microBYTES

HARDY DIAGNOSTICS
A Culture of Service™

PHARMA MICRO
VOL.1

Our heroes wear bunny suits.

PHARMA MICRO
VOL.1

microBYTES

Contents

A PHARMACIST'S SON MAKES A NAME IN CULTURE MEDIA MANUFACTURING

Hardy Diagnostics may have had humble beginnings, but has since grown to become one of the top producers of culture media in the country. Not only that, Hardy Diagnostics has the unique distinction of being a 100% employee owned company. The Hardy Diagnostics ESOP was created in 2012, and in October 2015, Jay Hardy sold the remainder of his majority share in Hardy Diagnostics back to his employees. Hardy Diagnostics now operates as a 100% Employee Owned ESOP.

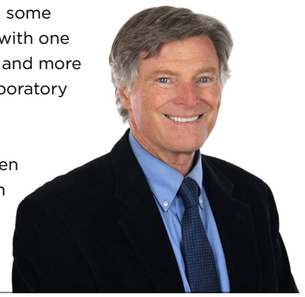
So how did we get here? Meet our founder and president, Jay Hardy.

"The year was 1980, and I had just finished a one year internship at a hospital in Santa Barbara to train as a Medical Technologist. The requirements are a bachelor's degree and a rigorous year of practical training in the hospital lab. After finishing and passing the California State Board exams, my dream had been realized and I had finally become full-fledged Medical Technologist.

However, there were no jobs available at the time! Having come from the LA area, spending a year in the Central Coast of California was like paradise to me, so I very much wanted to stay in Santa Barbara. Disappointed and dejected about not being able to find work in my new profession, I was talking to my friend who had also completed the internship. We did not know which way to turn, but somehow came up with the idea of making culture media. My father was an entrepreneurial pharmacist who operated many drug stores during his career, so starting a new business seemed to be a somewhat natural path for me to follow.

My friend and I started our fledgling business on a shoestring budget. We rented two small rooms in what had once been a motel in Santa Barbara. After borrowing $10,000 from each of our Dads, and rescuing some antiquated equipment from a trash heap, our little business was ready to be launched. We started with one customer, which was the hospital where we had trained. Over the years, we began to service more and more hospitals in Central California, and eventually grew to a company that now supplies over 10,000 laboratory customers worldwide with over 13,000 products that are used in the laboratory.

I often stop and wonder how different my life would have been if I had gotten my wish and had been offered a job back in 1980. I am constantly reminded of one of my favorite sayings when faced with adversity, which is the Marines' motto: 'Adapt, Improvise, Overcome.' We did just that, and I'm now enjoying the ride with no regrets!"



Jay Hardy, CLS, SM(NRCM),
Co-Founder and President

ACTIVE ENVIRONMENTAL AIR SAMPLING

ARE TWO HEADS REALLY BETTER THAN ONE? WHAT ABOUT THREE?

The Advantages of Microbial Air Monitoring Using Instruments with Multiple Aspirating Heads

Lazzaro Spallanzani, in the 1700s, and Louis Pasteur, in the 1800s, were the two scientists who first demonstrated the presence of airborne microorganisms after several years of multiple-step experimentation using broth and flasks. Three centuries later, it is possible to perform the same test in just a few minutes with portable microbial air samplers, introduced over 45 years ago.

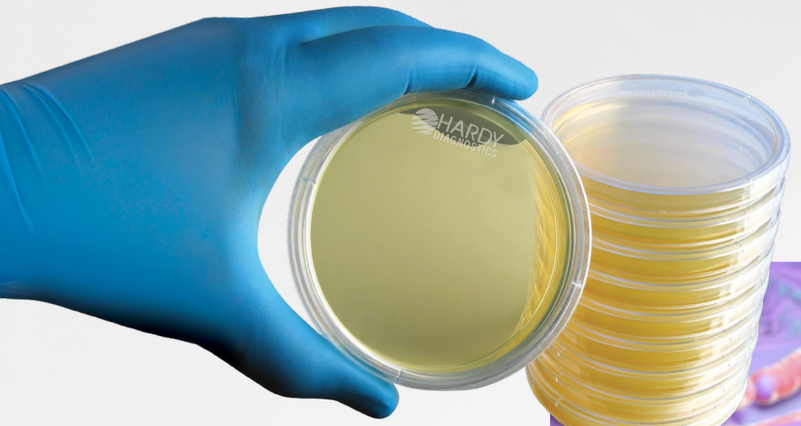
These instruments have made a big impact for industries with controlled environments needing to assess the microbial quality of the air. Air sampling methods must include the use of active devices, according to Federal Drug Administration and United States Pharmacopeia guidelines. The most well-known and prolific instrument type is the single intake aspirating head format, which conducts sampling by impact method on an agar plate.

