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# Cleaning Aseptic Fill Areas

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*For pharmaceuticals that cannot be given terminal sterilization at the end of production, the alternative of aseptic fill is available, requiring sterile materials placed in sterile containers in a cleanroom. Nonviable particles >10 µm and viable particles must be kept away from the product. Part of contamination control involves wiping the walls, tables, and equipment so as to remove contamination. Another part is disinfection. The cleaning of aseptic fill areas must be done in a systematic manner, described here, and audited for effectiveness. Removal of contaminants aids disinfection and is usually carried out by chemical means, using sterile wiping materials.*

Advanced methods of contamination control are essential to successful pharmaceutical manufacturing, especially pharmaceuticals that do not receive terminal sterilization but rather are packaged using aseptic fill techniques in cleanrooms. The rooms typically contain automated equipment and some personnel, filling vials, IV pouches, etc., with measured amounts of a given drug. The strategy is simple: sterilize the containers, sterilize everything that enters the containers, then perform the filling in a virtually sterile environment. Planning and building such cleanrooms was covered in a recent article by Hansz and Linamen[1]. Useful details and standards concerning various aspects of sterilization are available in the recent book by the Association for the Advancement of Medical Instrumentation, covering ethylene oxide, steam, other chemical sterilants; gamma radiation; and electron beam radiation[2]. Background information on cleanroom microbiology is available in a new book by Carlberg[3].

Unfortunately, personnel and their garments cannot be made and kept sterile, so one must rely on clean airflow, garments, gloves, and cleaning to protect the products from biological contamination and isolate the workers from the products. To provide cleanliness and sterility, clean and sterile materials are introduced into cleanrooms or clean zones that are controlled to Federal Standard 209E, Class 100 or better, for air cleanliness. This means the air can contain no more than 100 particles >0.5 µm per cubic foot. The level of viable particles in the air is much lower than this, and efforts are made to prevent even one viable particle from being incorporated into (or onto) the product being made. Much useful information on microbial sampling of air and surfaces (including an extensive tabulation) and the control of microorganisms in the cleanroom is available in IES Recommended Practice 23.1.[4]

Contaminants enter the cleanroom in gases and liquids and on materials, clothing, and skin. They are also generated by processes and personnel actions inside the room. Once they settle on a surface, particles <10 µm are unlikely to be blown off, but can be transferred by contact to other surfaces and eventually

to the products. Particles may even remain on cleaned vials, ampuls, and closures, and they may be generated by friction during handling of these elements, as well as depositing from other sources within the cleanroom. Particles may form by flaking of glass or plastic or may be caused by reactions after filling, such as with leachates from glass containers or rubber stoppers, but better cleaning of the cleanroom cannot reduce the magnitude of these sources.

Cleaning the aseptic fill area is made more difficult by the variety of surfaces that need attention: barrier curtains, walls, windows, floors, ceilings, table tops, and machinery with complicated inner and outer surfaces. In what follows, general principles of cleaning and making surfaces sterile are put in the context of cleaning aseptic fill rooms.[5, 6]

