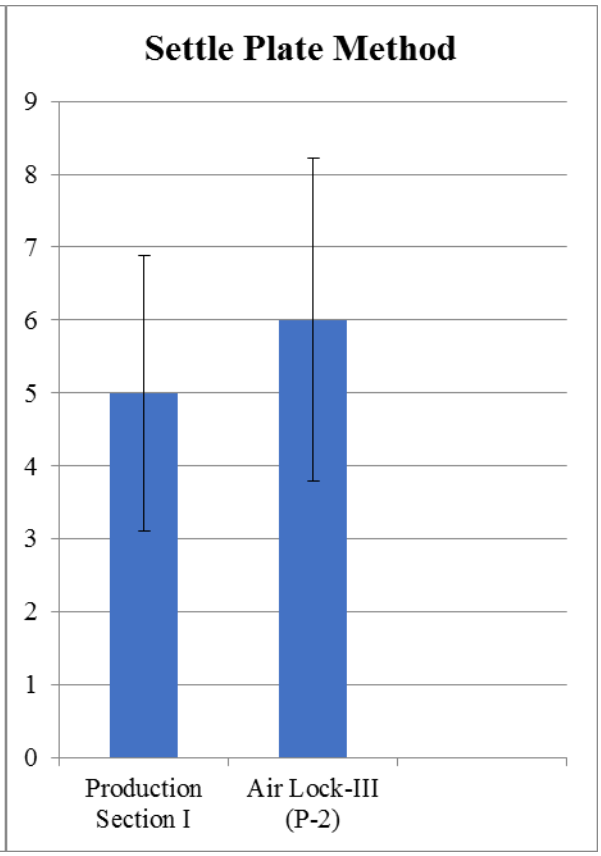


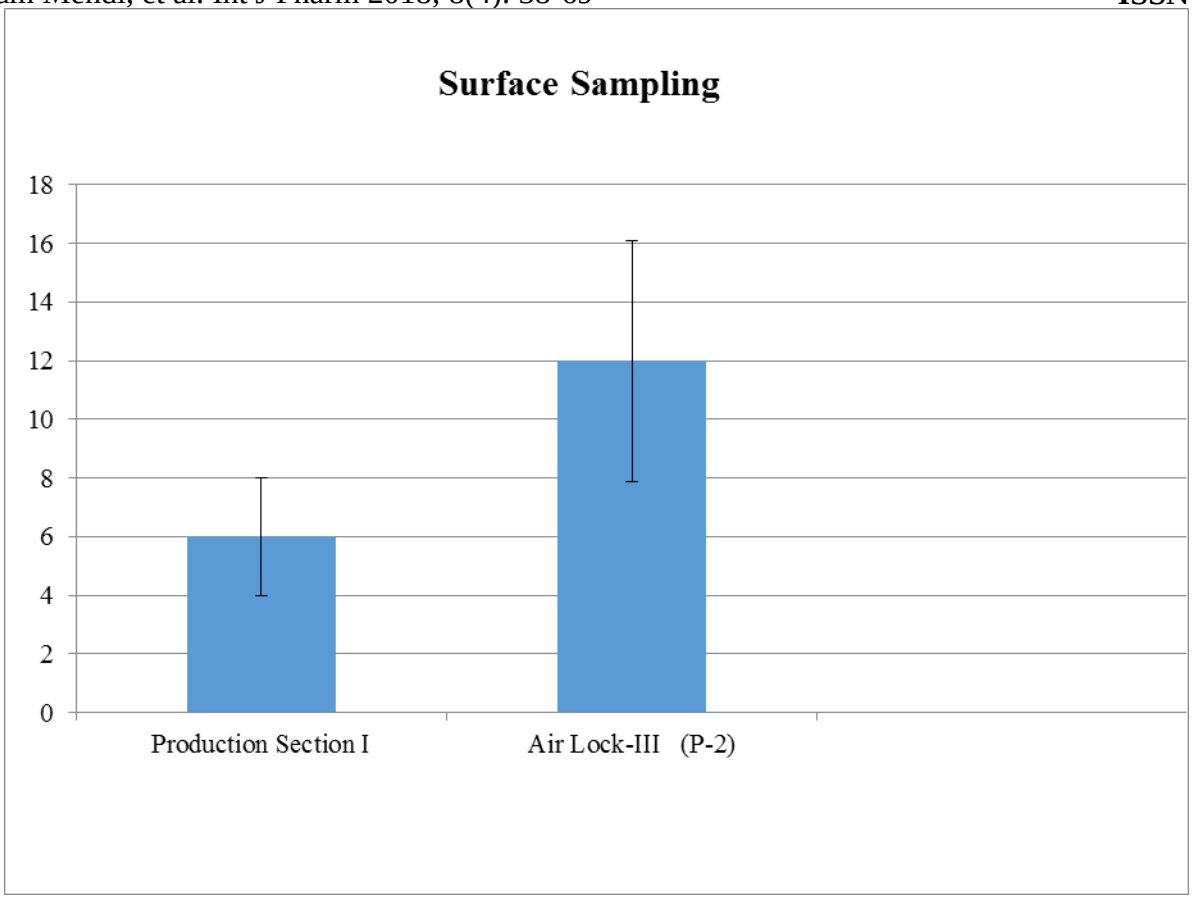




