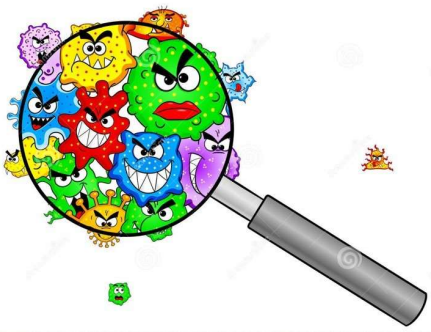
New England Chapter
Parenteral Drug Association
Presentation – March 15, 2017

Effective Microbial Contamination Control from a Microbiologist's Perspective



Ed Balkovic, PhD
Consulting Microbiologist

Adjunct Associate Professor
Dept. of Cell & Molecular Biology
University of Rhode Island

508-450-2502
edbalkovic@gmail.com

MicroBio Technical Support
Biologics Biotechnology Microbiology Virology Contamination Control

DISCLAIMER

"The views and opinions expressed in this presentation are those of the presenter.

This presentation includes intellectual property that belongs to the presenter, which is protected under the copyright, trademark and other laws of the United States of America and other countries. All rights are reserved."

OBJECTIVES

- Sources
- Prevention
- Selection of decontaminating agents & their proper use
- Understanding types of microbes expected to be recovered

3

MICROBIOLOGIST PERSPECTIVE - SOURCE

Clinical Virologist / Microbiologist:

- Hospital Diagnostic Virology Labs – Rhode Island and Pennsylvania
- Influenza Research Center – Baylor College of Medicine
- National Virology Reference Lab – U.S. Dept. of Veterans Affairs Medical Ctrs.
- NIH Antiviral Contract – Yale Univ. School of Medicine

Biologics / Biopharmaceutical Microbiologist:

- **Quality Control / Research & Development** – Major Vaccine Manufacturer (Connaught Labs) and Microbiological Media Manufacturer (Scott Labs)
- **Operations** - Contract Cell Culture Testing Lab (Tufts Vet School / Charles River Labs Partnership)
- **QC / QA / Regulatory Affairs / Facility Operations** – small Biotech Companies (OraVax, Cambridge Biotech)
- **Subject Matter Expert Microbiologist** (Sanofi Genzyme)

4

MY MICROBIOLOGIST MENTORS / COLLEAGUES

Clinical / Academic Microbiologists:

G. D. Hsuing	Howard Six	Phil Wyde	Robert Couch
Julius Kasel	Andy Onderdonk	Marie Landry	Tom Monath

Industry Microbiologists:

Gary du Moulin	Jeanne Moldenhauer	Jim Polarine	Marsha Hardiman
Tony Cundell	Scott Sutton	Art Vellutato Jr.	Michael Miller
Jim Agalloco	Jim Akers	Dona Reber	Dawn McIver
Mary Griffin	Tim Sandle	Dennis Guilfoyle	*Many more……*

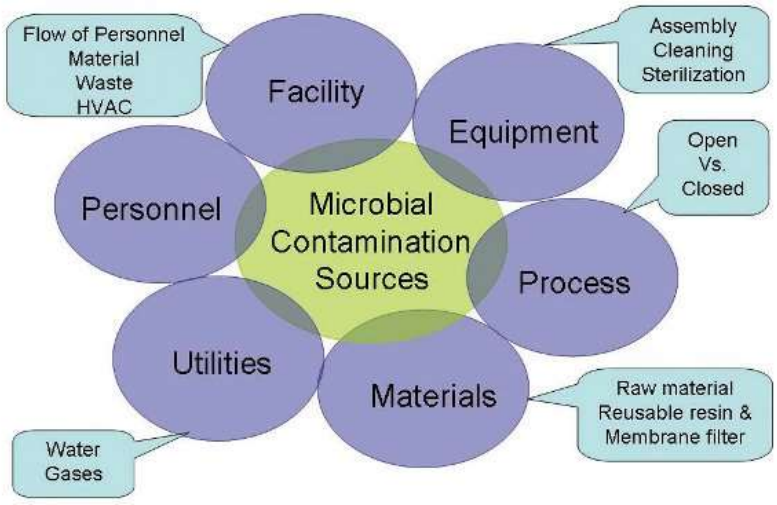
Regulatory Microbiologists (FDA):

