INSTITUTE OF ENVIRONMENTAL SCIENCES AND TECHNOLOGY

Contamination Control Division Recommended Practice 018.3

IEST-RP-CC018.3

Cleanroom Housekeeping: Operating and Monitoring Procedures

INSTITUTE OF ENVIRONMENTAL SCIENCES AND TECHNOLOGY

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Please note that in regard to references herein to *Federal Standard 209 (FED-STD-209), Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones,* the U.S. General Services Administration issued the following NOTICE OF CANCELLATION of FED-STD-209 on November 29, 2001: "Federal Standard 209E dated September 11, 1992 is hereby canceled and superseded by International Organization for Standardization (ISO) Standards. International Standards for Cleanrooms and associated controlled environments, ISO 14644-1 Part 1: Classification of air cleanliness; and ISO 14644-2 Part 2: Specifications for testing and monitoring to prove continued compliance with ISO 14644-1."

Copies of ISO Standards 14644-1 Part 1, and 14644-2 Part 2, may be obtained from the Institute of Environmental Sciences and Technology (IEST), 5005 Newport Drive, Suite 506, Rolling Meadows, IL 60008-3841. Phone: 1-847-255-1561, Fax: 1-847-255-1699, Website: www.iest.org, E-mail: publicationsales@iest.org.

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1 SCOPE AND LIMITATIONS

1.1 Scope

This IEST Recommended Practice (RP) provides guidance for maintaining a cleanroom at the level for which it was designed. This document is intended to be used as a guide for establishing appropriate housekeeping procedures. In addition, test procedures are provided for use in establishing the frequency and monitoring the effectiveness of the housekeeping.

The test methods also are designed to help determine appropriate levels of surface cleanliness for specific cleanrooms.

1.2 Limitations

This RP does not specify acceptance limits, but does provide standard terminology for specifying desired limits.

Note: References to "cleanroom" in this document are to be construed, where appropriate, as including clean zones and clean-air devices.

2 REFERENCES

The editions of the following documents are incorporated into this Recommended Practice to the extent specified herein. Users are encouraged to investigate the possibility of applying the most recent editions of the references.

2.1 Applicable documents

American Society for Testing and Materials. *ASTM F24-00: Standard Method for Measuring and Counting Particulate Contamination on Surfaces.* West Conshohocken, PA: American Society for Testing and Materials, 2000.

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Institute of Environmental Sciences and Technology. *IEST-RP-CC003.2: Garments System Considerations for Cleanrooms and Other Controlled Environments.* Mount Prospect, IL: IEST, 1993.

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Institute of Environmental Sciences and Technology. *IEST-RP-CC005.2: Gloves and Finger Cots Used in Cleanrooms and Other Controlled Environments.* Mount Prospect, IL: IEST, 1996.

Institute of Environmental Sciences and Technology. *IEST-RP-CC006.2: Testing Cleanrooms.* Mount Prospect, IL: IEST, 1997.

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Institute of Environmental Sciences and Technology. *FED-STD-209E: Airborne Particulate Cleanliness Classes in Cleanrooms and Clean Zones.* Mount Prospect, IL: IEST, 1992.

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International Organization for Standardization. *ISO 14644-3/DIS Cleanrooms and associated controlled environments—Part 3: Metrology and test methods.* Mount Prospect, IL: IEST, forthcoming.

International Organization for Standardization. *ISO/DIS 14644-5 Cleanrooms and associated controlled environments—Part 5: Cleanroom operations.* Mount Prospect, IL: IEST, 2001.

International Organization for Standardization. *ISO/DIS 14698-1: Cleanrooms and associated controlled environments—Part 1: Biocontamination control—General Principles.* Mount Prospect, IL: IEST, 1999.

International Organization for Standardization. ISO/DIS 14698-2: Cleanrooms and associated controlled environments—Part 2: Biocontamination control—Evaluation and interpretation of biocontamination data. Mount Prospect, IL: IEST, 1999.

O'Hanlon, J. F.; S. M. Collins; R. G. Cates; L. A. DeShane; and J. A. Rice. "Cleanroom Cleaning Procedures Studies." In *CleanRooms '96 East Proceedings.* Nashua, NH: Pennwell Publishing Company, 1996.

O'Hanlon, J. F.; A. Arif; R. G. Cates; L. A. DeShane; and J. A. Rice. "Cleanroom Cleaning Procedures Studies II." In *CleanRooms '97 East Proceedings.* Nashua, NH: Pennwell Publishing Company, 1997.

O'Hanlon, J. F.; A. Arif; R. G. Cates; L. A. DeShane; and J. A. Rice. "Cleanroom Cleaning." *CleanRooms: The Magazine of Contamination Control Technology* 11, No. 5 (1997): 28-31.

United States Pharmacopeial Convention. *USP24-NF19: The United States Pharmacopeia and National Formulary.* Rockville, MD: United States Pharmacopeia, 2000.

2.2 Sources

American Society for Testing and Materials (ASTM)
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3 TERMS AND DEFINITIONS

adhesion
Force exerted across a surface of contact between liquids and solids, or solids and solids, which resists their separation.

clean zone
Defined space in which the concentration of airborne particles is controlled to specified limits.

cleanroom
Room in which the air filtration, air distribution, utilities, materials of construction, equipment, and operating procedures are specified and regulated to control airborne particle concentrations to meet ap-

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propriate airborne particulate cleanliness classifications, as defined by *FED-STD-209* (see section 2.2).

cleaning agent
Any liquid that has the ability to aid in the removal of surface contamination, is compatible with the product and process, and does not contribute substantially to the contamination of the cleanroom.

classes of air cleanliness
Airborne particle concentration levels defined by *FED-STD-209* (see section 2.2).

contamination
Act or result of the deposition of contaminants on a surface.

housekeeping
Maintenance or cleaning performed to preserve the specified cleanliness of a cleanroom.

particle
Solid or liquid object that, as a general rule, measures from between 0.001 µm to 1000 µm in size.

particle burden
Number of particles in the relevant size range per unit area.

primary surface
Surface that is in direct contact with the product.

secondary surface
Surface that is not in contact with the product but indirectly affects the product.

residue
Any substance left on a surface after cleaning.

surface contaminant
Any unwanted substance present in or on a surface.

viable particle
Particle capable of living or growing.