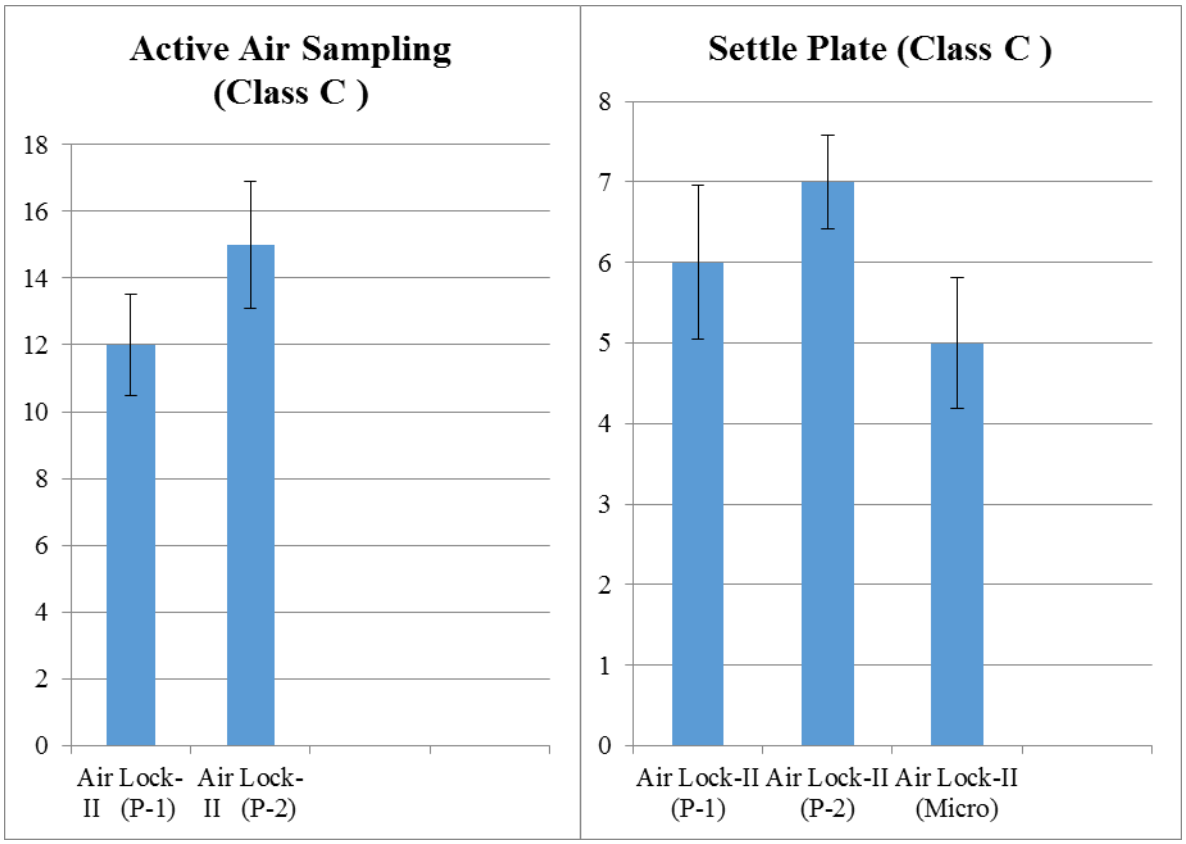
Figures 36-38: Viable counts in air and surface monitoring of class A.

