Environmental Microbial Monitoring (EMM)

環境微生物監控

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USP Regulations

- <51> Antimicrobial effectiveness testing
- <61> Microbiological examination of nonsterile products: microbial enumeration tests
- <62> Microbiological examination of nonsterile products: tests for specified microorganisms
- <71> Sterility tests
- <1113> Microbial characterization identification and strain typing
- <1116> Microbiological control and monitoring of aseptic processing environments
- <1117> Microbiological Best Laboratory Practices
- <1227> Validation of microbial recovery from pharmacopeial articles

Purpose of EM

- Critical process within the pharmaceutical industries.
- Determines the microbial and particulate content of cleanroom air and surfaces.
- Highlights conditions contributing to excessive microbial & particulate levels due to ineffective cleaning, or personnel/equipment issues (Trending).
- Alerts to conditions exceeding classifications.
- Proactive tool for Quality Assurance.

Who Does It?

- Quality Control
  - Demonstrate product safety
  - Environmental Monitoring
  - Testing

- Quality Assurance
  - Oversight responsibilities – ensure compliance with GMPs
  - Review and Approve all Records, Reports, written procedures, specifications
  - Audit methods, results, systems and processes

To be monitored

- Non-viable airborne particulates
- Viable airborn particulates
- Viable surface bound particulates on cleanroom surfaces and personnel

- Contamination Sources
  - People ~75%
  - Ventilation ~15%
  - Room Structure ~5%
  - Equipment ~5%

USP 39 <1116>

- Studies indicate that gowned humans slough particulate and microbial contamination at a rather consistent rate.
- Where equipment is the primary source of particulate matter, the resulting particulates are essentially all nonviable.
- It is not possible to clearly distinguish between background total particulate contamination generated largely by mechanical operations and the total particulates contributed by personnel. →both a total particulate component and a microbiological component.

Classifications & Control Level

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Table 1. Airborne Total Particulate Cleanliness Classes[a]

ISO Class[b]	Particles ≥0.5 µm/m3
ISO 5	3520
ISO 6	35,200
ISO 7	352,000
ISO 8	3,520,000

a Taken from ISO International Standard 14644 Part 1, published by the International Organization for Standardization, May 1999.

b The four ISO 14644-1 classes correspond closely to former U.S. Federal Standard 209E classifications. The relationships are
ISO 5/Class 100, ISO 6/Class 1000, ISO 7/Class 10,000, and
ISO 8/Class 100,000.

USP34 <1116> Microbial considerations and Action levels for Controlled Environment

Table 3. Air Cleanliness Guidelines in Colony-Forming Units (cfu) in Controlled Environments (Using a Slit-to-Agar Sampler or Equivalent)

Class*		cfu per cubic mete	cfu per cubic feet of air
SI	U.S. Customary		
M3.5	100	Less than 3	Less than 0.1
M5.5	10,000	Less than 20	Less than 0.5
M6.5	100,000	Less than 100	Less than 2.5

* As defined in Federal Standard 209E, September 1992.

** A sufficient volume of air should be sampled to detect excursions above the limits specified.

FDA(2004)
1
10
100

Table 4. Surface Cleanliness Guidelines of Equipment and Facilities in cfu in Controlled Environments

Class		cfu per Contact Plate*
SI	U.S. Customary	
M3.5	100	3 (including floor)
M5.5	10,000	5
		10 (floor)

* Contact plate areas vary from 24 to 30 cm^2. When swabbing is used in sampling, the area covered should be greater than or equal to 24 cm^2 but no larger than 30 cm^2.

Table 5. Surface Cleanliness Guidelines in Controlled Environments of Operating Personnel Gear in cfu

Class		cfu per Contact Plate*	
SI	U.S. Customary	Gloves	Personnel Clothing & Garb
M3.5	100	3	5
M5.5	10,000	10	20

* See in *Table 4* under (*).