4 BACKGROUND

4.1 Particles on surfaces: adhesion and removal

Mechanisms by which particles are held to surfaces in a cleanroom include London-van der Waals forces (the dominant mechanism when surfaces are not wetted), electrostatic forces (which usually are small if the facility has been properly shielded), and capillary adhesion. Removal of particles is aided by the use of a liquid because such usage decreases the London-van der Waals forces by several orders of magnitude, dissipates electrostatic forces, and, if a surfactant is present in the liquid, decreases capillary adhesion through a lowering of surface tension.

Ionic surfactants alter surface electrical potentials and thus can also reduce electrical forces. Liquids also permit better coupling of the mechanical energy used during cleaning, as well as provide an opportunity for the removal of soluble contaminants through dissolution.

Individual sub-micron particles are extremely difficult to remove, and even an optimum cleaning procedure will not completely eliminate them from cleanroom surfaces (see section 2.1, Bowling). It is likely that only those particles larger than 5 µm will be significantly affected by the cleaning procedures presented in this Recommended Practice.

See the appendix for a detailed discussion of particle adhesion and removal.

4.2 Validation of cleaning

If cleaning is being undertaken by or for a manufacturing process, where the control of viable organisms is critical, (e.g. pharmaceutical, biologic, or medical devices manufacturing,) the cleaning will probably be under the control of a regulatory agency such as the FDA, in which case the validation of cleaning is essential.

The FDA's formal definition of such validation can be summarized as:

"To establish documented evidence that provides a high degree of assurance that a specific [cleaning] process will consistently produce a product [result] meeting its predetermined specifications and quality characteristics."

Validation, usually undertaken as a project prior to full-scale operation, starts with the creation of the Validation Master Plan. Most often, it contains three sections: Strategy, Protocols, and Evaluation. "Strategy," or the design phase, sets out the objectives and specifies the results to be achieved. Results are specified as permitted residual limits. "Protocols" covers the implementation stage and specifies how the goals will be achieved. "Evaluation" describes the means and methods, either visual or sampling and analytical tests, which will be used to analyze the results of the process.

Sampling techniques should be able to collect contamination, the residues, in a form that is detectable by the analytical methods of choice. That choice will depend on the sampling site, the nature and properties of the residues, and the type of facility and its equipment.

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5 PROCEDURES

5.1 Equipment and supplies

All tools, equipment, and supplies used in the cleanroom for housekeeping purposes should be clean and, if appropriate, sterilized or disinfected prior to being brought into the cleanroom. Equipment designated for cleanroom service should be restricted to this use.

The following is a list of recommended supplies and equipment for use in housekeeping. It should be noted that this list is not all-inclusive. The responsible person should select equipment and supplies that are compatible with the cleanroom, cleanroom surfaces, the product, and related processes.

5.1.1 Vacuum cleaning system

A central plant vacuum (wet or dry) with attachments, or a portable vacuum cleaner with HEPA/ULPA filtered exhaust and accessories.

5.1.2 Tacky roll mop

A mop that removes particles by means of an adhesive contact surface.

5.1.3 Bucket

A bucket made of plastic or stainless steel—single, dual, or triple.

5.1.4 Wet mop

A mop that has a head constructed of foam, nylon, or low-linting material, and handles constructed of stainless steel, aluminum, reinforced plastic or an equivalent material.

5.1.5 Cleanroom wiper

A cleanroom wiper selected in accordance with recommendations of *IEST-RP-CC004* (see section 2.1).

5.1.6 Stepladder

A stepladder constructed of aluminum, stainless steel, or fiberglass-reinforced plastic.

5.1.7 Cleanroom apparel

Cleanroom apparel compatible with the cleaning agents and the cleanroom classification, as recommended in *IEST-RP-CC003* and *IEST-RP-CC005* (see section 2.1).

5.1.8 Water

Water, such as filtered tap, distilled, reverse osmosis, or deionized water, or water suitable for injection.

The water used in healthcare cleanrooms is delivered in one of two forms: Purified Water or Water for Injections, commonly called WFI. Water originates from the mains supply, as delivered by the water supply company, or from an artesian well, and is processed [purified] to specification (see section 2.1, United States Pharmacopeia).

a) Purified Water

   Purified water represents the bulk of water used in cleanroom applications. The water purification process includes pre-treatment to remove gross impurities, which may then be followed by the reverse osmosis process or deionization, or both in sequence.

   The test for the purity of water is chemical and depends on its conductivity, measured in microSiemens per cm. The Total Organic Carbons content is another measure of purity. Dependent on the quality specified, purified water is mostly free of solid contaminants.

b) Water for Injections

   WFI is used in biotechnological and pharmaceutical manufacturing. The difference between Purified Water and WFI is that the latter must be free of viable organisms and pyrogens as well as solid contaminants.

   WFI may be produced by deionization, or, frequently, for extra security, as a third stage in the purification process by distilling the already purified water.

c) Mains Supply

   Water feedstock from the water supply company is delivered in potable, or drinkable, form but is not required to be bacteria-free, pyrogen-free, or contamination-free.

5.1.9 Cleaning agent

Cleaning agents that are aqueous (acid and alkaline, both with and without detergents and disinfectants) and those that are based on solvents. Cleaning agents that are dispersed as aerosols or sprays usually are not acceptable for cleanroom use.

Note: Toxic or corrosive substances require special handling precautions, tools, and disposal procedures.

5.1.10 Squeegees

A squeegee that has a blade made of a material that contributes minimal contamination. It should have a support and handle made of stainless steel or reinforced plastic or sealed with a durable, acceptable coating.

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5.1.11 Vacuum-controlled, steam cleaning systems

A cleanroom compatible system that utilizes steam to clean surfaces. Steam is applied directly to the surface, trapped and removed by a vacuum head to avoid escape to the cleanroom environment.

5.1.12 Vacuum-controlled floor scrubbing systems

A cleanroom scrubbing system that utilizes rotating brushes to clean or buff cleanroom floors. Built-in vacuum system, enhanced by shrouds, traps any particles residue created by the cleaning action and removes them to a HEPA filtered container.

