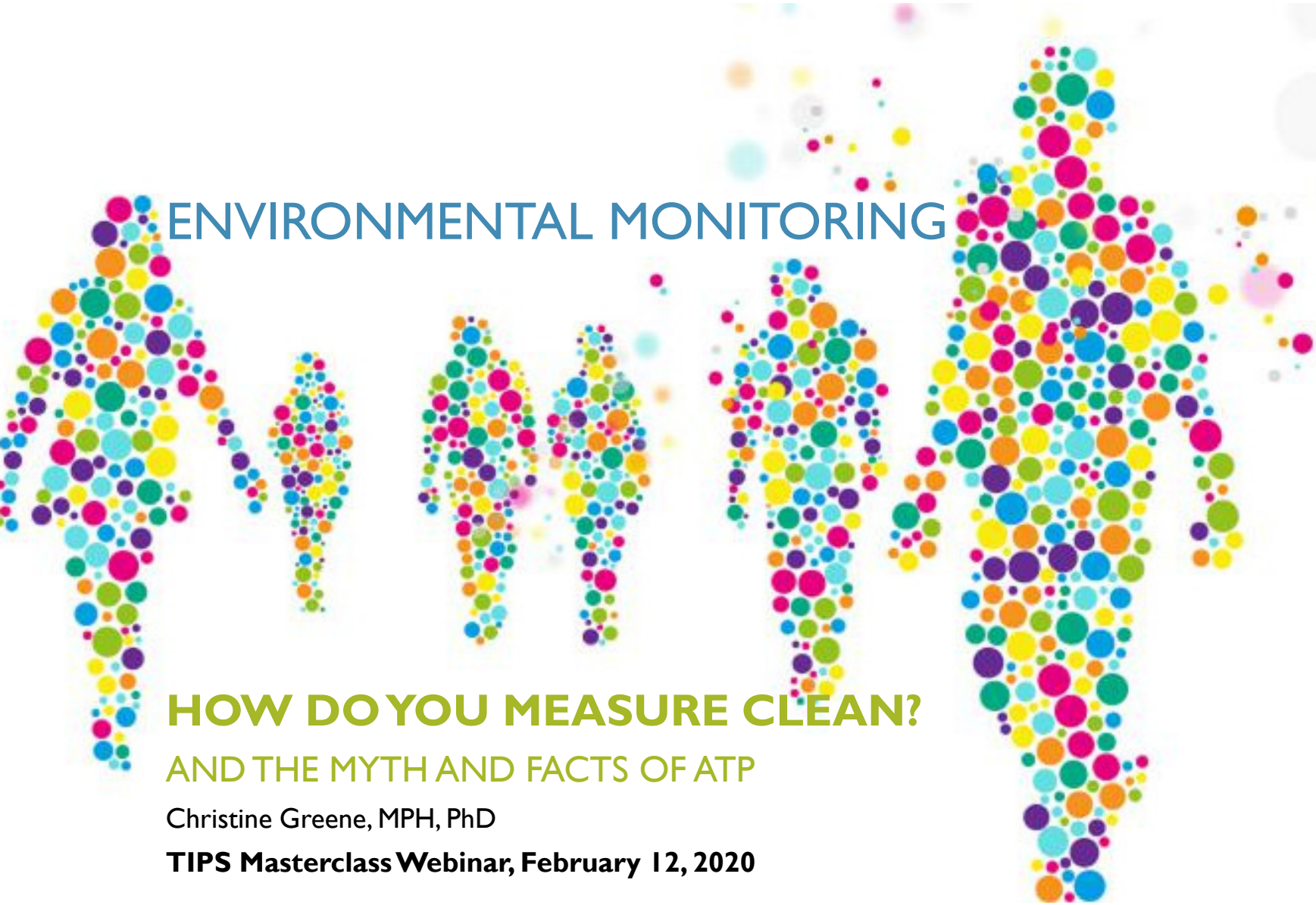

ENVIRONMENTAL MONITORING

HOW DO YOU MEASURE CLEAN?