Tom Arista	Marla Stevens Riley	Bryan Riley	Rick Friedman
Pat Hughes	Sharon Thoma	Lyn Ensor	John Metcalfe

Dennis Guilfoyle (former)

5

POTENTIAL SOURCES OF MICROBIAL CONTAMINATION



FROM: K. Suvarna et al. Am. Pharm. Rev. 2011

8

Potential Sources of Microbial Contaminants

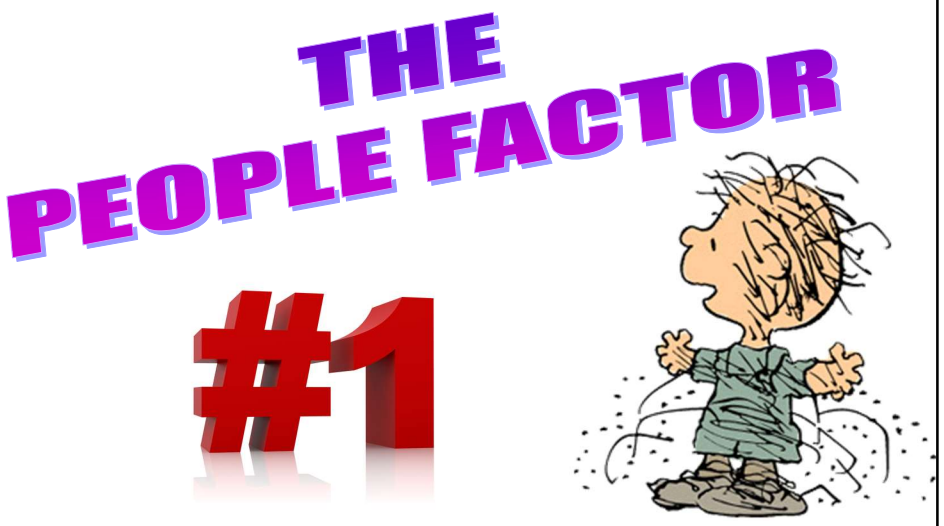
- **Air**
  - 500 - 700 viable microbes / m^3 of typical indoor office air

- **Water / Fluids**
  - Incoming City Water - up to 100 viable microbes per mL

- **Equipment**
  - Varying levels of viable microbes
    - transferred during preparation and transportation prior to use

- **Raw Materials / Components**
  - Most not sterile when purchased
  - Must be tested to assure they meet acceptance standards for microbial bioburden

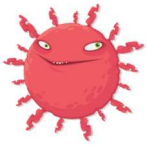
Potential Sources of Microbial Contaminants



MICROBES AND YOU

- Number of microbes in/on body (normal flora)
  Approx. 10^{14} (hundred trillion)
- ~100,000 bacteria / cm^2 of skin
- ~1 kg in weight of bacteria in gut
- ~10^{11} microbes / gram of feces

Large part of "you" (mass of your body): bacteria, viruses and fungi



11

WHAT GERMS ON YOUR HANDS ACTUALLY LOOK LIKE

12

GROWTH OF MICROBES

- Presence of Microbe or its spores
- Appropriate Atmosphere
- Temperature
  - *most of concern - grow best at 20° - 37°C*
- Food and Water (moisture)

13

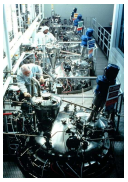
CONTROL AND REDUCE RISK OF MICROBIAL CONTAMINATION?

