Reducing Cost by Reducing Frequency of Submitting to Identify Microorganisms Isolated from Non-critical Areas in a Parenteral Manufacture

Jennifer M. Almodóvar Rodríguez
Master's in Manufacture Competitiveness
Miriam Pabón, Ph.D.
Industrial Engineering Department
Polytechnic University of Puerto Rico

Abstract *— A microbiology laboratory identifies all the microorganisms isolated from non-critical rooms with a frequency of every three months, in order to establish a microbial profile of the microorganisms present in the areas.*

The purpose of the project is to reduce costs by 50% by decreasing the frequency to biannual. Identifying each microorganism using the MicroSEQ has a cost of $86.18. Data from March, June, September, and December 2019 were evaluated. The data identified 305 microorganisms, which represented a cost to the laboratory of $26,284.90. The most common microorganisms in those months were Micrococcus luteus, Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus haemolyticus, *and* Staphylococcus hominis. *By obtaining similar microorganisms each month, a reduction in frequency, every six months instead of every three months, can be recommended without damaging the areas' microbial profile. The reduction of these annual expenses can give more visibility to the laboratory as well as more competitiveness for new projects.*

Key terms *— cost reduction, environmental monitoring, manufacture rooms, microbiology*

INTRODUCTION

Background

A parenteral plant was approved for the manufacturing process of a biological drug product. The manufacturing suite is divided into four classified areas: Grade A (ISO 5), Grade B (ISO 7), Grade C (ISO 8), and some Grade D (ISO 9) adjacent to a controlled non-classified surrounding area. Areas were classified according to ISO 14644-1 [1]. The Grade A cleanroom is the local zone for high-risk operations like filling zone, stopper bowls, and aseptic connections. The Grade B area is the background environment for Grade A. The Grades C and D areas (non-critical) are used for performing less critical tasks that are carried out during less critical stages in the manufacturing process.

This parenteral plant has a validated environmental monitoring (EM) program and the sampling points were selected following ISO 14644 standards together with USP <1116> [2]. The environmental monitoring program measures the on-going effectiveness of the cleaning and sanitization procedures, personnel behavior, the production environment, and the engineering control system. The Microbiology Laboratory is responsible to conduct the Viable and Non-Viable monitoring in this facility.

Microbiology environmental monitoring is the collection of data of microorganisms present in a cleanroom. These microorganisms are recovered via viable monitoring from surfaces, air, and people (i.e. gowning). The result of the samples is compared against the alert and action levels established on procedures. All microorganisms isolated from Grade A and B areas will be fully identified by genus and species. For microorganisms isolated from the non-critical areas, (Grades C and D) full identification is not required, except when alert and action levels are exceeded. It only requires gram and spore stain methods. These stain methods entail applying a sample of bacteria grown in culture media onto a glass slide. Then, they are treated with a special stain in order to distinguish and classify bacteria based on the physical properties of their walls and whether the bacteria have endospore. A trained microbiology analyst examines this slide under a

microscope [3]. In addition, the microorganisms isolated from non-critical areas are submitted for full identification every three months (quarterly) in order to establish a microbial profile of the microorganism present in the areas.

The laboratory uses the MicroSEQ Microbial Identification System to identify all microorganisms requiring full identification. The MicroSEQ Microbial Identification System is ideal for environmental monitoring, contamination investigation, root-cause analysis, raw material testing, and microbial identification in biopharmaceutical manufacturing [4]. This system is a microbial identification tool for bacteria and fungi. It compares genetic information from an unknown organism against a validated library. Materials and the reagent kit are expensive; the laboratory's manager wants to reduce these expenses.

Problem

The microbiology laboratory evaluates the identification of the microorganisms isolated from non-critical areas every three months (March, June, September, and December). This way, the laboratory has data on the microflora of the different areas. The laboratory uses the MicroSEQ Microbial Identification System to classify all microorganisms that require full identification. Currently, the total cost of identifying each microorganism is approximately $86.18. This total cost represents $35.61 in resources and $50.57 in materials. In 2019, the laboratory submitted for identification approximately 305 microorganisms isolated from non-critical areas on March, June, September, and December. This action represented $26,284.90 of the annual expenses. High expenses in the laboratory reduce visibility and competitiveness when compared to other manufacturing plants of the company.

