Improving Your Microbiological Investigations Playbook

Marc Glogovsky, MS, S.M. (NRCM) – ValSource, Inc.

Creation of PDA TR #88

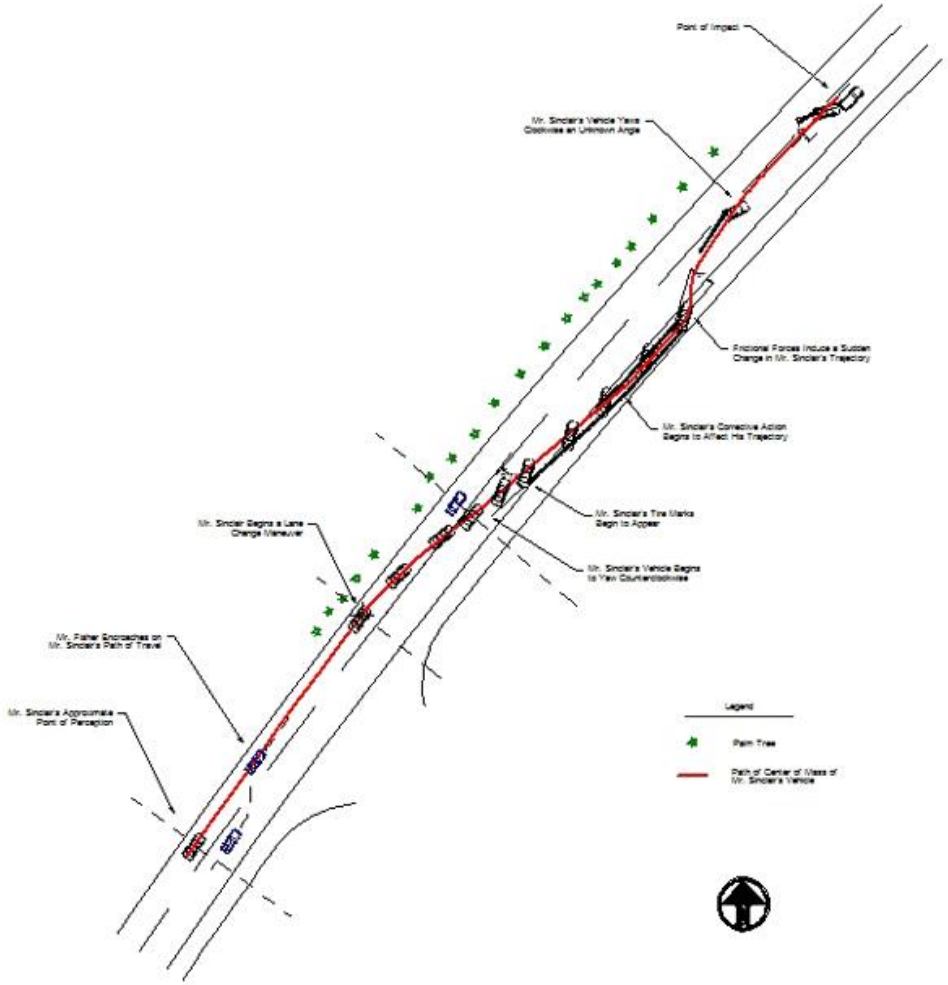
TR #88 presents a holistic approach for performing a microbiology investigation, providing a framework to focus on areas that may contain or contribute to the root cause of data deviations.

I should point out:

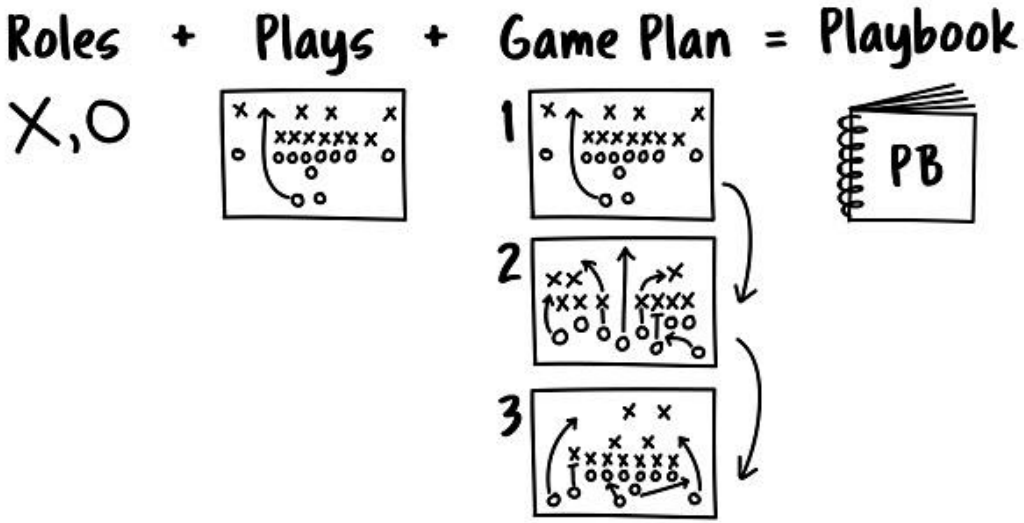
- Almost 20 authors, 3 co-chairs
- 7 years
- 812 peer comments, 241 advisory board comments, 68 director comments

When Things Go Wong

- 1st Time is chance
- 2nd Time is a coincidence
- 3rd Time is a pattern



Starting Your Investigation



Initiated to:

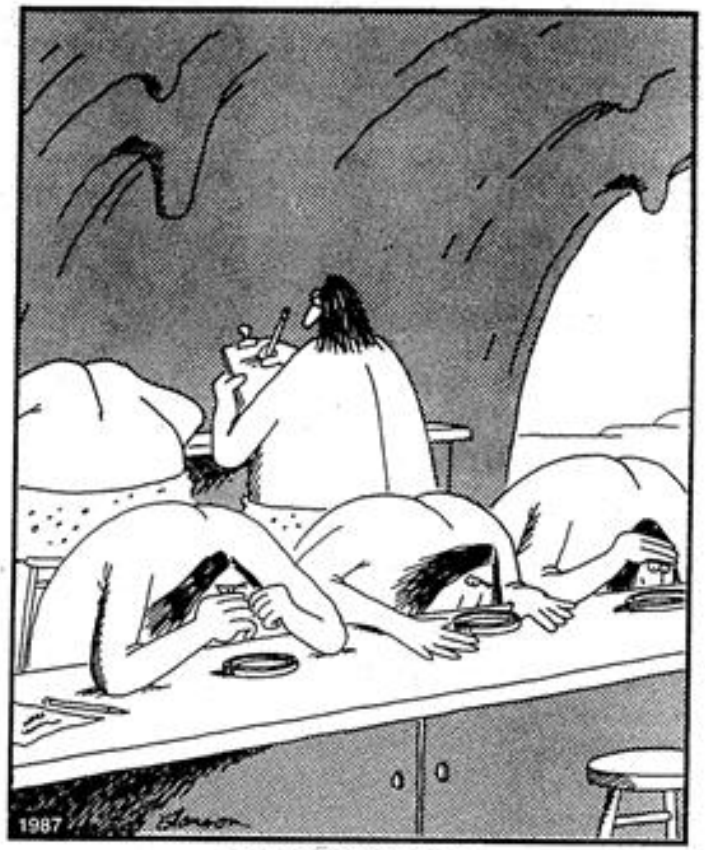
- Address an action level excursion.
- Address an adverse trend.
- Determine a cause-and-effect relationship.

Steps should be pre-specified in a written plan

- Should define the level of investigation required regarding multiple or sequential excursions.
- Should include product impact assessment and risk evaluation for other products manufactured in the same time frame.

Document investigations and follow-up

Now What?



