Volume 7 • Number 1 Autumn 2005

BIOSCIENCE WORLD

THE NEWSLETTER OF BIOSCIENCE INTERNATIONAL

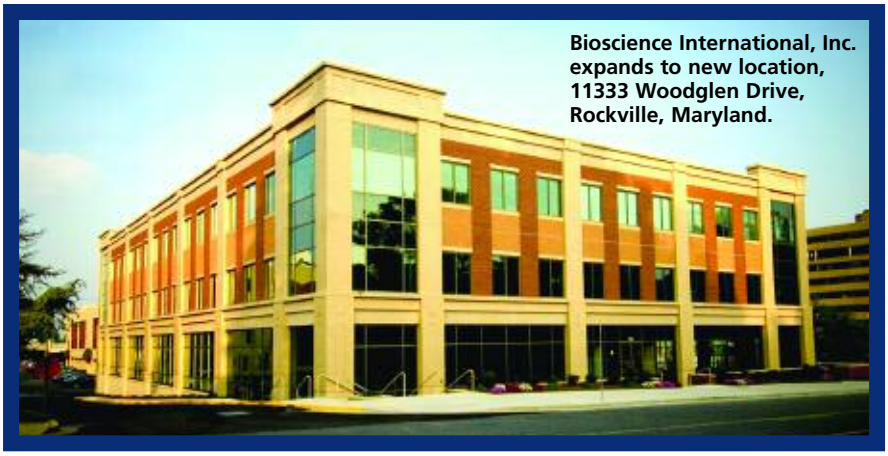
Environmental Testing for USP 797

Andrew Streifel
Hospital Environment Specialist
University of Minnesota

THE NEW USP 797 PROVIDES GUIDANCE FOR ENVIRONMENTAL TESTING and training for safe manufacturing of compounds for patients. Sterility risk to patients and the level of compounding risk dictate quality control efforts.

Source management is essential to manage asepsis. For air, classifications of rooms from ISO 3 to 8 are part of risk assessment. Verification of airborne contamination must follow procedures that assure existing conditions provide contamination control.

In the pharmacy compounding environment, air handling is essential for comfort due to biological safety cabinets and clean benches. Due to recirculation of discharge air, air quality is often found at ISO 6 in compounding rooms. With HEPA (99.97% @ 0.3µm diameter particles), air discharged into the room is particle-free. In several pharmacy compounding area evaluations of the >0.5µm particles, we found 0 p/ft^3in the BSC where antibiotics were mixed in front of the clean bench <100 p/ft^3. In the ante area, <100,000 p/ft^3 (ISO 8) was observed. Observations were collected before construction of pharmacy compounding area.



Bioscience International, Inc. expands to new location, 11333 Woodglen Drive, Rockville, Maryland.

PHOTO: JBG COMPANIES

At Fairview University Medical Center pharmacy, a dual headed SAS Super 180 Microbial Air Sampler was used to collect 500 liters in the compounding area and 200 liters outside.

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Innovative Sampling of Injured Microbial Cells

by Daniel Y. C. Fung, Kansas State University

WHEN MICROBIAL CELLS ARE TREATED with heat, cold, chemicals, dehydration, radiation, or pressure, three populations of cells usually exist. One survives; another dies; a third survives but is injured. These injured cells can repair under favorable conditions, and later grow and metabolize as healthy cells. If we do not use the proper method to resuscitate injured cells, we may underestimate the existence of potentially pathogenic organisms in environmental samples.

At Kansas State University, I led a group of researchers to work on a one-step Thin Agar Layer (TAL) method to recover injured cells. A layer of selective agar is first placed at the bottom of the Petri dish to solidify. Then, a layer of non-selective agar is poured over the selective agar. After solidification, the population of healthy and injured cells is spread above the thin agar layer. In three hours, the injured cells on top will repair while the inhibitory compounds from the selective agar at the bottom migrate upward. When the compounds reach the top of the non-selective agar, injured target cells are repaired and resist toxic effects of the selective agar.

The TAL, when used with the SAS Air Sampler, can improve the study of aeromicrobiology.

