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Microbial Air Sampling Plans in Cleanrooms According to the USP

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Abstract

USP guidelines for Cleanrooms state that the contamination monitoring is performed during the normal working day activity of each shift.

Using a single head active microbial air sampler requires Operators to perform several manipulations regarding preparation and culture plate transfer.

Work time and the risk of contamination can be reduced with the use of a two or three aspirating head format sampler. These samplers can be prepared and programmed prior to starting daily activity, with the culture plates collected at the end of the shift.

This paper presents several examples of protocol to reduce operator contamination risk, save time, and apply recommended regulatory compliance standards.

Keywords: *Air Sampler; Aspirating Chamber; Aspirating Head; At Rest; Cleanroom; CFU; Contamination; Bacteria; Culture Plate; Delay; Disinfection; End of Shift Activity; Fraction Time; Fraction Number; Incubation; In Operation; Microorganisms; Petri Dish; Run; Standard; Sterilization; Unidirectional Laminar Flow*

Definitions

A Cleanroom is an enclosed space in which airborne particulates, contaminants, and pollutants are kept within strict limits. In the pharmaceutical industry, cleanrooms ensure an environment is free of bacteria, viruses, or other pathogens.

Microbial air samplers are devices used to sample a measured amount of air in a specified time to quantitate the particulate or microbiological status of air within a controlled environment.

Standard Operating Procedures (SOP) are step-by-step instructions compiled by an organization to help workers carry out complex routine operations. SOPs aim to achieve efficiency, quality output, and uniformity of performance, while reducing miscommunication and failure to comply with industry regulations.

A sampling plan is a detailed outline of which measurements will be taken at what times, on which material, in what manner, and by whom.

Shift work indicates a type of work schedule in which groups of workers rotate through set periods throughout the day, typically performing the same kind of operations.

Introduction

USP guidelines for Cleanrooms state that contamination monitoring is performed during the normal working day activity of each shift. The volume of 1000 liters of air should be collected at each sampling point. Air samples should be collected "at rest", "in operation", and "after process" [1].

Sampling plan SOP

The sampling plan SOP should be written prior to beginning an ongoing environmental program. A robust air sampling program is developed based upon the size of the area, number of hoods, and after a risk assessment. In order to trend the number and type of microorganisms on a day/weekly/monthly basis, it is necessary to sample with the same method/protocol each time. Areas to be sampled are indicated on a floor plan of the Cleanroom and should include laminar flow benches, BSC, Isolators, RABS, buffer zones, gowning areas, and anteroom areas. The SOP (Standard Operative Procedure) should include: Number and Identification, Title, Purpose, Responsibility, Glossary, Safety rules, Standard, Materials, Protocol, Non-Conformity, Corrective Actions.

"Shift working time" differs from company to company, and with cyclical processing circumstances. In this document, we report several examples using two and three aspirating heads air samplers to demonstrate sampling flexibility. In addition to the programming samples provided, instrument start times may also be programmed with the "delay" function of the sampler.

Materials

TRIO.BAS DUO, TRIO.BAS DUO CABLE microbial air sampler (200 l/m air aspiration).

90 mm Petri dish with TSA agar culture media.

"Daily Shift" sterile certified aspirating heads.

Figure 1: *Mycolic acids structure.*

Protocol

Longer sampling spans can be programmed by selecting various "delay", "fraction time", and "fraction number" functions. With "delay", "fraction number", and "fraction time" functions utilized, the pre-programmed volume of monitored air is reported as a continuous single sample.

This paper reports several examples of 1,000 liters of air sampled during different working shift runs.

The cycles are stored in the air sampler memory and then can be transferred to a P.C., via a data transfer accessory option.

Two aspirating heads with a 200 liters/minute format air sampler

- **1st Case**: 1,000 liters of air in 2 fractions = each run is 500 liters = 2 samples.

  Total Working Shift 2h.20m.

  Starting "At rest" at 09:00 a.m. / "At end" at 11:20 a.m.

- **2nd Case**: 1,000 liters of air in 3 fractions = each run is 333 liters = 2 samples.

  Total Working Shift 3h.21m.

  Starting "At rest" at 09:00 a.m. / "At end" at 12:21 p.m.

- **3rd Case**: 1,000 liters of air in 3 fractions = each run is 333 liters = 2 samples.

  Total Working Shift 4h.21m.

  Starting "At rest" at 09:00 a.m. / "At end" at 01:21 p.m.

