Adventures *in the* Environmental World *and* Environmental Microbiology Sampling *of* Air *for* Pharmaceutical Sterile Compounding

Roberto Ligugnana

Abstract Chapter <797> issued by the United States Pharmacopeial Convention, Inc. is the standard for sterile compounding. It is designed to reduce the number of patient infections due to contaminated pharmaceutical preparation. This regulation applies to all staff who prepare compounded sterile preparations and all places where they are produced, including hospitals, clinics, pharmacies, and physician's offices. This article provides the history of environmental microbiology and provides a discussion on environmental microbiology sampling of air for pharmaceutical sterile compounding.

History of Environmental Microbiology

The history of environmental microbiology (EM) began with Francesco Redi, a Tuscan doctor, in 1668, and Lazzaro Spallanzani, an Italian biologist, in 1768, who both paved the way for Louis Pasteur to debunk the theory of the spontaneous generation of microbes almost a century later. Pasteur invented the swan-necked flask to create an environment known not to grow microorganisms. After sterilizing a nutrient broth in these flasks, he removed the swan necks of the samples in the control group. Microorganisms grew in the control group, but not the experimental group, thus supporting biogenesis and rejecting "spontaneous generation."

After three centuries, it is now possible to perform the same test in a few minutes with the latest generation of microbiological air samplers.

My "adventure" in the field of EM started at the end of 1970 when I was involved in the contamination control of dairy factories producing Parmesan Cheese and yogurt. The method to detect the air presence of molds was to expose a Petri dish with culture media to the air for 60 minutes. The results were quite inconsistent, and my idea was to find another system, easily portable in the field, and combine an agar plate to test the air. The idea to simplify the laboratory activity and use for air sampling the same agar plates used for surface sampling resulted in the first portable air sampler prototype, designed with my brother Sandro. The prototype was made from cardboard and then later the system was made from polyvinyl chloride (PVC) and was patented.



LOUIS PASTEUR EXPERIMENTING IN HIS LABORATORY

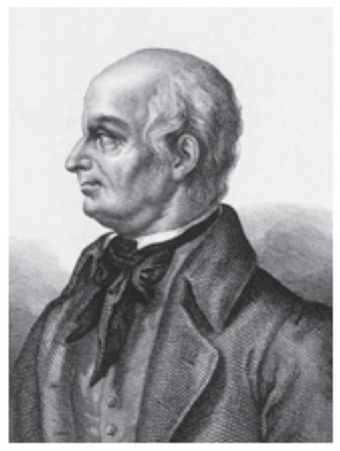
The first major step was to have a portable instrument with a battery pack. At that time, the batteries were the same type used for cars and, therefore, the sampler was quite heavy and the aspirating chamber was separated from the battery. With new technology, we were able to produce a lighter battery and a more compact unit.

As one can imagine, from the perspective of personal satisfaction, it was gratifying when our "invention" was used on board a Russian spaceship to assist with astronaut health monitoring and also for restoration of the Sistine Chapel in the Vatican. Furthermore, the 1997 edition of the *United States Pharmacopeia* reported that our impact method to detect viable airborne particles.[1] It is quite unusual that a governmental document cites the commercial name of an instrument. Some friends jokingly asked me how much I paid them.

The next evolution of the air sampler, around 1980, was to replace the metal body with a plastic body to produce a sampler that was lighter and easier to handle. Roughly 10 different



