Advances in Sterility Assurance: Single Use Devices and Rapid Microbiological Tests

Abstract

The last decade brought an impressive list of technological advances, ensuring the sterility and quality of a myriad of new drug and biological products, in addition to medical devices. These advances bring new treatments, improve quality of life and extend life expectancy for millions of patients. This paper summarizes some of those new technologies and focuses on a few examples that clearly enhance sterility assurance in the manufacturing of pharmaceuticals.

Introduction

It has been more than ten years since the Food and Drug Administration (FDA) released its first progress report on a major initiative concerning the regulation of drug product quality. The two-year initiative, *Pharmaceutical cGMPs (current Good Manufacturing Practices) for the 21st Century: A Risk-Based Approach* [1], launched on August 21, 2002, applied to human and veterinary drugs and had several objectives:

1. To encourage the early adoption of new technological advances by the pharmaceutical industry.
2. To facilitate industry application of modern quality management techniques, including implementation of quality systems approaches, to all aspects of pharmaceutical production and quality assurance.
3. To encourage the implementation of risk-based approaches that focus both industry and Agency attention on critical areas.
4. To ensure that regulatory review and inspection policies are based on state-of-the-art pharmaceutical science.
5. To enhance the consistency and coordination of the FDA's drug quality regulatory programs, in part, by integrating enhanced quality system approaches into the Agency's business processes and regulatory policies concerning review and inspection activities.

By positioning the early adoption of new technological advances by the pharmaceutical industry as the first bullet in the list of objectives for this initiative (see above), the FDA emphasized the need to modernize the industry by seeking and incorporating new technologies into the manufacturing process.

Regulatory Guidance Encouraging the Adoption of New Technologies to Modernize the Pharmaceutical Industry

Numerous new and updated programs and regulatory documents followed in the years after the release of the abovementioned initiative in 2002. Those relevant to this paper include the FDA-issued final report of the *Pharmaceutical cGMP for the 21st Century* (2004), the FDA *Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing-Current Good Manufacturing Practice* [2], and the European Commission guidance on the *Manufacture of Sterile Products* [3].

The FDA guidance [1, updated 2015] recommends "building quality into products" through science-based facility, equipment, process, and system design for sterile drug manufacture. The guidance emphasizes the importance of the pharmaceutical industry's adoption of new technological advances. It underscores the advantages offered by automation and isolation concepts in protecting the exposed sterile drug product during its aseptic manufacture, and encourages the use of modern microbiological testing methods that are more accurate and precise. It also advocates a risk-based and quality system framework that stresses contamination prevention, describing the roles played by personnel, design, environmental control, and media fills in an aseptic processing operation.

Similarly, other publications support the adoption of standard cGMPs and new technological advances. Notably, the chapters published by the USA and European Pharmacopoeias supporting validation and implementation of alternative and rapid microbiological methods [4, 5], recommendations on new practices in the microbiological control of aseptically produced products [7], and the Parenteral Drug Association technical report 13 [6] all play an important role in encouraging the adoption of rapid microbiological methods in numerous QC pharmaceutical tests.

Finally, on December 23rd, 2015, the FDA released a draft guidance on the *Advancement of Emerging Technology Applications to Modernize the Pharmaceutical Manufacturing Base* [8]. The guidance discusses a new FDA program that allows pharmaceutical companies to present pre-submission questions and proposals about the use of innovative technology to a group within the Center for Drug Evaluation and Research (CDER), entitled the Emerging Technologies Team (ETT). Interestingly, the ETT will work with pharmaceutical companies to evaluate proposals, serving as the primary point of contact within FDA for companies interested in implementing new manufacturing technology. To participate in the new FDA program, applicants must submit a written request for a Type C meeting to CDER-ETT@fda.hhs.gov. Requests can include an explanation of new testing, processes, or proposed technology.

The latter FDA initiative offers the direct support and involvement of a specialized CDER team to pharmaceutical companies for the purpose of evaluating and incorporating new technologies into their drug manufacturing process. This and previous references strongly suggest manufacturers should invest in the incorporation of novel approaches and technologies.

Technological Advances in Pharmaceutical Manufacturing and Sterility Assurance

As mentioned previously, the last decade or so has seen incredible growth in the availability of new or improved technologies that increase the sterility assurance of pharmaceutical products. Some of these advancements are listed below.

