

BIOSCIENCE

THE NEWSLETTER OF BIOSCIENCE INTERNATIONAL

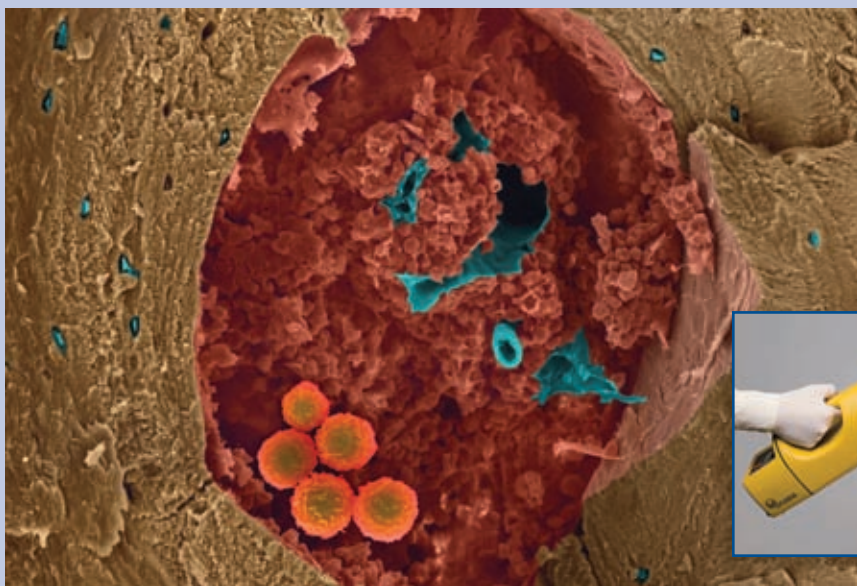
WORLD

Viable Cell Counts

By Daniel Y. C. Fung
Professor of Food Science
Kansas State University

THE WELL-KNOWN viable cell count has been serving the field of applied microbiology effectively for more than a century. Simply stated, on or in the appropriate agar medium, one living cell or a few living cells grouped together can grow to a mass visible to the human eye, usually as a round colony. The number of organisms in one visible colony is actually about one billion cells (1×10^9 or log 9 cells). Since a round visible colony could have been developed from one or more individual cells, it is referred to as a Colony

— Continued on pg. 2, col. 1



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Active or red marrow is dominated by the production of red blood cells. With age, marrow becomes less active and is inhabited by adipocytes (fat cells). (See "Sampling Critical Environments", pg. 3)

USP 797—Best Practices

By Laura A. Thoma
Professor of Pharmaceutical Sciences, University of
Tennessee Health Science Center

IN THE REVISED Chapter of USP 797 Pharmaceutical Compounding – Sterile Preparations, the requirement of the frequency of environmental monitoring has been greatly reduced.

The chapter states that active air viable monitoring is to be done at a minimum of every 6 months, as part of the re-certification of facilities and equipment, after service of facilities and equipment and when problems with end-products, staff technique or work practices are identified. Glove fingertip sampling shall be done three times initially during evaluation of garbing and gloving competency with re-evaluation occurring along with the annual or semi-annual media fills and surface sampling shall be performed periodically.

These stated frequencies are the very minimum required. More frequent sampling will give a better picture of microbial bioburden and allow for earlier detection of a problem or loss of control in the facility. The data generated from air viable monitoring should be reviewed on a regular basis and used to identify a

loss of environmental control and to locate and correct the problem. Surface

sampling is a good way to evaluate the state of your facility, the effectiveness of your cleaning and disinfection program and the work practices of employees.

It is necessary to develop a sampling plan based on the risk of the compounding activities being performed. Choose sites in each ISO Class 5 environment and at nearby locations in the ISO Class 7 or 8 areas with the greatest risk of contamination. A good sample plan must include the locations to be sampled, method of collection, frequency of sampling, volume of air to be sampled, the time of day as related to activity and the action level. Sampling must occur frequently enough to be useful and the data must be reviewed.