FRANCESCO REDI



LAZZARO SPALLANZANI



LOUIS PASTEUR

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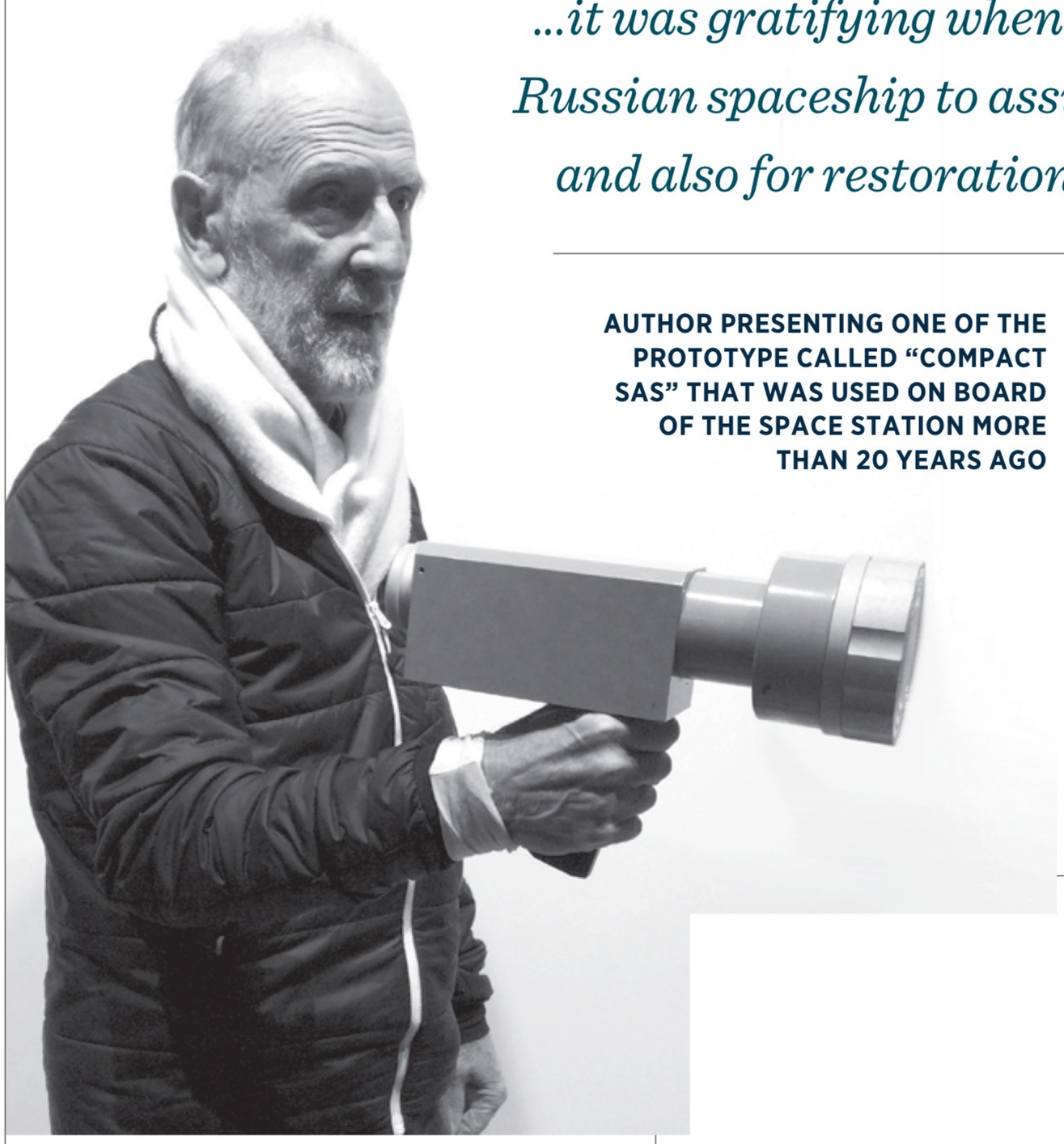
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...it was gratifying when our "invention" was used on board a Russian spaceship to assist with astronaut health monitoring and also for restoration of the Sistine Chapel in the Vatican.



AUTHOR PRESENTING ONE OF THE PROTOTYPE CALLED "COMPACT SAS" THAT WAS USED ON BOARD OF THE SPACE STATION MORE THAN 20 YEARS AGO

models came from our research and development and manufacturing plant.

Twenty years ago, the NATO Defense authorities were concerned about the possibility of bacteriological warfare. We were involved with the Italian and Dutch governments to produce a system to detect the air presence, in "almost real time," of dangerous microorganisms (e.g., anthrax [*Bacillus anthracis*]) used by enemy armies. The project lasted eight years, resulting in the final production of a sampler for use on board an army tank for the collection of microorganisms in liquid and subsequent identification by the polymerase chain reaction (PCR) method. The total time of identification was about 60 minutes.The project also involved the development of an 8-meter "wind tunnel" specifically designed for the validation and calibration of air samplers, as well as gamma irradiated plastic aspirating heads as an optional addition to the metal heads. During this time, I had the opportunity to visit the factories producing army tanks. It was an amazing experience for two reasons: first, to see how technology had advanced, and second, seeing the amount of investment in "guns"!

For the past 20 years, the pharmaceutical industries have been strictly controlled by the U.S. Food and Drug Administration (FDA) and, consequently, the FDA has focused on the problems of the sterile production environment, with cleanroom production managers stressed for the presence of a single microorganism. Unfortunately, microbiology is not an exact science, and the microorganisms are not doing what the microbiologist would like!

Microbiology Sampling of Air

During the 1990s, the typical cleanroom started to be replaced by isolators and restricted access barrier systems (RABS) to reduce the possibility of human contamination. A new type of air sampler with the command unit separated from the aspirating head (satellite unit) was presented with success on the market.