Sterilization and Disinfection

An increased regulatory focus placed on sterilization practices encourages pharmaceutical and medical device companies to re-examine sterilization methodologies, not only to remain regulatory compliant but also to promote a more effective and safe manufacturing process. Sterilization and disinfection are critical processes used in multiple aspects of pharmaceutical manufacturing process, such as materials, packaging, process equipment, isolators, medical devices, and cleanrooms [9]. Steam and dry-heat sterilization are used for process equipment and for sterile drug products, but these processes can potentially degrade heat- or moisture-sensitive materials.

The emergence of novel sterilization technologies such as vaporized hydrogen peroxide, gamma, x-ray, and electron beam irradiation and ethylene oxide sterilization offer the opportunity to drive innovation in the safety and effectiveness of pharmaceutical related products.

Other technologies with advantages for certain applications are emerging, such as:

- **Nitrogen dioxide** (NO_2) gas generators which are under development for isolator decontamination and cleanroom applications.
- **Chlorine dioxide** (ClO_2) gas which can be used for decontamination in a wide range of pharmaceutical manufacturing situations, from isolators or process equipment to cleanrooms and entire facilities.
- **Carbon dioxide** (CO_2) which is used in precision cleaning and disinfecting processes for medical instruments and devices, and cleaning silicone rubbers and polymers prior to bonding, coating, or assembly for use in stringent environments (i.e. cleanrooms), biomedical devices, or aerospace. A significant benefit of CO_2 as a sterilization technique is that it can both clean and disinfect a surface, while other processes leave behind debris, which can include biological endotoxins [9]. Additionally, sterility assurance engineers and microbiologists are motivated to streamline sterilization methods through cycle optimization and reliable risk management programs.

Automation and Robotics

Automation is becoming an increasingly important part of pharmaceutical manufacturing. The many benefits of automation include efficiency, saving workers from the need to perform repetitive tasks or work in hazardous environments, reducing training overhead, eliminating human error, and increasing repeatability and reproducibility. In addition, automation has benefited inspection and packaging operations, and diminished or removed the potential for human contamination in cleanrooms [10].

A robotic system is a type of automation that has multiple axes of motion and can be programmed to perform a function. Following that definition and with multiple avenues of development, there are currently robots specialized in filling, inspection, packaging, and production of personalized immunotherapies. In addition, robotic technologies are ideal for cleanroom processes, such as aseptic filling, because it eliminates human contamination risk. At the microbiology lab level, a robotics platform has been successfully used in rapid microbiology to automatically handle agar plate incubation and early detection and counting of microbial colony forming units [11].

Single Use Technology

Single use systems benefit both upstream and downstream manufacturing processes, and production of buffer solutions and cell-culture media, reducing the time required to perform cleaning and cleaning validation. They also allow manufacturers to more quickly turn over from one product to another, or from one batch to another batch. In addition, single use systems minimize hold time and enable continuous processing. Finally, single use systems are shown to reduce overall operating costs by minimizing or eliminating the need for clean in place (CIP)/sterilize in place (SIP), reducing analytical quality control costs and improving facility utilization time. The processes benefiting most are those around contamination and cleaning, reducing water consumption, the ability to run multi-molecules in the same facility, and small-scale commercial production with low numbers of batches per year [12]. Recently, a commercially available single use impactor combined with an integrated sterile agar plate was launched. This product (BioCapt® SU, [13]) is briefly described in the next section.

Rapid Microbiology Methods

Go to **Real Time Airborne Particle and Microbial Monitoring** and **Surface Sampling in ISO 5 Environments** sections for more information.

A Single Use Impactor in ISO 5 Environmental Monitoring Environment

Environmental Monitoring (EM), particularly in Pharmaceutical manufacturing facilities, where the risk of microbial contamination is controlled through aseptic processing, comprises both physical and microbiological test methods. **Table 1** lists the most common devices and instruments used to collect samples of airborne microorganisms from cleanroom environments.