USP39 <1116> Suggested Initial Contamination Recovery Rates(CRR) in Aseptic Environments

Room Classification	Active air Sample (%)	Settle Plates(9cm) 4hr Exposure (%)	Contact Plates or Swab(%)	Glove or Garment (%)
Isolator/Closed RABS (ISO 5 or better)	＜0.1	＜0.1	＜0.1	＜0.1
ISO 5	＜1	＜1	＜1	＜1
ISO 6	＜3	＜3	＜3	＜3
ISO 7	＜5	＜5	＜5	＜5
ISO 8	＜10	＜10	＜10	＜10

EU and FDA recommended limits for microbial contamination

PIC/S Annex 1 PE 009-13(2017) (top) ,FDA Pharmaceutical CGMPs (2004) (bottom)

Grade	Air Sample cfu/m^3	Settle Plates (Ø90mm), cfu/4hrs	Contact Plates (Ø55mm), cfu/plate	Glove Print 5 Fingers, cfu/glove
A	＜1	＜1	＜1	＜1
B	10	5	5	5
C	100	50	25	-
D	200	100	50	-

Clean Area Classification (0.5µm particles/cfu)	ISO Designation	Air Sample cfu/m^3	Settle Plates (Ø90mm), cfu/4hrs
100	ISO 5	1	1
1000	ISO 6	7	3
10000	ISO 7	10	5
100000	ISO 8	100	50

Sampling Plain

- Frequency
- Time
- Sites
- Method

Sampling Frequency

- The frequency of sampling depends on the manufacturing process conducted within an environment.

- Classified environments in which closed manufacturing operations are conducted, including fermentation, sterile API processing, and chemical processes, require fewer monitoring sites and less frequent monitoring because the risk of microbial contamination from the surrounding environment is comparatively low.

Sampling Frequency (cont.)

- It is not possible to recommend microbial control levels for each type of manufacturing environment. →depending on the production activities
- Monitoring should reflect the microbiological control requirements of manufacturing or processing activities.
- Formal risk assessment techniques can result in a scientifically valid contamination control program.

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ESTABLISHMENT OF SAMPLING PLAN AND SITES

Table 2. Suggested Frequency of Sampling for Aseptic Processing Areas[a]

Sampling Area/Location	Frequency of Sampling
Clean Room/RABS	
Critical zone (ISO 5 or better)	
Active air sampling	Each operational shift
Surface monitoring	At the end of the operation
Aseptic area adjacent critical zone	
All sampling	Each operating shift
Other nonadjacent aseptic areas	
All sampling	Once per day
Isolators	
Critical zone (ISO 5 or better)	
Active air sampling	Once per day
Surface monitoring	At the end of the campaign
Nonaseptic areas surrounding the isolator	
All sampling	Once per month

a All operators are aseptically gowned in these environments (with the exception of background environments for isolators). These recommendations do not apply to production areas for nonsterile products or other classified environments in which fully aseptic gowns are not donned.

Sampling Time

- Sampling should take place with the facility in the operational condition (personnel present and normal operations being carried out).
- Microbial sampling should occur when materials are in the area, processing activities are ongoing, and a full complement of personnel is working within the aseptic processing environment.
- The operational condition for sterile hoods and transfer devices can be considered to be when an operator is working in any part of the clean air device.
- Sampling in the static condition should be performed at an agreed frequency to monitor baseline contamination levels.

Sampling Sites

- Microbial monitoring of manufacturing clean rooms, RABS, and isolators should include compressed gases, surfaces, room or enclosure air, and any other materials and equipment that might produce a risk of contamination.

- The location and movement of personnel within the clean room correlate with contamination risk to the environment and to the processes conducted within that environment.

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Methods of EMM

- Air
  - Air sampler(active method): testing of the number of organisms per volume of air sampled.
  -Settling plate(passive method): settling plates can be used as qualitative, or semi-qualitat

- Surface
  -Contact plate: product contact surface, floors, walls, and equipment should be tested on a regular basis.
  -Swabbing

Air Sampler
(Viable airborne particulates)

Active Air Monitoring

ISO 14644, Fed Std-209E, USP <1116>

- Used to sample a defined volume of air, embedding viable particulates onto sterile media strips.
- The media strips are incubated to promote the growth of viable particulates
- The microorganisms are counted and results are reported as the number of CFU per volume of air sampled.