14

Proper Facility and Utility Design

FACILITY

- Appropriate **Materials of Construction**
  - Resists chipping, flaking, oxidizing, compatible with disinfectants, durable and easy to maintain such as stainless steel work surfaces, epoxy floors, & plastic laminates
- Layout **Work Areas** for single passage workflow
  - Define ingress and egress for Personnel, Components, In-Process Materials, and Waste

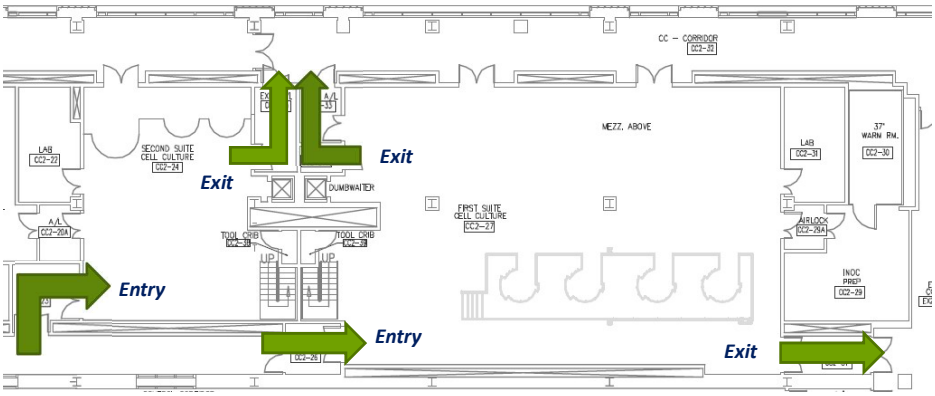


UTILITIES

- Adequate **Heating, Ventilation, & Air Conditioning System (HVAC)**
  - Control temperature & humidity and provide appropriate pressure differentials
- **Water Systems, Compressed Gas Systems** - appropriate materials of construction, use of filters, and controls to prevent contamination
- **Monitoring** performed to verify chemical, microbial & particulate quality

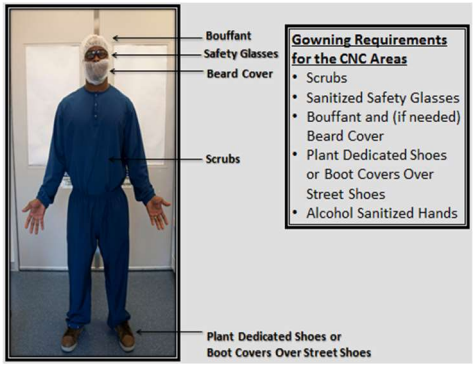
15

FOLLOW DESIGNATED MATERIAL AND PERSONNEL TRAFFIC FLOW PATTERNS



16

PROPER GOWNING PRACTICES



Gowning Requirements for the CNC Areas

- Scrubs
- Sanitized Safety Glasses
- Bouffant and (if needed) Beard Cover
- Plant Dedicated Shoes or Boot Covers Over Street Shoes
- Alcohol Sanitized Hands

Follow your site specific Gowning SOPs

Gown in Accordance With MF-082-12

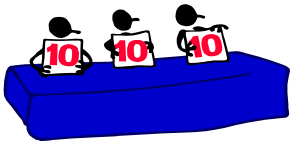
Additional Gowning for the Grade D Areas

- Coverall
- Boot Covers
- IPA Sanitized Gloves

17

PROPER BEHAVIOR IN CLEAN ROOMS

- Human intervention kept to minimum
- No disruption of laminar airflow
- Slow movement
- Minimal communication
- Restrict entry into and out of CR during processing
- Frequent sanitization/changeout of gloves
- If you have a question - ask first