5.1.13 Contamination control mats and flooring

Semi-permanent and permanent cleanable contamination control mats, usually made from polymers that attract and hold contamination tracked by the feet, are effective.

When used in pharmaceutical and other healthcare manufacturing and cleanroom applications where control of viable organisms is critical, particular attention should be paid to drying the underside after mopping because microorganisms can readily breed in such damp areas.

5.2 Housekeeping procedures

The housekeeping practices recommended herein provide a comprehensive program for the removal and control of viable and nonviable contamination. It is recognized that not all of these practices are necessary for all cleanrooms.

Cleanrooms in which viable contamination is of primary concern may require different cleaning steps or a different sequence of steps.

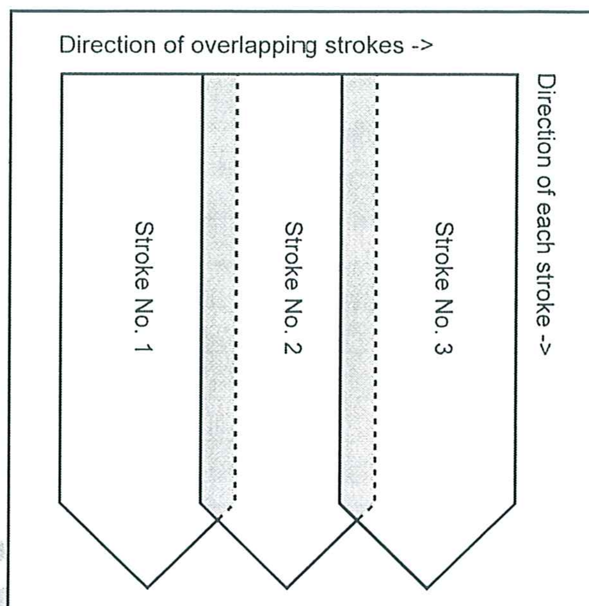
In general, cleaning should begin in the areas requiring the most critical level of cleanliness and proceed toward areas of less critical requirements.

Note: Safety precautions must be followed during any housekeeping activity. Personal protective equipment may be required. Waste disposal procedures should follow all local safety codes and environmental requirements.

5.2.1 Ceilings

Caution: If an ionization system is present, it must be deactivated prior to cleaning the ceiling.

Figure 1. Recommended wiping sequence



a) Ceilings in which HEPA filters are located adjacent to each other

Where HEPA filters are located adjacent to each other, only the grid should be cleaned, by dry vacuuming, to protect the filter. Strokes used in vacuuming should *follow the grid pattern* in one direction only.

b) Ceilings consisting of surfaces other than HEPA filters

Ceilings consisting of surfaces other than HEPA filters should be vacuumed and wiped with a cleanroom wiper or mop wetted with a cleaning agent. Cleaning should be performed in a straight-line fashion (see Figure 1). Circular and scrubbing motions should be avoided

c) Light fixtures

Light fixtures should be wiped with a cleanroom wiper moistened with a compatible cleaning agent (to prevent etching the lens). The inside of the lens should be cleaned in a similar manner.

d) Sprinkler heads and alarm system.

Accessories such as sprinkler heads and alarm systems should be wiped, using a cleaning agent and a cleanroom wiper.

Caution must be exercised when cleaning sprinkler heads to prevent activating them by contact.

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Figure 2. Wall-cleaning stroke method

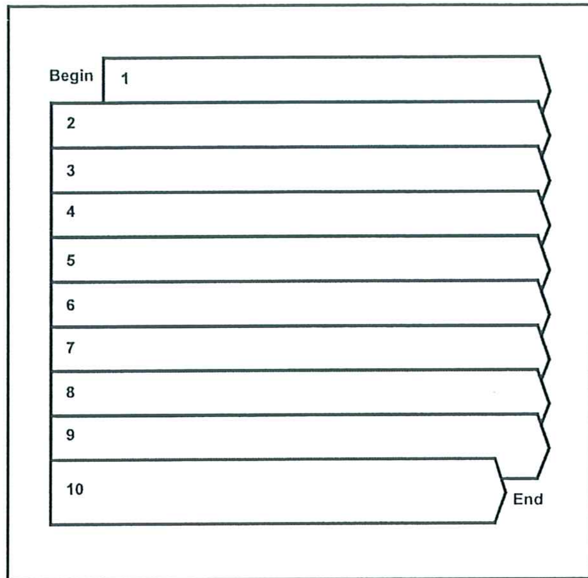
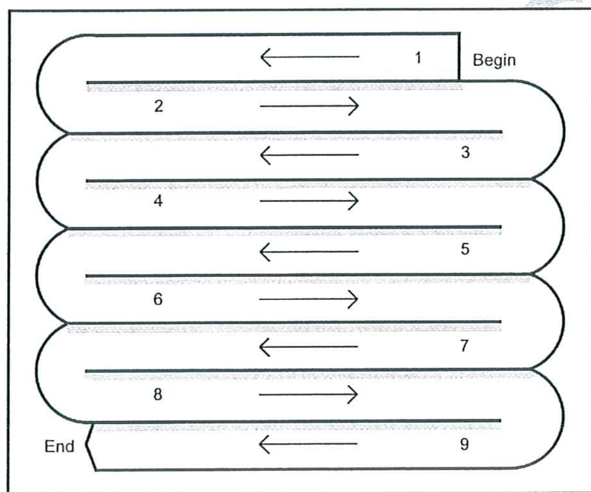


Figure 3. Floor-cleaning stroke method



e) Ionization systems

Ionization systems should be cleaned according to the manufacturer's instructions. Because particles are generated during this procedure, no product should be present in the cleanroom and no process should be performed while cleaning is taking place in the cleanroom. Using a vacuum cleaning system for the collection of particles as they are generated may minimize particle dispersion. After the ionization system has been cleaned, the entire cleanroom should be cleaned because contamination may be generated during the ionization system cleaning.

5.2.2 Walls

Walls should be cleaned beginning at the ceiling and working in vertical lines toward the floor. First, the walls should be cleaned with a vacuum apparatus or a tacky mop, using overlapping strokes (Figure 1), followed by wiping or damp mopping. The wipers or mop should be wet with a cleaning agent. Rinsing maybe required, depending upon the cleaning agent selected.