Dr. Crozier-Dodson modified the TAL method for use in the SAS Super 180 Microbial Air Sampler and collected data on the recovery of microorganisms in the air of beef, poultry, and swine confinement units. Typically, microbes in the air are injured due to lack of nutrients and dehydration. It is difficult to recover Gram-negative organisms directly from air due to the injury. Gram-positive organisms are more resistant to the air environment and can be recovered more easily. So, the recovery of airborne bacteria mostly consists of Gram-positive bacteria. In fact, many Gram-negative bacteria may be present and not recovered due to lack of a proper technique. Dr. Crozier-Dodson's research found that the use of selective agar alone greatly underestimated the amount of

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SAS is Twenty-five

By Roberto Ligugnana
Managing Director, International PBI

THE SURFACE AIR SYSTEM (SAS) MICROBIOLOGICAL AIR SAMPLER was invented by brothers, Roberto and Sandro Ligugnana, directors of Pool Bioanalysis Italiana Company (PBI) in Milan, Italy in 1979.

The SAS concept was to improve an existing non-portable impaction-style microbiological air sampler that required glass petri plates and connection of a main 110 Volts power supply and a heavy separate vacuum pump. The SAS provided a more efficient portable sampler using a standard disposable contact plate. With contact plates, the SAS provided a more direct impaction of the sampled air onto the agar surface for greater collection efficiency.

A cardboard pre-prototype of the SAS paved the way for the first SAS prototype made from PVS and the final prototype cast in anodized aluminum. In a few years, lead battery packs were upgraded to compact NiCad batteries and later to NiMH battery packs to provide a light enclosed power supply with seven-hour battery life.

The first microbiological test of SAS was performed in a small laboratory at PBI using a cosmetic sprayer to distribute an aerosol of *Bacillus subtilis* in the air. Validation tests were conducted at the microbiological laboratories of the international company, Farmitalia. In 1980, the first lot of 25 SAS units was produced in the Monza factory of PBI. The SAS has been used by cosmonauts and astronauts in the international space stations for the past ten years. Since its inception twenty-five years ago, more than twenty different SAS models have been produced to serve the pharmaceutical, food, clinical and industrial sectors worldwide. ■

PHOTO: INTERNATIONAL PBI

PHOTO: NASA

Clockwise from top left: Brothers, Sandro (left) and Roberto Ligugnana – Inventors of SAS Air Sampler. Roberto Ligugnana and grandson, Andrea, at PBI anniversary ribbon cutting ceremony. Cosmonaut with early model SAS on the space station.

Testing for USP 797

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Samples collected on Malt Extract Agar and Plate Count Agar were incubated at 25C and 35C respectively. MEA has a bacterial inhibitor for fungal air quality and the PCA allows for bacteria and fungal growth. Most fungi grow at 25C and are inhibited at 35C. Isolates were counted after 72 hours and counts adjusted to colony forming units per cubic meter. Opportunistic fungal pathogens, e.g. *Aspergillus* fumigatus, grow at body temperature. (see chart)

cfu/m^3		
Location	25C fungi	35C bacteria + fungi
Outdoors	700	80
Ante area	16	6
BSC	<2	<2
Fill area	18	8

The area has a 90% efficient filter system with area pressurized airflow to less clean areas. During renovation, pressure relationship is not ideal at 0.003 inches of water column and should be adjusted to provide 0.01" wc, which represents air velocity of 400 linear feet per minute.

The expectation is cleanest areas with lowest counts. Data should represent a rank order reduction of airborne microbial particles reflecting clean-cleaner-cleanest air quality compared to outside. Cleanest area should be the biological safety cabinet.

Interpretation of Healthcare Air Sampling Data

Rank order analysis
- Lowest counts of microbes in the areas with best ventilation
- Ventilation parameters: filtration, air exchanges and pressure
- Comparison data necessary with outdoor control

Qualitative analysis
- Pathogen recovery *(Aspergillus)*
- Homogeneous population (versus heterogeneous isolates)

Indoor outdoor ratio
- I/O <1 normal (seasonal considerations)
- I/O >1 potential problem
- Local aerosols can skew daily results

Temperature selectivity
- Pathogens grow best at >35C (<1 cfu/m^3 pathogens)
- Filtration efficacy determined at 25C (lowest counts = best filtration)

In a relatively clean environment, larger air samples are necessary to validate cleanliness. Microbial growth media selection for air quality/surface testing should consider fungi, bacteria or both. Incubation temperatures assure differentiation of fungal versus bacterial contamination.