EXAMPLE OF 2h.20min WORK SHIFT						
SAMPLER CFG: VOL - 1000 LTS - OK - DELAY - 0 -OK - FRACTION NUM. - 2 -OK - FR. TIME - 60 - OK - HEAD MODE SQS - OK - GO - HEAD 1&2 - OK						
DUO 100 L/M PROVA 2 FRACTION - 1000 L/M						
HEAD	FRACTION	STARTING HOUR	TIME TEST	SAMPLER %	L/M SESSION	END OF TEST
1	A	9.00	5,00 MIN	50%	500	9.05
1	B	10.05	5,00 MIN	100%	500	10.10
2	A	10.10	5,00 MIN	50%	500	10.15
2	B	11.15	5,00 MIN	100%	500	11.20

EXAMPLE OF 3h.21min WORK SHIFT						
SAMPLER CFG: VOL - 1000 LTS - OK - DELAY - 0 - OK - FRACTION NUM. - 3 -OK - FR. TIME - 45 - OK - HEAD MODE SQS - OK - GO - HEAD 1&2 - OK						
DUO 100 L/M PROVA 3 FRACTION - 1000 L/M						
HEAD	FRACTION	STARTING HOUR	TIME TEST	SAMPLER %	L/M SESSION	END OF TEST
1	A	9.00	3,30 MIN	33%	333	9.03
1	B	9.48	3,30 MIN	66%	333	9.52
1	C	10.37	3,30 MIN	100%	333	10.40
2	A	10.40	3,30 MIN	33%	333	10.44
2	B	11.29	3,30 MIN	66%	333	11.32
2	C	12.17	3,30 MIN	100%	333	12.21

EXAMPLE OF 4h.21min WORK SHIFT						
SAMPLER CFG: VOL - 1000 LTS - OK - DELAY - 0 - OK - FRACTION NUM. - 3 -OK - FR. TIME - 60 - OK - HEAD MODE SQS - OK - GO - HEAD 1&2 - OK						
DUO 100 L/M PROVA 3 FRACTION - 1000 L/M						
HEAD	FRACTION	STARTING HOUR	TIME TEST	SAMPLER %	L/M SESSION	END OF TEST
1	A	9.00	3,30 MIN	33%	333	9.03
1	B	10.03	3,30 MIN	66%	333	10.07
1	C	11.07	3,30 MIN	100%	333	11.10
2	A	11.10	3,30 MIN	33%	333	11.14
2	B	12.14	3,30 MIN	66%	333	12.17
2	C	13.17	3,30 MIN	100%	333	13.21

Figure 2

Three aspirating heads in 200 liters/minute format air sampler

- **4th Case: 1,000 liters of air in 3 fractions = each run is 333 liters = 3 samples.**

  Total Working Shift 4h.30m.

  Starting "At rest" at 09:00 a.m. / "In operation" at 12.17 p.m. / "At end" at 01:31 p.m.

- **5th Case**: 1,000 liters of air in 3 fractions = each run is 333 liters = 3 samples.

  Total Working shift 6h.30m.

  Starting "At rest" at 09:00 a.m. / "In operation" at 01.17 p.m. / "At end" at 03:31.

EXAMPLE OF 4h.31min WORK SHIFT						
SAMPLER CFG: VOL - 1000 LTS - OK - DELAY - 0 -OK - FRACTION NUM. - 3 -OK - FR. TIME - 30 - OK - HEAD MODE SQS - OK - GO - HEAD 1&2&3 - OK						
TRIO 100 L/M PROVA 3 FRACTION - 1000 L/M						
HEAD	FRACTION	STARTING HOUR	TIME TEST	SAMPLER %	L/M SESSION	END OF TEST
1	A	9.00	3,30 MIN	33%	330	9.03
1	B	9.33	3,30 MIN	66%	330	10.37
1	C	11.07	3,30 MIN	100%	330	11.10
2	A	11.10	3,30 MIN	33%	330	11.14
2	B	11.44	3,30 MIN	66%	330	11.47
2	C	12.17	3,30 MIN	100%	330	12.21
3	A	12.21	3,30 MIN	33%	330	12.24
3	B	12.54	3,30 MIN	66%	330	12.58
3	C	13.28	3,30 MIN	100%	330	13.31

EXAMPLE OF 6h.31min WORK SHIFT						
SAMPLER CFG: VOL - 1000 LTS - OK - DELAY - 0 -OK - FRACTION NUM. - 3 -OK - FR. TIME - 60 - OK - HEAD MODE SQS - OK - GO - HEAD 1&2&3 - OK						
TRIO 100 L/M PROVA 3 FRACTION - 1000 L/M						
HEAD	FRACTION	STARTING HOUR	TIME TEST	SAMPLER %	L/M SESSION	END OF TEST
1	A	9.00	3,30 MIN	33%	330	9.03
1	B	10.03	3,30 MIN	66%	330	10.07
1	C	11.07	3,30 MIN	100%	330	11.10
2	A	11.10	3,30 MIN	33%	330	11.14
2	B	12.14	3,30 MIN	66%	330	12.17
2	C	13.17	3,30 MIN	100%	330	13.21
3	A	13.21	3,30 MIN	33%	330	13.24
3	B	14.24	3,30 MIN	66%	330	14.28
3	C	15.28	3,30 MIN	100%	330	15.31

Figure 3

Conclusions

A detailed, complete, and exhaustive microbial air monitoring plan is a must. It should be prepared and written with great care so that it is easily applied and it can be defended during a regulatory authority inspection and compliance audit.

Bibliography

1. European Standard Draft prEN 17141. "Cleanrooms and associated controlled environments-Biocntaminaton control". English version (2017).

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