Early microbiologists

- Identify the microorganism(s)
- Talk to the operators - Observe the operators, support personnel, areas and facility (GEMBA)
- Review historical data: EM trends, flora, disinfectant effectiveness tests, cleaning and disinfection logbooks (contact time), sterility test results, equipment logs, sterilization records, deviation reports, other investigations, etc.
- Identify possible sources: PEER/PEMME
- Perform targeted sampling to pinpoint source
- ICH Q9 R1: Level of assessment (investigation) proportional to impact on product

When to Perform Identifications

Regulation	Microorganism
EU Annex 1 (2022)	• Microorganisms detected in the grade A and grade B areas should be identified to species level and the potential impact of such microorganisms on product quality (for each batch implicated) and overall state of control should be evaluated. Consideration should also be given to the identification of microorganisms detected in grade C and D areas (for example where action limits or alert levels are exceeded) or following the isolation of organisms that may indicate a loss of control, deterioration in cleanliness or that may be difficult to control such as spore-forming microorganisms and moulds and at a sufficient frequency to maintain a current understanding of the typical flora of these areas.
FDA Guidance	• Monitoring of critical and immediately surrounding clean areas as well as personnel should include routine identification of microorganisms to the species (or, where appropriate, genus) level • Establishing an adequate program for differentiating microorganisms in the lesser-controlled environments, such as Class 100,000 (ISO 8), can often be instrumental in detecting such trends (migration of microorganisms into clean areas)
USP <1116>	• A successful environmental control program includes an appropriate level of identification of the flora obtained by sampling. • A knowledge of the flora in controlled environments aids in determining the usual microbial flora anticipated for the facility and in evaluating the effectiveness of the cleaning and sanitization procedures, methods, agents and recovery methods. • The information gathered by an identification program can be useful in the investigation of the source of contamination, especially when recommended detection frequencies are exceeded.

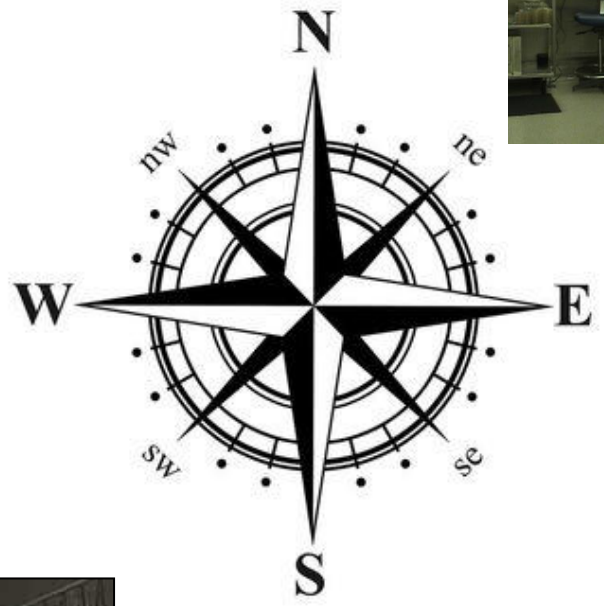
Let's Simplify…

Extent (minimum) of Identification	Contamination Event
Identification to genus	Grade C & D - alert level excursions
Identification to species	• Grade A & B - alert/action level excursions • Grade C & D - action limit excursions
Strain typing or molecular fingerprinting	Significant product failure (e.g.) • media fills • sterility test • significant adverse trends

“Investigatory Compass”