Objectives

This investigation focuses on the following objectives:

- Reducing 50% of the cost in changing frequency in submitting to identification the microorganisms isolated from non-critical areas, from every three months to biannually.
- Determining the impact of reducing the frequency in submitting to identification the microorganisms isolated from non-critical areas.

Contribution

Enabling the reduction of frequency in submitting to identification the microorganisms isolated from non-critical areas will decrease yearly expenses. Reducing some expenses will give more visibility to the laboratory at a senior management level. The goal of the microbiology laboratory at this site is to be the main center, where samples can be received and processed from other sites of the company. It is also beneficial for the laboratory because it can obtain more funds for new projects, where managers and supervisors can delegate to the analysts for their professional development.

Literature Review

Environmental Monitoring Program

Parenteral industries base their environmental monitoring program on USP <1116> Microbiology Control and Monitoring of Aseptic Processing Environments, and with ISO 14644 Cleanrooms and associated controlled environments. There are four grades or levels of manufacturing requirements when manufacturing sterile products: Grade A (ISO 5), Grade B (ISO 7), Grade C (ISO 8), and Grade D (ISO 9). The Grade cleanroom A is the local zone for a high-risk operation like filling zone, stopper bowls, and aseptic connections. The Grade B rooms are background environments for Grade A zones. Grades C and D rooms are used for performing less critical tasks that are carried out during less critical stages in the manufacturing process [5].

The purpose of USP<1116> is to maintain and control the microbiological quality of controlled environments. The environmental monitoring program measures the ongoing effectiveness of the

cleaning and sanitization procedures, personnel behavior, production environment, and the engineering control system. The Microbiology Laboratory is responsible for conducting the Viable and Non-Viable monitoring in the facility. A non-viable particle is a particle that does not contain a living microorganism, but acts as a transport for viable particles [6]. A viable particle is a particle that contains one or more living microorganisms (e.g., bacteria, yeast, or mold). These can affect the sterility of the pharmaceutical product and generally range from ~0.2µm to ~30µm in size [6].

Viable monitoring consists of surface and air viable (passive and active) monitoring. Viable air sampling involves collecting air samples on growth media; the samples are then incubated so that the viable particles can germinate, grow, and form colonies. There are two methods of viable air sampling [7]:

- **Air samplers (active):** An air sampler draws in a fixed volume of air over a sterile media plate.
- **Settle plates (passive):** A petri dish containing sterile growth media is kept in the open air for an established time, for example, 4 hours.

The surface sampling for viable microorganisms is used to evaluate the effectiveness of the disinfection and cleaning process in the controlled area and is used to monitor the gowning of the personnel. It uses contact plates with sterile growth media and is used on a flat surface. The swabs method is used for irregular surfaces where contact plates may be difficult to apply. All microorganism colonies obtained from viable monitoring will be transferred to a different type of media for identifying and classifying the species and groups.

Microbiology Identification

An effective environmental control program needs an adequate program of microorganism identification to know the flora obtained from sampling. Knowing the microflora can evaluate the effectiveness of the sanitation procedures, agent, and recovery methods. Identification of isolates from critical and immediately adjacent areas should take precedence over the identification of microorganisms from non-critical areas [8].

Almost all top pharmaceutical companies use genotypic methods to identify microorganisms. Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques [9]. Genotypic testing methods such as the sequencing approach utilized by the MicroSEQ rapid microbial identification system are known as the gold standard in the identification of bacteria and fungi. This system has many benefits, such as accuracy, validated bacterial and fungal libraries, reduced retesting, and decreased dependency on outside services.

The Applied Biosystems MicroSEQ Rapid Microbial Identification System is ideal for environmental monitoring, contamination investigation, root-cause analysis, raw material testing, microbial identification in small-molecule, biopharmaceutical manufacturing, and services laboratories. The MicroSEQ uses a five-step workflow that ranges from an isolated colony to results: retrieving grown culture, extracting DNA, performing PCR, sequencing DNA, and identifying the microorganism. The MicroSEQ System combines the benefits of PCR and DNA sequencing technologies to enable highly accurate results [8]. The system includes the largest fully validated bacterial and fungal libraries. The bacterial library includes over 2,000 species, including *Staphylococcus spp.*, *Bacillus spp.*, coryneform, mycobacteria, and Gram-negative non-fermenters. The library for fungal species includes over 1,100 entries. Both libraries are frequently updated and expanded with new entries [10].