**Figure 1:** *Sterility testing an aseptic fill area by performing filling with nutrient broth.*

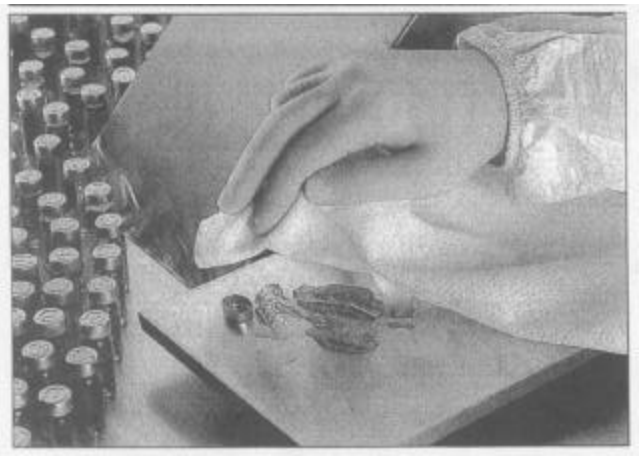

**Figure 2:** *Liquid and fragments from a broken vial being cleaned up under cleanroom conditions.*

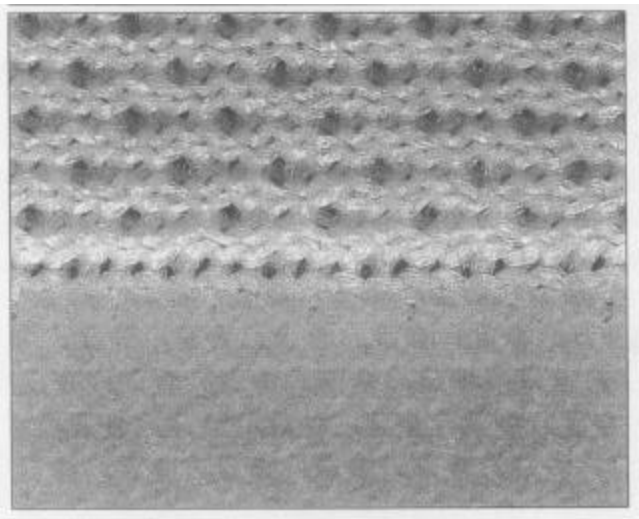

**Figure 3:** *Magnified photograph of a portion of a laundered, sealed-edge, knitted polyester cleanroom wiper.*

**Aseptic Fill Cleanrooms**

Figure 1 shows sterility testing of an aseptic fill area. The material in production will not undergo terminal sterilization, so special efforts are made to keep the product sterile. Filtered air bathes the production equipment and personnel, who wear special cleanroom garments. Hiraoka showed that the fewer personnel present, the fewer contaminant particles were found in the product, and the number of insects present decreased by a factor of 10 for every door between the cleanroom and outdoors, with five doors needed for a complete absence of insects.[7] Materials entering the room are sterilized.

Van Gestel described how his organization carried out aseptic fill operations.[8] He emphasized the following elements: design, materials, separation of areas, product flow, progression in cleanliness, and protection against insects and rodents. First under the design heading was easy cleaning, disinfection, and maintenance: smooth surfaces, corners that are covered,

lighting that is flush-mounted and sealed, lack of recessed zones where dust can accumulate and be hard to clean, and a minimum of piping present in the room itself. Heat, humidity, and nutrients foster biological growth, so these should be minimized by avoiding porous materials and materials containing carbon that might become metabolized, and by a cool, dry airflow in the room. The essence of good product flow is to keep contaminated and uncontaminated regions clearly delimited and to prevent interchange. Van Gestel recommends four levels of increasing cleanliness, progressing from the entrance on the street to areas corresponding to U.S. Federal Standard 209 Class 100k, Class 10k, and finally Class 100, the aseptic fill area. Required utilities include warm and cold sterile pyrogen-free water, dry and filtered and oil-free compressed air, clean nitrogen and oxygen, and steam. Raw materials, water, filters, etc., should undergo periodic testing for organisms and pyrogens. Personnel must be monitored routinely. Walls, ceilings, and floors and equipment surfaces can be monitored with contact plates. Periodic filling with media designed to support biological growth should be done to check for sterility. Personnel must be trained and periodically tested.

Although sterility is the primary concern in the aseptic cleanroom, prevention of nonviable particle contamination is an important concern, too. Gallelli and Groves wrote, “It is widely recognized that the level of particulate matter in an injectable product is one measure of quality, directly reflecting the success with which the manufacturer applies good quality control.”[9]

Factors that make particles difficult to remove include capillary forces, electrostatic forces, hardened chemical bridges, thermal solidification, tackiness, van der Waals forces, deformation and embedding, surface roughness, and gravity.

Aseptic cleanroom cleaning supplies (adapted from Thompson, 1994), all sterilized and dedicated to the cleanroom only

- Wipers and swabs
- Cleaning and disinfecting solutions
- Dispensing bottles
- Stools or ladders
- Vacuum cleaner (central system or HEPA-filtered cooling and exhaust)
- Buckets with wringers
- Mops
- Trash receptacles

**Cleaning Strategy**

Areas from the building entrances to the equipment interior need to be cleaned using different techniques and materials. Contamination control requires frequent conventional cleaning of entrances, halls, and offices. Areas immediately adjacent to the cleanroom, including the service cores, need more thorough and frequent cleaning. The garment changing room and the cleanroom need the most careful and frequent cleaning. The cleanroom floors should be cleaned toward the entrance and from there to the changing/gowning room. Cleaning of equipment may require dismantling, cleaning, then reassembling.