Agent Selection

Properties of ideal antimicrobial agent

Fast-acting

Acts against many microbes without harming surfaces or persons

Good penetrating power

- will improve if dirt and debris are first removed

Compatible with detergents

Inexpensive

Easy to prepare

Chemically stable

Inoffensive odor

Not harmful to the environment

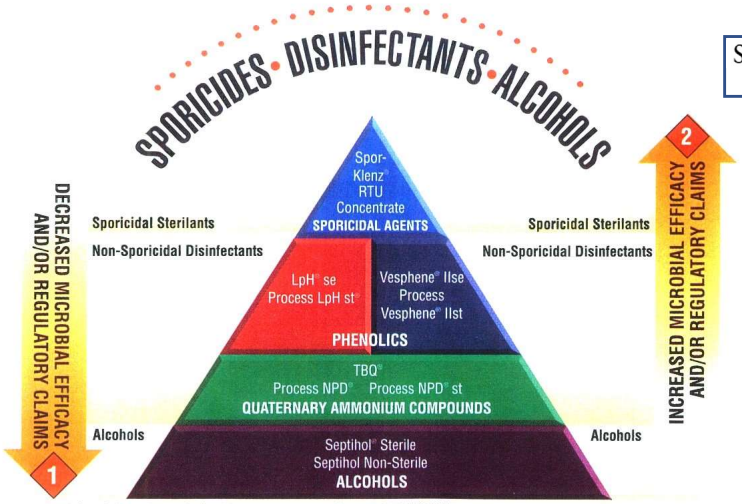
19

THE AGENTS

- Halogens
- Phenolics
- Heavy Metals
- Alcohols
- Quaternary Ammonium Compounds (Quats)
- Aldehydes
- Peroxygen Compounds

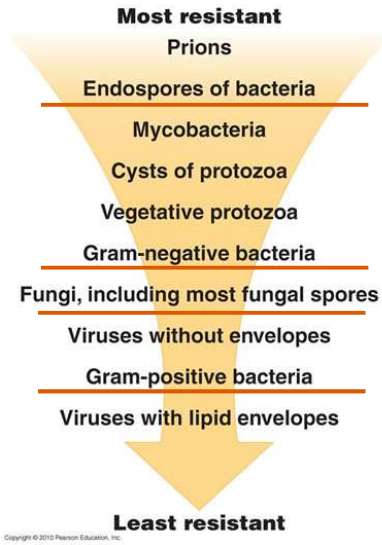
20

AGENT PYRAMID



1. PRODUCTS AT THE BASE OF THE PYRAMID ARE MOST FREQUENTLY USED AND ARE GENERALLY NOT SPORICIDAL.
2. PROGRESSION UP THE PYRAMID INDICATES STRONGER PERFORMANCE OVERALL AND A BROADER SPECTRUM OF CLAIMS.

INTRINSIC RESISTANCE TO DISINFECTANTS



THE PROCESS

Cleaning / Disinfection Practices

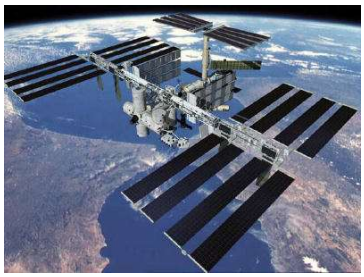
23

CLEAN???? CONTAMINATED???

24

Microbes are Everywhere

"Generally speaking, microbes are invisible, and so people just don't think of them as much as you do some other things."



Duane L. Pierson, Ph.D.
NASA Johnson Space Center

25

CLEAN???? CONTAMINATED???