Understanding appropriate control measures for respective microbial sources coupled with training and environmental sampling provide a quality control program with a safe patient care environment in pharmacy compounding areas.

Andrew J. Streifel, MPH, REHS

For thirty years, Mr. Streifel, Hospital Environmentalist at the University of Minnesota Department of Environmental Health and Safety, has published and lectured extensively and served over 200 hospitals worldwide on air quality and patient care environments for solid organ and bone marrow transplant areas. Appointed to the Revision Task Force American Institute of Architects Guidelines for Construction of Hospitals, he assists industry leaders in design of critical care environments. ■

Andrew J. Streifel, MPH, REHS

Aerobiological Methodology: Limits and Uses of Viable Testing

By Bryce Kendrick, PhD, DSC, FRSC

IN GENERAL, TECHNIQUES OF INVESTIGATIVE MOULD SAMPLING are divided into two main categories, which indicate two very different approaches to the problem of finding out what is there and what it might be doing. These are called 'viable' and 'non-viable' sampling methods.

The term 'viable' is used in the aerobiological context to describe propagules (whether spores, spore-bearing structures or hyphal fragments) which will germinate and grow on some common laboratory media (and sometimes, by extension, inside people whose immune systems are deficient or compromised). These propagules are often collected by drawing air onto the surface of a plate (Petri dish) containing a solid nutritive medium.

There are several situations in which 'viable' and 'non-viable' methods can be truly complementary. (1) Perhaps the most important is the ability of culturing to resolve *Penicillium* from *Aspergillus*...

It is clear that the so-called 'viable' methods will detect only a fraction of the spores in the air that are actually viable, that many of the visually identifiable spores in the air are actually non-viable, and that the results of such methods, unsupported by other approaches, is bound to be misleading.

There are situations in which it matters whether the spores are alive. These are: (1) in hospitals, where patients may be either immunocompromised, or immunosuppressed as a result of their medical condition or their treatment, or (2) in the homes of immunodeficient people. In those cases, mould spores belonging to thermotolerant or opportunistic species may be able to establish themselves within the patient and lead to serious illness and even death.

There are several situations in which 'viable' and 'non-viable' methods can be truly complementary. (1) Perhaps the most important is the ability of culturing to resolve *Penicillium* from *Aspergillus*, and to be precise about the species involved. Identification of, for example, *Aspergillus fumigatus* (which can cause disease in humans), may be very important. (2) If spores in samples are proven to be viable, at least some of those spores may remain viable for an extended period, and can affect future sampling. (3) Certain kinds of spores, especially those which are small and colourless, are often difficult or impossible to identify visually. (4) In some situations it may be helpful to know which species of a particular genus are present inside and outside a building.

It is clear that both 'viable' and 'non-viable' methodologies are valuable.

For the sake of clarification, I would like to see the term 'viable' replaced by 'culturable'. I would also like to drop the term 'non-viable', and call such tests 'broad-spectrum'.

Bryce Kendrick, PhD, DSC, FRSC

Author of twelve books including The Fifth Kingdom, *three textbooks and over 300 other publications, Dr. Kendrick is Technical Advisor for Aerobiology Laboratory Associates, Inc.* ■

Bryce Kendrick, PhD, DSC, FRSC

Switching to SAS

By Paul E. Blankenship
Metrology Technical Specialist
Monsanto

ENVIRONMENTAL MONITORING IS ESSENTIAL TO OUR MONSANTO PROCESSING FACILITY, the world's largest automated bulk protein production operation. It produces Posilac, a sterile injectable pharmaceutical used to treat dairy cows to increase milk output.