Alternate method—vacuum and mop walls horizontally. Horizontal strokes should be a maximum of 4 feet in length, using overlapping strokes (Figure 2) (see section 2.1, Foarde, O'Hanlin).

5.2.3 Doors, frames, and components

First, the top and side edges of the doors should be cleaned while they are ajar, using the appropriate vacuum tool or tacky mop, followed by wiping. The wiper should be moistened with a cleaning agent and the surface rinsed, if required by the cleaning agent. The procedure described for cleaning walls in section 5.2.2 should also be used to clean doors and associated surfaces.

5.2.4 Windows

Windows should be cleaned using a wiper wet with a suitable cleaning agent. Windows should be wiped from top to bottom with vertical strokes. After each stroke, the wiper should be folded to expose an unused portion.

In a non-aseptic environment, a dry wiper or squeegee should be used to dry the window from top to bottom with vertical strokes, as described in the previous paragraph.

5.2.5 Floors

The entire floor should first be vacuumed or mopped with a tacky mop, using the following procedure:

The vacuum attachment or mop roller should be placed on the floor, pulled toward the operator, then lifted and moved, so that the next stroke starts adjacent to and slightly overlapping the first stroke (see Figure 1).

The floor should then be damp-mopped with a cleaning agent, using a series of slightly overlapping strokes. Rinsing may be required, depending on the type of cleaning agent used.

An alternate stroke method is the modified figure "8." The floor is mopped from right to left, then left to right with overlapping strokes. Stroke distance should not exceed 3 feet (see Figure 3) (see section 2.1, Foarde, O'Hanlin).

5.2.6 Mats or floor coverings with adhesive surfaces

Permanent mats and flooring with an adhesive surface should be cleaned in accordance with the manufacturer's instructions.

The sheets of renewable mats that have removable sheets should be changed whenever necessary. The soiled sheet should be peeled from the four corners and lifted in the form of a bag or pocket. The action should be performed slowly so that the accumulation of static charge and the spread of already deposited contamination are minimized.

5.2.7 Workstations

Wiping should begin at the rear of the work surface with a folded cleanroom wiper moistened with a cleaning agent. The wiping motion should be in one direction only, from one side to the other (horizontally), and a fresh area of the wiper should be used for each stroke. Each stroke should slightly overlap the previous stroke.

5.2.8 Waste receptacles

If trash liners are acceptable, the liners should be removed from the waste receptacles by folding the top of the liner closed without evacuating the trapped air. The liners should be removed from the cleanroom immediately. A new liner should be placed inside the receptacle and the edges peeled open around the circumference. The liner should not be inflated.

If trash liners are not used, the receptacle should be removed from the cleanroom work area. The trash should be emptied and the waste receptacle cleaned with a cleaning agent before it is returned to the cleanroom area.

Note: Follow all safety procedures for handling waste.

5.3 Housekeeping records

To ensure that all housekeeping procedures in the cleanroom are performed as scheduled, the specified activities should be tallied as they are accomplished. Figure 4 is an example of a checklist that may be used for this purpose.

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Figure 4. Cleaning Checklist

CLEANING CHECKLIST		
DATE:	DIRECTIONS: Write in the time each item is completed.	
ITEM	TIME	NAME/EMPLOYEE #
Gownroom		
Vacuum Floor		
Tacky Mats		
Clean Walls		
Empty Waste Receptacles		
Parts Cleaning Area		
Tacky Mats		
Wet-Mop Floor, Airlock		
Cleanroom		
Dry Vacuum		
Tacky Mop		
Wet Mop		
Cleanroom		
Cleanroom		
Cleanroom and Gownroom		
Dust Lockers, Wire Racks, Etc.		
Wash Windows and Doors		

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Table 1. Cleaning Frequency ISO 5 – Class 100

Surface	Shift	Daily	Weekly	Monthly
Floors	X			
Walls			2x	
Windows			2x	
Doors		x		
Ceilings				x
Trash removal	as needed			
Equipment	X			
Furniture	X			

5.4 Cleaning frequency

The frequency of cleaning is determined by the process, equipment, level of activity, number of people, and the cleanliness requirements of the product and cleanroom. Table 1 is an example of the frequency ofcleaning for an average cleanroom operation, Class 100 (ISO 5).

6 TEST METHODS

This section recommends test methods for use in assessing the cleanliness of surfaces. The methods are classified according to the type *(i.e.,* viable or nonviable) and size of the particle. Inspection by ultraviolet light or by high-intensity, oblique-angle white light, as well as vacuum sampling, are insufficient in themselves to verify cleanliness. The operations listed in Table 2 are performed in a particular sequence, as required to determine the information desired, in order to constitute a valid certification.

All tools and equipment used in the test pro-cedures should be cleaned thoroughly before and after each use with the appropriate methods and supplies (see section 5), unless otherwise indicated.

6.1 Test methods for nonviable particles

Visual inspection techniques depend on the physical characteristics of the particles, the contrast with background surfaces, the wavelength and intensity of light used, and the visual acuity and experience of personnel. As a general rule, these kinds of inspections provide a quick assessment of surface conditions involving contaminants greater than 50 µm. It is also possible to detect particles much smaller if conditions are optimum.

6.1.1 Ultraviolet light inspection

Ultraviolet (365 nm) light causes certain organic materials to fluoresce. Care therefore should be taken to distinguish this fluorescence from the blue light reflected by pol-ished metal surfaces. Not all fluorescent material is contamination. Most conformal coatings and many aseptic cleaners will fluoresce under ultraviolet light. Nor will all contamination fluoresce. For example, metals and most types of glass do not fluoresce.

Sizing particles under ultraviolet light is difficult because of differences in the ability of materials to fluoresce and darkened conditions during inspection. A particle that is highly fluorescent may appear to be larger than a similarly sized particle that is only slightly fluorescent.