AND THE MYTH AND FACTS OF ATP

Christine Greene, MPH, PhD

TIPS Masterclass Webinar, February 12, 2020

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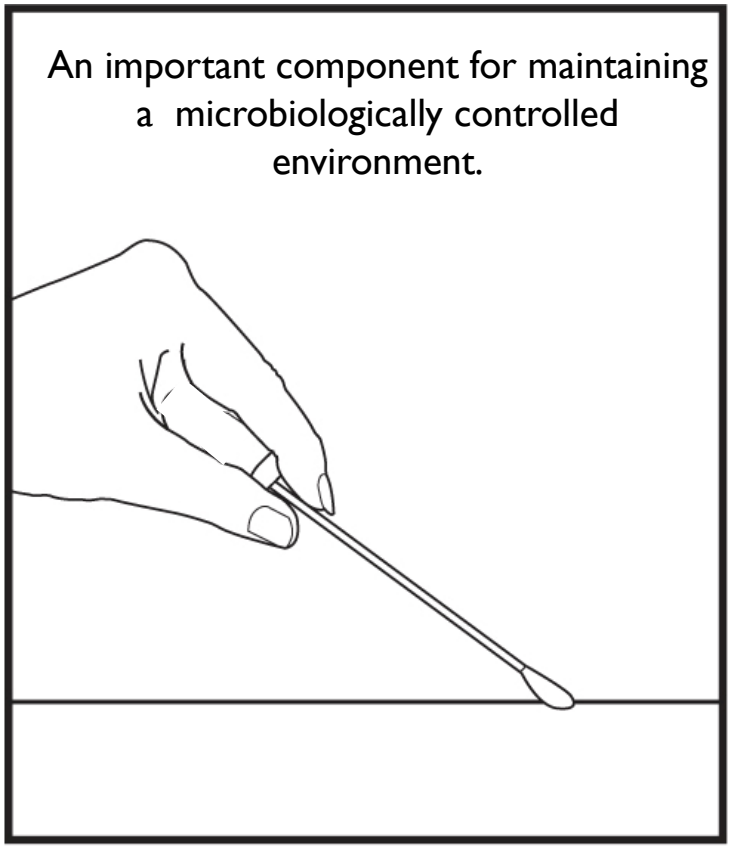
THANK YOU!

DISCLOSURES

- TSG, Regulatory Microbiologist
- EVS Protects, LLC, Co-Owner
- HITS Consortium, President
- TIPS, Board Member

WHAT IS ENVIRONMENTAL SURFACE MONITORING?

- Describes the processes and activities that needed to characterize and monitor the effectiveness of your disinfection/cleaning procedures.
- Traditionally
  - A surveillance system for **microbiological control** of critical areas
  - Involves the detection and enumeration of bacteria, and sometimes yeast and mold.



WHY MONITOR?

- Public health and safety & regulatory requirements
- Multiple sources of contamination

- Pathogens can be come resistant to biocides
- Can survive on surfaces for very long periods of time

Organism	Survival time
Clostridium difficile (spores)	5 months
Acinetobacter spp.	3 days to 5 months
Enterococcus spp. including VRE	5 days – 4 years (!)[1]
Pseudomonas aeruginosa	6 hours – 16 months
Klebsiella spp.	2 hours to > 30 months
Staphylococcus aureus, inc. MRSA	7 days – 7 months
Norovirus (and feline calicivirus)	8 hours to > 2 weeks[2]
SARS Coronavirus	72 hours to >28 days[3]
Influenza	Hours to several days[4]

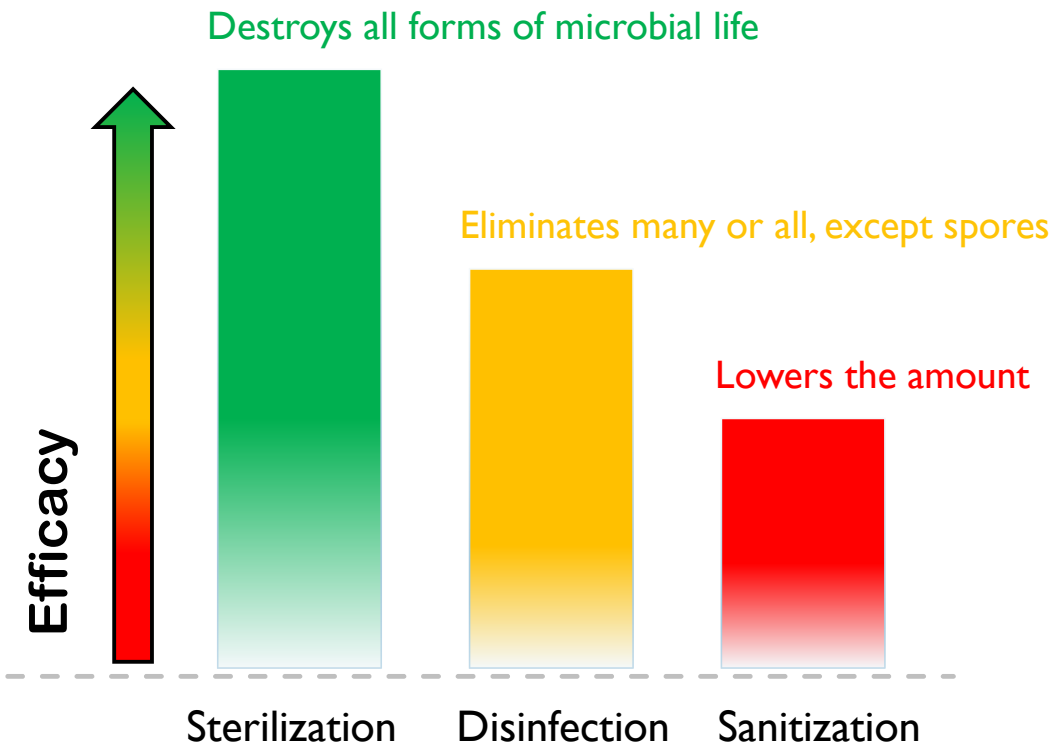
Adapted from Kramer *et al. BMC Infect Dis* 2006;6:130.