Table 1. Passive and Active Monitoring Methods

MONITORING TYPE	MONITORING METHOD
Passive	• Settling Plates
Active	• Slit-to-Agar (STA) Air Sampler (Air through narrow slit, rotational agar plate) • Sieve Impactors (Air through a perforated plate/s) • Single-Stage (contact plates or Petri dishes) • Multi-Stage Cascade (Stacked perforated plates) • Sterilizable Atrium (Stainless Steel head collection device) • Single Use Sterile Atrium (Disposable) • Centrifugal Propeller Sampler (Agar coated strip) • Filtration (Polycarbonate, cellulose acetate, gelatin filters) • Impinger (use of liquid medium for particle collection) • Real Time Laser-Induced Fluorescence Systems

One of the most commonly used instruments for active microbial airborne monitoring is the single stage sieve impactor. With this instrument, the air sample is taken through a perforated plate and impacts on an agar surface, on which any potential microbial contaminant present in the air can be recovered, enumerated and identified upon proper incubation conditions (for a full description of these instruments see reference 13).

FIGURE 1. MINICAPT® MOBILE MICROBIAL AIR SAMPLER AND MINICAPT REMOTE MICROBIAL AIR SAMPLER

Single-Stage Sieve Impactors (Air through a perforated plate/s): The sample of air is drawn through slits in the sampling head using a vacuum pump. The microorganisms are impacted on the agar surface in the pattern designed on the sampling head. Air flow is 25, 50 or 100 liters per minute (LPM). The exhaust air is HEPA filtered. Mobile and remote devices, kits for compressed gases and isolators, and connectors for remote use with stainless steel or single use atriums are commercially available.

- **Sterilizable Atrium** (Stainless Steel head collection device): Composed of a head with 20 slits, a base with pins to locate an agar plate, and a connector to a vacuum pump. It requires autoclaving.
- **Single Use Sterile Atrium** (Disposable polystyrene device combination of impactor and agar plate): The incorporated agar plate cannot be accidentally touched by the operator, reducing or eliminating the risk of contamination by improper handling (false positives) and costly investigations.

FIGURE 2. BIOCAPT STAINLESS STEEL

FIGURE 3. BIOCAPT SINGLE USE

There are multiple advantages to using the BioCapt Single-Use active air microbial sampler. Using a unique radial slit impactor design, the product complies with ISO 14698-1. The single use unit comes fully-prepared and sterilized by gamma irradiation, eliminating agar plate preparation and extensive operator manipulations before and during sampling collection. This product achieves sampling with minimal air disruption at 25 or 50 LPM.

Rapid Microbiology Methods: Real Time Airborne Particle and Microbial Monitoring

Nonviable particulate and viable microbiological surveillance are used to evaluate the design and control of a cGMP-manufacturing environment. The nonviable particulate monitoring program plays an important role as it is used on a routine basis to verify the maintenance of air classifications. It is a common assumption that if fewer total particulates are present in a cleanroom, it is less likely that airborne microorganisms will be present. This is true only if human operators are the main source of particulate matter in the air. However, it is not possible to clearly distinguish between background total particulate contamination generated largely by mechanical operations and the total particulates contributed by personnel. Thus, it is routine for cleanroom environmental monitoring programs to consist of both a total particulate component and a microbiological component.

It should be noted that the microbial monitoring within an EM program does not provide an exact quantity and quality of the microorganisms present in the manufacturing area. Numerous studies have shown that there is a large proportion of microorganisms that are viable but unable to grow on the traditional agar media. Therefore, these microorganisms, known as viable but not culturable (VBNC) are not detected using the traditional methodology. In addition, traditional methods are unable to sample every particular location and time. This methodology only provides observational windows of time. Consequently, the microbial monitoring program is not a way to guarantee the sterility of a given batch by collecting counts under defined specifications, but rather it helps by showing that the manufacturing process is in a continuous state of control.

A Real Time Laser-Induced Fluorescence System uses a type of device that continuously monitors viable (microbial) and non-viable particles in real time (see **Figure 4**). Extremely sensitive, the limit of detection is down to 1 microbial cell. It provides both total particulate and viable counts (see next section).