METHODOLOGY

The purpose of the project is to prove the benefits of reducing the frequency in submitting to identification the microorganisms isolated from non-critical areas. This reduction has a positive financial impact on the company. The microbiology laboratory uses the MicroSEQ rapid microbial

identification system to have full identification of isolated microorganisms. The total cost to identify each microorganism is $86.16: $50.57 for the cost of materials and $35.61 for resources. The microbiology laboratory's analyst submits to full identification the microorganisms isolated from non-critical (Grades C and D) areas every three months (March, June, September, and December) to have data on the areas' microflora.

The project consisted of evaluating data of full identification of microorganisms isolated from non-critical areas. The period in evaluation will be March, June, September, and December 2019. The microorganisms' data from environmental monitoring of rooms where less critical tasks are performed and fewer stages from the manufacturing process occurred will be collected. Particularly, the data was documented in the original controlled forms of the manufacturing process. Some examples of these rooms included storage rooms, hallways, equipment wash, clean preparation, material entrance, and exit. An MS Excel spreadsheet was used as a tool for data entry, tables, and graphs. To have a successful project, several metrics had to be evaluated and were vital for this research, such as environmental monitoring evaluation, determining microorganisms' isolation frequency, and the most common type of microorganisms recovered from non-critical areas.

Results and Discussion

This project evaluated data of microorganisms that were submitted for full identification every three months (quarterly) in 2019 from rooms with less critical tasks. The main purpose of this research was to establish a microbial profile of the microorganism present in the manufacturing areas. These microorganisms were recovered via viable monitoring from surfaces and air. The result of the samples was compared against the alert and action levels established on procedures. From the total of 2,337 samples (air and surface) collected during those four months, zero environmental monitoring excursions were reported that resulted in reaching the Alert/Action levels. Table 1 shows the viable samples collected from non-critical (Grades C and D) rooms in 2019.

Table 1
Summary of viable data collected from non-critical rooms in 2019

Months	Total of Samples Collected	Samples with Alert/ Action Growth	% Overall Samples within Alert/ Action Levels
March	623	0	100.00%
June	589	0	100.00%
September	603	0	100.00%
December	522	0	100.00%

The laboratory uses the MicroSEQ Microbial Identification System to identify all microorganisms that require full identification. The total cost of identifying each microorganism using this method is approximately $86.18: $50.57 for the cost of materials and $35.61 for resources. A total of 305 full microbiology ID was performed every three months in 2019. This represents an approximate yearly cost of $26,284.90 to the laboratory. Table 2 shows all full ID counts and costs on March, June, September, and December 2019. There is no monthly variability of Full ID counts and costs.

Table 2
Summary of full ID count and costs per months in 2019

Months	Full ID Count	Costs
March	81	$ 6,980.58
June	71	$ 6,118.78
September	77	$ 6,635.86
December	76	$ 6,549.68
Grand Total	**305**	**$26,284.90**

Table 3 shows microorganisms isolated from non-critical rooms on March, June, September, and December 2019. The typical habitats for this group of microorganisms are human skin and the environment. The most frequently isolated microorganisms in 2019 were *Micrococcus luteus* (23.28%), *Staphylococcus epidermidis* (18.69%), *Staphylococcus capitis* (10.82%), *Staphylococcus haemolyticus* (9.51%), and *Staphylococcus hominis* (8.85%). These five recovered microorganisms are associated with human-borne microorganisms and

represent 71.15% of all microorganisms isolated on the months evaluated.

Table 3
Summary of microorganism isolated in March, June, September and December 2019