1. Wagenvoort et al. J Hosp Infect 2011;77:282-283.
2. Doultree *et al. J Hosp Infect* 1999;41:51-57.
3. Rabenau *et al. Med Microbiol Immunol* 2005;194:1-6.
4. Bean *et al. J Infect Dis* 1982;146:47-51.

CDC'S DEFINITION OF "CLEAN"

- **Cleaning removes germs**, dirt, and impurities from surfaces or objects.
- Involves soap (or detergent) and water to physically remove germs from surfaces.
- This process does not necessarily kill germs, but by removing them, it lowers their numbers and the risk of spreading infection.

- https://www.cdc.gov/flu/school/cleaning.htm



In Environmental Microbiology Clean is defined in terms of microbial removal (efficacy)

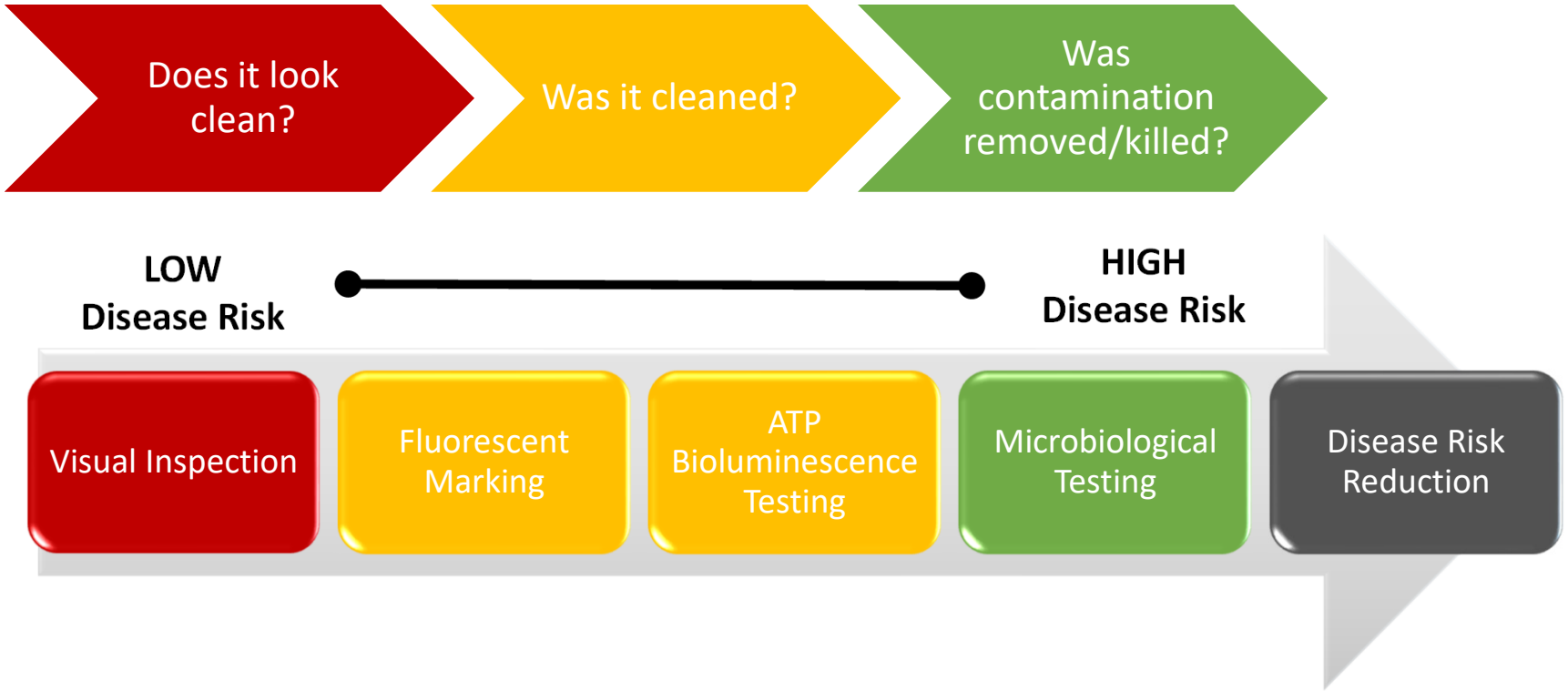
https://www.cdc.gov/infectioncontrol/guidelines/disinfection/introduction.html