Real Time Microbial Monitoring using Laser-Induced Fluorescence (LIF)

The use of Trypticase Soy Agar (TSA), also known as Tryptone Soya Agar (TSA) or Casein Soya Bean Digest agar) constitutes a common practice in the microbial monitoring of pharmaceutical environments. The results are obtained after enough days of incubation allow the development of colonies to be counted by visual inspection. The results are then expressed as colony forming units (CFU) per unit of sample volume or mass. Due to the length of time necessary to develop results, the traditional TSA plate-based microbial monitoring method is seriously disadvantaged in comparison to total particulate monitoring, which can be continuously run with results obtained in real time. Moreover, it is well known that a large number of microorganisms found in natural and man-made environments (e.g., manufacturing areas) do not necessarily grow on the media under incubation conditions recommended in the pharmacopoeia references. If provided with the right media and conditions, many of these microorganisms would be able to grow. Ideally, it would be good to have instrumentation capable to detecting more diverse types of microbial contaminants (including damaged, stressed, dormant, and VBNC cells) that usually do not grow on the media described in the guidelines but could potentially grow as an opportunistic infection under special conditions found in clinical patients.

FIGURE 4. BIOLAZ® REAL-TIME MICROBIAL MONITOR

The development of such instrumentation capable of providing an evaluation of microbial monitoring on a continuous and real time basis has been facilitated by the application of the laser-induced fluorescence (LIF) technology. LIF instruments utilize a high intensity light source (e.g. 405 nm laser) to induce light scatter and fluorescence in the particles passing through an appropriate detection cell, resulting in the real time detection of both inert particles and biologics like bacteria, yeasts, and molds. One such instrument, used in pharmaceutical cleanroom applications, is shown in **Figure 4**. The sample is drawn through the optical chamber and illuminated by a 405 nm laser source. Light scattering occurs when any particle crosses the laser beam inside the particle flow path detection chamber. Only particles containing fluorescent molecules can give a signal at the second detector. Filters ensure only the fluorescence of the appropriate wavelength is detected on the second detector and that the general particle scattered light does not interfere with this signal.

The fluorescence is derived from internal fluorophores naturally present in molecules such as NADH and riboflavins, which are found in all microbial cells. Additionally, dipicolinic acid (DPA), a molecule found in microbial spores, is also detected by this technology. Typically, particles in the 0.5 to 50 micron range are detected. Some of the advantages of the LIF-based monitoring instruments are:

- Data is reported in Biologic/Fluorescent counts, not CFU.
    - However, if a trend is observed in the air microbial sampler, it will likely be observed much earlier (in real time) in the LIF monitor.
    - The limit of detection of a LIF instrument is down to just 1 cell per a certain volume of air or liquid.
    - Both CFU-based plate count monitoring and Bio counts monitoring should be run in parallel for microbial monitoring (at least through validation and implementation).

- Change in environmental conditions is much more apparent because data is generated in real time and continuously.
    - Actions can be taken immediately
    - LIF helps to decrease risk and accelerates investigations and re-training.