Settling Plates
(Viable airborne particulates)

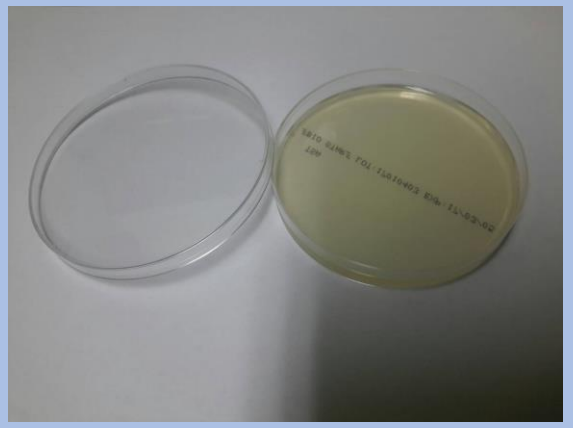
Passive Air Monitoring

ISO 14644, Fed Std-209E, USP <1116>

- Settling plates filled with media are used to sample the microbial fallout over time.
- The plates are incubated to promote growth
- Microorganisms are counted and results are reported as the number of CFU per time sampled.
- The average size of microbial particle will deposit, by gravity, onto surfaces at a rate of approximately 1 cm/s.

Sampling Sites for Settling Plates

- Areas where there is little air movement (i.e. "dead spaces") or where airflows converge or are excessively turbulent. These conditions are most likely to occur:
  - adjacent to doors
  - in pass through hatches
  - at low level return air grilles
  - between HEPA's in clean rooms
  - in corners of rooms



- Areas within the clean room where there is personnel activity or where specific operations are carried out.

RODAC Plates
(Viable, Surface-Bound Particles)

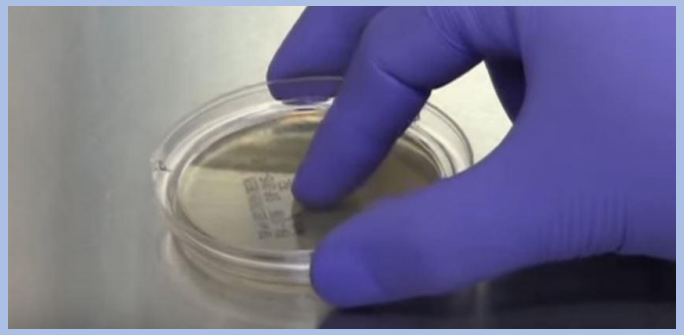
Surface Monitoring

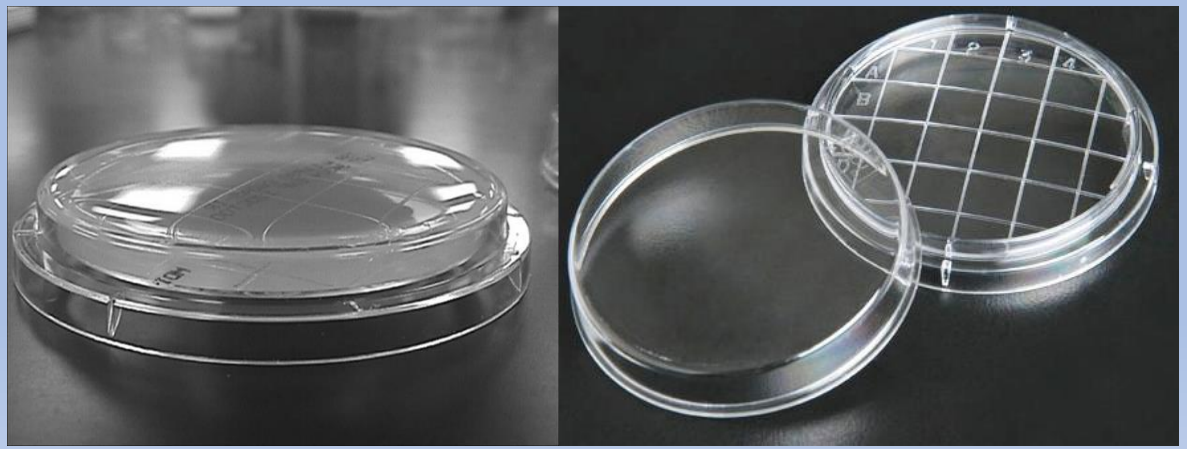
ISO 14644, Fed Std-209E, USP <1116>

- Contact plates (RODAC Plates) filled with media are used to sample tabletops, walls, benches, floors, garments, and gowned personnel.
- Measure the number of microorganisms per area sampled.
- Plates are incubated to promote growth, the microorganisms are counted and results are reported as the number of CFU per area sampled.