Another interesting story from my air microbial experience occurred several years ago. A technician called me reporting that after several samplings there was no growth of microorganisms on the Petri dish. The problem was solved after several questions and answers.



HE [MICROBES] USES WATER, AIR, HUMANS, ANIMALS, FOOD, FOR HIS SPREADING IN THE WORLD AROUND HIM



HE [MICROBES] IS ABLE TO SURVIVE IN DIFFICULT SITUATIONS ON ANY TYPE OF SURFACE



MAN CAN LIVE ON EARTH THANKS TO THE INTENSE ACTIVITY OF MICROBES AND IT IS THEREFORE PROPER TO RAISE A MONUMENT IN THEIR HONOR

It turned out this technician was using a sterile Petri dish—without the medium.

I have, in fact, noted during my career that the world of microbiology is an "unknown world" for many involved in activities where the microbial population is an important factor. Therefore, with the help of an illustrator, I prepared a teaching tool in the form of "bacteria comics" presenting the typical life of bacteria, three of which are shown within this article.

Environmental Sampling of Air (Viable Airborne Particles) for Pharmaceutical Sterile Compounding Regulation *USP* Chapter <797>

Chapter <797> of the *United States Pharmacopeia–National Formulary (USP–VF)*, issued by the United States Pharmacopeia, is the standard for sterile compounding. It is designed to reduce the number of patient infections due to contaminated pharmaceutical preparation. This regulation applies to all staff who prepare compounded sterile preparations (CSPs) and all places where they are produced, including hospitals, clinics, pharmacies, and physician's offices.

ENVIRONMENTAL SAMPLING

The sampling of air and surfaces within compounding areas is a key requirement for *USP* <797> compliance. The areas to be considered are International Organization for Standardization (ISO) Class 5, buffer areas, ante areas, and segregated compounding areas.

The current *USP* <797> compliant microbial air sampling frequency is at least every 6 months for air and more frequently for surfaces and hands. *USP* <797> revisions are calling for active air sampling of all ISO-classified areas to occur once every month during typical operating conditions. For educational purposes and to establish a reference point, the frequency for sampling should be increased beginning with the establishment of the clean environment.

The adoption of the 55/60 mm contact plate can simplify the microbiological laboratory activity because the same plate can be used for air, surface, and personnel monitoring.

TABLE 1. RECOMMENDED ACTION LEVELS FOR AIR MICROBIAL CONTAMINATION.

ENVIRONMENT CLASSIFICATION	COLONY FORMING UNIT/ 1000 LITERS OF AIR
ISO Class 5	>1
ISO Class 7	>10
ISO Class 8 or over	>100

ISO = International Organization for Standardization

FIGURE 1. EXAMPLE STANDARD OPERATING PROCEDURE.

SOP No.: _____

Title of SOP: Microbial Air Sampling Procedure to Apply *United States Pharmacopeia* Chapter <797> Regulations

Original: Yes __ No __ Revision: Yes __ No __ Revision No.: _____

RESPONSIBILITY:

Cleanroom Manager (or Quality Assurance Manager)

PURPOSE:

The purpose of this standard operating procedure is to specify the precise use of the active impact air sampler placed in areas of greatest contamination risk to obtain correct microbial results, to maintain contamination control, and reduce the probability of patient infections due to contaminated pharmaceutical preparation.

BACKGROUND:

The sampling of air and surfaces within compounding areas is a key requirement for *United States Pharmacopeia (USP)* Chapter <797> compliance. The areas to be considered are International Organization for Standardization (ISO) Class 5, buffer areas, ante areas, and segregated compounding areas. *USP* Chapter <797> is the standard for sterile compounding. It is designed to reduce the number of patient infections due to contaminated pharmaceutical preparation. This regulation applies to all staff who prepare compounded sterile preparations (CSPs) and all places where they are produced, including hospitals, clinics, pharmacies, and physician's offices.

PROTOCOL:

- Introduction of the air sampler into the cleanroom
  Follow Good Aseptic Practice.
- Introduction of poured contact plates into the cleanroom
  Use irradiated double- or triple-bagged contact plates.
- Introduction of sterile disinfectant spray into the cleanroom
  Use irradiated triple-bagged disinfectant spray.
- Identification of contact plates
  Each plate must be identified by sampling site (XXXX), date (XX / XX / XX), hour (XX.XX).
- Disinfection of air sampler
  Follow the Good Disinfection Practice.
- Use of air sampler
  Follow the instruction manual.
- Air sampler positioning inside the cleanroom
  The microbiological air sampler can be positioned:

  a) directly on the surface, close to the area considered most critical
  b) fixed on tripod, at the height considered most critical
  c) fixed on the wall, using a wall arm
- Air sampler programming
  The volume of air to be sampled and the sampling program are a choice of the Cleanroom Manager. The suggested amount of air per each plate in a cleanroom per USP Chapter <797> should be 1000 litres of air.
- Sampling data downloading
  Each sampler should be connected to the printer for sampling data downloading (date, operator identification, sampler identification, sampling site, volume of air).
- Transfer of contact plate to incubation
  At the end of sampling operation, each identified contact plate must be protected by its sterile bag and sent to the lab for incubation.