Warning: Do not shine unfiltered ultraviolet light into the eyes. Eye protection is required; eye shields with the proper optical filters should be used.

a) Equipment for ultraviolet light inspection

   The following equipment is needed to perform ultraviolet light inspection:

   1) Ultraviolet light– A light source with a 100-w to 125-w ultraviolet lamp, having a wavelength of 365 nm.
   2) Probe– Any device that is used to ascertain by mechanical action whether a suspected particle is a contaminant. The suitability of a probe for use on certain surfaces should be determined on a case-by-case basis.
   3) Vacuum device– A device used primarily to remove particles that are loosely held to a surface. It also can be used to collect particles when a suitable filter is placed downstream of the vacuum inlet. Particles collected in this manner can be sized according to *ASTM F24* (see section 6.1.3).

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Table 2. Application of test methods according to type of contamination

Test method		Nonviable	Viable
6.1.1	Ultraviolet light inspection	>50 μm	
6.1.2	High-intensity, oblique white light inspection	>20 μm	
6.1.3	Counting and sizing particles with an optical microscope	> 5 μm	
6.1.4	Automated particle fallout test and optional fallout characterization	> 5 μm (optical microscope) > 0.2 μm (laser)	
6.1.5	Surface particle detector method	> 0.3 μm	
6.1.6	Wipe visual tests	>50 μm	
6.2.1	Contact plate method (for flat surfaces)		X
6.2.2	Swab method (for non-flat surfaces)		X

4) Solvent– An agent used to dislodge particles by reducing surface tension and neutralizing electrostatic charges. The solvent must be filtered to the appropriate level of cleanliness and must be compatible with the surface materials.

Note: This method could dissolve the contaminants and therefore give false verification.

b) Procedure for ultraviolet light inspection

Ultraviolet light inspection should be performed according to the following steps:

1) Allow the ultraviolet light to warm up for at least 5 min to reach full operating output.

Note: If the lamp is extinguished for any reason, the bulb must cool before it will restart. This will take 5 to 10 min. To avoid delays and to prolong bulb life, leave the lamp on while performing intermittent inspections.

2) Perform the inspection with the lighting in the cleanroom reduced to as low an intensity as possible in order to see small fluorescent particles. Allow 2 to 3 min for the eyes to adapt to the darkened area.

3) Inspect all surfaces with the ultraviolet lamp, using a probe, with solvent if necessary, or using a vacuum device to aid in dislodging suspected particles. If particle analysis is necessary, remove the particle from the probe onto a suitable sampling apparatus.

6.1.2 High-intensity oblique white light inspection

Warning: This type of inspection can cause eyestrain if exposure is prolonged. Shining the light beam directly into the eyes or indirectly off reflective surfaces into the eyes should be avoided, as it may have a cumulative detrimental effect.

This technique makes use of the reflection of light, contrast of color, and shadow effects that will be caused by any particles on relatively smooth surfaces. The angle at which the light is held is critical to detecting very small particles. Angles of from 15° and to 45° relative to the surface being inspected are considered to be the most effective. Angles greater than 45° can cause particles to become lost in the general background reflection as a result of loss of the shadow effect and low color contrast between particle and surface.

Highly contrasting colors or large particle size can make it possible to use greater angles. Angles of less than 15° can highlight aberrations that exist in all but the smoothest surfaces, making detection of small particles extremely difficult. In general, the smaller the particle, the smaller the angle needed to detect it.

a) Equipment for high-intensity oblique white light inspection

The following equipment is needed to perform high-intensity white light inspection:

- A variable-intensity white light source (0 to 600 foot-candles at 1.17 cm). A fiber-optic light pipe with a 150-w halogen lamp will provide a cool light at the point of use that can easily be manipulated to the desired angle and direction.
- A probe (see section 6.l.la)
- A vacuum device (see section 6.1.1a)
- A solvent (see section 6.1.1a)

b) Procedure for high-intensity oblique white light inspection

To perform this test, the light level in the area to be inspected should be approximately 50% of normal intensity.

High surrounding light levels tend to diminish the effective contrast produced by the

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high-intensity light. Very low surrounding light levels, those approaching darkness, can narrow the field of vision to the small area illuminated by the high-intensity light source. Eyestrain can result after prolonged periods of time.

High-intensity oblique white light inspection should be performed by inspecting all surfaces with high intensity white light and varying the distance and the light as needed to detect all particles in the relevant size ranges. A probe (with solvent, if necessary,) or vacuum device should be used to aid in dislodging suspected particles. If particle analysis is necessary, the particle should be removed with the probe onto a suitable sampling apparatus.

6.1.3 Counting and sizing particles with an optical microscope

This method should be performed in accordance with *ASTM F24* (see section 2.1). After particles are removed, this method involves counting and sizing them under a light microscope. The method is useful for particles greater than 5 μm.

6.1.4 Automated particle fallout test and optional fallout characterization

This method provides data that indicate relative levels of surface contamination from the settling out of airborne particles and fibers. Fallout samples are collected on polished glass sample plates and a numerical rating of the level of contamination on each plate is obtained photometrically in response to the obscuration or scattering of light reflected from the surface of the sample plate. The determination of relative levels of surface contamination from fallout can be used as a basis for selecting appropriate methods and for planning schedules for cleaning surfaces in specific areas of the cleanroom or clean zone.

This test method is applied most effectively where levels of contamination from fallout are more pronounced and is generally inappropriate where such contamination is negligible, unless used as a routine monitoring test to provide evidence of random events.

The photometer employed in this test method also provides an opportunity for visual examination and characterization of the fallout, especially materials of a fibrous nature.

a) Procedure

1) Determine locations for sampling. The locations may be related to known or suspected sources of airborne contamination. Collection of data for historical baseline record keeping may also prove valuable, even if no objectionable levels of contamination are expected or found. Keep a detailed record of the locations, using appropriate descriptions or a map with a logical numbering scheme.

2) Mark an appropriate number of clean sample plates with location designations or symbols so that test data can be properly recorded.

3) Take each sample plate to its designated sampling location and slide open the cover, being careful to avoid contaminating the surface of the sample plate. Record the date and the time of day when exposure is initiated for each plate.

4) At the end of the predetermined sampling period (or when a significant contaminating event is suspected to have occurred), carefully close the cover on the sample plate, being careful to avoid contribution of additional fallout on the sample surface. Record the time when the cover was closed.

5) Transport the sample plate(s) to the particle fallout photometer, turn on the power to the photometer and allow it to warm up, then slide each plate into the photometer in turn to obtain the measurement of its respective contamination level.