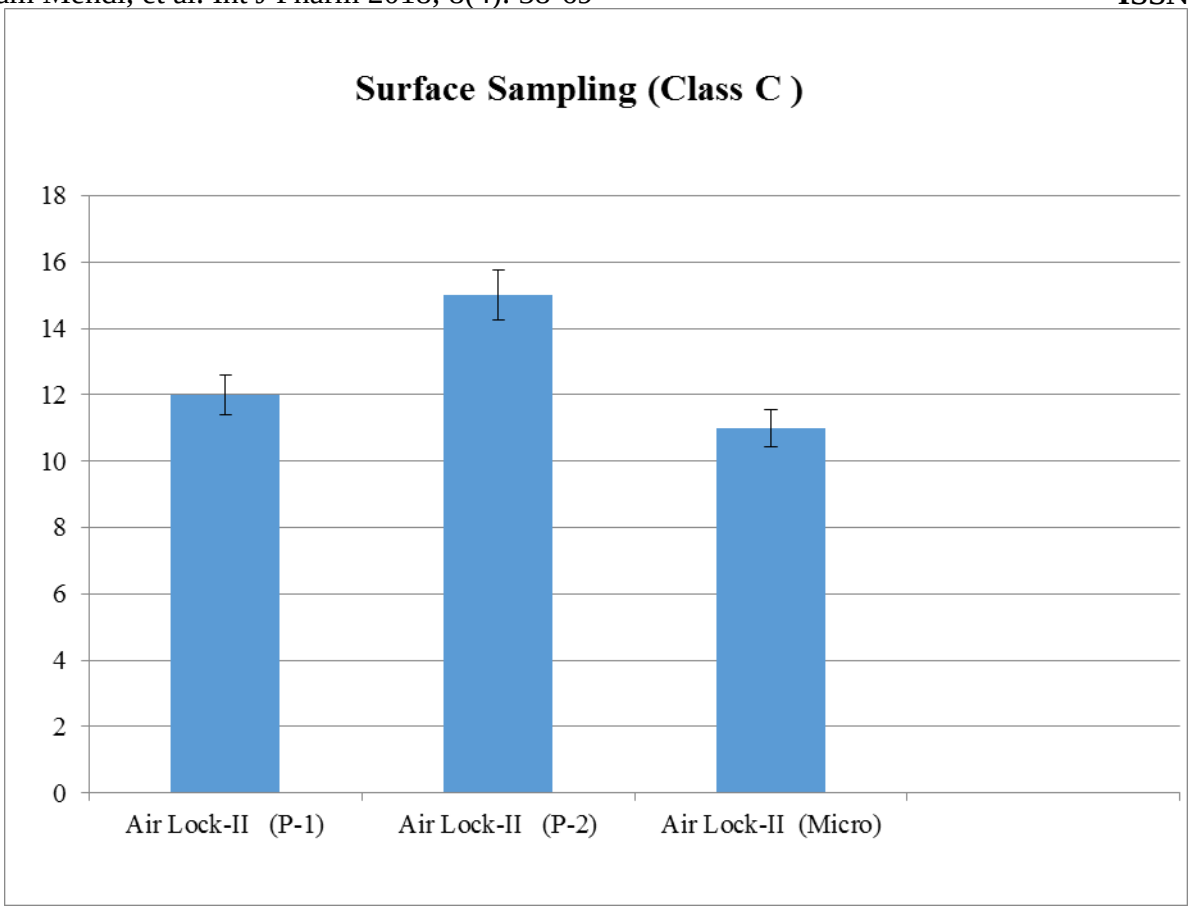




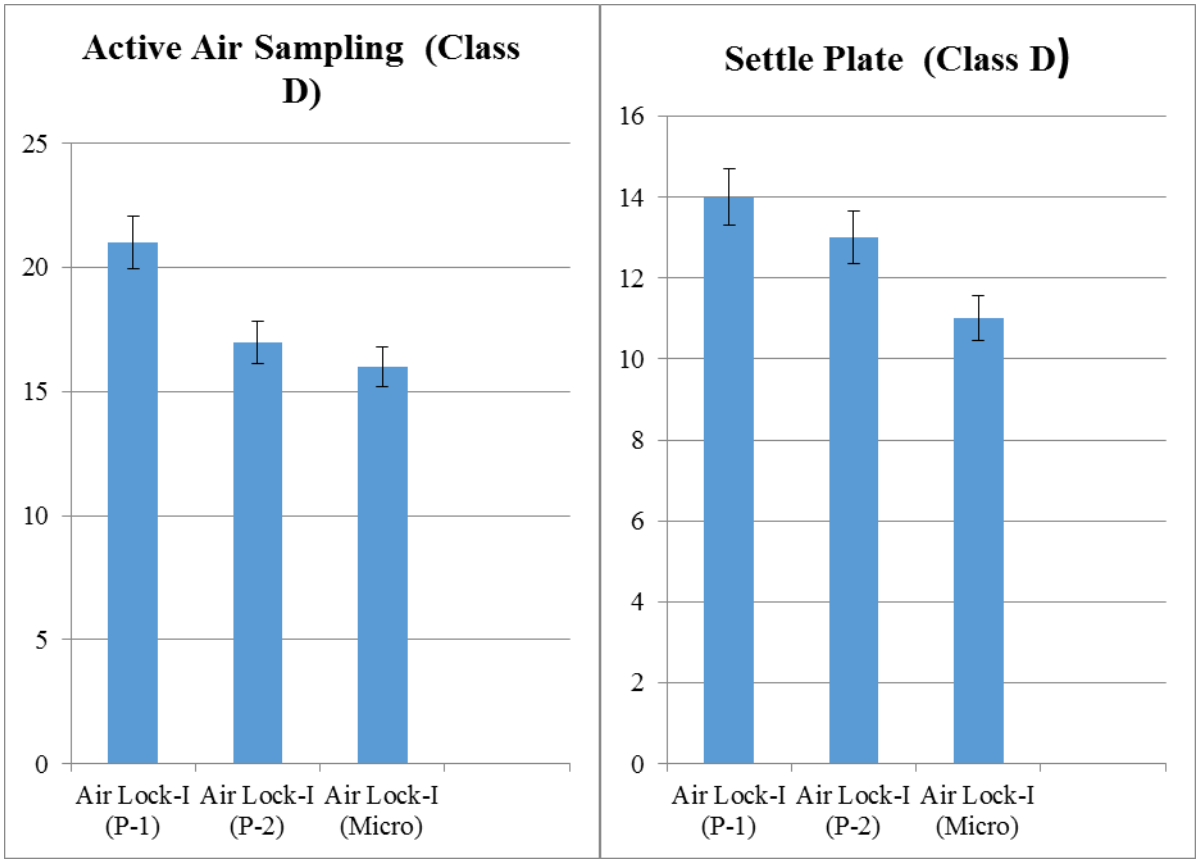