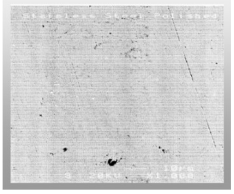
26

CLEANING VS. DISINFECTION

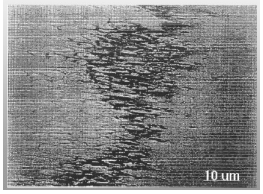
27

SEM View of Clean Room Surfaces

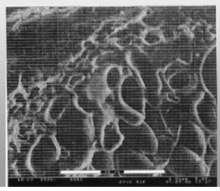
Stainless Steel Surface



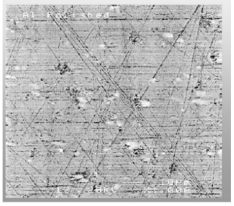
Epoxy Surface



Plastic Curtains Surface #1

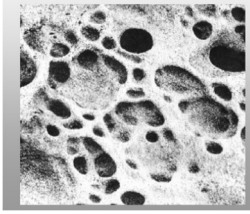


Aluminum Surface



Vinyl Surface

Plastic Curtain Surface #2



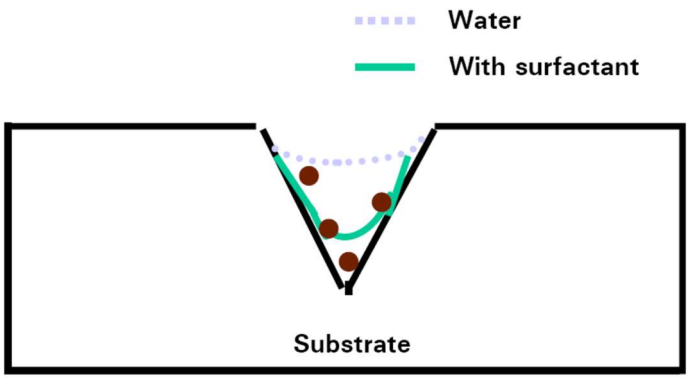
SOURCE: Art Vellutato, Jr. - Veltek Assoc., Inc.

28

Surfactants

STERIS

Access to microbes



29

Comparison of Methods

CSI ClorDiSys Solutions, Inc.

Summary

Issue	Spray / Wipe /Mop	Fogging	Formaldehyde Gas	Hydrogen Peroxide Vapor	Chlorine Dioxide Gas
Equipment Cost	Low	Low	Low	High	Moderate
Labor Costs	High	High	High	Low	Low
Consumable Costs	Low	Low	Low	Low	Low
Facility Downtime Costs (cycle time costs)	High	High	High	High	Low
Corrosiveness	Low-High (agent specific)	Low-High (agent specific)	Low	Low (unless condensation)	Low
Total Cycle Time	1-2 days	1-2 days	9 to 15 hours + clean up	4 hours (small) 12 hours (large)	1.5 hrs (small) 5 hrs (large)
Residues	High	High	High	Low	Low
Concentration Monitoring	No	No	No	Yes (not integrated to equipment)	Yes
EPA approvals	Yes (agent specific)	Yes (agent specific)	No	Yes (Isolators & Small Chambers only)	Yes
Scalability	Yes??	Yes	Yes	Yes??	Yes

14

30

For Efficacy…..Think - "TACT"

Efficacy of disinfectants are dependent upon the following factors:

- **T**emperature
- **A**pplication
- **C**oncentration
- **T**ime

31

POINTS TO CONSIDER

- Microbes have not been shown to become resistant to standard disinfectants and sporicides

- No need to rotate between two or more disinfectants
- Do not rotate Phenolics with Quats

- Sporicide should be rotated with disinfectant
  - Only used, as needed - corrosive & potentially hazardous to workers
- Increased spore detection – increase sporicide use

- Mold spore detections – look for moisture source
- Personnel are key source of microbial contamination

- Airlocks, pass-throughs, gown rooms may require more frequent decontamination to prevent microbial entry

32

Potential Trouble Spots

- Effectiveness of Process only as good as diligence of workers
- Rushing the Process - Process considered not important
- Activity is often delegated to most recent hires
- Most outsourced to contractors
  - May be high turnover in contractor's staff
- Workers may not be fluent in English
  - SOPs only in English…..Language of workers may change
- Activity is often performed during night shift
  - Minimal supervision
- Workers may not be trained in microbial awareness

33

Management Responsibilities

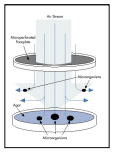
- **Educational Materials:**
  - Have educational / training materials (SOPs, MSDSs, etc.) available for all employees -- *especially new hires*
- **Training:**
  - Take time to train all staff members on proper use of your disinfectants
- **Surveillance:**
  - Follow-up with routine surveillance programs for documents, users, equipment, water quality, etc.
  - Continually analyze data generated