People

Water

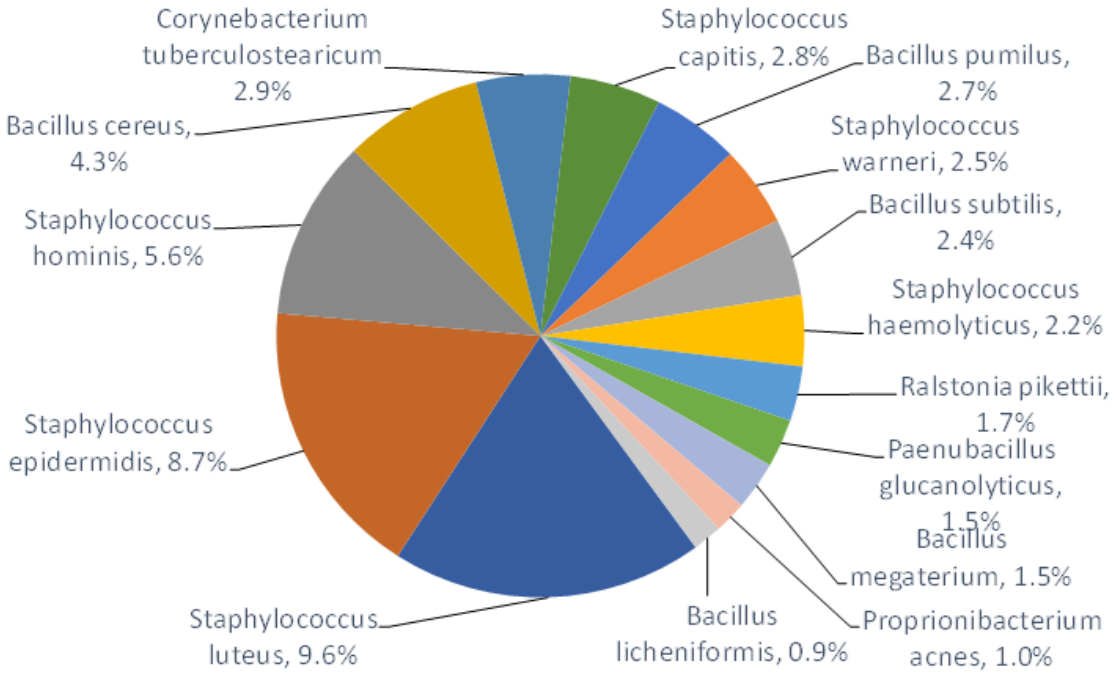


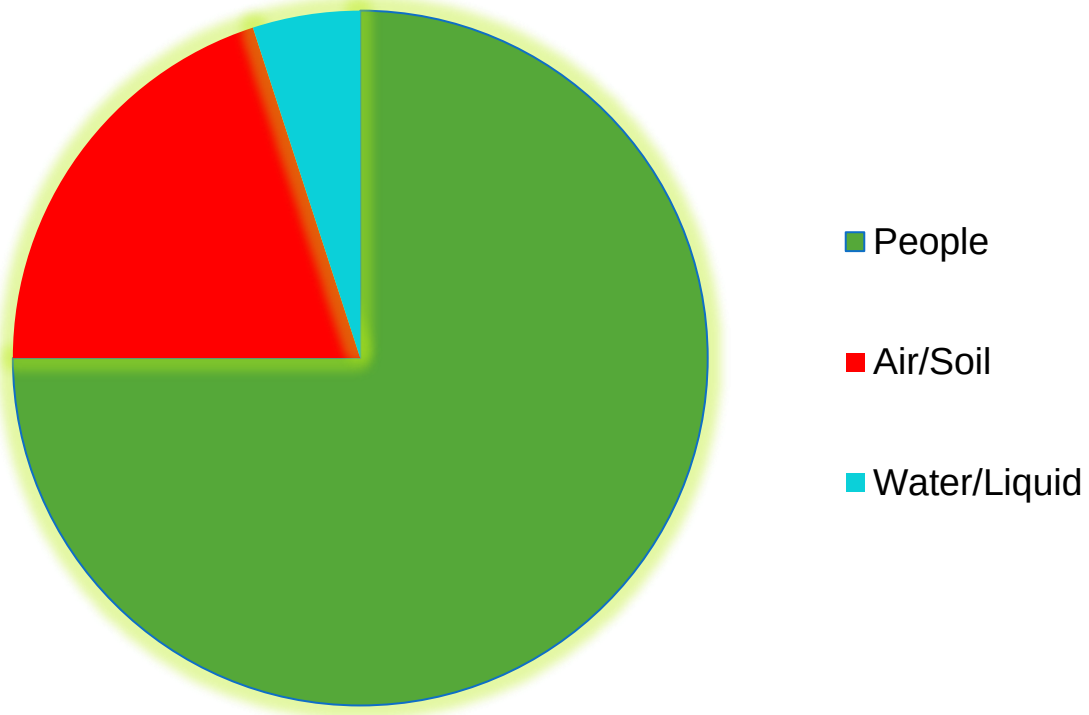
Equipment

Raw Materials

“PEER” or “PEMME”

Which Direction to Choose?