Are you currently evaluating your Environmental Control Plan, making sure it is as robust as it needs to be? Would you like to discover more efficient methods, or find more time in the day?

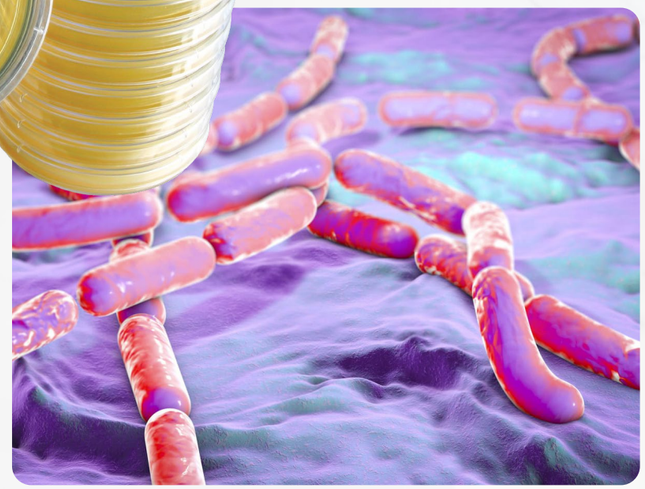
Sampling with multiple aspirating heads has notable advantages, and should be a consideration when designing or reassessing an active air monitoring program.

Here, we break down some key reasons to consider a microbial air sampler with more than one aspirating head:

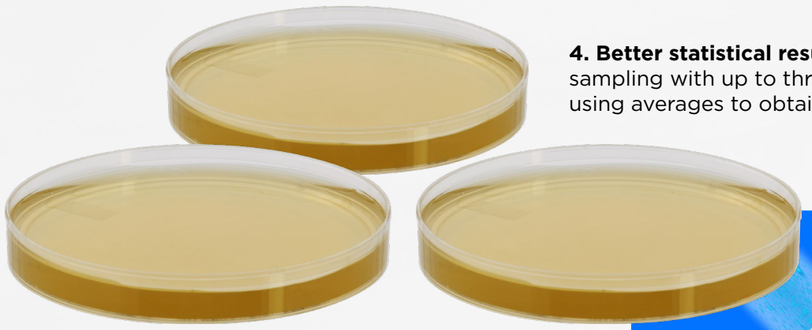
1. Sampling with multiple aspirating heads allows **continuous environmental monitoring** by programming fractions and intervals within a single sample. This method provides a “video” of the sampled environment during an entire working shift, vs. a single 5 or 10 minute “snapshot” one would take with a single aspirating head instrument.



2. Use **two or more types of culture media at the same time**, such as TSA and SabDex, thus avoiding the need to run two separate samples.



3. Microorganisms aren't predictable or distributed evenly in the air. Obtain a **more realistic sample of the environment** by sampling two or three culture media plates at once.

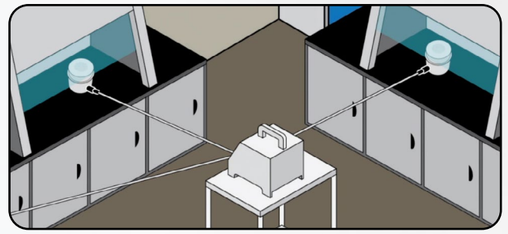


4. Better statistical results can be obtained by sampling with up to three agar plates at once and using averages to obtain sample trends.

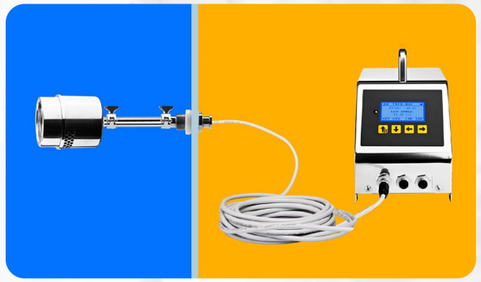
5. Achieve **faster sample times** when several aspirating heads perform simultaneously.