34

Environmental Monitoring Program

Types of Sampling in Controlled Environments

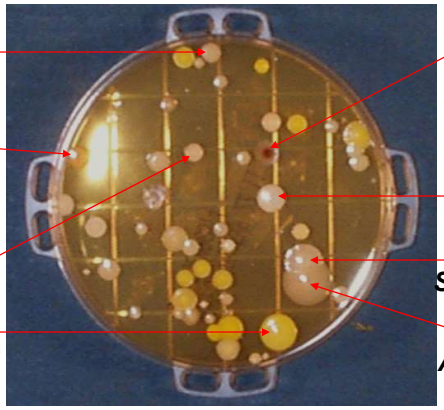
- **Air Sampling**
  - Non-viables
  - Viables



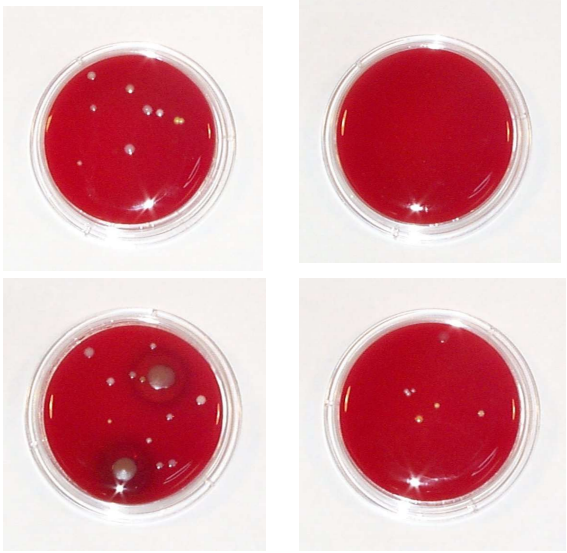
- **Surface Sampling**
  - Work surfaces (benches, walls, floors, etc.)
  - Equipment
  - Personnel

Identification of Representative Colonies from Environmental Air Sample Plate

Grade D Cleanroom



Environmental Sites - Lab Surfaces



Environmental Sites - Personnel

Shirt

Pants

Shoe Bottom

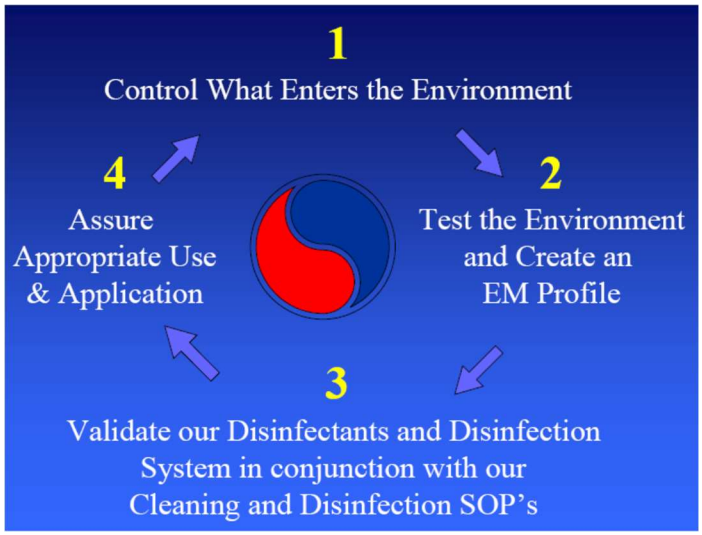
Forehead

Hand

% Recovery of Microbial Isolates from Environmental Samples - 8 Years

Organisms	Air	Person	Surface
Bacillus species	7.6	8.7	36.4
Coryneform species	10.8	11.5	8.4
Micrococcus species	22.3	25.1	13.0
Staphylococcus species	39.5	41.0	17.5
TOTAL (>6,600 isolates)	**80.2**	**86.3**	**75.3**

Contamination Control Life Cycle



Art Vellutato, Jr.
Veltek Assoc., Inc.

41

From my Microbiologist's Perspective

So - what is the most important item needed for Effective Microbial Contamination Control?

42

Experienced Microbiologists – Expertise / Skills

Technical & Professional

Investigational

Inter-Personal

Educational

43

Thank You --- Any Questions?

44