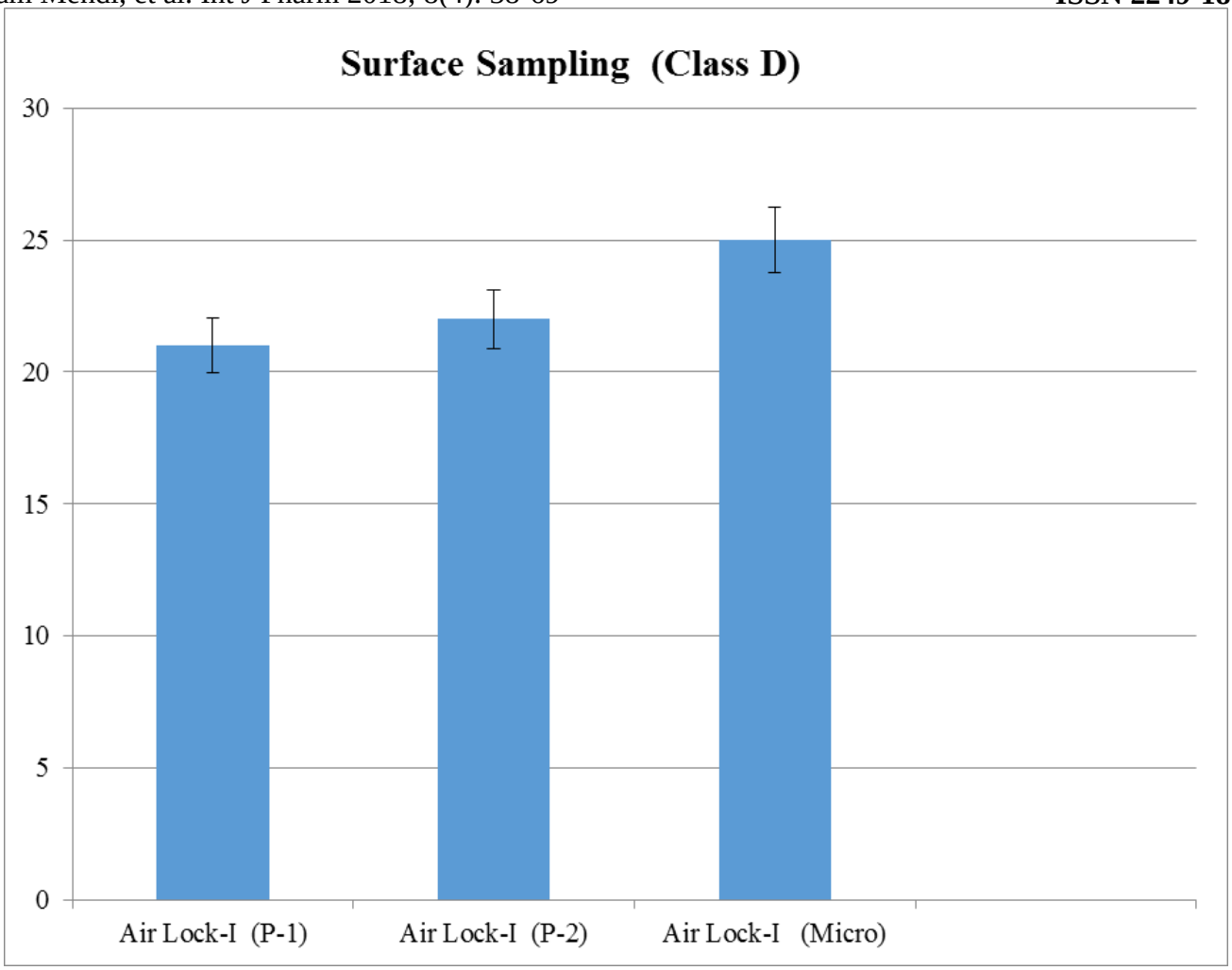
Figures 39-41: Viable counts in air and surface monitoring in class B.