6. More efficient sampling = **better use of Operator Time**, which saves the company money and increases the ability to maintain a robust environmental control program.

7. The ability to load several culture media plates during one activity, and to position remote SATELLITES inside RABS or Isolators **reduces human interaction and contamination risks.** The adoption of sterile, disposable Daily Shift Heads further protects the environment from risk.



8. RABS Isolator and MULTIFLEX formats allow unmatched flexibility and **positioning of satellites in several high-risk points at once.**



9. Save space inside equipment with limited room: TRI CLAMP remote system satellites can be placed horizontally and vertically within walls of RABS and isolators.

10. Customization of satellites is possible with the RABS Isolator format: choose Standard, HEPA Filter-equipped, or TRI CLAMP options (must use three of the *same* satellite format; satellite types cannot be "mixed").

Air Sampling

TRIO.BAS™
BIOLOGICAL AIR SAMPLER

TRIO.BAS™ Impact Air Samplers introduced the newest generation of microbial air sampling. These ergonomically designed instruments combine precise air sampling with modern connectivity to help you properly assess the air quality of your laboratory and simplify your process.

RABS ISOLATOR and MULTIFLEX Systems specifically designed to meet pharmaceutical and bio-pharmaceutical cleanroom regulatory requirements and standards.

TRIO.BAS™ RABS ISOLATOR

Each kit includes:
TRIO.BAS™ RABS ISOLATOR air sampler, batter charger and cable, satellite aspirating head, cover heads, 5 meter long satellite attachment cable, hard shell carrying case, calibration certificates, and IQ, OQ, PQ fillable documents. Add up to two additional satellite kits.

100 liters/min., Petri plate	**BAS269K**
100 liters/min., contact plate	**BAS268K**
200 liters/min., Petri plate	**BAS271K**
200 liters/min., contact plate	**BAS270K**

TRIO.BAS™ MULTIFLEX 1+2

Each kit includes:
TRIO.BAS™ MULTIFLEX 1+2 air sampler, batter charger and cable, three satellite aspirating heads, cover heads, two 5 meter long satellite attachment cables, hard shell carrying case, calibration certificates, and IQ, OQ, PQ fillable documents.

100 liters/min., Petri plate	**BAS475K**
100 liters/min., contact plate	**BAS474K**
200 liters/min., Petri plate	**BAS477K**
200 liters/min., contact plate	**BAS476K**

TRIO.BAS™ MONO

Each kit includes:
TRIO.BAS™ MONO air sampler, battery charger and cable, aspirating heads, cover heads, hard shell carrying case, calibration certificates, and IQ, OQ, PQ fillable documents.

100 liters/min., Petri plate	**BAS201K**
100 liters/min., contact plate	**BAS200K**
200 liters/min., Petri plate	**BAS205K**
200 liters/min., contact plate	**BAS205K**

TRIO.BAS™ DUO

Each kit includes:
TRIO.BAS™ DUO air sampler, battery charger and cable, aspirating heads, cover heads, hard shell carrying case, calibration certificates, and IQ, OQ, PQ fillable documents.

100 liters/min., Petri plate	**BAS221K**
100 liters/min., contact plate	**BAS220K**
200 liters/min., Petri plate	**BAS226K**
200 liters/min., contact plate	**BAS225K**

Air Sampling

TRIO.BAS™
BIOLOGICAL AIR SAMPLER

TRIO.BAS™ TRIO

Each kit includes:
TRIO.BAS™ TRIO air sampler, battery charger and cable, aspirating heads, cover heads, hard shell carrying case, calibration certificates, and IQ, OQ, PQ fillable documents.

100 liters/min., Petri plate	**BAS241K**
100 liters/min., contact plate	**BAS240K**
200 liters/min., Petri plate	**BAS243K**
200 liters/min., contact plate	**BAS242K**

TRIO.BAS™ DAILY SHIFT HEAD

For use on the MINI, MONO, DUO, TRIO and MULTISTATION units. Technopolymer aspirating heads are ready for immediate cleanroom use. Individually packaged, sterile by irradiation, and includes a certificate of sterility assurance.