RODAC
(Replicate Organism Detection and Counting)

- flat agar surface is above the edges of the dish (so you can press it on flat surfaces) and a grid, allowing counting of cfu per cm^2.





RODAC Plates

- One objective of surface sampling is to determine the efficiency of routine cleaning procedures in removing contamination.
  - Sampling is done before and after cleaning.
  - The medium in the plates contains neutralizing agents, which inactivate residual disinfectants on the surface to be tested, allowing comparative results before and after cleaning.

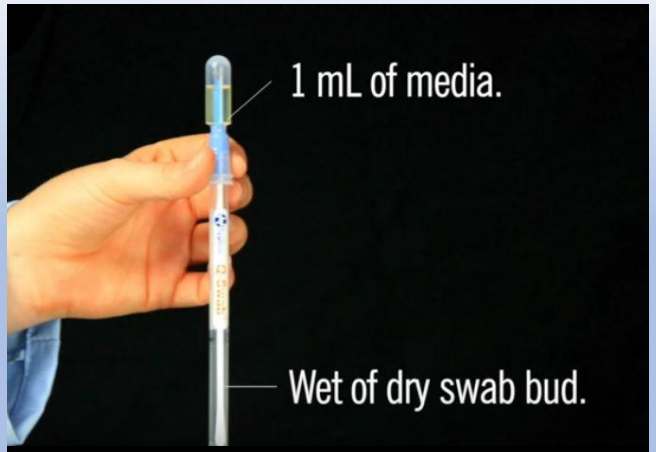
- RODAC plates are also used to monitor the contamination level of personnel gowns and Personal Protective Equipment (PPE) before or during manufacturing production.

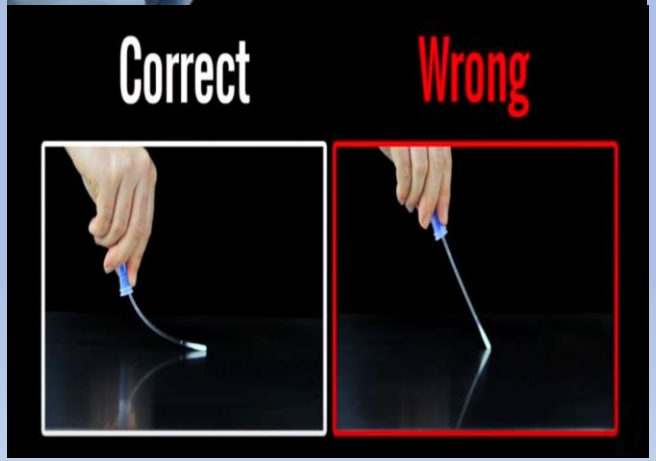
Swabbing method
(Viable, Surface-Bound Particles)