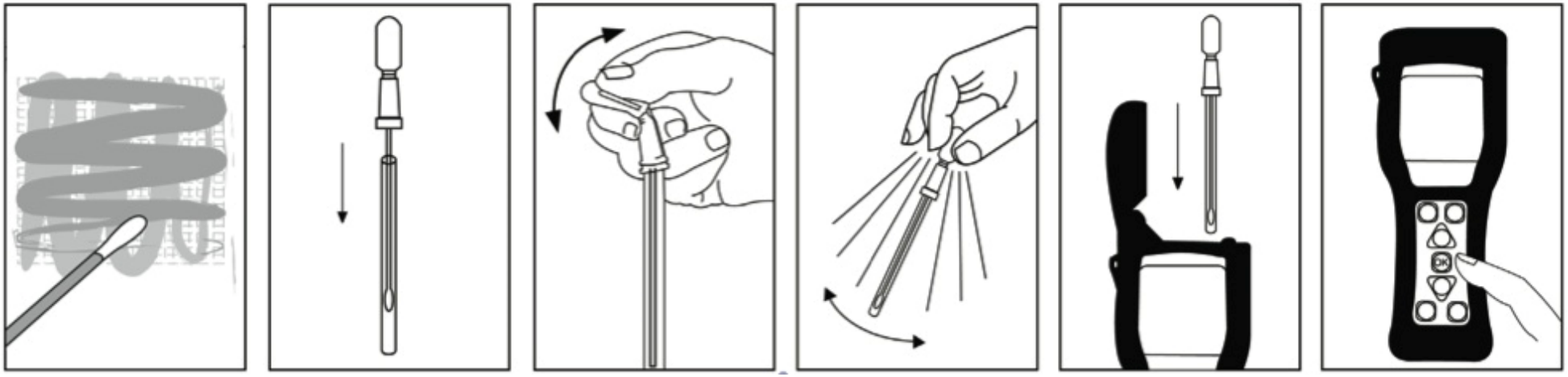
How do we measure “clean”?