6) Record the numeric reading shown on the instrument display for each plate. Also record the location of sampling and the date, times, and duration of sampling.

7) Prepare the sample plates for the next round of testing by cleaning them in accordance with the manufacturer's instructions. To verify surface cleanliness of the plates, insert them into the photometer and obtain a reading of less than 5 on the display. Clean any plates yielding a photometer reading of 5 or more again, and recheck cleanliness; repeat until cleanliness is verified.

b) Data reporting

Report the results of testing to appropriate designated personnel. If limits have been established for numerical test values at specific locations or in general, notify the responsible personnel. Limits may be established in the form of numeric control values (as read from the photometer) or as normalized values with respect to the duration of sampling (e.g., test value per hour, per day, or per shift).

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6.1.5 Surface particle detector method

This method provides a means of rating or ranking cleanliness levels on flat surfaces, based on counts of loosely adhering particles removed from these surfaces.

The method provides for the use of a scanning head, which is drawn over the surface being rated. The head directs a continuous supply of filtered air onto the surface and dislodges the particles, which are drawn into the head by means of a continuous vacuum. The area concentration of particles collected over a designated time interval is expressed in terms of particles per cm^2 (or per $in.^2$) by laser based particle-sensing instrumentation.

a) Equipment

To employ the surface particle detector method, a surface particle detection sampler is needed. It should consist of a scanning probe with a remote actuator switch and connecting tubing and a laser-based count system with a 0.3-µm sensitivity threshold. Air filtered to 0.2 µm is supplied to the probe of the sampler, and a like volume is collected and returned to the laser system for automatic determination and display of particle concentration.

b) Procedure

The surface particle detection method should be performed using the following steps:

1) Establish an initial background count by holding the probe inlet in clean air issuing from a HEPA-filtered supply, until the count rate reaches zero or other acceptable background level.

2) Press the actuator ("run") switch on the head and scan the test surface at a rate of 2 in./sec (3m/min) for a period of 6 sec. (A light touch is recommended for reproducible results, taking advantage of the fluidizing action of the air underneath the head.) Record the particle concentration.

Note: An alternative scan duration may be chosen, where appropriate, based upon the size of the area being sampled, the number of tests to be performed, or other considerations. Sampling periods such as 3 secs or 1 sec may be used, for example, provided that all surfaces to be compared in a given series of tests are done in the same manner.

3) Repeat the scan of the area under test at least five times *(i.e.,* a minimum of six readings should be taken for each location) making certain that the same exact test location is not in use. Testing small areas may result in only one test being run.

c) Reporting

Reporting the results of the surface particle detection method should be done as follows:

1) For each test area sampled, record the maximum reading, and the average of all readings taken.

2) As desired, a file of historical data may be maintained to provide the basis for a routine monitoring program for each test area.

Note: If the sampler has optional particle size capability, test data may be sorted into ranges so that information may be gained regarding size distribution and trends in the distributions. Such data may aid in the determination of sources of particulate contamination.

d) An optional, in-line capture filter may be employed with the surface particle detector. The device consists of a removable 47-mm membrane filter and housing located downstream of the laser optics and before the vacuum pump. The membrane filter captures the particles being measured for later identification and analysis using various laboratory analytical methods. The housing and filter assembly should be cleaned and prepared under very stringent laboratory conditions to avoid contamination of the sample. The surface particle detector should be purged to a zero-count reading level before inserting the particle capture device.

6.1.6 Wipe visual tests

A simple and effective visual cleaning test method is to wipe a brand new white or black wipe over the surface to be tested and to inspect closely for signs of contamination. This method will often demonstrate contamination if the wipe shows sign of discoloration.

6.2 Test method for viable particles

This Recommended Practice presents two test methods for assessing viable microbiological populations on surfaces. However, other methods may have benefits in certain applications.

The discussion of these two methods specifies materials, testing criteria, growth media, and conditions for sterilization and incubation. Alternatives or variations in these may be appropriate, depending upon the needs of the user.

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No biological recovery method is absolute, and the user must be aware that results measure only a proportion of the living organisms on the surfaces. Test results are affected by several recovery variables, such as:

- the transfer of an organism from the test surface to the test system;
- the survival of an organism during the transfer;
- the growth of an organism to a visible colony on the test media.

The organism's metabolic state during the time of removal can affect recovery. A physically or chemically stressed organism may find nutrients in the test media to be toxic.

Organism growth factor requirements, relative to test conditions, will have a pronounced effect on the detection. Length and temperature of incubation, growth media constituents, and the presence of oxygen and carbon dioxide are all critical to organism survival and growth.

Organism distribution on the surface can have a bearing on detection. Organisms usually are found on particulate matter, and the particulate matter may have several organisms clinging to it. If several organisms originate from one particle, some may have growth factor requirements that are inhibited by their neighbors, and thus they do not form visible colonies.

In summary, it is important to understand the basic limitations of viable particle testing. There is a considerable amount of variability not only between recovery procedures, but also within a given procedure from sample to sample. Techniques and methods should remain as constant as possible when viable surface populations are measured and compared. When properly used, these procedures are sensitive enough to detect major shifts in surface contamination levels and population types.

6.2.1 Contact plate method (for flat surfaces)

The contact plate method is used for detecting microorganisms that may be present on flat surfaces in relatively low numbers. Samples of heavily contaminated surfaces will result in the growth of colonies and unreadable plates. A plate with an area of 25 cm^2 is applied to a surface to pick up a high proportion of organisms that are present due to the convex agar meniscus.