ID	Isolation Frequency	Isolation Frequency %
Micrococcus luteus	71	23.28%
Staphylococcus epidermidis	57	18.69%
Staphylococcus capitis	33	10.82%
Staphylococcus haemolyticus	29	9.51%
Staphylococcus hominis	27	8.85%
Corynebacterium tuberculostearicum	11	3.61%
Staphylococcus warneri	9	2.95%
Corynebacterium sp.	8	2.62%
Staphylococcus cohnii	8	2.62%
Brachybacterium conglomeractum	4	1.31%
Staphylococcus saprophyticus	4	1.31%
Kocuria marina	3	0.98%
Kocuria rhizophila	3	0.98%
Sphingomonas sp	3	0.98%
Staphylococcus sciuri	3	0.98%
Bacillus gibsonii	2	0.66%
Bacillus megaterium	2	0.66%
Bacillus sp.	2	0.66%
Corynebacterium amycolatum	2	0.66%
Corynebacterium lipophiloflavum	2	0.66%
Corynebacterium mucifaciens	2	0.66%
Staphylococcus lugdunensis	2	0.66%
Bacillus altitidinis	1	0.33%
Bacillus circulans	1	0.33%
Bacillus flexus	1	0.33%
Brevundimonas intermedia	1	0.33%
Corynebacterium propinquum	1	0.33%
Corynebacterium striatum	1	0.33%
Corynebacterium xerotis	1	0.33%
Cupriavidus sp.	1	0.33%
Kocuria koreensis	1	0.33%
Kocuria palustris	1	0.33%
Kocuria sp.	1	0.33%
Kytococcus schroeteri	1	0.33%
Kytococcus sedentarius	1	0.33%
Lysinibacillus boronitolerans	1	0.33%
Lysinibacillus contaminans	1	0.33%
Lysobacter soli	1	0.33%
Scopulariopsis brevicaulis	1	0.33%
streptococcus salivarius	1	0.33%
Grand Total	**305**	**100.00%**

Upon evaluating each month (figures 1, 2, 3, and 4), the predominant microorganisms are the same five that were obtained in the summary of microorganisms isolated in 2019. These five common microorganisms are *Micrococcus luteus*, *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus haemolyticus*, and *Staphylococcus hominis*. The frequency of these isolated microorganisms was 72.84% in March, 64.78% in June, 75.33% in September, and 71.05% in December.

When microorganisms were submitted for identification every three months, similar microorganisms were obtained each month. The five most frequently isolated microorganisms were the same each month.

CONCLUSION

This project attempted to evaluate whether it is feasible to reduce the frequency of the identification of microorganisms isolated from non-critical rooms. After evaluating the data of the microorganisms isolated every three months, it is recommended to reduce the identification frequency, since it was shown that the same common microorganisms are obtained each month. By reducing the frequency from quarterly to biannually, the microbiology laboratory can collect important data and establish a microbial profile of microorganisms present in the areas. By implementing a biannual frequency, the microbiology laboratory will reduce 50% of its expenses in materials and resources required to identify microorganisms using the MicroSEQ Rapid Microbial Identification System. To implement this new frequency, training for employees as well as proper documentation in the laboratory procedures are required.

This project can contribute to the microbiology laboratory's goal of being the main center workroom, where samples can be received and processed from other sites of the company. The reduction of some expenses will give more visibility to the laboratory at a senior management level and will make the company more competitive.