Gross cleaning can be done with vacuum cleaners (centralized systems or portable units with filtered exhausts) and squeegees, brushes, and sticky rollers. The main airflow and any cooling airflow for the vacuum cleaners must be directed outside of the cleanroom or be filtered. Cleaning heads must themselves be cleaned. Precision cleaning needs to be done, whether or not there has been gross cleaning. Figure 2 shows liquid and fragments of a vial being cleaned up under cleanroom conditions. Precision cleaning almost always requires fabric wipers or swabs. These need to be among the cleanest available, given the value of the products involved and the impact of contamination problems if cleaning is not successful. The sidebar on this page shows materials needed for cleaning in the aseptic fill cleanroom.[10]

**Cleaning Materials**

The cleanest wiping fabrics are made from continuous filament yarns (long, unbroken fibers) rather than from staple yarns (short fibers), either natural or chopped synthetic. Options for fabric formation include felting the staple yarns, knitting, or weaving. Knitting seems to produce a more open and absorbing structure than weaving. Materials used for wipers include natural materials, such as cotton and other cellulosic materials (including rayon), and synthetic materials such as nylon, polypropylene, and polyester. These synthetic materials are more readily made very clean. The recent concern over the EtO-sterilization-resistant fungus *Pyronema*, found in some cotton products from China, underscores the problems with fabrics based on natural fibers. The bioburden, measured in colony-forming units per wiper, depends on many factors, primarily on the handling of the wiper material during conversion to wipers and the quality of the water in which they are laundered. The least contaminating wipers tend to be those with edges that have been sealed, as cutting the fabric into wipers tends to create particles and fibers that can migrate to equipment and product surfaces. Laundering the fabric gets rid of most of these particles. Figure 3 is a magnified view of a portion of a laundered, sealed-edge, knitted polyester cleanroom wiper. An aqueous solution is used, followed by dewatering, rinsing, dewatering, and so on, and the cleaned product is then dried with hot air. For the most rigorously cleaned products, the water is deionized and filtered before use.

Wipers designed for aseptic fill areas are often purchased presterilized, typically by gamma radiation at a dose level adequate to ensure a <1 in one million chance of having a viable organism on any particular sterilized wiper. Such doses are typically near 25 kGy (2.5 Mrad), which is not a problem for polyester but is too high for cotton and perhaps too high for nylon. Sometimes the purchaser will sterilize the wipers by autoclaving with steam at 2 atm and 121 °C. However, even dead bacteria can be a problem as pyrogens, the fever-causing outer surfaces of Gram-negative bacteria. Sterilization by steam or radiation, the two most common methods, will not destroy pyrogens, so they must be minimized by clean manufacturing before any terminal sterilization process. Double and triple bagging allow the wipers to be passed through contaminated areas before reaching the site of use, with contaminated bagging layers removed as needed.

In summary, the important qualities for wipers include

- absorbency: ability to hold liquid
- cleanliness: low levels of particulate, chemical, and viable contaminants
- robustness: ability to resist wear and tear

- chemical compatibility: resistance to chemical attack during cleaning or disinfecting
- sterilizability: ability to be sterilized chemically, by wet or dry heat, or by radiation
- ease of use: ease of folding, sliding, or compressing to release liquid
- electrostatic properties: minimal generation of static electricity
- abrasiveness: sufficient roughness but no tendency to scratch other surfaces.

Tests for some wiper qualities have been agreed on through committee work done with The Institute of Environmental Sciences, which has published IES-RP-CC004.2, “Evaluating Wiping Materials Used in Cleanrooms and Other Controlled Environments,” including tests for particles released in liquids under minimal stress and under biaxial shaking, tests for extractable matter using various solvents, tests for sorbent capacity and rate, and a reference for testing electrostatic properties of wipers (IES-RP-C022). Steps can be taken to increase the particle counts found in testing cleanroom materials, if desired. To increase extraction efficiency, a surfactant can be added to the extraction liquid. If the material can withstand it, ultrasonic cleaning can be used. Where available, the use of a scanning electron microscope allows detection of particles that are a fraction of a micrometer in size and that have an effective index of refraction close to that of water, which may be undercounted or undersized in the usual optical liquid-borne particle counter. Disposability may be an issue as well, especially if there is concern about biohazards from special pharmaceuticals.

