DESIGN OF AN ENVIRONMENTAL MONITORING PROGRAM FOR A NEW BIOTECHNOLOGY FACILITY

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ABSTRACT

A new biotechnology facility in Puerto Rico is in need of an Environmental Program to ensure the stability of room conditions for the manufacturing of their products. This environmental baseline study provided documented evidence through various testing and verification activities that the viable and non-viable environmental conditions for the Manufacturing areas and laboratories were established. Each room was monitored for viable (total CFU count) and non-viable (total particle count) during two (2) weeks. The monitoring process consisted of environmental sampling during the first (1) week for three (3) consecutives days under static conditions and the second (2) week for three (3) consecutives days under dynamic. Identification of microorganisms was performed and provided knowledge of the microbial flora collected. The result of this baseline study provided an environmental monitoring program for this new facility and assured that this one complied with the San Jose, California (main facility) established criteria.

INTRODUCTION

Establishing an "Environmental Monitoring Program" is vital for the quality assurance of the in-process and final product.

This baseline study will be the first evaluation of the environmental conditions in this new facility. Finding any contaminants present will help determined if there will be any impact to our products.

A cleanroom is a room in which the concentration of airborne particles is controlled; this could be accomplished by controlling the air flow of the room, installing HEPA (high efficiency particulate air) filters and airlocks.

In a cleanroom the temperature and humidity are also monitored because these factors are relevant in controlling the particulate counts in a room.

Parameters such as airflow, microbiological, particulate quality of air, equipment surfaces, other room surfaces, and personnel equipment (including gowns, boots, and masks), are monitored and compared to standards or targets. [7]

Clean area control parameters should be supported by microbiological and particle data obtained during qualification studies. [6] Initial cleanroom qualification includes, in part, an assessment of air quality under as-built, static conditions. [6] It is important for area qualification and classification to place most emphasis on data generated under dynamic conditions (i.e., with personnel present, equipment in place, and operations ongoing). [6]

The International Organization for Standardization is a worldwide federation of national standards bodies (ISO member bodies) which provide Environmental Monitoring Guidelines. [4] These guidelines will provide useful information in the development of the environmental program in a new facility.

There will be equipment and materials needed in our Environmental Monitoring Program, a particle counter, centrifugal air sampler, RODAC plates and TSA strips.

For a particle counter the sensitivity of the equipment is very important. This determines the smallest size of particle the machine is able to

detect. A typical particle counter has sensitivities of 0.1, 0.3 or 0.5 μm.

Bioaerosols are viable living particulates, such as bacteria, spores, molds and yeast. [8] These organisms can be combined with airborne particulate such as dust, sprays. [8] The best way to monitored these is using specialized devices called impactors. This equipment must include a nutrient agar that can trap the organisms collected form the air sampled. [8] After incubation at appropriate temperatures these organisms are able to grow and can be counted and identified.

These instruments must be calibrated in order to provide consistency in the testing. [8] A test method validation must be performed and it also has to be incorporated in an Environmental Monitoring Program. [8]

The objectives of this baseline study are to establish an environmental monitoring program. Have a standard operating procedure that includes the number of sampling points, locations and frequency of the monitoring. Collect the baseline data for viables and non viables particulate counts and establish the microbial flora of the laboratories by performing identification of the microorganisms.

METHODOLOGY

For the baseline study each room will be monitored for viable and non-viable (total particle count) particles during two (2) weeks.

ISO standards allows to perform calculation of non- viable sampling points in a room using a formula that takes in consideration the size of the room to be monitored. Using ISO standards helps in the creation of standard operating procedures which specifies sampling locations using maps, frequency and alert/action control limit.

Classification level is the process of specifying or determining the level of airborne particulate cleanliness applicable to a cleanroom or cleanzone, expressed in terms of an ISO Class N, which represents the maximum allowable concentration (in particles per cubic meter of air) for considered sizes of particles. [4] The concentrations are determined by using equation: [4]

$$Cn = \frac{10^N \times (0,1)^{2,08}}{D} \qquad (1)$$

ISO 14644-1 is a worldwide standard for classifying the cleanliness of the air in clean areas. [4] For example, in an ISO Class 8 classification the maximum particle concentration allowed for 0.5 μm is 3,520,000 particles and for 5.0 μm is 29,300 particles. [4]

For this facility will resemble a classification ISO Class 8. Not to become certified as a cleanroom ISO Class 8, just have a controlled environment representative of an ISO class 8 cleanroom.

Rooms will be classified in accordance to its criticality. For example, rooms in which the product will be exposed are class one (C1), rooms where product may not be exposed but critical test are being performed and its integrity must be assured are considered class 2 (C2), and rooms in which the product is not exposed at all are considered class 3 (C3). C1 and C2 rooms will be monitored for viable and non viables twice a month. C3 rooms will be monitored for non viables twice per month.

The sampling of the rooms, location and frequency will be based on the Standard Operating Procedure of the facility. This document will be originated and maps will be included with the sampling location in each one of the laboratories. The minimum number of sampling point locations will be derived from the equation in Annex B of ISO 14644-1:

$$N_L = \sqrt{A} \qquad (2)$$

N_L = is the minimum number of sampling point locations (rounded up to a whole number).