Increased Emphasis on Trending

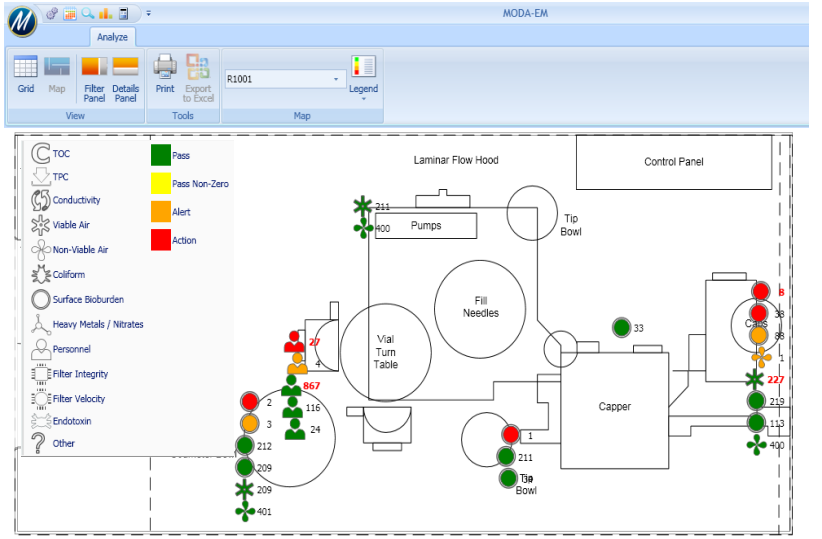
Monitoring procedures should define the approach to trending. Trends should include, but are not limited to:

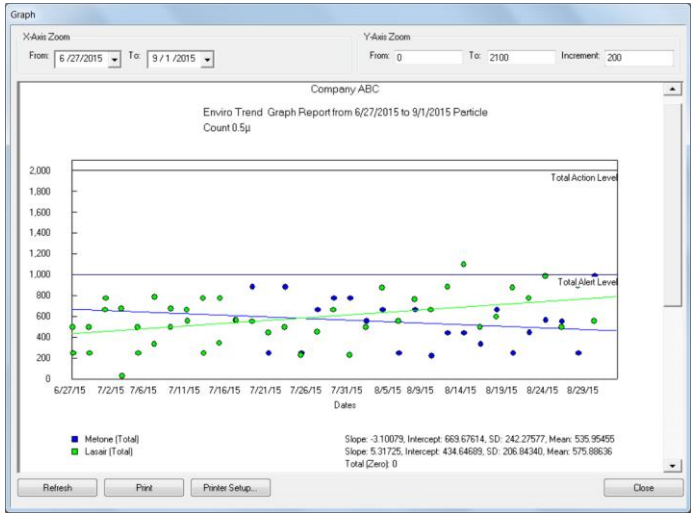
i. Increasing numbers of excursions from action limits or alert levels.

ii. Consecutive excursions from alert levels.

iii. Regular but isolated excursion from action limits that may have a common cause, for example single excursions that always follow planned preventative maintenance.

iv. Changes in microbial flora type and numbers and predominance of specific organisms. Particular attention should be given to organisms recovered that may indicate a loss of control, deterioration in cleanliness or organisms that may be difficult to control such as spore-forming microorganisms and molds.





How Are They Getting In Here!?

Environment	Low temperature
	Low humidity
	Low nutrients
	Frequent disinfection

How do they survive?

How Dirty Are We?

AREA	NUMBER OF CFU/cm²
Scalp	1 million
Saliva and nasal fluid	10 million/gram
Back	100 million
Groin	1 - 20 million
Forehead	100 million - 1 billion
Hand	10,000 - 100,000
Armpit	1 - 10 million
Feet	1 million

Source: Clean Room Primer, 1985, J.J. Nappi Jr. Liberty Industries Inc. USA.

Gross! So, What Happens When We…?

Activity	Number of particles generated (0.5 micron and larger per minute)
Sitting or standing still	100,000
Sitting, small movement of arms or head	500,000
Sitting, moving arms, legs or head	1,000,000
Standing Up	2,500,000
Walking slowly	5,000,000
Walking normally	7,500,000
Walking ~ 5.5 MPH	10,000,000
Performing a workout	15,000,000 - 30,000,000

Source: Encyclopedia of Cleanrooms, Bio-Cleanrooms, and Aseptic Areas, July 2000, Philip R. Austin

Everyone Understands Microbiology (Contamination)!