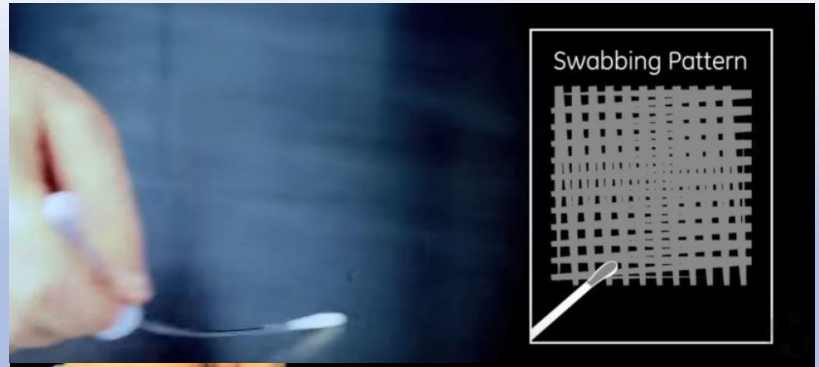
Surface Monitoring

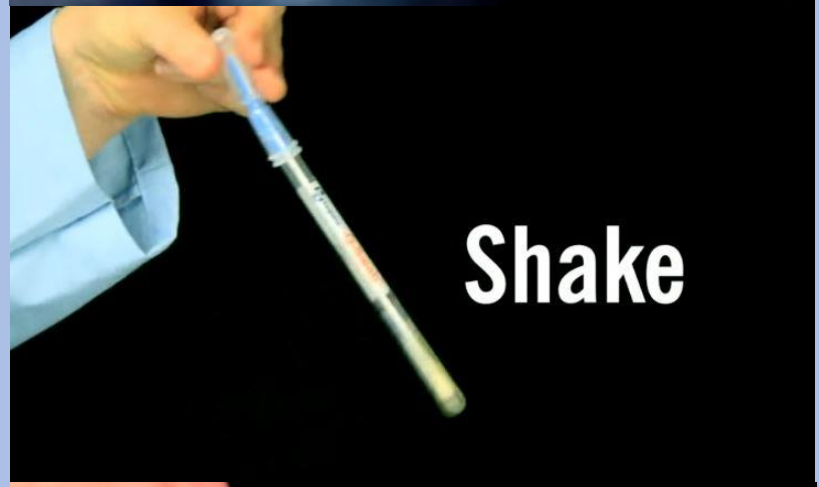
ISO 14644, Fed Std-209E, USP <1116>

- The swabbing method can be used to supplement contact plates for sampling of irregular surfaces, especially irregular surfaces of equipment.
- The area that will be swabbed is defined with a sterile template of appropriate size.
- In general, it is in the range of 24–30 cm^2.
- After sample collection the swab is placed in an appropriate diluent or transport medium and is plated onto the desired nutrient agar.


1 mL of media.
Wet of dry swab bud.

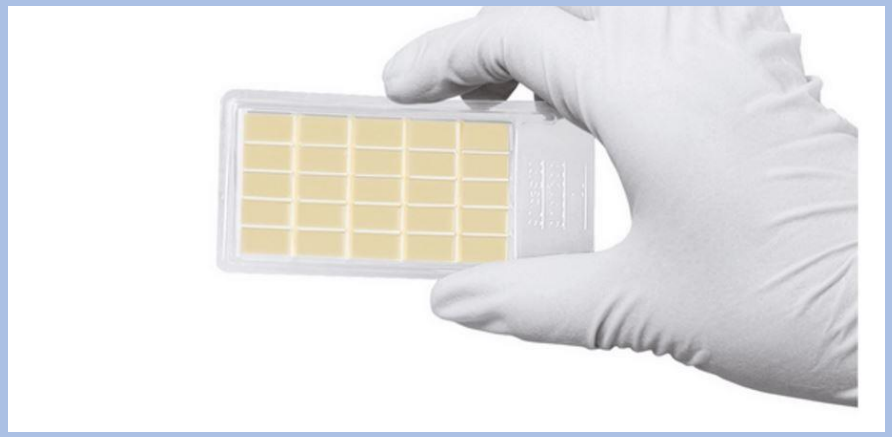

Correct
Wrong


Swabbing Pattern

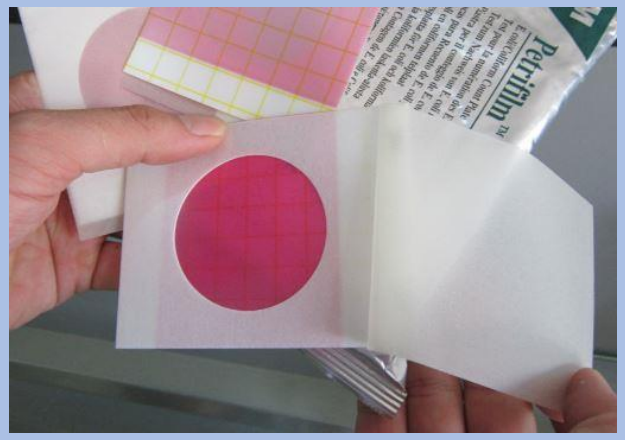

Shake

Rapid Microbiological Methods

Contact Slide(25cm^2)