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SAS Isolator Model

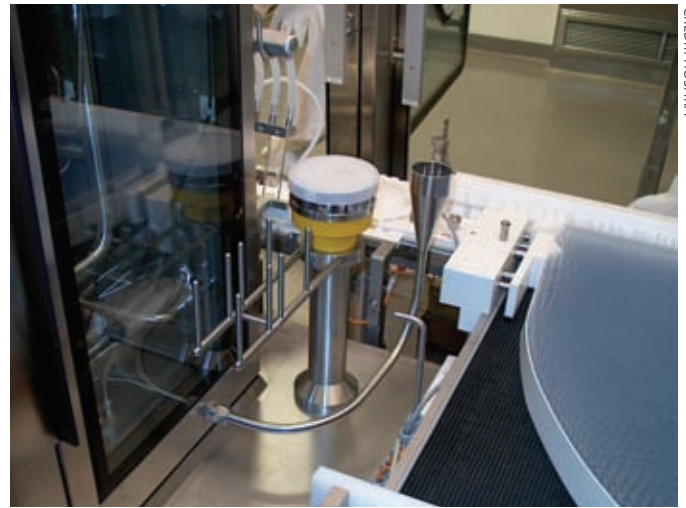
THE SAS Super Isolator Air Sampler, with separate sampling head and autonomous program control unit, is specifically designed for monitoring in isolators and barrier environments.

A primary benefit of the SAS Super Isolator, said Jayma Alcala, Biological Quality Supervisor, Hospira, is the interval sampling program. "In our Class Four Cleanrooms, we use the multi-mode program to program our seven SAS Isolator Air Samplers to sample several times over each eight to twelve hour shift for a volume of 1000 liters per interval using DE neutralizing contact plates."

"The main reason we chose

the SAS Super Isolator Air Sampler was its separate computer program control outside the isolator", said the Manager at a global pharmaceutical account using SAS. "Secondly", she said, "unlike all of the other eight systems we

"SAS stops the air inside the isolator with no air tubing that goes outside the isolator."



CREDIT: HOSPIRA

SAS Isolator Model inline installation using stainless steel pedestal to manage connections.

evaluated, the SAS stops the air inside the isolator with no air tubing that goes outside the isola-

tor. In other sites, we had tubing outside and were concerned with a break with integrity and that the product would be at risk; as well as analysts being at risk outside the enclosure." ■

Viable Cell Counts

Continued from pg. 1

Forming Unit or CFU.

Just knowing the number of CFU's on or in an agar medium has very little meaning unless the number is related to per volume of liquid (ml), per weight of matters (g), per area of a surface (cm square), or per volume of air (cubic meter). After more than 30 years of working in applied and food microbiology, I have developed the Fung Scales for CFU for general and food microbiology.

These scales are for general and normal environmental microbial pop-

ulations. No frank pathogen, such as *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, etc., is allowed in any ready-to-eat foods or drinks for consumption or for food preparation environments. On the other hand, in fermented foods and drinks, high numbers of desirable microorganisms, such as, *Lactobacillus acidophilus*, *Streptococcus thermophilus*, *Saccharomyces cerevisiae*, etc. are encouraged.

To obtain microbial counts from the air, an impactor air sampler, such as the SAS Microbial Air Sampler, is used to pull a known volume of air and impact the particles which may contain live microbes on an agar medium and later incubate the agar plate at the appropriate temperature for about 24 to 48 hours to let the organisms grow to visible colonies and then convert the CFU into cubic meters by knowing the volume of air impacted over the agar plate.

Viable cell counts of

normal microbial flora and of specific target pathogens in/on air, food, water, surfaces and the general environment are important information for the protection of humans, animals and plants.

Long Live Viable Cell Count! We can Count on You.

(An expanded version of this article, which covers standards for surfaces, may be requested from Bioscience International.)