A= is the area of the room or zone in square meters.

This monitoring process consists of environmental sampling during three (3) consecutives days for static and another three (3) consecutive days under dynamic conditions.

The tests will be performed using the following instruments; Biotest CAS "Centrifugal Air

Sampler" which collects viable particles, Climate CI-450t Particle Counter which collects non-viable particles and RODAC plates for viable surface sampling.

For total particle count collection (using the CI-450t Particle Counter), at least one sample is to be taken within 4-5 feet of open product or filling activity. One (1) minute samples will be taken each at different locations throughout the room.

For non-viables counts the following formula will be used in order to calculate the average of the sample taken during the study:

$$\text{Average} = \frac{\sum(X1+X2+X3)}{n} \qquad (3)$$

Where; X= Sample at each value point
n = Total # of samples

The Centrifugal Air Sample will be used for viable counts taking samples for 4 minutes in each location. Viable particles are to be taken in close proximity, 18-24 inches to working locations.

The samples will be incubated at 30° to 35°C for 48 hours for total bacteria count. Then the sample will be transferred and re-incubated for 5 more days at 20° to 25°C for a total of seven (7) days for mold and yeast counts.

The CAS "Centrifugal Air Sampler" is used for viable counts. In the test samples taken during the study the following formula is used:

$$\text{CFU} / \text{Ft}^3 \frac{\#\text{Colonies}}{[(0.0353\,\text{ft}^3/\text{l})(40\,\text{L/min})(\text{Sampling Time})]} \qquad (4)$$

Contact plates (RODAC) which will be use to monitor surfaces are to be taken on work stations near exposed product; such as work surface, container, equipment and personnel laboratory coats. A minimum of four (4) samples will be taken.

The samples will be incubated at 30° to 35°C for 48 hours for total bacteria count. Then the sample will be re-incubated for 5 more days at 20° to 25°C for a total of seven (7) days for mold and yeast counts.

To calculate the CFU/cm^3 in the surface viables counts used in testing the samples during the study, the following formula is used:

$$CFU/cm^3 = \frac{\#\text{Colonies}}{(26.42\,cm^2)} \qquad (5)$$

Microorganisms recovered from viables samples will be identified using the API System. API is a standardized test with an extensive database that provides accurate results.

RESULTS AND DISCUSSION

Results of this study provided information of whether or not this new facility complies with the environmental conditions needed for the manufacturing of their product, using as a guide the established environmental criteria in a plant in California. Refer to Table 1.

Table 1: *Environmental Monitoring Acceptance Criteria*

Limits for Rooms	Alert Limits	Action Limits
Non Viable Particles:		
0.5 µm or larger	>75,000Particles	>100,000 Particles
5.0 µm or larger	> 500 Particles	> 700 Particles
(All Rooms)		
Viable Particles:		
Rooms C1	≥ 4.95 CFU/ft³	
Rooms C2	≥ 12.87 CFU/ft³	≥ 14.00 CFU/ft³
Surface Viables:		
Rooms C1	≥0.41CFU/cm²	≥ 0.55 CFU/cm²
Surface Viables:		
Personnel	≥ 1.50 CFU/cm²	≥ 2.00 CFU/cm²
Rooms C1		

The Base-Line study provided documented evidence through various testing and verification activities that the viable and non-viable environmental conditions for the Manufacturing areas, Quality Control and Microbiology laboratories at In-vitro Diagnostics Facility complies with the requirements and specifications established by Facility. The following sections summarize the activities performed during the Base-Line study execution.

Table 2 represents the Maximum, Minimum and Average Value obtain for each room for Static and Dynamic Conditions for non viable particle counting:

Table 2: *Maximum, Minimum and Average Value*

Units: Particles/ft³	Static Condition		Dynamic Condition	
	0.5µ	5.0µ	0.5µ	5.0µ
A Room (C2)				
Max.	3291	274	2113	168
Min.	1523	48	660	24
Average	2282	136	1452	98
B Room (C2)				
Max.	3098	289	3875	246
Min.	390	21	909	83
Average	1358	123	2040	143
C Room (C1)				
Max.	3835	176	3411	392
Min.	272	1	452	20
Average	1080	64	1559	148
D Room (C2)				
Max.	3174	352	3941	352
Min.	311	11	518	30
Average	875	90	1447	135
E Room (C2)				
Max.	1986	254	2535	415
Min.	825	44	804	68
Average	1384	118	1701	232
F Room (C2)				
Max.	2809	160	2230	216
Min.	601	18	698	30
Average	1692	96	1601	123
G Room (C3)				
Max.	2349	338	5088	627
Min.	789	34	510	28
Average	1263	94	1463	149
H Room (C3)				
Max.	4805	4614	5379	503
Min.	174	102	1366	95
Average	2363	1184	3052	258

Results obtained for all the rooms were within acceptance criteria for non viable total particle counts. We can see from the data a slight increase of particles in the room (except in room A) between the static and dynamic conditions. This can be attributed to the fact that as equipment and personnel began working in the laboratory and increase in particle counts was noticed.