Figures 42-44: Viable counts in air and surface monitoring of class C.





Figures 45-47: Viable counts in air and surface monitoring of class D.

Personnel monitoring

Total 43 personnel from different areas were included in finger DAB test for personnel monitoring. 11 workers from Production Section II, 9 from Production Section I, 7 from Microbiology Laboratory, 8 from quality control Laboratory, 5 from Incubation room and 3 from Quarantine room were involved in personnel monitoring. The average colony forming unit (CFU) of 11 workers in Production Section II was zero and of nine workers in Production Section I was 4 and of 7 workers from Microbiology laboratory was 3 and of 8 workers from quality control laboratory was 13 and of 5 workers from Incubation room was 20 and of 3 workers from Quarantine room was 19 in personnel monitoring as shown in Table 6.

Table 6: Personnel monitoring by finger DAB test in all sampling sites.

Working sites of personnel	Finger DAB test (5 fingers CFU/gloves)
Production section I	4
Production section II	0

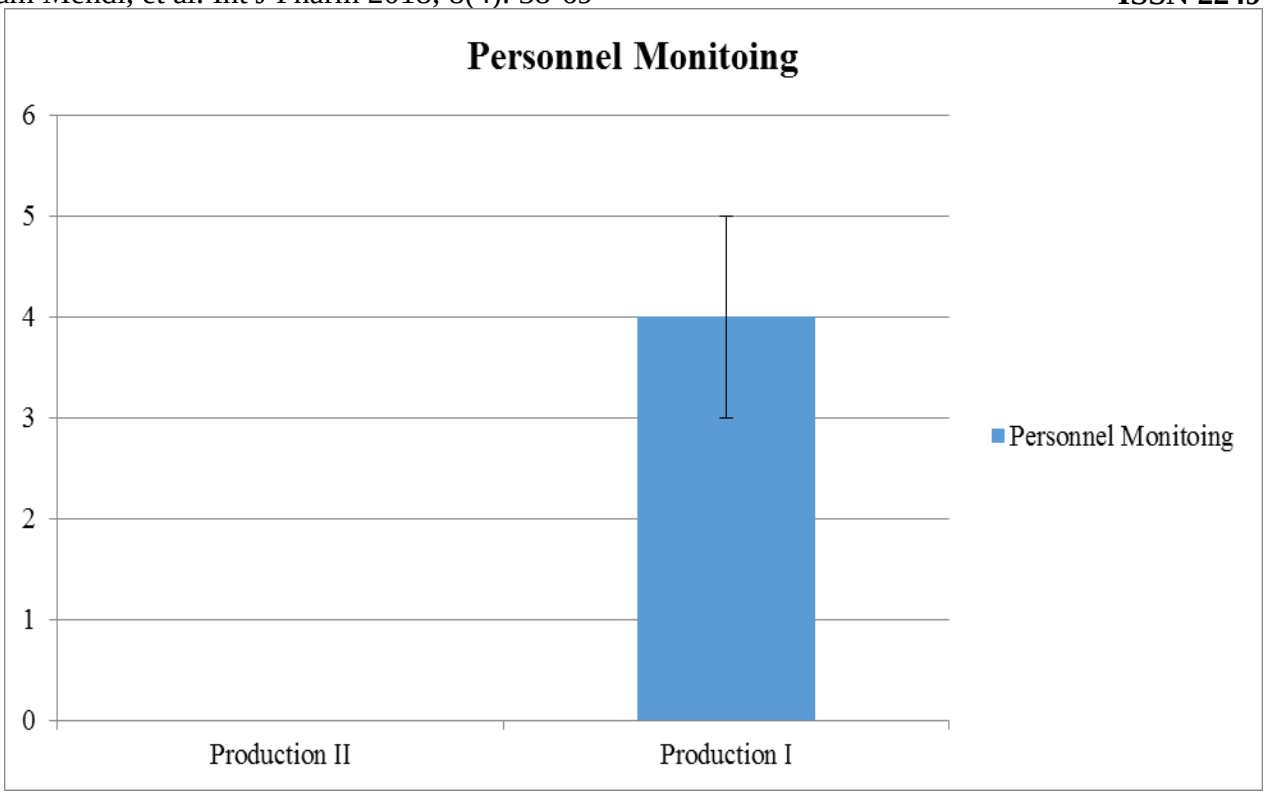


Figure 48: Personnel monitoring in each section of vaccine manufacturing plant.

3.4.

Differential air positive pressure

The differential air positive pressure Production Section II is 0.20 inches of water and Production Section I have 0.15 inches of water inside the room as shown in Table 7.

Table 7: Differential positive pressure among clean zones relative to adjacent area.

Sections	Differential positive pressure
Production section II	0.20
Air lock-I (P-2)	0.15
Air lock-II (P-2)	0.10
Air lock-III (P-2)	0.05
Production section I	0.15
Air lock-I (P-1)	0.10
Air lock-II (P-1)	0.05

DISCUSSION

This research has discussed the implications of Environmental monitoring in manufacturing quality pharmaceutical products. The quality of product depends on environmental controls in manufacturing area. As current research illustrated that an adequate environmental monitoring system requires to obtain a pharmaceutical product free of contamination which includes testing, identification and elimination of bioburden to ensure the quality of product in critical