Investigation Elements

System	Investigation Elements
Facility Surfaces	Perform investigation for possible sources of contamination.
	Evaluate sanitization/disinfection practices, review preparation of disinfectants, cleaning records, and training records of individuals performing sanitization/disinfection.
	Review possible unusual events during manufacturing operation.
	Examine areas during usage.
	Review close circuit video (if applicable).
	Verify that controls were not circumvented.
	Review risk of product contact.
	Review isolates for occurrence in other types of tests.
	Evaluate integrity of the room (e.g., peeling paint, cracks in ceiling, walls, and floor).
	Examine endotoxin and water chemistry data for system.

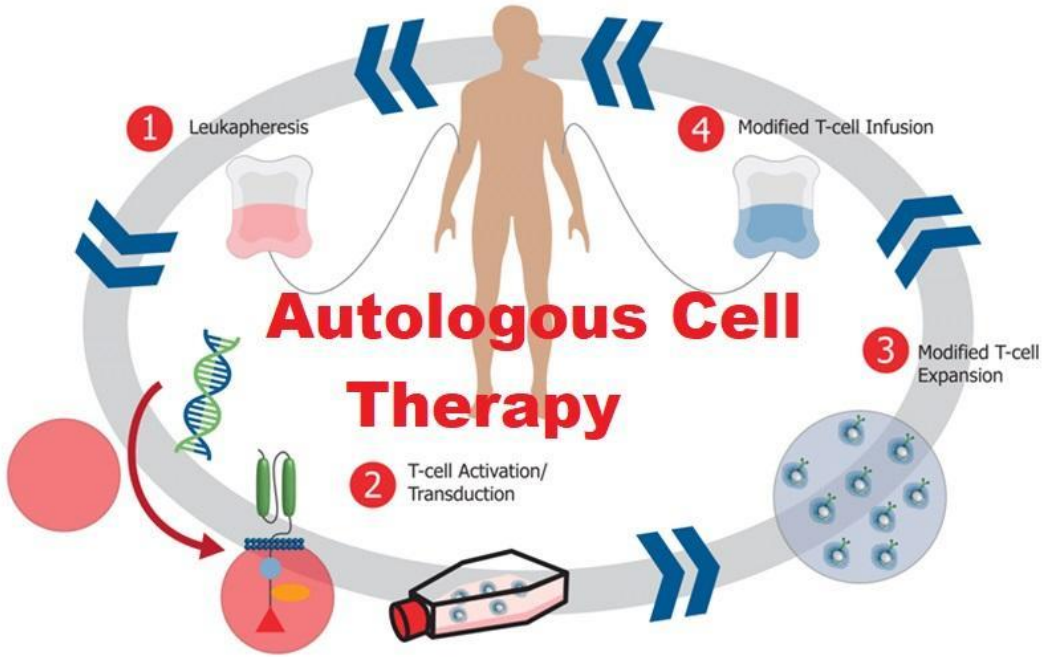
Investigation Elements (continued)

System	Investigation Elements
High Purity Water System (WFI, clean stream, purified water)	Examine bioburden data for other samples or sites in system – port contamination vs. system contamination.
	Review efficacy of sanitization procedure and schedule.
	Inspect system preventive maintenance records. Evaluate impact to product.
Personnel gowning (gowning and gloves)	Evaluate possible operator impact upon product.
	Review environmental monitoring data and sterility test data.
	Review preparation and expiration dates for disinfectants used on gloves.
	Identify all morphologically unique isolates (human vs. environmental).
	Interview operator for potential cause and retrain or re-quality operator. Check the system for the integrity of gloves (isolators and RABS).
	Evaluate training of operator. Review sanitization/disinfection records of area.
	Review closed circuit video (if applicable).