Petrifilm plate



- USP<61> <62> <71> <1227>

Growth Media

- Use a growth medium with low selectivity i.e. capable of supporting a broad spectrum of microorganisms including bacteria, fungi, yeast and molds.
  - **SCDM(TSA)** is suitable for environmental monitoring in most cases because it supports the growth of a wide range of bacteria, yeast, and molds.
  - **Sabouraud Dextrose Agar (SDA)** supports yeast and fungal colonies.

Growth Media

- When necessary to detect or search for a particular type of microorganism a selective culture medium should be used.
  - Malt Extract Agar(MEA) is used as a general purpose growth media to isolate and cultivate yeasts and molds from clinical samples, as well as a wide range of environmental sources.

Culture Condition

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- Typically, for general microbiological growth media such as SCDM, incubation temperatures in the ranges of approximately 20 –35℃ have been used with an incubation time of not less than 72 hours.

- The temperature ranges given above are by no means absolute.

- For many mesophilic organisms, recovery is possible over a range of approximately 20℃ .

Culture Condition

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- In the absence of confirmatory evidence, microbiologists may incubate a single plate at both a low and a higher temperature. Incubating at the lower temperature first may compromise the recovery of Gram(+) cocci that are important because they are often associated with humans.

- Typically, for general microbiological growth media such as SCDM, incubation temperatures in the ranges of approximately 20–35℃ have been used with an incubation time of not less than 72 hours.

  - TSA plates are incubated at 30-35°C for 3 days.
  - SDA plates are incubated at 20-25°C for 5 days.

(*A review of cleanroom microflora: Types, treans, and Pattens. PDA J Pharm Sci Technol ,2011*)

Identification

- FDA Pharmaceutical CGMPs, 2004

B. Microbiological Media and Identification

Characterization of recovered microorganisms provides vital information for the environmental monitoring program. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigation. Monitoring critical and immediately surrounding clean areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level. In some cases, environmental trending data have revealed migration of microorganisms into the aseptic processing room from either uncontrolled or lesser controlled areas. Establishing an adequate program for differentiating microorganisms

- FDA Pharmaceutical CGMPs, 2004

Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques. These methods are especially valuable for investigations into failures (e.g., sterility test; media fill contamination). However, appropriate biochemical and phenotypic methods can be used for the routine identification of isolates.

The goal of microbiological monitoring is to reproducibly detect microorganisms for purposes of monitoring the state of environmental control. Consistent methods will yield a database that allows for sound data comparisons and interpretations. The microbiological culture media used in environmental monitoring should be validated as capable of detecting fungi (i.e., yeasts and molds) as well as bacteria and incubated at appropriate conditions of time and temperature. Total aerobic bacterial count can be obtained by incubating at 30 to 35°C for 48 to 72 hours. Total combined yeast and mold count can generally be obtained by incubating at 20 to 25°C for 5 to 7 days.

- USP 39 <1116> Identification of microbial isolates

A successful environmental control includes an appropriate level of identification of the flora obtained in sampling. A knowledge of the flora in controlled environments aids in determining the usual microflora anticipated for the facility and in evaluating the effectiveness of the cleaning and sanitation procedures, methods, agents and recovery methods. The information gathered by and identification program can be useful in the investigation of the source of contamination, especially when recommended detection frequency are exceeded.

Identification of isolates from critical and immediately adjacent areas should take precedence over identification of microorganisms from noncritical areas. Identification methods should be verified and ready to use kits should be qualified for their intended purpose.

Gowning Procedure

4 Personnel

1st – eliminate the source of contamination !