areas including clean-rooms for drug fill or finish, formulation rooms, clean zones, compounding areas and sterile packing. Futuristic aspect of current study is practical implementation of statistical sampling model to gain appropriate numbers of sampling locations by an imaginary boundary (grid) that splits total area of respective room into area units proposed by revised ISO 14644-1 for airborne particles quantification in cleanroom. Each sampling location is considered independently for viable and non-viable sample collection from air, surfaces and Personnel.

The current study revealed that airborne particles the size of 0.5 µm per cubic meter was observed at rest state were ranged from 11 to 40 from sampling sites including Filling line, Homogenizer, Vial Trolley, Aspiration Assembly, Production Section II Room and Bio Safety Cabinet placed in Microbiology Laboratory which are considered as cleanroom class A are in agreement with WHO which reported that the maximum number of permissible airborne particles the size of 0.5 µm per cubic meter at rest state are 3,520 in cleanroom class A [13]. Similarly, Sandle who narrated that 3,520 airborne particles the size of 0.5 µm per cubic meter are permitted at operational state in cleanroom class A which supported the outcomes of current study which were ranged from 23 to 92 at operation state with regarding particles the size of 0.5 µm per cubic meter in each sampling sites declared as class A of WHO cleanroom standard [14].

Similarly, the number of airborne particles the size 5.0 µm at rest state from all sampling sites which deemed as class A was ranged from 1 to 7 is harmonized with ISO which explained that the maximum number of airborne particles the size of 5.0 µm at rest state is 29 in ISO Cleanroom Class 5 which correspond to WHO Cleanroom Class A [15]. The data obtained in the current study with regarding to airborne particles the size 5.0 µm which was ranged from 8 to 20 at operation state from sampling sites declared as class A is in line with WHO which stated that 20 are the maximum acceptable number of particles the size of 5.0 µm at operation state in cleanroom Class A [13].