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. ■

Fung Scales for CFU

FOR AIR

0-100 CFU/cubic meter	Acceptable level, no concern
100-300 CFU/cubic meter	Intermediate level, slight concern
>300 CFU/cubic meter	Serious concern, need corrective actions

The above scale is for general food processing environments. For hospitals and clean room operations, more stringent requirements are needed.

FOR SOLID, LIQUID FOOD, OR FOOD SURFACES

Log 0 to Log 2 CFU/g, ml, 10 ²	Low count, no concern
Log 3 to Log 4 CFU/g, ml, 10 ²	Intermediate count, slight concern
Log 5 to Log 6 CFU/g, ml, 10 ²	High count, serious concern
Log 7 CFU/g, ml, 10 ²	Index of Spoilage, food will soon spoil
Log 8 CFU/g, ml, 10 ²	Odor development, not acceptable
Log 9 CFU/g, ml, 10 ²	Slime development, highly unacceptable
Log 10 CFU/g, ml, 10 ²	Discard immediately

Sampling Critical Environments

By Andrew Streifel
Hospital Environmental Specialist
University of Minnesota

CERTAIN HOSPITAL ROOMS provide a safe air quality environment for patients with suppressed immune systems. Air sampling for fungi is part of a process to validate the cleanliness of certain patient care areas.

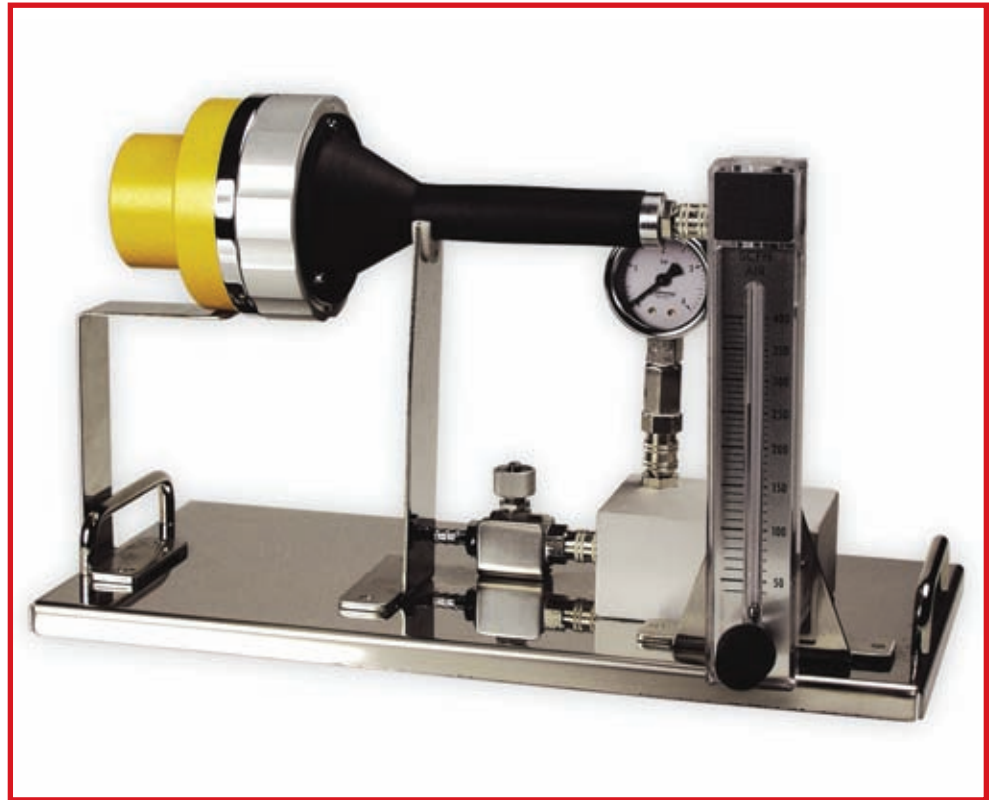
The critical environments, such as a Bone Marrow Transplant Room or Surgery Suite, should provide a safe environment with minimal presence of airborne microbes.