- No recognized standard method for measuring cleanliness

Aesthetic vs Bacterial cleanliness

At opposite ends in terms of ability to assess risk of disease transmission

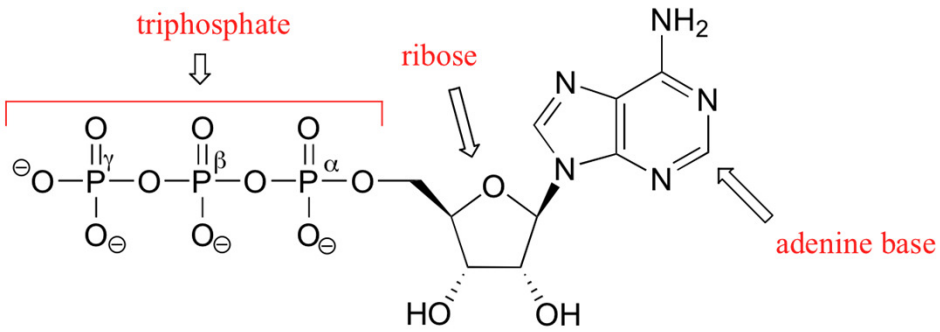




ATP SAMPLING

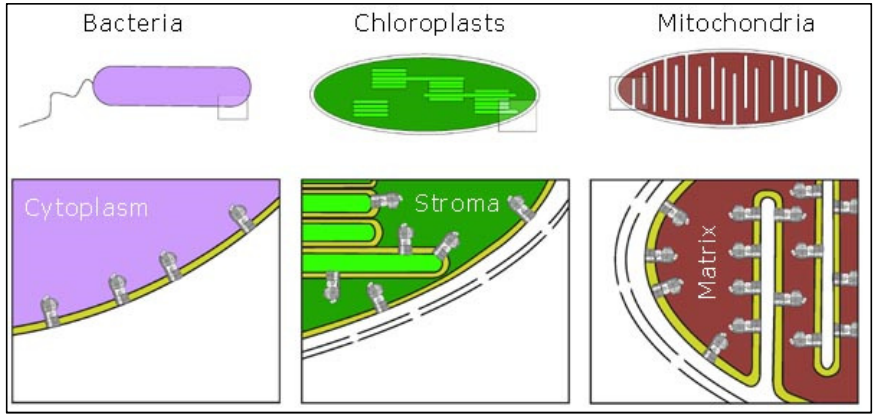
WHAT YOU NEED TO KNOW…

An estimate of the amount of adenosine triphosphate present



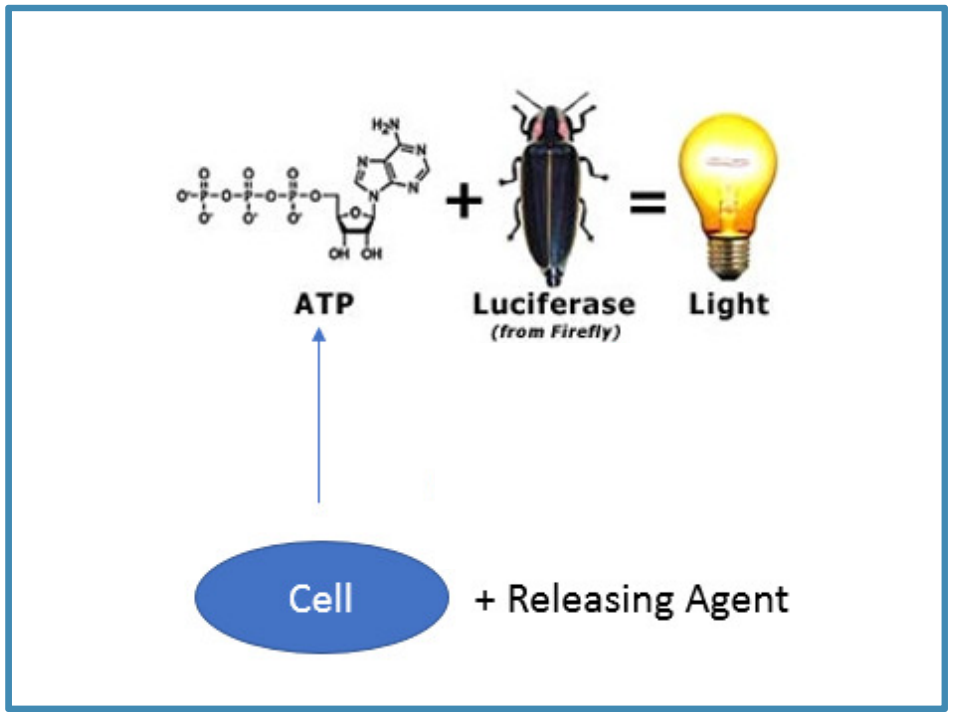
adenosine triphosphate (ATP)

ATP is a molecule found in all cells



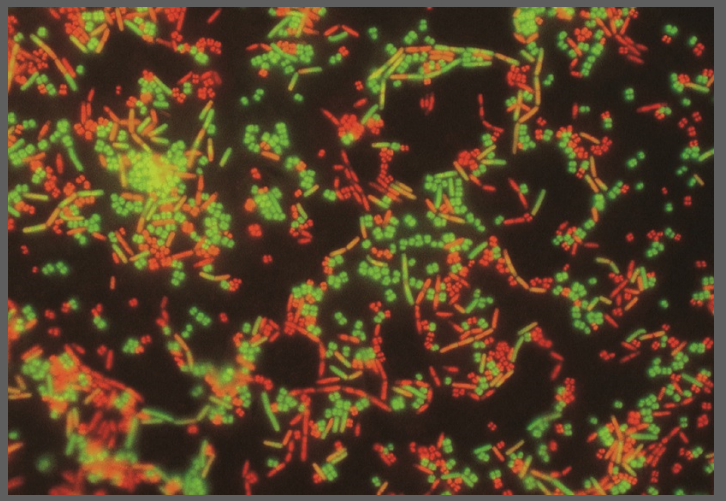
https://www.atpsynthase.info/old/FAQ.html#ATP_synthase_location

How ATP Systems Work



- ATP monitoring systems contain 2 important chemicals:
  - **ATP releasing agent –** break open cells to release the ATP.
    - Similar in chemistry to detergents
  - **Enzyme Luciferase** - reacts with ATP to generate light
    - measured as relative light units (RLU) using a luminometer.
- light emitted is directly proportional to amount of ATP present.