Table 3 and Table 4 present the results for the environmental monitoring static and dynamic conditions, respectively.

Table 3: *Environmental Monitoring Results Viables C1 & C2 (Static Conditions – Day 1, 2 &3)*

Room (Class)	Centrifugal Air Sampler (Sampling Time=4min)		
	CFU/Ft³ 1 Week Day 1	CFU/Ft³ 1 Week Day 2	CFU/Ft³ 1 Week Day 3
A (C2)	2.30	4.60	1.24
B (C2)	4.78	1.42	0.89
C (C1)	1.59	1.42	0.71
	2.30	3.01	2.83
	1.59	1.06	0.18
D (C2)	1.77	3.54	1.95
	3.19	2.30	0.89
	1.77	5.67	2.12
E (C2)	10.62	3.54	0.18
F (C2)	0.18	0.35	0.71
	1.95	0.70	0.53

Table 4: *Environmental Monitoring Results Viables C1 & C2 (Dynamic Conditions – Day 1, 2 & 3)*

Room (Class)	Centrifugal Air Sampler (Sampling Time=4min)		
	CFU/Ft³ 1 Week Day 1	CFU/Ft³ 1 Week Day 2	CFU/Ft³ 1 Week Day 3
A (C2)	4.78	1.95	1.06
B (C2)	0.88	3.01	2.49
C (C1)	1.59	1.95	1.77
	3.72	1.59	2.12
	2.30	4.43	0
D (C2)	1.59	2.12	1.06
	4.43	2.49	2.49
	4.60	3.36	0.70
E (C2)	11	7.97	2.83
F (C2)	0.71	0.53	4.07
	0.71	1.42	1.59

Under static conditions no personnel were present inside the manufacturing and laboratory areas. Results for air viables were within acceptance criteria. For rooms A, B, & C results are $\geq$ 4.95 CFU/ft³ (acceptance criteria, Table 1). Rooms D, E & F are $\geq$ 12.87 CFU/ft³ (acceptance criteria).

For dynamic conditions laboratories and manufacturing areas had from one (1) to five (5) analyst in the rooms. When compare to static results we can see an increase for dynamic viable results, as expected. No relation is foreseen between the number of analysts, CFU and room classification. Results for air viables are within

acceptance criteria. For rooms A, B, & C results are ≥ 4.95 CFU/ft^3 (acceptance criteria). Rooms D, E & F are ≥ 12.87 CFU/ft^3 (acceptance criteria).

Table 5 and Table 6 present the results of surface sampling for the environmental monitoring static and dynamic conditions, respectively.

Table 5: *Environmental Monitoring Results Viables for Surface sampling C1 (Static Conditions – Day 1, 2 & 3)*

Room (Class)	Location	RODAC Sample TSA Plate		
		CFU/cm^3 1 Week Day 1	CFU/cm^3 1 Week Day 2	CFU/cm^3 1 Week Day 3
C (C2)	Bench	0.04	0	0.08
	Container	0.08	0.26	0.15
	Shelf	0.04	0	0
	Hood#3	0	0	0

Table 6: *Environmental Monitoring Results Viables for Surface sampling C1 (Dynamic Conditions – Day 1, 2 & 3)*

Room (Class)	Location	RODAC Sample TSA Plate		
		CFU/cm^3 1 Week Day 1	CFU/cm^3 1 Week Day 2	CFU/cm^3 1 Week Day 3
C (C2)	Bench	0.08	0	0.19
	Container	0.11	0	0
	Lab. Coat	1	0.04	0.11
	Hood	0	0.04	0

Under static conditions all results were within acceptance criteria ≥ 0.41CFU/cm^2. Laboratory coat sample was not performed since monitoring was under static conditions and no personnel were in the rooms. Instead another surface was used in the monitoring. In dynamic conditions all results were within acceptance criteria ≥ 0.41CFU/cm^2. Laboratory coat sample were performed since monitoring was performed under dynamic conditions.

Microorganism recovered and identification from the viable monitoring was performed. Some were identified to the species level using the API System and others were identified by morphology.

Some of the microorganisms recovered from this study were; *Staphylococcus haemolyticus*, *Staphylococcus capitis*, black cottony molds, white cottony molds, yeast and Gram (+) rods.

CONCLUSION

An Environmental Monitoring Program was established. An SOP with the sampling points, location and frequency was created. Baseline data for viable and non viable counts was collected. The microbial flora in the laboratories and manufacturing areas were obtained and identified.

The environmental monitoring specification of California's facility was challenged and approved in the new facility of Puerto Rico. For future investigation the environmental monitoring specification for Puerto Rico should be created.

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Elizabeth Queiro Santiago se graduó del programa de Maestría en Manufactura Competitiva en la colación de grados de 2009. La señora Queiro posee un grado de bachillerato en Biología y sus intereses en investigación van alineados al área de Gerencia de Calidad.