While there isn't a standard relating to hospital air quality, there are ISO Standards that can serve as a guideline. Once the hospital is occupied, the maintenance of low levels of air quality becomes a challenge without focused control measures. (For information on acquiring the ISO Standards, contact Bioscience International.)

We need to assure an environment free of environmental infectious agents, such as, *Aspergillus* species and other fungal organisms capable of causing Aspergillosis, *Aspergillus fumigatus*, *A. flavus*, *A. niger*, *A. nidulans* and other opportunistic fungal species.

We look for these fungi using selective media and an air sampler. The SAS Air Sampler is used to compare outside with inside microbial air quality. The tendency is to sample for fungal spores. The culture method is preferred because visual identification of fungal spores is indistinguishable when they are small and round. The size at 2-4 microns looks like a variety of species, which range from *Gleocladium*, *Penicillium* to *Aspergillus* species.

The use of the air and surface sample capability will provide assurance of air and surface microbial sanitation. Often microbes found on surfaces end up in air samples. Interpretation guidelines for such evaluations need to be established before sampling begins. Opening of bone marrow units have been delayed because of environmental contamination found on the floors and in the air.



Pinocchio II Compressed Gas Test Fixture for accurate and easy testing for viable microorganisms.

After the cleanest samples are taken, move to similar area (with HEPA if bone marrow transplant), lobby area and outside for those comparisons.

Typical data in Minneapolis MN in summer can be seen in the chart on page 4.

Outside data varies greatly depending on area climate (desert, agriculture, urban or forest), so results require a ranking of the samples demonstrating the cleanest area where the controls area greatest have

— Continued on pg. 4, col. 1

Ninety-nine percent of the SAS Air Samplers sold in the USA since 1979 are still in use.



Celebrating 50 years serving science and 30 years monitoring environments with SAS. Ninety-nine percent of the SAS Air Samplers sold in the USA since 1979 are still in use. Peter Pratt, CEO, Bioscience International; Roberto Ligugnana, President, International PBI.

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Sampling Critical Environments

Continued from pg. 3

the lowest counts. This would rank the BMT unit as best air quality with the lobby being the least controlled.

HOSPITAL LOCATION	CFU/M ³	PARTICLES/FT ³	PRESSURE	COMMENT
Outside	1289	234000	Na	Cloudy/warm
BMT Rm 1 HEPA	2	260	.032	Airflow out
BMT Rm 2 HEPA	4	420	.021	Airflow out
BMT nurse/suite HEPA	14	1280	.01	Airflow out
Nurse area medicine	21	2100	na	na
Med Rm 1	19	2300	na	na
Lobby	54	3400	na	na

- Colony forming units per cubic meter
- Particles per cubic foot at >0.5 micron diameter
- Pressure in inches water column

The data below would be typical of an unoccupied area while the Nurse area would be high because it is occupied with minimal control.

(An expanded version of this article may be requested from Bioscience International.)

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. ■

USP 797—Best Practices

Continued from pg. 1

More information obtained about the bioburden in the facility leads to a better understanding of the overall state of control.

Laura A. Thoma, Pharm.D.

Laura A. Thoma, Pharm.D., is Professor of Pharmaceutical Sciences and Director of the Parenteral Medications Laboratories, University of Tennessee Health Science Center.

Dr. Thoma is responsible for the University's sterile product manufacturing and training programs. She has over 15 years of experience in training pharmaceutical and biotechnology industry personnel



in all procedures and methods used in the aseptic preparation and quality control of sterile products. She is currently a member of the Parenteral Drug Association (PDA) Board of Directors, the PDA Strategic Planning Committee, and the Program Advisory Board. She is currently serving a 5 year term on the USP Sterile Product Compounding Committee. ■



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.

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Innovative Microbiology Products

11333 Woodglen Drive • Rockville, Maryland • 20852
301.231.7400 • Fax: 301.230.1418
www.biosci-intl.com