**Liquid Cleaning Agents**

Wet wiping is valuable not only for any disinfectant effect of the liquid, but also because the liquid can dissolve and weaken bonds between the particles and the surface being cleaned. It is important to remove dust particles because they often harbor microbes or shield microbes from the disinfectant. A damp wiper will have a greater affinity for particles than a dry wiper, but getting the wiper fully wet leads to some redeposition of the particles on the surface.

Freshly prepared deionized water can be an effective cleaner, especially for removing ionic contaminants, but usually it is augmented with various other chemicals. The liquid must remain sterile. It is not simple to obtain and maintain sterile water; organisms can live in deionized water. Favero and Bond reported that these levels can be as high as 1000 to 10 million cells per milliliter.[11] Filtration takes cells out of the water, but after some time, they can grow through the filter and become waterborne again. Water and isopropyl alcohol are two liquids commonly applied to wipers for cleaning cleanroom surfaces, but because various spores can survive in them, solutions of isopropyl alcohol and water are not relied on for sterilization for pharmaceutical production. Filtered, sterilized (gamma irradiated) IPA/water solutions are available at 91% (v/v) (the azeotrope) and 70% (v/v). Favero and Bond noted that diluting with water below 50–60% (v/v) can reduce the antimicrobial activity substantially. The liquid put into the dispenser should be filtered through a hydrophilic 0.2-µm pore filter. Heat or radiation may be needed to ensure sterility despite these precautions. The air that enters as the container is emptied should be filtered, with a 0.2-µm pore hydrophobic filter. The opening of the container should not be allowed to touch other surfaces.

Favero and Bond distinguished between sterilization (killing all microorganisms with a margin of safety) and disinfection (killing almost all microorganisms except perhaps spores). They listed various liquid chemical agents and their effectiveness against various organisms and also distinguished appropriate chemicals by the uses to which the surface being cleaned is to be put: the most critical surfaces contact patients' blood; semicritical devices contact various membranes of the body but not the bloodstream; noncritical devices do not contact patients, or only contact their skin, and can be cleaned with detergents and low-level disinfectants.

Common disinfectant aqueous solutions include bleach (sodium hypochlorite), quaternary ammonium compounds, peracetic acid, hydrogen peroxide, phenols, etc., often as part of proprietary commercial mixtures. It is good practice to rotate among two or more solutions, to prevent the perpetuation of strains of organisms that have become resistant to any one type. Information about actions and the testing of disinfectants is available.[12,13,14] The Institute of Environmental Sciences compared alcohols, phenolics, chlorine, glutaraldehyde, quaternary ammonium chloride, and iodine for ionic contamination, activity in the presence of organic contamination, EPA registration as a disinfectant or sterilant, water solubility, residual activity, staining, and efficacy against bacteria, tuberculosis, bacterial spores, fungi, and lipophilic and hydrophilic viruses, concluding that "the ideal disinfectant does not exist."[4] That publication outlined the following options for cleanroom disinfection: wiping and mopping, flooding and vacuuming, fogging, and the submersion of small objects.

Desirable attributes of a liquid cleaner include the following:

- reduces surface tension to wet surfaces
- has an aqueous component, a hydrocarbon solvent, and an alkaline component to help dissolve ionics and oils and greases
- evaporates rapidly
- leaves minimal residue after evaporation
- contains minimal metals, halogens, and volatile organics
- has an acceptable odor
- is not toxic, flammable, or ozone-depleting.

Presaturated wipers may reduce the consumption of cleaning solutions, reinforce good technique (wetting the wiper rather than the surface), avoid the mixing of chemicals, ensure the right chemical concentrations in the cleaning liquid, prevent wiper—liquid incompatibility, and simplify sterilization. They should be provided in containers that allow easy removal singly, avoid contamination of those remaining, and limit the evaporation of the liquid.

**Cleaning Methods**

Cleaning personnel should be garbed as are other personnel in the cleanroom, presumably with boots, coveralls (typically woven polyester), masks, hoods, and gloves.