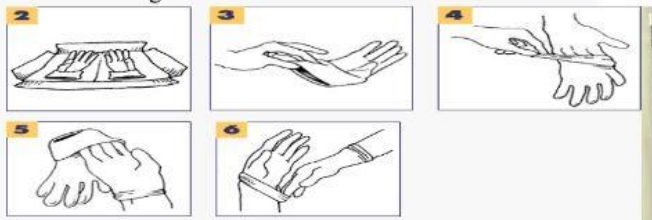
2nd - Reduce the Risk of contamination through:

Sterile barriers

Aseptic technique

Environmental monitoring

3rd Gowning / Gloves

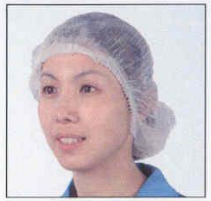


-Frequent disinfection of gloves shoud done during operations

Sterile Gowning Chart

Spiriclens or similar sterile alcohol should be used to swab gloved hands between two to three times during the gowning process.

1. Wash hands with an appropriate skin disinfectant. Dry hands with a non-linting wiper.



2. Don disposable headcover prior to entering airlock. Ensure that ears and hair are completely covered.

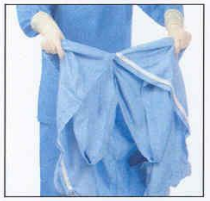
3. Don disposable shoe covers. Take three steps across tacky mat before entering airlock.

4. Enter airlock and don sterile gowning gloves.

5. Select packaged garments. Check the indicator and pouch seal to confirm sterility.

6. Wipe gowning bench with Spiriclens or similar sterile alcohol, using a non-linting wiper.

7. Remove hood from pouch without touching outside surface. Don hood and fasten clip closures.



8. Remove coverall from sterile pouch. Take hold of garment at hip level, ensuring that it does not touch the floor.

9. Insert one leg raised into the coverall, careful not to touch the outside. Repeat for other leg.

10. Insert arms into sleeves of coverall without touching the outside of garment.

11. Carefully ease zip up, ensuring that the hood is tucked inside the coverall. Snap shut clip closures.

12. Sit on gowning bench with both feet in grey zone. Lift one leg and don overshoe. Make sure that the overshoe covers the leg of coverall.

13. Swing this foot over to white zone (clean side) of gowning bench. Repeat for other foot.

14. Don mask, ensuring that it adequately covers the nose and mouth. Don safety glasses if required.

15. Don second pair of sterile gloves. Check yourself in mirror to ensure skin and hair are adequately covered.

Enter cleanroom when fully gowned, as illustrated.

Clean Room Garments Pty Ltd Ph (02) 8437 4600

/www.pda.org/pda-europe/news-archive/full-story/2015/05/27/usp-1116-and-its-implications-for-measuring-microbial-recovery-rates

Case Study: Garment Contamination Rates

Garment samples at a large European manufacturing facility were tabulated and trended on an annual basis. There were approximately 100 samples collected per quarter (horizontal axis of **Figures 1** and **2).** Two annual evaluations are shown in Figure 1 (2013 and 2014). In this example, noncontaminated samples were assigned a value of 1, and the samples that were contaminated with cfu values lower than the action limit were assigned a value of 2 (vertical axis of Figures 1 and 2). Samples with values equal or above the Action Level were not observed. In this study, the rate of contaminated samples for 2013 and 2014 were 4% each (Figure 1). It is important to consider that in terms of garment limits, for EU GMP Grade B/ISO class 7 areas, the industry understanding is often to adopt the same limits as per the limits applied to finger plates. Following this common understanding, the Action Level for gowns is ordinarily 5 cfu/25cm2, and the facility complied based on cfu results (all positives were <5 cfu/sample) (as shown in Table 1, top, for the European microbial limit). In addition, this facility also complied based on CRR (Table 2). All positives analyzed on an annual basis presented an incidence rate of 4%—which is a value complying with the <1116> recommendation of <5% limit (Table 2) for grade B.

Questions

USP39 <1117>

- Special care should be taken with media that is used in environmental monitoring studies. Media used for environmental monitoring of critical areas should preferably be double-wrapped and terminally sterilized. If terminal sterilization is not performed, media should be subjected to pre-incubation and 100% inspection prior to use within a critical area. This will prevent extraneous contamination from being carried into controlled environments and will prevent false-positive results. A raised agar level for surface contact plates should be verified.