FIGURE 1. **EXAMPLE STANDARD OPERATING PROCEDURE CONTINUED.**

EQUIPMENT/SUPPLIES REQUIRED:

- Calibrated microbial air sampler (specific model listed here)
- Battery charger for air sampler
- Certified sterile aspirating head (or autoclavable head with autoclave process listed)
- Tripod or stand for air sampler
- 60-mm (or 100 mm Petri) culture poured contact plate for bacterial population (e.g., Tryptic Soy Agar)
- 60-mm (or 100 mm Petri) culture poured contact plate for fungal and yeast (e.g., Sabouraud Dextrose Agar/Malt Extract Agar)
- Sterile gloves
- Sterile Spray and disinfectant wipes

GLOSSARY OF TERMS:

- Action level
- Agar plate
- Alert level
- Aseptically
- Bacterial population
- Bio-aerosol
- Colony-forming unit (CFU)
- Compounding
- Contact plate
- Contamination
- Corrective action
- Culture plate
- Disinfection
- Good Aseptic Practice
- Good Hygiene Practice
- Good Laboratory Practice (GLP)
- Good Manufacturing Practice (GMP)
- Incubation
- Installation Qualification
- International Organization for Standardization (ISO)
- Irradiation
- Isolator
- Mold
- Non-conformity
- Nutrient media
- Operation and Operator Qualification
- Petri dish
- Quality assurance
- Quality control
- Restricted access barrier system
- Sterility
- Sterilization

REGULATION:

USP Chapter <797>

SAFETY:

Apply the indication in force at the production site.

NON-CONFORMITY:

Contaminated contact plates	Do not use them.
Expired contact plates	Do not use them.
Torn plastic bag	Do not use them.
Low-battery warning signal	Charge the air sampler.

Approved by _____ Date _____

Implemented by _____ Date _____

The microbial air sampler should be of the "active impact" agar type. It should be light, easy to handle, and programmable with passwords to ensure the operators involved in air sampling are using it properly to avoid false results. The sampling system should hold *data integrity*, a subject of increasing importance, with the ability to transfer unalterable sampling data in a "technologically safe" way.

If air and surface sampling cycles are operated by non-microbiologists, it is imperative to educate them in this specific task.

The volume of air to be aspirated by the air sampler in the clean area is 1 cubic meter (1000 liters).

The culture media are typically Tryptic Soy Agar for the Total Bacterial Count and Sabouraud Dextrose Agar or Malt Extract Agar for fungal count.

The microbial air sampler should be officially calibrated every 12 months.

The installation qualification (I.Q.) and operation qualification (O.Q.) performances should be conducted before beginning the activity after the purchasing of the air samplers.

If the microbial population exceeds the "action level," it is necessary to apply basic measures such as wiping surfaces with sterile disinfectant and re-evaluating proper garbing, hand hygiene practice, and a review of the air-filtration system. Recommended action levels for air microbial contamination are provided in Table 1.

This article provides the history of EM and provides a discussion on EM sampling of air for pharmaceutical sterile compounding. Also provided in this article is an example SOP, a necessary and integral part of any pharmacy.

STANDARD OPERATING PROCEDURES

To be sure that the air samples are collected correctly by all involved personnel, a specific standard operating procedure (SOP) should be prepared by the Cleanroom or Quality Assurance Manager in cooperation with the Microbiological Laboratory Manager (if applicable). An example SOP is provided in Figure 1.

Conclusion

Chapter <797> issued by the *United States Pharmacopoeia* is the standard for sterile compounding. It is designed to reduce the number of patient infections due to contaminated pharmaceutical preparation. This regulation applies to all staff who prepare compounded sterile preparations and all places where they are produced, including hospitals, clinics, pharmacies, and physician's offices. This article provides the history of EM and provides a discussion on EM sampling of air for pharmaceutical sterile compounding. Also provided in this article is an example SOP, a necessary and integral part of any pharmacy.

Reference

1. United States Pharmacopeial Convention, Inc. *United States Pharmacopeia–National Formulary*. Rockville, MD: US Pharmacopeial Convention, Inc.; 1997, Vol. 23, No. 6: 5284.

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