First, don gloves, which is normal cleanroom procedure. Otherwise, skin oils and flakes can transfer to the surfaces being cleaned. Depending on the cleaning liquid, if any, to be used, this protection may be needed for safety, as well.

Next, fold the wiper, as several clean wiper surfaces can be obtained this way, unless the wiper is wet, in which case, some liquid with contamination may

transfer through the wiper layers. The folded wiper also yields more uniform pressure from the hand and fingers.

Wipe in a regular pattern of parallel strokes with enough overlap to ensure no area goes unwiped, changing the surface of the wiper or the wiper itself at the beginning of each stroke, which should start at the cleaner end of the planned path. A rule of thumb is to change the wiper surface every 10 wiper lengths of wiping (more if the surface is visibly dirty).

For disinfection, use a nearly saturated wiper and wipe with sufficient pressure to leave a visible disinfectant film.

Wiping a surface transfers contaminants to the wiper roughly in proportion to the areal density of the contaminants and transfers contaminants from the wiper roughly in proportion to the contamination on the wiper. The transfer rate is modeled as the difference between *K(M/A)*, a rate of pickup proportional to the mass per unit area on the surface being cleaned, and *k(m/a)*, a rate of redeposition proportional to the mass per unit area on the wiper. (The factors *K* and *k* will depend on the wiper, the surface, any cleaning fluid, and the contaminant.) One wants to keep the wiper as clean as possible while cleaning, so the pattern for wiping should be from the cleaner regions to the dirtier regions. Thus, one wipes ceilings and walls starting from filters. Ceilings are wiped before walls, which should be wiped before floors. Similarly, for drying spills, wiping should be from dryer regions to wetter regions. Disinfecting solution is to be applied from the cleanest area to the least clean and allowed to air dry. For disinfection, the wiper typically has to be wet enough to leave a substantial film of liquid on the surface being wiped, a film that will reside there long enough to provide sufficient lethality. The effectiveness of chemical disinfection depends on the organisms involved, the chemical used, time, temperature, concentration, and mechanical agitation; more of each leads to more thorough disinfection (unless the temperature is so high as to damage the chemicals or surfaces).

For floors it is best to use two buckets: one with the cleaning solution and a second in which the dirty mop is rinsed before being put into the first bucket. Floors should be left dry to prevent someone from slipping and falling.

It is safer to make cleaning solutions by putting the chemical into the water, rather than the water into the chemical (which might cause splashing of the concentrated chemical). To conserve cleaning liquid, put the liquid onto the wiper rather than onto the surface being cleaned. Cross-contamination can be prevented by not immersing the wipers in the cleaning liquid one after another.

Additional information is available in IES-RP-CC018.2, where the necessary equipment is listed along with recommendations on cleaning ceilings (filters, nonporous areas, fixtures), walls, doors, windows, floors, adhesive mats, work stations, and waste receptacles.[15] The principles are to use an orderly progression from cleanest to least clean areas, in parallel strokes with slight overlap, and avoiding recontamination of cleaned areas. A daily cleaning checklist is provided.

**Cleaning Efficiency Audits**

Successful cleaning requires audits to find any areas missed and to highlight problems of materials, techniques, or personnel. Methods of detecting

contaminants on surfaces may be used or methods of determining product suitability, such as product appearance or bioburden. Steps in determining the causes for unusually high bioburden readings were presented by Hansen et al., and they include the manufacturing area as well as the laboratory (bioburden-measuring) area.[16]

The following techniques can be used to check the cleanliness of cleanroom surfaces: bright light; ultraviolet light; wiping with a dark or light wiper or swab, subsequently evaluated by naked eye or microscope; use of sticky tape, subsequently evaluated by naked eye or microscope; vacuum head connected to optical particle counter or to microscope filter; rinse of the area and analysis of the liquid by optical or electron microscope; or by liquid particle counter or by biological assay (colony counting and identification, Gram stain, polymerase chain reaction, gel electrophoresis) or by chemical assay (atomic absorption, chromatography, capillary electrophoresis, specific ion electrode, nonvolatile residue analysis), by radiological assay, or by use of a contact plate with sterile medium touched to the surface and then incubated. More information on the following tests is available in IES-RP-CC018.2: UV light, high-intensity oblique white light, optical microscopy, witness (deposition) plate, surface particle detector (scanning beam, light scattering measurement), contact plate for viable particles, swab for viable particles.