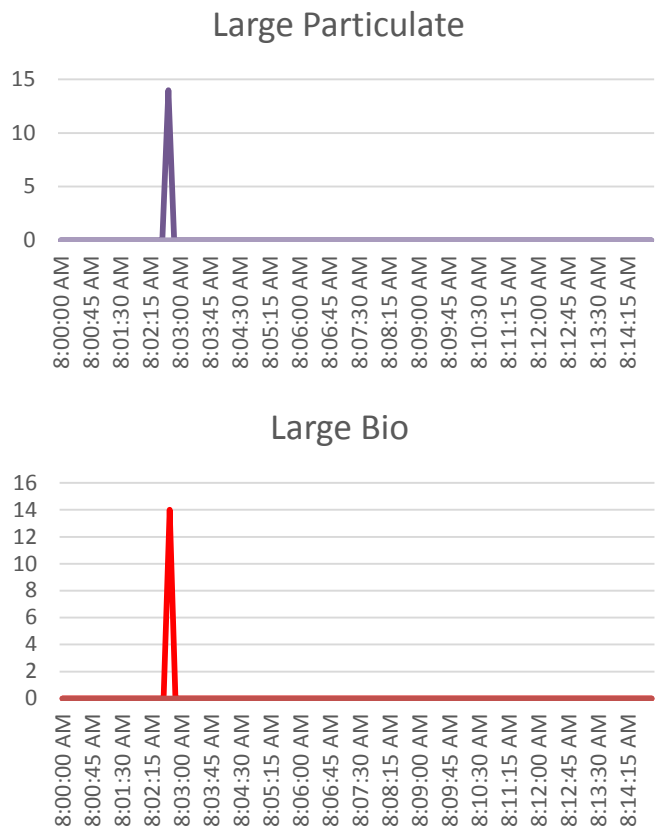

FIGURE 5. REAL TIME AIRBORNE PARTICLES MONITORING RESULTS IN A GRADE B AREA FOR BOTH ≥ 2 µM TOTAL PARTICULATES (TOP) AND ≥ 2 µM TOTAL BIOLOGICAL (BOTTOM). THE AREA WAS AT REST, COMPLETELY CLOSED, AND MONITORED FOR 3 MONTHS. THERE WAS ONLY ONE REGISTERED INCIDENT (THREE CONSECUTIVE POSITIVE SAMPLES) IN EACH CHANNEL (TOTAL PARTICULATES [BIO PLUS INERT] AND BIO) AS SEEN IN THE FIGURE. THE INCIDENT WAS DUE TO AN INCORRECT INGRESS OF A DELIVERY PERSON INTO THE ROOM THAT LASTED APPROX. 27 SECONDS (INVOLVING THREE CONSECUTIVE MEASUREMENTS OF 9 SECONDS EACH BY THE INSTRUMENT).

Figure 5 demonstrates one of the great capabilities of a real time monitoring system where an incident that lasted only 27 seconds was detected over the baseline due to a human intervention.

Some points to emphasize related to the use of LIF technology and real time monitoring are:

- The Pharmaceutical Industry is moving toward **continuous manufacturing and control** to ensure full understanding of the aseptic manufacturing process and product quality.
- Environmental monitoring trend analysis provides an enhanced way to detect process changes **before** they impact the product.
- Used in conjunction with **rapid microbiological test methods**, such as those based on laser-induced fluorescence for air monitoring, valuable process information can be obtained in real time or in a short period of time.

Rapid Microbiology Methods: Surface Sampling in ISO 5 Environments

The surface sampling of equipment, facilities, and personnel are important components of the microbial control program in the environmental monitoring of controlled manufacturing areas. Both contact plates and swabbing methods for surface sampling are commonly used [7]. There are several advantages of the swabbing method over contact plates, particularly in an ISO 5/ Grade A areas. A cleaning validation is required when the contact plates leave an agar residue on the testing surface, which is not usually needed when swabs are used for testing. In addition, swabs are particularly useful for sampling irregular surfaces of Grade A areas such as those frequently found in cleanroom equipment (e.g., filling machine). The advantages of using a swabbing method over contact plates become obvious when performing a risk analysis of the critical point during manufacturing in a Grade A area. Interestingly, a rapid microbiological method (RMM) that combines swabbing sampling with detection of microbial contamination by oxygen consumption has recently been brought to the market.

Sampling Microorganisms on Surfaces and Rapid Testing

SurCapt® is an oxygen-depletion based rapid microbiology assay to test surface microbial contamination in cleanroom environments. Other applications of this technology include testing microbial bioburden in raw materials, excipients, drug products, pharmaceutical water, and environmental monitoring applications in the pharmaceutical industry. The system detects microbial contamination based on measurements of oxygen depletion upon time of incubation in a pharmacopoeia-recommended liquid broth, such as TSB.

The technology is based on the growth of the contaminating microbial culture, and follows this process:

1. Bacteria grow in a sample (incubated in a SurCapt vial) and consume dissolved O2.
2. The polymer sensor attached to the inside of the vial bottom reacts to the O2 depletion.
3. The measure of bacterial O2 consumption equates to microbial load. The greater the initial microbial load, the faster the result. When O2 concentration increases, it causes a reversible decrease in the phosphorescence signal. Inversely, when microbial respiration is active, the oxygen concentration in the media decreases and the sensor produces a larger phosphorescence signal.

$$\downarrow O_2 = \uparrow \text{Phosphoresence Signal}$$

The graphical representation of the phosphorescence signal versus time mimics a typical microbial growth curve.

The results of validation studies show that this rapid microbiology technology is reliable, sensitive, and equivalent to standard compendia tests.