Contact plate	**BAS340**
Petri plate	**BAS341**

BIOLOGICAL AIR SAMPLER SOFTWARE (BAS)* DATA TRANSFER SOFTWARE

Designed to facilitate paperless record keeping of all aspects of environmental monitoring, BAS software is the ideal TRIO.BAS instrument accompaniment for cleanroom environments. One software license per PC is required. Multiple instruments can be used with a single software license.

*For Bluetooth capable Windows systems

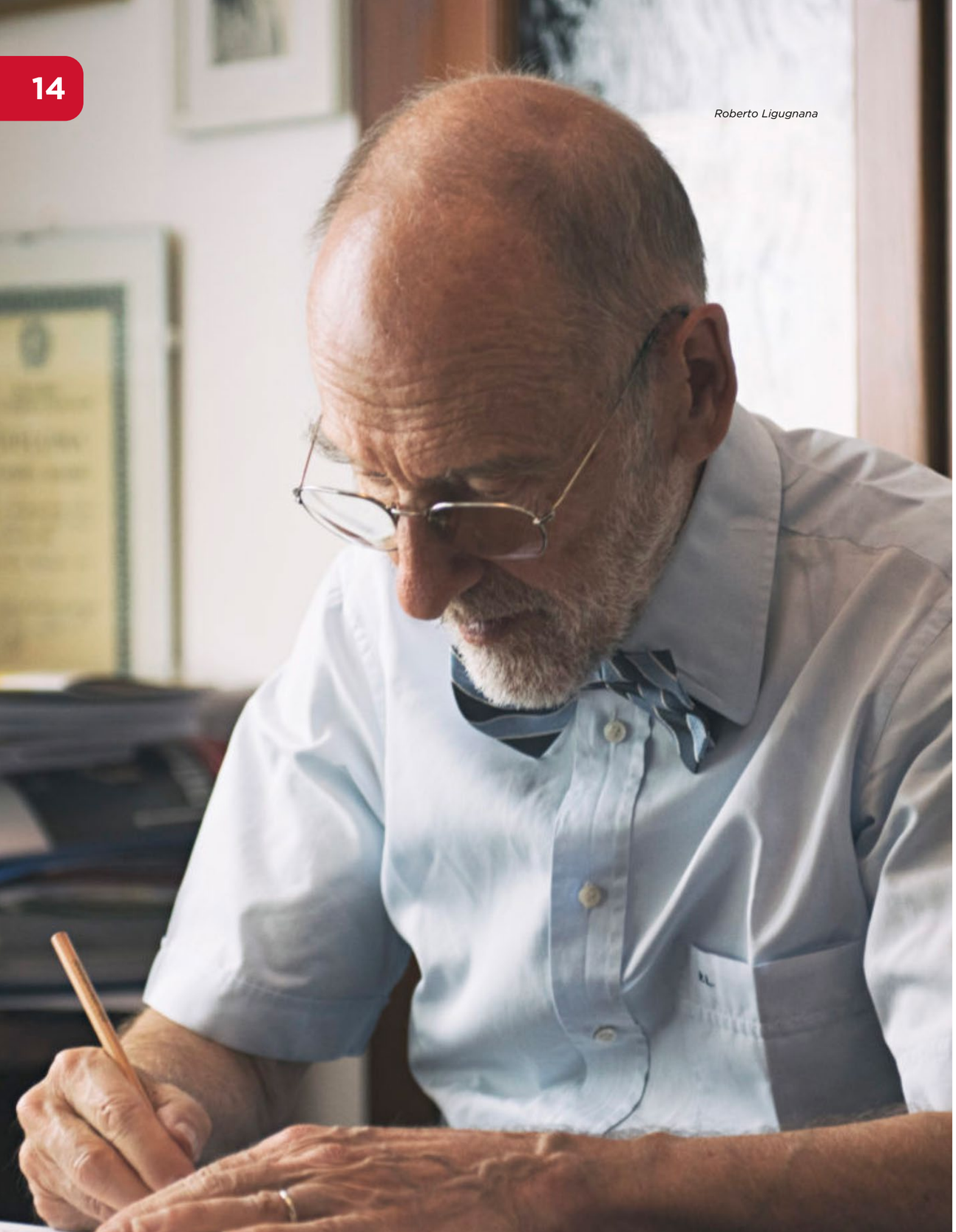
Each	**BAS296**

TRIO. SETTLE FOR PASSIVE AIR MONITORING

The TRIO.SETTLE ideally standardizes the position of the culture plate reducing the risk of contamination during passive sampling.