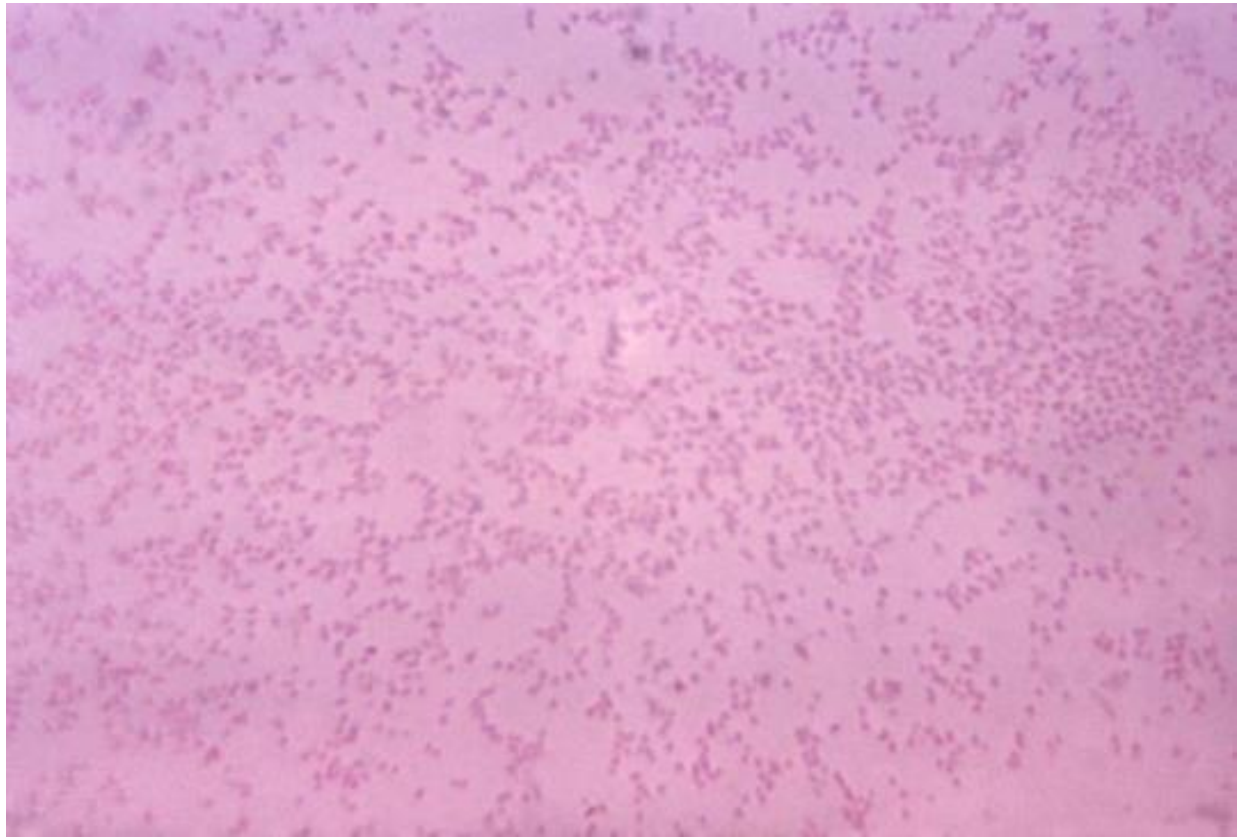
Investigation Elements (continued)

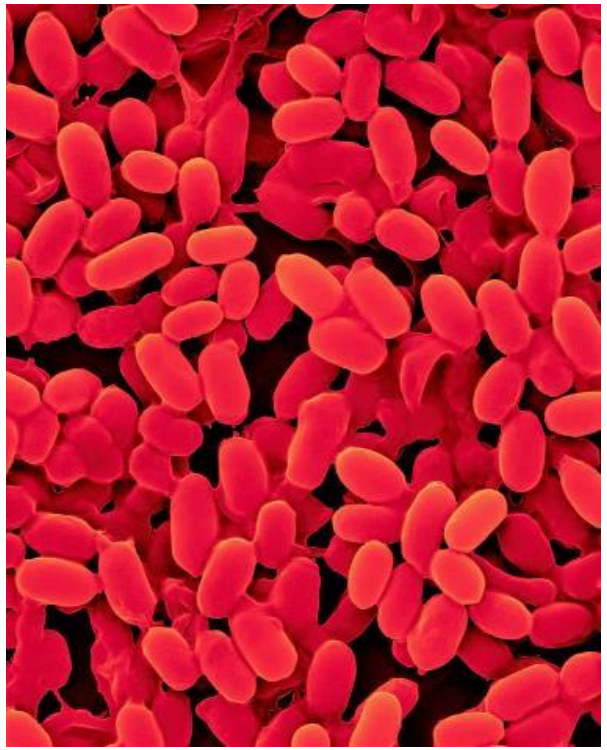
System	Investigation Elements
Compressed Gas System	Repeat test immediately. Perform filter integrity testing.
	Replace filter if excursion confirmed on retest.
	Evaluate impact upon processed component and/or product.
Room Air/HVAC	Review level of personnel activity.
	Review/perform air flow patterns/HEPA integrity tests.
	Review aseptic technique of personnel and training records.
	Review gowning procedures and requirements for area.
	Review trends and any incidents of HVAC outages, if they occurred.
	Inspect incoming air filters for leaks and pressure differential across filter.
	Review room disinfection/sanitization procedures, sanitization intervals, and disinfectant efficacy. Review training records of individuals performing sanitization/disinfection.
	Check area pressure differentials, particularly with respect to the last sanitization.
	Evaluate mechanical equipment in area as possible source of contamination.
	Review relevant, recent data at the same sites and subsequent monitoring results available.