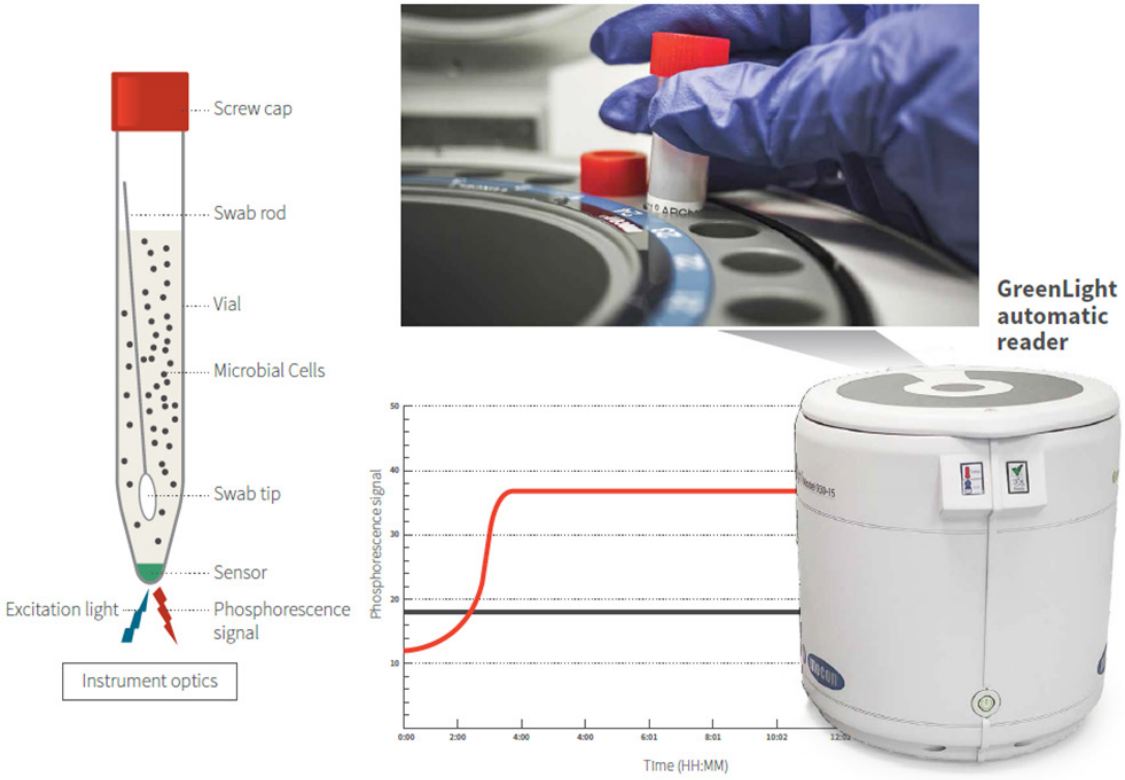

FIGURE 6. THE SURCAPT AND GREENLIGHT® SYSTEM

Some advantages of this rapid method are listed below.

- The SurCapt Microbial Surface Detection Kit and GreenLight® system represent a reliable and sensitive rapid microbial detection technology applied to pharmaceutical surface samples.
- The SurCapt Kit is comprised of high recovery flocked swabs, an oxygen-sensitive sensor and the traditional TSB liquid media for a ready-to-use disposable test. (Refer to the PMS GreenLight Operations Manual for further information.)
- The use of flocked swabs promotes high recovery and optimal releasing of surface microbial contaminants.
- Objective detection level results are as low as 1 cfu.
- The automatic reader eliminates human error in reading results and can sequentially operate up to six carousels, each carrying 24 surface samples.
- GreenLight's integrated barcode reader offers improved traceability of vials and carousels.
- The method is non-destructive. The sample is available and intact for any further identification steps, if required.
- The time taken to reach a preset threshold signal is faster than other methods, allowing a Presence/Absence result typically within 24 hours for surface samples from a Grade A/ISO 5 environment.

Conclusion

The pharmaceutical industry has been called upon to continually raise its sterility assurance standards through ongoing increased global regulatory standards, especially over the last ten years. Various regulatory agencies expect faster and more complete microbial contamination control.

In response to this expectation, suppliers have found a variety of approaches and solutions to meet these needs in a way that both satisfies regulatory requirements and helps pharmaceuticals save time and money by efficiently and accurately providing effective air and surface microbial monitoring. Top solutions for pharmaceutical contamination control meet at least one of the following criteria: sterility requirements, automation, and single use.

Author

Claudio Denoya, Ph.D.
Particle Measuring Systems

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