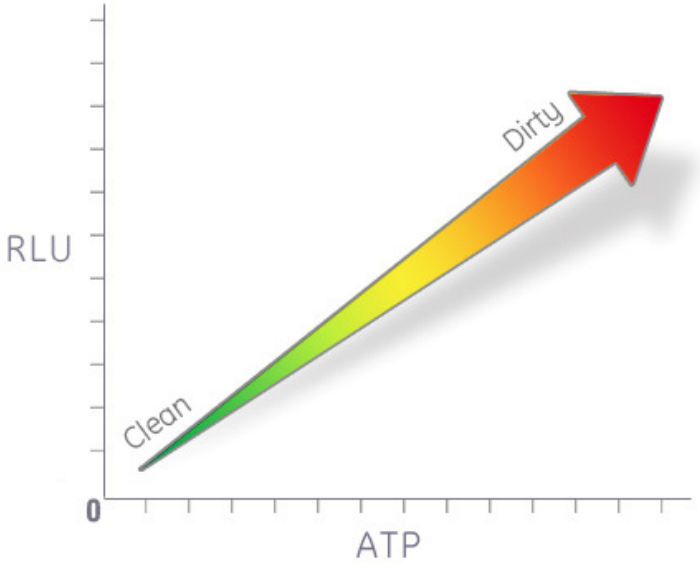
POLLING QUESTION:



ATP is measured in terms of RELATIVE LIGHT UNITS (RLUs)

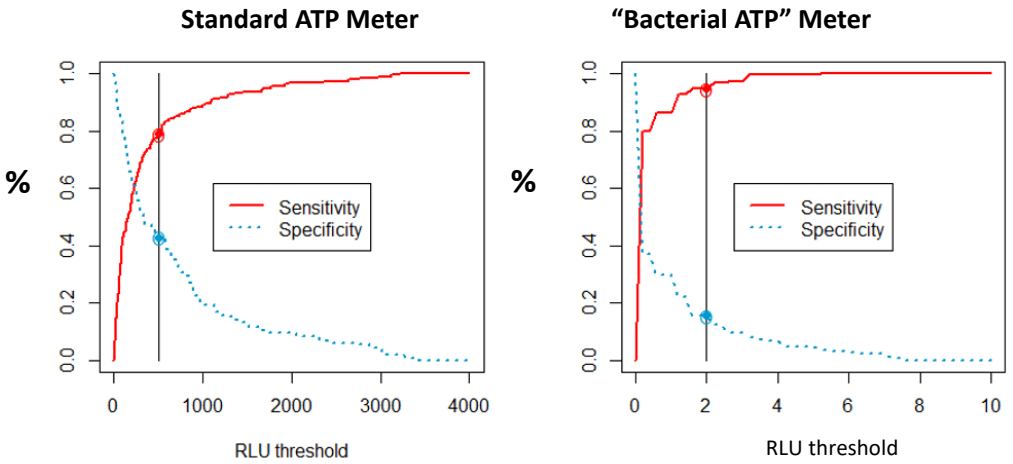
RLUs ≠ Cells

- RLUs are a measure of luminescent units, ***not cells.***
- The luminometer detects bioluminescence from ATP, ***a cell constituent***.
- ATP Concentration varies from cell to cell!
  - ATP produced by a cell depends on
    - cell type
    - mechanism of cellular respiration
  - Extracellular ATP outside of cells from dying cells



Independent Correlation Study

NSF International Unpublished data from 2018 study at hospital; n=394 samples per group



	Sensitivity	Specificity
ATP	0.7894737	0.4277108
Bacterial ATP	0.9479167	0.1556886

"clean" = 2.5 cfu/cm2

Found No correlation between ATP & CFUs

- Standard ATP System
  - Identifies true negatives* ~79% of time
    - ~ 21% of samples identified as dirty had no contamination
  - Identifies true positives** ~43% of the time
    - ~ 57% of the samples identified as clean had contamination
- Bacteria Specific ATP System
  - Identifies true negatives* ~95% of time
    - ~5% of samples identified as dirty had no contamination
  - Identifies true positives** ~15% of the time
    - ~ 85% of samples identified as clean had contamination

*true negative= RLU ≤ 500 (or ≤ 2) for a surface that is truly clean.
**true positive = RLU ≥ 500 (or ≥2) for a surface that is truly dirty.