After appropriate incubation, the microbial contaminants will grow into macroscopic colonies that can be enumerated. Colony-forming units are commonly expressed per 25 cm^2.

a) Materials and equipment

- Trypticase (or Soybean Casein Digest) agar with lecithin and polysorbate 80 contact plates
- Sterile wipes
- 70% 2-propanol or other suitable disinfectant
- An indelible marking pen
- An incubator capable of reaching temperatures of 30° to 35°C (86° to 95°F)
- An incubator capable of reaching temperatures of 20° to 25° C (68° to 77° F)
- A refrigerator capable of reaching temperatures of 2° to 8°C (35.6° to 46.4°F)
- An autoclave

b) Preparation for testing

1) Pre-incubate the agar plates to ensure they are sterile by removing the plates from the refrigerator and placing them in the incubator at 30^0 to 35^0 C (86^0 to 95^{0}F) for 18 hr to 24 hr.
2) Inspect each pre-incubated plate for contamination. Discard those with visible contamination.
3) Label each plate with the location to be sampled and the test date.

c) Sampling

1) Transport contact plates, sterile wipes, and 70% 2-propanol (or other suitable disinfectant) to the area to be tested.
2) Hold the rim of the lid of the agar plate with the fingertips of one hand and remove the cover with the other hand, making sure not to touch the agar surface.
3) Apply the plate's agar surface directly to the test surface with a rolling motion, exerting moderate pressure. Replace the cover carefully.
4) To remove any residual agar on the test surface, wipe the surface (see section 5.2.7) with a sterile wipe saturated with 70% 2-propanol. Leave as little disinfectant as possible on the test surface.

d) Incubation

All plates should be incubated at 30° to 35°C for a minimum of 48 hr. If mold is of primary interest, the plates should be incubated at least 72 hr at 20° to 25°C (68° to 77°F).

e) Reading and recording results

1) After incubation, count the number of discrete colony-forming units (CFU) on each plate and record the number on the appro-

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priate form. Separate colony counts may be tabulated for mold and bacteria.

If CFU are not discrete entities or are too numerous to count (usually > 300 CFU), record the result as "TNTC" (too numerous to count).

If one type of CFU tends to grow in a spreading manner, count this as "one spreading colony" and record as such.

2) If organisms are to be identified and/or Gram stained, store plates under refrigeration.

3) To discard, place the plates in an appropriate receptacle in the autoclave for 60 min at 121°C (249.8°F).

6.2.2 Swab method (for other than flat surfaces)

The swab method involves collecting microorganisms by rubbing a sterile swab, moistened with sterile water, a nonnutritive medium, or both, in several directions over a standardized test area. Attention should be given to the selection of either a nonnutritive medium that contains a surfactant, or a disinfectant inactivating agent, or both, so that the organisms sampled are not destroyed.

Once collected, the organisms can be counted in several ways. They maybe streaked directly onto an agar plate with a premoistened swab, or the swab can be placed into a sterile collection solution and either dissolved, agitated, or sonicated to transfer the microorganisms from the swab into a liquid collection medium. The collection medium may then be tested by a most probable number method (MPM), membrane filtration method, or direct plating method.

a) Materials and equipment

- Sterile water (minimum 10 ml)
- Sterile swabs
- Trypticase (or Soybean Casein Digest) agar with lecithin and polysorbate 80 plates
- An indelible marking pen
- An incubator capable of reaching temperatures of 30° to 35°C (86° to 95°F)
- An incubator capable of reaching temperatures of 20° to 25° (68° to 77°F)
- A refrigerator capable of reaching temperatures of 2° to 8°C (35.6° to 46.4°F)
- An autoclave
- Autoclave bags and tape
- Sterile snippers
- 70% 2-propanol or other suitable disinfectant

b) Preparation

1) Place swabs in an autoclave bag. Tape the bag closed with autoclave tape and label it with the contents. Alternatively, swabs can be placed in individually capped test tubes, to which a little moisture should be added prior to sterilization. Label each bag with the location to be sampled and the test date.

2) Sterilize the bags or tubes for 20 min at 121°C (249.8°F) in the autoclave or other validated cycle.

3) Sterile swabs also may be purchased.

4) Pre-incubate agar plates according to 6.2.1b.

c) Sampling

Sampling should be performed as follows:

1) Obtain bottles of sterile water, sterile swabs, and agar plates and transport them to the test area. If premoistened swabs in test tubes are used, eliminate the next step.

2) Aseptically remove the cap of the sterile water bottle. Remove one swab from the packet, making sure the other swabs in the packet do not become contaminated. Carefully dip the swab into a sterile water bottle. The swab should not touch the outside of the rim of the bottle. Do not supersaturate the swab.

3) Streak the swab in a rotating manner across approximately 25 cm^2 of the test surface.

4) Remove the lid from the appropriate plate and streak the swab in a rotating manner across the entire plate. Replace the lid.

5) Discard the swab in an appropriate receptacle. Wipe the surface with a sterile wipe saturated with 70% 2-propanol. Leave as little disinfectant as possible on the surface.

d) Incubation

All plates should be incubated at 30° to 35°C (86° to 95°F) for a minimum of 48 hr. If mold is of primary interest, plates should be incubated for a minimum of 72 hr at 20° to 25°C (68° to 77°F).

e) Reading and recording

1) After incubation, count the number of CFU (see section 6.2.1e) on each plate and record the number on the appropriate form. Separate colony counts may be tabulated for bacteria and mold.

If CFU are not discrete entities or are too numerous to count (TNTC) (usually > 300 CFU), record them as TNTC.

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If one type of CFU tends to grow in a spreading manner, count this as "one spreading colony" and record it as such.

2) If organisms are to be identified and/or Gram stained, store plates under refrigeration.

3) To discard, place the plates in an appropriate receptacle in the autoclave for 60 min at 121°C (249.8°F).

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APPENDIX: PARTICLE ADHESION AND REMOVAL

The first part of this appendix reviews the most common forces that bind a particle to a surface—the particle adhesive forces. The second part of the appendix discusses forces for removing particles from a surface—the cleaning forces.

1. ADHESIVE FORCES BETWEEN A PARTICLE AND A SURFACE

The forces that dominate particle adhesion to a surface are not necessarily the same forces that cause particles to deposit onto that surface. There are differences in the range of attraction of the various forces. As the separation distances between the particle and the surface vary, so does the influence of the different forces. Long range forces dominate particle deposition; short range forces dominate particle adhesion. This discussion is mainly about adhesive forces and assumes the particles have already been captured by the surface.