Position culture media (settling plates) for best articulation to provide a quantitative analysis of airborne microorganisms deposited over a set period of exposure.

TRIO.SETTLE table plate stand	**BAS367**
TRIO.SETTLE floor plate stand	**BAS368**



Roberto Ligugnana

THE EVOLUTION OF ENVIRONMENTAL MICROBIAL AIR SAMPLING

A conversation with Roberto and Sandro Ligugnana

The evolution of environmental monitoring traces back to Italian scientists Francesco Redi and Lazzaro Spallanzani, and Frenchman Louis Pasteur, who demonstrated, through experimentation with a swan-necked flask, that life does not arise spontaneously as originally postulated with the medieval idea of "spontaneous generation." Thanks in part to Roberto and Sandro Ligugnana, the principle of the swan- necked flask can be repeated today in just minutes with active impact air sampling instrumentation.

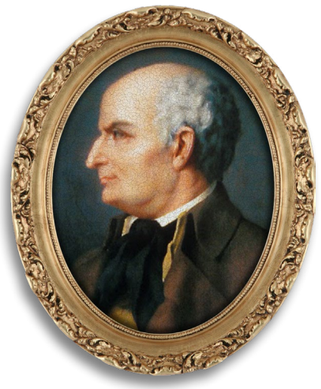
Along with their father, Elio, Engineer Rino Tressoldi, and Microbiologist Lawrence Whittard, the Ligugnana brothers set out to find a more reliable and consistent solution to the more random environmental method of sampling air quality using "settling plates." The United States Pharmacopeia (USP) states the use of settling plates alone is not adequate to assess the quality of air in a controlled environment.

We visited with Roberto and Sandro Ligugnana, brothers and founders of Orum International, at their villa in Milan, Italy to hear about their journey in the production of active air sampling equipment.

Q: Roberto, what is your background and why were you motivated to discover a better way to conduct air sampling?

My background is in dairy microbiology. My first position was in Quality Control at a yogurt and Parmesan cheese production facility. The operation included every aspect of production; from the farm to the dairy and cheese factory. During that time, I spent my life with microorganisms and their positive and negative effects. The microbial air contamination of dairy products was important to learn about during the 1950's and '60's. Finding molds inside yogurt, butter, and cheese at that time was a frequent occurrence. At that time, the methods to discover and mitigate against molds involved using Petri dishes, open and exposed to the environment for 4 hours, and the use of stationary six-stage Anderson air samplers. The first method, using "settling plates", was quite non-consistent. The second method, using the Anderson method, was complex, and the equipment was not portable. We decided to develop, and patent, a portable and easy-to-handle instrument applying the Anderson principle.

Francesco Redi



Lazzaro Spallanzani

Louis Pasteur

Sandro Ligugnana

Q. Sandro, there have been many versions since your original design 40 years ago. Your original patented invention has taken on many forms over the past 45 years. Would you please take us through history?

It is a long story spanning over 45 years. We started out by engineering a sampler with a separated battery pack that was quite heavy. We eventually produced a "gun-like" sampler which was much smaller and easier to handle for remote activities inside the MIR station. The years passed with around twenty other models and formats, bringing us to present day. We now have the most ergonomic, light, and technologically advanced version. TRIO.BAS represents the most complete line of active microbial air sampling instruments available. TRIO.BAS air samplers are sold in every continent, from Arctic regions to the desert, from Europe to the Far East, and from the North to the South.

Q. Sandro, how have you observed the industry changing over the years you've been in business?

In the 1960s, industrial microbiology was almost unknown. The Pharmaceutical industry was made up of small businesses mainly born in families' drugstores.

Companies were mainly artisanal, with solutions produced in smaller environments. There were no short shelf life concerns due to small scale production and rapid consumption of the products.