POLLING QUESTION:

DISINFECTANT RESIDUES LEFT BEHIND ON A SURFACE CAN IMPACT ATP MEASUREMENT

Depending on the cleaning chemistry, surface residues can have a negative or additive effect on the ATP result.

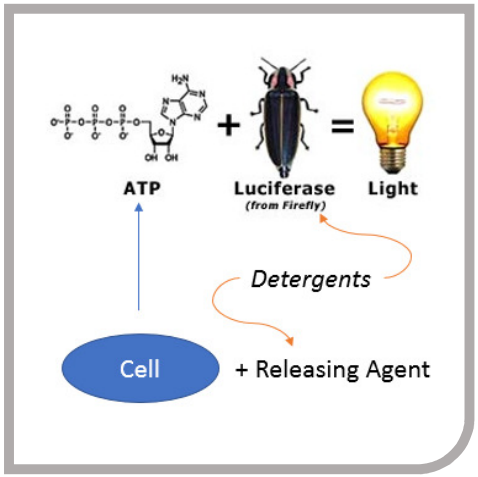
Table 4. Quenching and enhancing summary for tested disinfecting chemistries versus each tested ATP unit.

Product	Kikkoman	3M	Hygiene	Charm
CaviCide	23.18%	83.67%	62.80%	31.61%
PCS 1000	15.34%	53.49%	12.94%	13.71%
Sani-Cloth Plus	13.16%	52.23%	12.05%	14.78%
Accel TB	37.08%	65.69%	51.44%	44.48%
CleanCide	99.86%	89.64%	99.90%	-0.43%
Clorox Hydrogen Peroxide Wipes	51.40%	77.95%	92.83%	55.34%
Clorox Clean-up disinfectant	26.28%	93.54%	60.40%	77.02%
IPA, 70%	32.34%	32.20%	22.07%	24.24%
0.5% H2O2	−0.10%	−6.79%	5.43%	−2.80%
Ultra Clorox Bleach (1:10)	−0.20%	91.18%	27.35%	45.34%
Accel PREVention RTU	−5.69%	48.29%	36.11%	−38.80%
Virox 5 RTU	2.69%	23.34%	30.73%	−17.88%
BTC 50 (1:125)	14.79%	9.54%	9.54%	19.34%
Sporicidin	95.16%	40.14%	99.10%	80.15%

Enhancing is shown in negative values while quenching is in positive values.
doi:10.1371/journal.pone.0099951.t004

ATP Releasing Agent

Enzyme Luciferase



Luciferase is inactivated by high concentrations of ATP releasing agent.

Chemical composition of cleaning agents are often very similar to the ATP releasing agents!

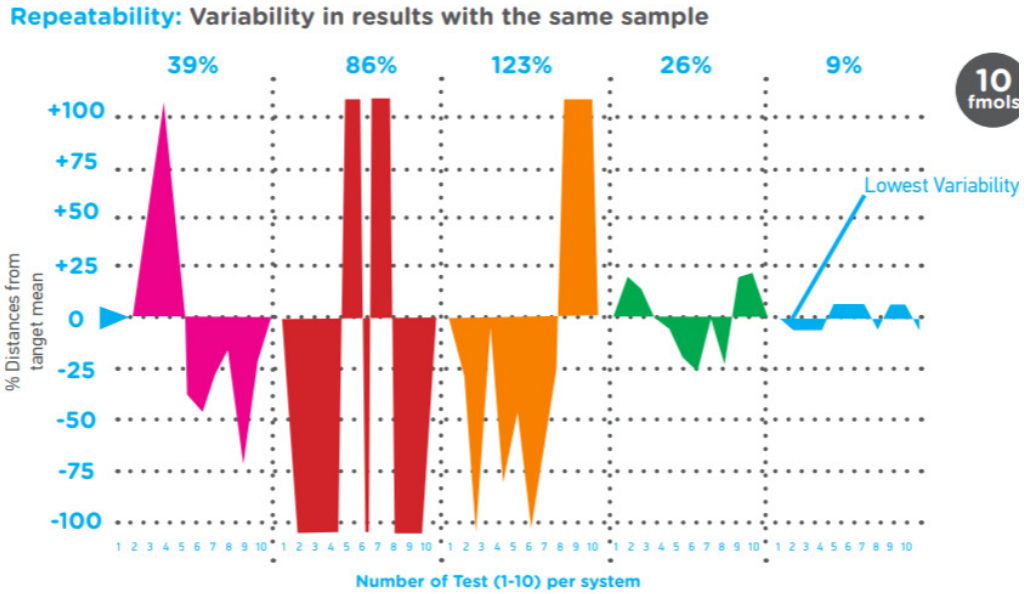
ATP systems have proprietary blends with a calculated concentration of releasing agent

- too little jeopardies ability to adequately break open the cells to release the ATP
- too high risks inactivating the enzyme Luciferase.