Correspondingly, The results data gained in the current study with respect to viable counts through Active air sampling technique was zero colony forming unit per cubic meter (CFU /m^3) in all sampling sites claimed as cleanroom class A coincides with ISO which stated that not a single colony forming unit per cubic meter (CFU /m^3) is allowed in ISO Cleanroom Class 5 which correspond to WHO Cleanroom Class A [15]. Likewise, the data obtained in the current study in aspect of viable count through settle plate method was zero colony forming unit per cubic meter CFU/m^3 is in line with WHO which informed that the minimum allowed number of colony forming unit per cubic meter CFU/m^3 is zero in WHO Cleanroom Class A [13]. In another study, the observation of PDA which reported that zero colony forming unit per cubic meter (CFU /m^3) is permissible with regarding to surface monitoring in Cleanroom Class A supports the outcomes of current study which showed zero colony forming unit per cubic meter (CFU /m^3) with regarding surface monitoring by swabbing method from sampling sites including Filling line, Homogenizer, Vial Trolley, Aspiration Assembly, Production Section II Room and Bio Safety Cabinet which are considered as Cleanroom Class A [16].

The current study discovered that airborne particles the size of 0.5 µm per cubic meter was observed at rest state were ranged from 38 to 46 from sampling sites including Air Lock-III of Production Section II, Production Section I and Microbiology Laboratory which are considered as cleanroom Class B are in agreement with WHO which reported that the maximum number of permissible airborne particles the size of 0.5 µm per cubic meter at rest state are 3,520 in cleanroom Class B [13]. Similarly, WHO which narrated that 352,000 airborne particles the size of 0.5 µm per cubic meter are permitted at operational state in cleanroom Class B which supported the outcomes of current study which were ranged from 81 to 119 at operation state with regarding particles the

size of 0.5 µm per cubic meter in each sampling sites declared as Class B of WHO cleanroom standard [8].

Likewise, the number of airborne particles the size 5.0 µm at rest state from all sampling sites which deemed as Class B was ranged from 5 to 17 is harmonized with ISO which explained that the maximum number of airborne particles the size of 5.0 µm at rest state is 29 in ISO Cleanroom Class 6 which correspond to WHO Cleanroom Class B [15]. The data obtained in the current study with regarding to airborne particles the size 5.0 µm which was ranged from 11 to 21 at operation state from sampling sites declared as Class B is in line with WHO which stated that 2,900 are the maximum acceptable number of particles the size of 5.0 µm at operation state in cleanroom Class B [13].

Correspondingly, The results data gained in the current study with respect to viable counts through Active Air sampling technique was ranged from 5 to 9 colony forming unit per cubic meter (CFU/m^3) in all sampling sites claimed as cleanroom Class B coincides with ISO which stated that 10 colony forming unit per cubic meter (CFU /m^3) are allowed through Active Air sampling technique in ISO Cleanroom Class 6 which correspond to WHO Cleanroom Class B [15]. Likewise, The data obtained in the current study in aspect of viable count through settle plate method was ranged from 2 to 5 colony forming unit per cubic meter CFU/m^3 is in line with WHO which informed that the minimum allowed number of colony forming unit per cubic meter CFU/m^3 is 5 through settle plate method in WHO Cleanroom Class B [13]. In another study, the observation of PDA which reported that 5 colony forming unit per cubic meter (CFU /m^3) is permissible with regarding to surface monitoring in Cleanroom Class B supports the outcomes of current study which showed 5 colony forming unit per cubic meter (CFU /m^3) with regarding surface monitoring by swabbing method from sampling sites including Air Lock-III of Production Section II, Production Section I and Microbiology Laboratory which are considered as Cleanroom Class B [16].

The current study discovered that airborne particles the size of 0.5µm per cubic meter was observed at rest state were ranged from 91 to 121 from sampling sites including Air Lock-II of Production Section II, Air Lock-II of Production Section I and Air Lock-II of Microbiology Laboratory which are considered as cleanroom Class C are in agreement with WHO., 2011 which reported that the maximum number of permissible airborne particles the size of 0.5 µm per cubic meter at rest state are 352,000 in cleanroom Class C [13]. Similarly, WHO., 2003 which narrated that 3520,000 airborne particles the size of 0.5 µm per cubic meter are permitted at operational state in cleanroom Class C which supported the outcomes of current study which were ranged from 234 to 267 at operation state with regarding particles the size of 0.5 µm per cubic meter in each sampling sites declared as Class C of WHO cleanroom standard [8].

Similarly, the number of airborne particles the size 5.0 µm at rest state from all sampling sites which deemed as Class C was ranged from 11 to 34 is harmonized with ISO which explained that the maximum number of airborne particles the size of 5.0 µm at rest state is 2,900 in ISO Cleanroom Class 7 which correspond to WHO Cleanroom Class C [15]. The data obtained in the current study with regarding to airborne particles the size 5.0 µm which was ranged from 56 to 78 at operation state from sampling sites declared as Class C is in line with WHO which stated that 29,000 are the maximum acceptable number of particles the size of 5.0 µm at operation state in cleanroom Class C [13].