As years passed, the growth of the market required quality improvements to extend product shelf life.

During these years, analysis laboratories for quality control were created to support the increased demand for production.
Due to these historic evolutions, and thanks to our constant presence and our close contacts with customers and the university academic world, we gained great experience and knowledge in the field.

Our greatest satisfaction is that for over 45 years we still remain a reference point for the market, and are always among the first to offer innovative instruments to the international markets.

Q. Roberto, you found great success with your original concept. Why were you and Sandro motivated, in your golden years, to make improvements upon your original design, sparking a new company? Will you please explain the main implementations you've made?

We are passionate about the subject of Environmental Monitoring and decided to develop a new generation of instruments with several technological improvements. The most important advancements can be summarized as: (a) the creation of instruments with two and three aspirating heads, in addition to the best known classic "MONO" format ; (b) Bluetooth capability for data transfer to a printer or PC; (c) an induction battery charger to eliminate an outer plug on the instrument, thereby making it more resistant to liquid cleaning agents penetration, as well as possible contamination; (d) IP65 certified protection from dust and water; (e) shockproof construction (f) cascade passwords; (g) quick bayonet manipulation of the much lighter stainless steel aspirating head to aid in loading and unloading culture media easily and aseptically; (h) Explosion proof certified instruments (in ATEX models) ; (i) 50% reduction of sampling time; (j) antibacterial treatment of the surface finish; (k) the option of certified sterile aspirating heads to save time in the Cleanroom;(l) the option of "remote" satellite units in several different formats for varied applications and sampling flexibility.

Q. When it comes to compliance, why is it important to have a robust sampling plan, possibly exceeding minimum requirements?

A sampling plan should be risk-based, with well-mapped sampling of the most critical areas of the cleanroom.
More frequent sampling produces more reliable trends. If something in the environment changes, appropriate action can quickly be taken.
Continuous microbial monitoring with multi-aspirating head samplers can capture sample data "at rest", "in operation", and "at the end" of the filling process to verify and apply the necessary "alert" and "action" interventions. Continuous monitoring activity is requested by the FDA in sterile drug manufacturing, but is a robust method for any cleanroom activity.

Q. This question is for each of you. In your long and illustrious careers, what is the most valuable lesson you have learned?

ROBERTO:
Our father, Elio, always told us we should come alongside the customer as valuable consultants. This is the reason we have produced a large amount of technical papers, application notes, bulletins, and have organized thousands of seminars and congresses around Italy and the world.

SANDRO:
Physical and mental discipline are very important ingredients to achieving goals.

I have always believed in technology and science as indispensable engines for growth. This is why, in our professional experience, we never fixed a final goal. We have always worked tirelessly, through knowledge, experience, and culture, to go further.
In the past 45 years, we've continued to create and update our instruments, adapting them to emerging industry needs. We're always looking for opportunities to adopt new technologies of mechanics, physics and electronics.

"Continuous growth" is the spirit that has driven my decisions; decisions always shared with my brother, Roberto.

WINNING THE WAR ON BIOFILMS

Can cinnamon help?

Just as we humans band together into communities for greater communication and protection from our enemies, some bacteria have the propensity to form biofilms, communal aggregates of colonized bacteria, that are encapsulated in a complex three-dimensional gelatinous, extracellular polymeric substance comprised of polysaccharides, DNA, and proteins.

Biofilm formation is a highly orchestrated process that requires extensive communication and coordination between cells within the commune. There is a division of labor amongst the cells too; some specialize in the production of surfactants, structural components, and enzymes for group motility, matrix production, and nutrient degradation. This mode of intricate organization increases the survival chances of a bacterial population exponentially. Without the biofilm commune, each individual bacterium on its own would be susceptible to most clinically available antibiotics; however, biofilms add a level of protection for these bacteria, making their ability to sustain disease significantly greater and thus presents greater challenges for clinicians to treat. Biofilms are often impermeable matrices that drugs have difficulty penetrating due to the inherent hydrophobic character and extensive layers of the film.