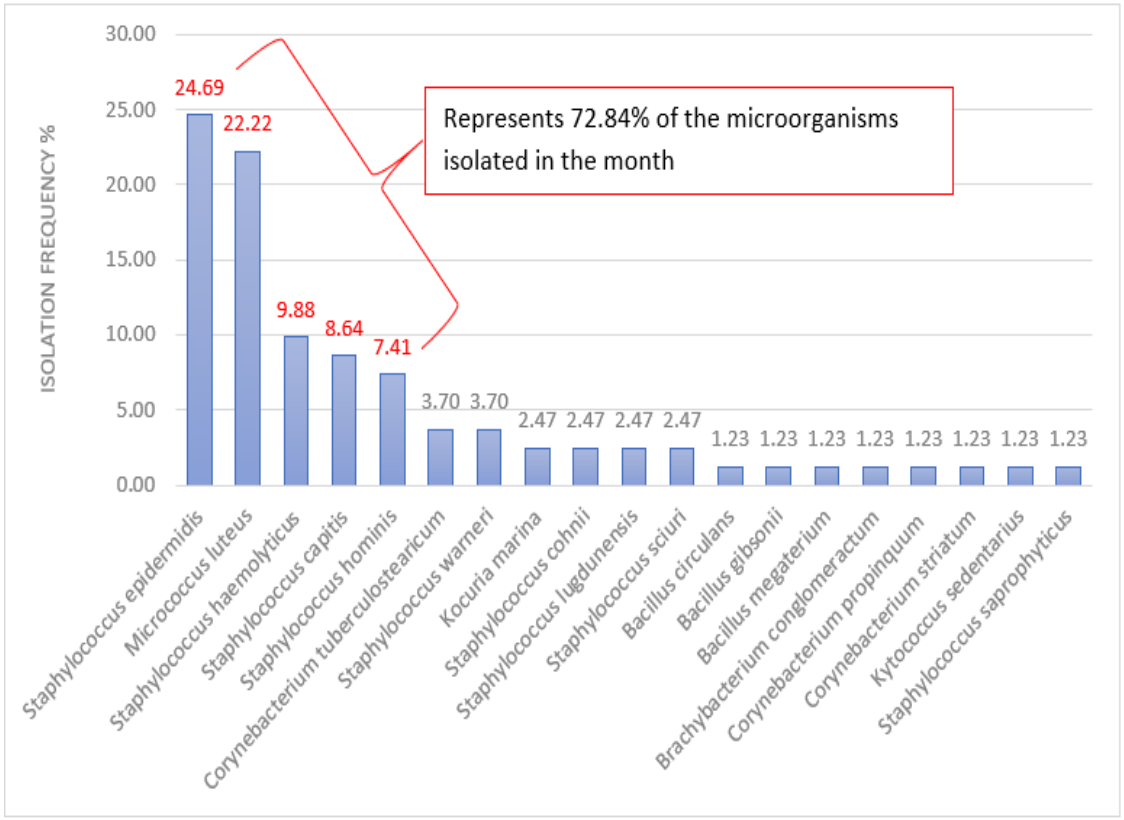

Figure 1

Isolation Frequency % of Microorganisms in March 2019

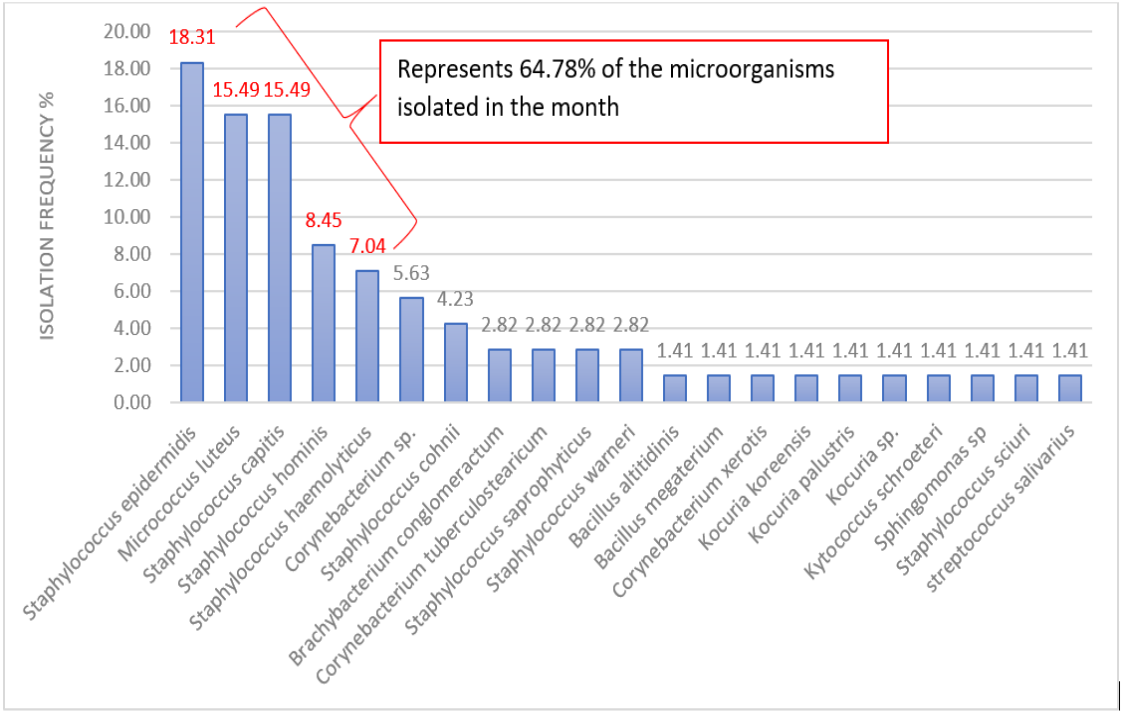

Figure 2

Isolation Frequency % of Microorganisms in June 2019

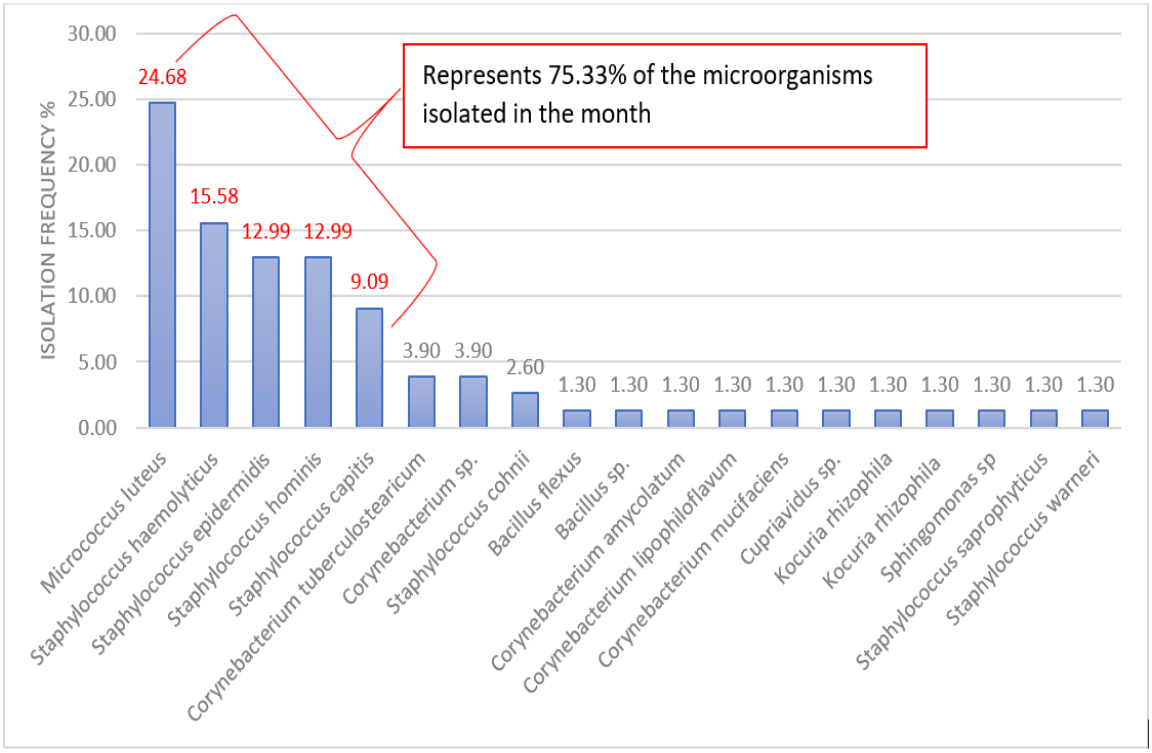

Figure 3
Isolation Frequency % of Microorganisms in September 2019

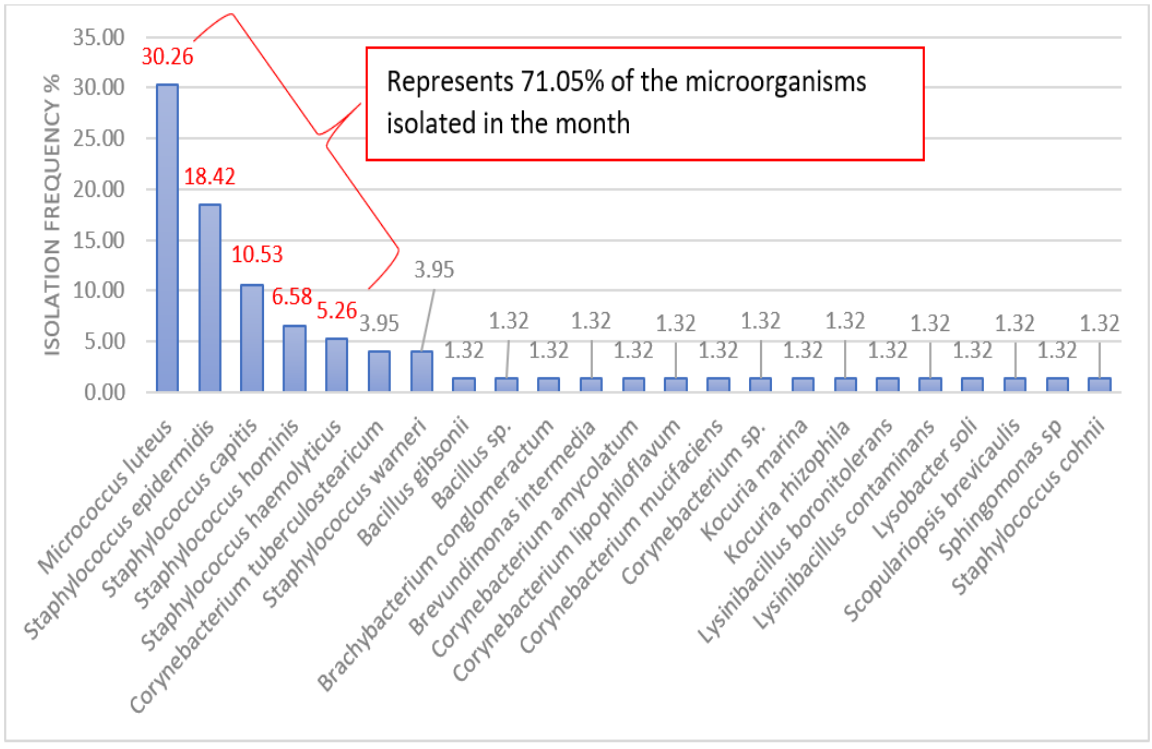

Figure 4
Isolation Frequency % of Microorganisms in December 2019

REFERENCES

[1] *Cleanrooms and associated control environments — Part 1: Classification of air cleanliness by particle concentration*, ISO 14644-1, Dec. 15, 2015. [Online]. Available: https://zoser.com.co/wp-content/uploads/2015/10/ISO%2014644-1%20Version%202015.pdf.

[2] *USP <1116> Microbiological control and monitoring of aseptic processing Eenvironments*, The United States Pharmacopeia Convention, May 1, 2012. [Online]. Available: https://www.dcvmn.org/IMG/pdf/usp_1116_em_for_aseptic_processing.pdf