Correspondingly, The results data gained in the current study with respect to viable counts through Active Air sampling technique was ranged from 9 to 13 colony forming unit per cubic meter (CFU /m^3) in all sampling sites claimed as cleanroom Class C coincides with ISO which stated that 100 colony forming unit per cubic meter (CFU /m^3) are allowed through Active Air sampling technique in ISO Cleanroom Class 7 which correspond to WHO Cleanroom Class C [15]. Likewise, The data obtained in the current study in aspect of viable count through settle plate method was ranged from and 5 to 7 (CFU/m^3) colony forming unit per cubic meter CFU/m^3 is in line with WHO which informed that the minimum allowed number of colony forming unit per cubic meter CFU/m^3 is 50 through settle plate method in WHO Cleanroom Class C [13]. In another study, the observation of PDA., 2002 which reported that 50 colony forming unit per cubic meter (CFU /m^3) is permissible with regarding to surface monitoring in Cleanroom Class

C supports the outcomes of current study which showed ranging from 11 to 15 colony forming unit per cubic meter (CFU /m^3) with regarding surface monitoring by swabbing method from sampling sites including Air Lock-II of Production Section II, Air Lock-II of Production Section I and Air Lock-II of Microbiology Laboratory which are considered as cleanroom Class C [16].

The current study discovered that airborne particles the size of 0.5µm per cubic meter was observed at rest state were ranged from 113 to 219 from sampling sites including Air Lock-I of Production Section II, Air Lock-I of Production Section I and Air Lock-I of Microbiology Laboratory which are considered as cleanroom Class D are in agreement with WHO which reported that the maximum number of permissible airborne particles the size of 0.5 µm per cubic meter at rest state are 3520,000 in cleanroom Class D [13]. Similarly, WHO which narrated that higher number than 3520,000 airborne particles the size of 0.5µm per cubic meter are permitted which numbers of particles is not defined at operational state in cleanroom Class D which supported the outcomes of current study which were ranged from 124 to 434 at operation state with regarding particles the size of 0.5 µm per cubic meter in each sampling sites declared as Class D of WHO cleanroom standard [8].

Similarly, the number of airborne particles the size 5.0 µm at rest state from all sampling sites which deemed as Class D was ranged from 123 to 194 is harmonized with ISO which explained that the maximum number of airborne particles the size of 5.0 µm at rest state is 29,000 in ISO Cleanroom Class 8 which correspond to WHO Cleanroom Class D [15]. The data obtained in the current study with regarding to airborne particles the size 5.0 µm which was ranged from 298 to 432 at operation state from sampling sites declared as Class D is in line with WHO which stated that higher number than 29,000 airborne particles the size of 0.5 µm per cubic meter are permitted which numbers of particles is not defined at operation state in cleanroom Class D [13].

Correspondingly, The results data gained in the current study with respect to viable counts through Active Air sampling technique was ranged from 16 to 21 colony forming unit per cubic meter (CFU /m^3) in all sampling sites claimed as cleanroom Class D coincides with ISO which stated that 200 colony forming unit per cubic meter (CFU /m^3) are allowed through Active Air sampling technique in ISO Cleanroom Class 7 which correspond to WHO Cleanroom Class D [15]. Likewise, The data obtained in the current study in aspect of viable count through settle plate method was ranged from and 11 to 14 (CFU /m^3) colony forming unit per cubic meter CFU/m^3 is in line with WHO which informed that the minimum allowed number of colony forming unit per cubic meter CFU/m^3 is 100 through settle plate method in WHO Cleanroom Class D [13]. In another study, the observation of PDA which reported that 100 colony forming unit per cubic meter (CFU /m^3) is permissible with regarding to surface monitoring in Cleanroom Class D supports the outcomes of current study which showed ranging from 21 to 25 colony forming unit per cubic meter (CFU /m^3) with regarding surface monitoring by swabbing method from sampling sites including Air Lock-I of Production Section II, Air Lock-I of Production Section I and Air Lock-I of Microbiology Laboratory which are considered as cleanroom Class D [16].

CONCLUSION

It is concluded that all sections of vaccine manufacturing unit are comply with WHO cleanroom standards. In each sampling site of all clean zones shows viable and non-viable counts within the limits set by local and international cleanroom standards. In risk assessment study, it is established that Personnel and external environment are the two risks for cleanrooms to affect the cleanliness of environment. Differential positive air pressure is a barrier to prevent the mixing the external air with cleanroom environment. It counters the air pressure of adjacent lower clean zone that separate the cleanroom environment from lower clean zone.

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