Adhesive forces can be of two general types: physical and chemical. Physical forces are discussed here. Chemical bonds, when present, are much stronger but are very dependent on the type of particle and surface and not easily discussed in general terms. Physical forces can be either universal when they are always present, or conditional, when they are present only when certain additional conditions are met. The three important physical forces binding particles to a surface are:

- London-van der Waals (Universal)
- Electrical (Conditional)
- Capillary (Conditional)

1.1 London-van der Waals forces

London-van der Waals forces are attributable to electron cloud interactions between adjacent molecules and exist between the molecules of a particle and those of an adjacent surface. London-van der Waals forces almost always create an attractive force between the particle and the surface. The magnitude of these attractive forces is directly proportional to particle diameter, i.e. it varies linearly with particle diameter and depends somewhat on the composition of both the particle and the surface. However, in virtually all cases, London-van der Waals forces are much weaker than chemical bonding forces and are always present. London-van der Waals forces are universal and, while their magnitude can be controlled to a degree, they can never be completely eliminated.

1.2 Electrical forces

Electrical forces between a particle and an adjacent surface can arise from two sources: 1) macroscopic charge on the particle, introducing an image charge in the surface, and 2) charge transfer across the particle to surface interface. *Macroscopic particle charge* is more important as a particle deposition force than a particle adhesive force. Once the particle is "deposited" (close enough for the London-van der Waals forces to dominate) it loses much of its macroscopic charge to the surface. This charge loss is more rapid and complete when the surface is a conductive material such as metal. However, even on poorly conducting polymer surfaces, the particle macroscopic charge eventually dissipates so that this source of an electrical adhesive force decreases or vanishes. The *charge transfer* origin of an attractive adhesive force between a particle and a surface arises from the electron transfer that takes place across the interface between any two dissimilar materials, creating a contact potential between the particle and the surface. This charge separation sets up an electrical double layer and an attractive Coulomb force between the two charge

Table A1. Effect of particle size

Particle size (µm)	Particle mass (g)	Adhesion force (dynes)	Acceleration required for removal (adhesion force expressed in terms of its gravity equivalent) (Gs)
10	2×10^{-9}	9×10^{-2}	4.5×10^{4}
1	2×10^{-12}	9×10^{-3}	4.5×10^{6}
.10	2×10^{-15}	9×10^{-4}	4.5×10^{8}

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sheets of that double layer, much like the force between the two electrodes of a parallel plate capacitor. Typical contact potentials, however, are 0.5 volts or less and the magnitude of the resultant adhesive force is usually small compared with that of the London-van der Waals forces.

1.3 Capillary forces

Capillary forces refer to the adhesive force contributed by a liquid layer present in the constricted region between the particle and the surface. Condensation of water vapor from the atmosphere is often the source of this liquid. Relative humidities as low as 65% can give rise to such a water layer. The capillary force depends on the surface tension of the liquid and, like the London-van der Waals forces, varies linearly with particle diameter. When present, the magnitude of capillary forces can be comparable to, or even larger, than London-van der Waals forces. Most cleanrooms operate at relative humidities below 65% so that the atmosphere is unlikely to be a source of a condensed water layer.

2 PARTICLE REMOVAL FROM CLEANROOM SURFACES

To remove surface particles requires overcoming the adhesive forces holding the particles to a surface.

Particle removal from large, fixed (immobile) surfaces such as cleanroom walls or equipment housings generally cannot take advantage of many of the successful cleaning methods used in wafer processing such as ultrasonics, megasonics, or surface acceleration (high speed spinners). Particle removal from these fixed cleanroom surfaces most often relies on aerodynamic or hydrodynamic drag forces. These forces vary with the surface area of the particle, i.e. the square of the particle diameter. Thus, the ratio of the adhesive force to the aerodynamic or hydrodynamic removal force varies as the inverse of particle diameter. As particle diameter decreases, the adhesive force decreases less rapidly than the removal force, consequently the smaller the particle the more difficult it is to remove from a surface by an aerodynamic or hydrodynamic cleaning method (Table A1).

Methods for removing particles from a cleanroom surface include:

- Vacuuming (wet or dry)
- Wiping (wet or dry)
- Lifting with a tacky roller

The first two of these methods rely on drag forces. The third removes particles by placing another surface adjacent to the particles that have greater particle adhesion forces than the surface being cleaned.

2.1 Vacuuming

"Vacuuming" implies the use of a suction pump to create a fluid flow past the surface in which surface particles become entrained. Vacuuming relies on drag forces to overcome the particle adhesion forces to the surface. Fluid drag depends not only on particle diameter squared but also the fluid velocity squared and the fluid density:

$$F_D \approx \rho_f d_p^2 v^2 \qquad \text{(A.1)}$$

where

F_D	=	fluid drag force
ρ_f	=	fluid density
d_p	=	particle diameter
v	=	fluid velocity with respect to the particle

The air velocity generated by vacuum systems is insufficient to remove particles much smaller than about 5 µm and is not very efficient up to about 50 µm. Liquids have much higher densities than gases, so that for a given fluid velocity, the drag forces exerted on an attached particle by a liquid are much greater. If a wet pick-up vacuum system is used, the additional drag forces available will substantially increase the collection efficiency. Immersing the surface in a liquid can also dissolve soluble particles (e.g., many salt particles are soluble in aqueous solutions). In addition, van der Waal's adhesion forces are typically smaller in liquids than in air.

A limitation associated with all fluid drag removal methods is due to the aerodynamic or hydrodynamic boundary layers. The transition regions in which the fluid velocity varies from zero at the surface to the value that is characteristic of the bulk fluid flow remote from the surface are much thicker than the diameter of the particles of concern. Boundary layer thicknesses are often in the 25 µm - 50 µm range, far greater than the micrometer- and submicrometer-sized particles of concern in many cleanroom applications. Thus, the velocity factor appropriate for particle removal in the drag Equation A.1 is less than, and often considerably less than, the velocity of the bulk fluid flow past the surface.

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2.2 Wiping

Wiping is another method of generating fluid drag forces in the vicinity of a surface. While very large particles can be physically contacted, moved, or captured by the fibers of the wiper, an important function of the wiper is to move the fluid over the surface, creating a drag force as in the vacuuming method. A wet wiper is more efficient than a dry one as the drag forces in the aqueous solution are greater.

2.3 Lifting with a tacky roller

The particle removal efficiency of a tacky roller is dependent on the magnitude of the adhesive force of the roller's surface. Other factors, such as the resilience of the roller material, will influence the efficiency, as will the particle size and heterogeneity.

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