Biofilms commonly have electrical charges associated with them, which can complicate matters more through electrostatic repulsions that can work against antibiotic treatment, rendering them ineffective. Additionally, bacterial cells localized deep within a biofilm are typically deprived of essential nutrients and oxygen, which allow them to divide at a very slow rate. Since antibiotics chiefly target rapidly-dividing cells, this is just another means for a bacterial commune to persevere. While a significant amount of resources are invested in the discovery of new antibiotics, research is now beginning to focus on targeting biofilms, specifically the prevention of biofilms. Most all antibiotics, both existing and current candidates, are natural products; these molecules are produced naturally and exhibit some level of bioactivity against a particular pathogenic bacterium or a wide breadth of bacteria.

In a study conducted by Dr. Sanjida Topa of Swinburne University of Technology, Australia, the natural product cinnamaldehyde, a constituent of cinnamon essential oils, has been observed to exhibit a level of bioactivity against *Pseudomonas aeruginosa*.

Cinnamaldehyde, in a concentration dependent study, successfully inhibited surface colonization and elicited biofilm degradation to a staggering degree. A biochemical analysis revealed significantly reduced levels of a pivotal secondary messenger in biofilm genesis, dimeric guanosine monophosphate, attributed to cinnamaldehyde's antimicrobial properties to the modulation of intracellular signaling cascades.

Cinnamaldehyde, the flavonoid that gives cinnamon its characteristic aroma, color, and distinctive taste, may pioneer the development of surface antimicrobial agents, most notably in the application of treating skin infections.

With the increasing emergence of antibiotic resistant bacteria, the control of biofilms has never been more important. The coupling of inappropriate prescriptions and extraneous applications, through improper diagnostics, has led to an unparalleled proliferation of multi-drug resistant bacteria that render many of the antibiotics ineffective in the treatment of such infections.

It is important that research is not only focused on the development of new, robust antibiotics but also in the development of preventive measures for biofilm formation.

Cinnamaldehyde, from the spice, cinnamon, has been shown to be active in the disruption of persistent biofilms.

Patricia Kienle is the Director of Accreditation and Medication Safety for Cardinal Health and a leading expert on regulatory issues. She is the recipient of the 2022 Harvey A.K. Whitney Lecture Award, health-system pharmacy's highest honor.

MICROBIAL ENVIRONMENTAL MONITORING FOR STERILE COMPOUNDING

Patricia C. Kienle, RPh, MPA, BCSCP, FASHP

Sterile compounding is a core component of hospital pharmacies. Other sites, including community pharmacies that specialize in compounding, clinics, physician offices, and others, are bound by the same standards if they compound sterile preparations.

The United States Pharmacopeia (USP) is an independent scientific organization that sets standards related to medicines. Several standards written by USP apply to sterile compounding. USP <797> Pharmaceutical Compounding Sterile Preparations sets the minimum standard for that activity. States and organizations that accredit hospitals, pharmacies, and other healthcare sites may have additional or more stringent requirements.

Two microbial environmental monitoring criteria are defined in <797>: electronic air sampling and surface sampling. Locations within IV hoods and within sterile compounding rooms must be monitored. The currently official <797> standard requires electronic air sampling every six months in each IV hood and room and surface sampling periodically.

USP <797> is currently in a revision process. The 2021 proposed revision of the standard (which underwent a public comment period and is being evaluated by a committee comprised of pharmacy practitioners and other experts) includes requirements to increase the required frequency for surface sampling to monthly for the vast majority of sites. Sites that will prepare compounded sterile preparations (CSPs) with longer expiry times, called extended beyond use dates (BUDs), have a proposed frequency of weekly and when batches of CSPs are mixed. The frequency for electronic air sampling remains at every six months for most sites. However, the proposed revision increases the frequency of electronic air sampling for those sites mixing sterile preparations with extended BUDs to monthly. In addition to frequency, USP <797> sets action levels for number of CFUs. Any CFUs detected require that the facility investigate the probable cause and implement corrective action. Results above the action levels require identification to at least the genus level and evaluation with the help of a microbiologist.

Most pharmacies and other organizations that compound CSPs have their certifier perform the electronic air sampling when the semiannual certification of the IV hoods and rooms are completed. However, an increasing number of compounding sites supplement this with the ability to do the sampling themselves. This provides the sites with the option to follow up when growth is detected. If compounding sites intend to perform some, or all, of the electronic air sampling themselves, they need a sampler, media, and a lab capable of incubating and identifying microbial samples. Similar media and lab resources will be needed for almost all compounding sites since the increased frequency for surface sampling will likely be done by personnel at the site.