The measuring technique should be sensitive to what is of practical interest. Particle counting is not likely to be sufficient if bioburden is the issue, although gross errors will likely be caught by monitoring airborne and surface particle counts. There is a wide variety of particle identification techniques available for use in determining the sources of particle contaminants.

Validation is a more formalized demonstration of the capabilities of the processing within the aseptic fill cleanroom, and this has been covered in useful detail by Leahy.[13] Short courses on cleaning validation are frequently presented by the Parenteral Drug Association (Bethesda, MD).

## Summary

Unless terminal sterilization is planned for the sterile product, aseptic processing will be needed, which involves a cleanroom and sterile materials, including sterile cleaning materials. Cleaning starts at the entrance to the plant, becoming more critical the closer one comes to the product. Cleaning is done from the cleaner regions to the less clean, with materials that themselves are clean. The materials can be made clean by careful choice of inputs and processing. Materials used in the aseptic fill room must also be sterile. The materials can be made sterile by using a terminal sterilization process, usually consisting of irradiation (gamma or electron beam) or autoclaving.

## References

1. T.E. Hansz and D.R. Linamen, “Planning, Programming, Designing, and Constructing a Cleanroom,” *MDDI* **17** (2), 73–86 (1995).

2. Association for the Advancement of Medical Instrumentation, AAMI Standards and Recommended Practices: Vol. 1: Sterilization, AAMI, Arlington, VA, 1992.

3. D.M. Carlberg, *Cleanroom Microbiology for the Non-Microbiologist* (Interpharm, Buffalo Grove, IL, 1995).

4. Institute of Environmental Sciences, *Microorganisms in Cleanrooms, IES-RP-CC023.1* (IES, Mount Prospect, IL, 1993).

5. D.W. Cooper, “Sterility Assurance for Cleanroom Wipers,” presentation at the annual technical meeting of the Institute of Environmental Sciences, Anaheim, CA, April 1995.

6. D.W. Cooper, “Cleaning Surfaces with Sterile Wipers,” presentation at Medical Design and Manufacturing East, New York, June 1995.

7. K. Hiraoka, “Particle Control in Form/Fill/Seal Systems,” in *Proceedings of the International Conference on Particle Detection, Metrology, and Control* (Institute of Environmental Sciences and the Parenteral Drug Association, Arlington, VA, 1990), pp. 626–635.

8. G. Van Gestel, “Clean Room Design and Operation,” in *Sterilization of Medical Products, Vol. IV*, E.R.L. Gaughran, R.L. Morrisey, and Y. Wang, Eds. (Polyscience, Montreal, 1986).

9. J.F. Gallelli and M.J. Groves, “USP Perspectives on Particle Contamination of Injectable Products,” *J. Pharm. Sci. Tech.* **47** (6), 289–292 (1993).

10. C.L. Thompson, “Cleanroom Cleaning,” presentation at Clean-Rooms ‘94 East, Philadelphia, March 1994.

11. M.S. Favero and W.W. Bond, “The Use of Liquid Chemical Germicides,” in *Sterilization Technology: A Practical Guide for Manufacturers and Users of Health Care Products*, R.L. Morrisey and G.B. Phillips, Eds. (Van Nostrand Reinhold, New York, 1993).

12. G.K. Bass, “Methods of Testing Disinfectants” in *Disinfection, Sterilization, and Preservation*, S.S. Block, Ed. (Lea & Febiger, Philadelphia, 1977).

13. T.H. Leahy, “Microbiology of Sterilization Processes,” in *Validation of Aseptic Pharmaceutical Processes*, F.J. Carleton and J.P. Agalloco, Eds. (Marcel Dekker, New York, 1986).

14. J.D. Wilson, “Validation of Sanitizers,” in *Validation of Aseptic Pharmaceutical Processes*, F.J. Carleton and J.P. Agalloco, Eds. (Marcel Dekker, New York, 1986).

15. Institute of Environmental Sciences, *Cleanroom Housekeeping—Operating and Monitoring Procedures, IES-RP-CC018.2* (IES, Mount Prospect, IL, 1992).

16. J. Hansen et al., “Investigating AAMI Radiation Audit Results,” *MDDI* **16** (5), 218–222 (1994).

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