[3] M. R. J. Salton and K. S. Kim, "Structure," in *Medical Microbiology*, 4th ed., S. Baron, ed. TX: The University of Texas Medical Branch at Galveston, 1996, ch. 2. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK8477/

[4] Thermo Fisher Scientific, "Microbial identification." Accessed September 8, 2019. [Online]. Available: https://www.thermofisher.com/us/en/home/life-science/bioproduction/contaminant-and-impurity-testing/microbial-identification.html?cid=bpd_ppa_pha_r01_cp1295_pjt0000_bpd00000_0se_gaw_bt_awa_microseq&ef_id=CjwKCAjwzdLrBRBiEiwAEHrAYqFOQi2XTVL-AT5c7RsATuq_4QD

[5] Blue Thunder Technologies, "Clean room grade ABCD explained," August 22, 2018. [Online]. Available: https://bluethundertechnologies.com/clean-room-grade-abcd-explained/

[6] R&D World, "Pharmaceutical industry cleanroom monitoring: viable and non-viable particle detection," January 1, 2005. [Online]. Available: https://www.rdworldonline.com/pharmaceutical-industry-cleanroom-monitoring-viable-and-non-viable-particle-detection/

[7] SanAir Technologies Laboratory, "What is viable air sampling?", March 29, 2017. [Online]. Available:https://www.sanair.com/what-is-viable-air-sampling/

[8] Thermo Fisher Scientific, "Microbial identification." Accessed October 2, 2019. [Online]. Available: https://www.thermofisher.com/pr/en/home/life-science/bioproduction/contaminant-and-impurity-testing/microbial-identification.html

[9] *Guidance for Industry: Sterile Drug Products Produced by Asseptic Processing — Current Good Manufacturing Practice*, FDA, Rockville, MD, Sept. 2004 September2004.[Online].Available:https://www.fda.gov/media/71026/download.

[10] Life Technologies, *MicroSEQ® Rapid Microbial Identification System*, CO141440111, 2011. [Online]. Available: https://www.thermofisher.com/content/dam/LifeTech/migration/files/bioproduction/pdfs.par.28967.file.dat/rapid-microbial-identification-system.pdf