Some direction for locations to sample is included in USP <797> but the compounding site supervisor needs to evaluate the appropriate spots and frequency to ensure safe practices. Surface sampling will be more of a local obligation since monthly (or weekly) sampling will likely be required. Many compounders have already embarked upon doing this themselves in anticipation of the proposed revised standards. Some plan to have the certifier perform surface sampling during the semiannual certification and will supplement with surface sampling performed by site personnel during the other months. In either case, the ability for the compounders at the site to use surface sampling for follow-up after detected growth will improve the ability to trend potential risk points.

Microbial sampling may be new to personnel at sterile compounding sites since most of this has been done by certifiers. Microbiologists, infection control practitioners, and risk managers will be valuable collaborators with compounders to ensure the sterile compounding facility is selecting the appropriate sites within the IV hoods and cleanrooms, providing education when needed concerning proper sampling techniques, and identification of microbes.

From Aseptic Technique Validation Kits for Sterile Compounders, to culture media formats for Pharmaceutical Aseptic Process Simulations, Hardy Diagnostics has it in the bag.

See our website for our full line of aseptic testing products.

HardyVAL™ Multiple Technician Aseptic Technique Kit.
Cat. no. HVMTK

media BAG™

HardyVAL™ CSP Medium Complexity Comprehensive Aseptic Technique Kit.
Cat. no. HVM1

HardyVAL™

Media-Bag™ Tryptic Soy Broth (TSB) bag, 700ml fill, for aseptic media fill processing.
Cat. no. HVB5

Hardy Diagnostics
DuraRack®

Petri and Contact Plate Rack for Transport and Storage

The DuraRack™ is a durable, autoclavable, epoxy coated, heavy gauge steel wire rack that allows for easy loading and unloading of culture media plates. Sturdy welded design and advanced long-lasting protective coating provides a plate rack that will last.

DURARACK®

Advanced epoxy coated steel wire is chemical and corrosion resistant and steam autoclavable. Six columns hold either 100mm or 65mm diameter size stacked plates. Bottom and top access opening allows for easy and safe removal of dish stacks

DURARACK™ holds sixty, 100mm Petri plates	**DURA60**
DURARACK™ holds eighty-four, 100mm Petri plates	**DURA84**
DURARACK™ CONTACT, holds sixty, 65mm contact plates	**DURA60C**

Prepared Media

LOK TIGHT™

Tryptic Soy Agar with Lecithin and Tween® 80, USP, Irradiated, Triple Bagged Contact Plates

For the cultivation and enumeration of microorganisms. Irradiated, triple bagged. Lok-Tight™ friction lid with locking feature.

Tryptic Soy Agar with Lecithin and Tween® 80 Contact Plate
Contact plate, 10/pk **P520**

STERIEM™ Sterile Plate for Environmental Monitoring

Tryptic Soy Agar with Lecithin and Tween® 80, USP, Irradiated, Triple Bagged Petri Plates

For the cultivation and enumeration of microorganisms. Validated for a Sterility Assurance Level (SAL) of 10-5.

Tryptic Soy Agar with Lecithin and Tween® 80 Petri Plate
Contact plate, 10/pk **W520**

Cleanroom Bag for Sterile Transport of Media Plates

Sterile bags for transporting Petri dishes, contact plates, swabs, or other objects outside of the cleanroom.

Cleanroom Bags

750 Bags/Case **BAS381**

Visual inspection at plate line

At Hardy Diagnostics, you're not just a number. You're not a figure on a graph in a quarterly report. At Hardy Diagnostics, you're a Partner. From laboratories that utilize our tests to diagnose illness, to our employee owners who ensure that every lot meets your rigorous expectations, Hardy Diagnostics is about a team of people coming together to better the world, one test at a time.

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100% Employee Owned

ESTABLISHED 1980

MADE IN USA

bsi. ISO 13485 Medical Devices Quality Management

FM 572526

Hardy Diagnostics maintains a Quality Management System that is certified to ISO 13485 and is an FDA licensed medical device manufacturer.

All referenced articles can be found in entirety and with citations at **Blog.HardyDiagnostics.com**

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