What About ATMPs?



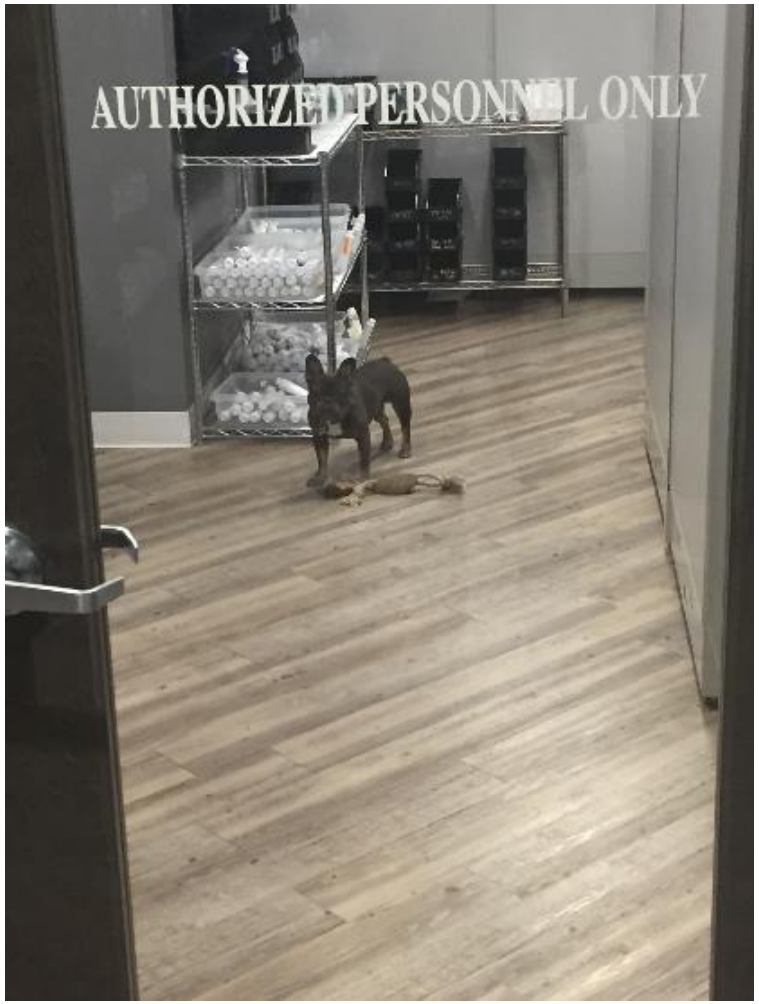
Sometimes it Doesn't Make ANY Sense…





Pasteurella multocida

Even When You Wish it Did!



Any Better?


LOST
CONFUSED
UNSURE
UNCLEAR
PERPLEXED
DISORIENTED
BEWILDERED

Questions?

MARC GLOGOVSKY, MS, S.M. (NRCM)

BUSINESS UNIT MANAGER/SENIOR CONSULTANT - MICROBIOLOGY

mglogovsky@valsource.com

M 609.432.1314

P 610.269.2808

F 610.269.4069

918A Horseshoe Pike

Downingtown, PA 19335

valsource.com