Six-month service delays and frequent false positives and deviations with media strips provoked a consultant formerly from the FDA to highly recommend we switch to the SAS Microbial Air Sampler. After installation of 36 SAS Super 180 units to monitor our ten isolators and two cleanrooms, we prevented all false positives and the fingerprint "phenomenon" we had with strips. Less expected were the extensive cost savings with SAS. Each SAS sampler paid for itself in just two months. In only six months, we saved $380,000 in media, preparation time and down time for calibrations

Each SAS sampler paid for itself in just two months. In only six months, we saved $380,000 in media, preparation time and down time for calibrations.

The 48-hour turnaround for calibration and service of Bioscience International allows me to keep my 24-hour-a-day production cycle of four continuously rotating shifts 365 days a year running strong. We have a joke here at Monsanto that as soon as we ship a unit to Bioscience for calibration and the delivery person takes it out the door, the return delivery person passes him in the doorway with the returned unit.

SAS also saves operating time because of its high speed of 180 lpm, the simplicity of inserting agar plates and the ruggedness of SAS to withstand sanitization. With our prior air samplers, when a technician sprayed the room during decontamination, liquid penetrated and ruined the circuit board. SAS keeps fluid out of the instrument.

Turbulence was another issue with prior samplers. Before purchasing the SAS, we conducted smoke studies with the SAS and observed that it greatly reduces turbulence and has a clean unilinear airflow.

In addition to cost-savings and method improvement, the SAS provides the benefit of being a reference sampler. In a recent FDA inspection, the inspector saw the SAS and immediately commented "great" without the more typical response of ten questions for other instrumentation. (Having been validated in the field since 1979, SAS is referenced in the ACGIH guidelines and the USP Section 1116.) ■

3M Targets Indoor Air

IN THE DEVELOPMENT LAB AT 3M in St. Paul, MN, John Horns, Technical Specialist, developed a test to measure the filter efficiency for fungi of industrial air handlers. The test can help determine indoor air quality in office buildings.

Using the Duo SAS 360 Microbial Air Sampler, he collected air from the upstream and downstream sides inside a fiberglass filter. With a large fan-like-blower, he simulated different air velocities from a minimum of 100 feet per minute to a maximum of 500 feet per minute.

Over a series of days, he collected 20 sample sets. With Malt Extract Agar plates in each head of the Duo SAS, he ran a 500-liter air sample downstream in less than three minutes and a 100-liter air sample upstream in less than thirty seconds. "I chose the Duo SAS 360 Air Sampler because it was straightforward to use and the double heads saved testing time," he said.

The Duo SAS 360 Air Sampler

In each sample set, one plate was cultured at 25 degrees C. simulating the temperature outside the body and the other plate was cultured at 37 degrees C. simulating body temperature. "The data of plates was the basis for our efficiency calculations," Horns said. For example, if the upstream sample is 100 cfu's. and the downstream sample is 50 cfu's, the filter efficiency is fifty percent. The test can be used to measure filter efficiency of air handlers used to cool, heat and move air in commercial space. ■

Microbial Cells

Continued from pg. 1

Gram-negative organisms. The TAL, when used with the SAS Air Sampler, can improve the study of aeromicrobiology.

This TAL method has been tested on a variety of food pathogens such as *Salmonella, Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica, E. coli* O157:H7, after these cells have been injured by heat, cold, organic acids, salt, radiation, and high pressure.

Daniel Y.C. Fung, Ph.D.

PHOTO: KANSAS STATE UNIVERSITY

Daniel Y.C. Fung, Ph. D.

A world renowned applied microbiologist in rapid methods and automation in microbiology, Dr. Fung has published nearly 600 publications and received many awards, including the International Award from The Institute of Food Technologists. In addition to teaching, research and university services, he has initiated and directed the internationally renowned Workshop in Rapid Methods and Automation in Microbiology from 1981 to the present. For reprints on research generated from the SAS Air Sampler or information on the workshop, contact Dr. Fung at DFUNG@OZ.OZNET.KSU.EDU. ■

SAS Super Isolator

The new SAS Super Isolator provides a control unit with separate sampling head for monitoring isolators and barrier environments. The head can be placed inside the isolator while the program control unit remains outside. A simple electric cable connects the head to the controls through the wall of the isolator or cleanroom.