Disinfectant residuals can severely affect Luciferase causing an artificially erroneous result.

High variability in sensitivity & reliability between different systems

- Proprietary chemical blends mean differences in:
  - Detection limits and software systems
  - Ability to detect gram negative bacteria
- Cost for mobile and instant results:
  - **Reduced Reliability**
  - No standard test, so do not have a true result to compare your results to.



Each system reads on a brand specific and arbitrary scale of RLUs. Studies show wide dissimilarities between the RLU scaling at standardized testing levels (pure ATP and bacterial cultures).

ESTABLISHING YOUR ATP PASS/FAIL CRITERIA

Each manufacture has own "quality standards" (RLU thresholds), ranging from 100-500.

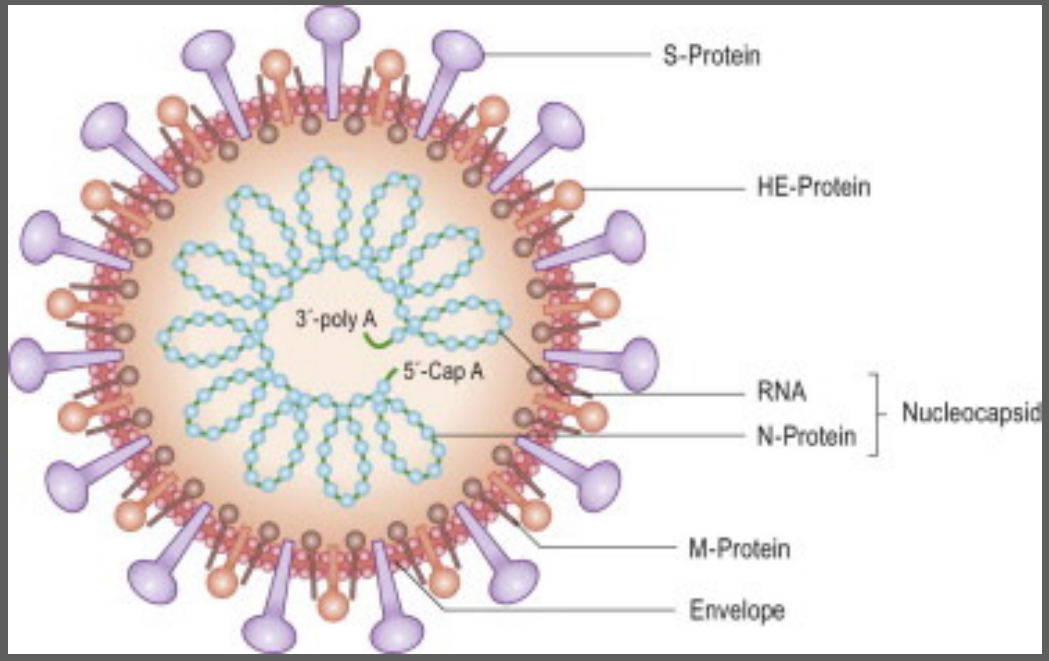
Using Sampling Algorithm to increase confidence in derived threshold: https://doi.org/10.1016/j.idh.2016.11.003

1) Clean the area
2) Collect reference samples

General assumption is that RLUs >2-3 times background indicates the area tested is soiled.

Ask manufacture for their sensitivity and variability data.

POLLING QUESTION:



https://www.sciencedirect.com/topics/neuroscience/coronavirus

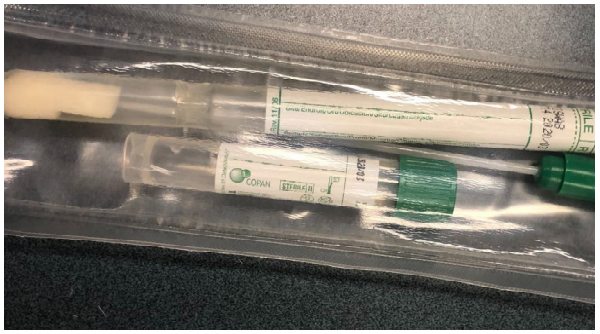
ONLY MICROBIOLOGICAL METHODS CAN ASSESS MICROBIAL RISK

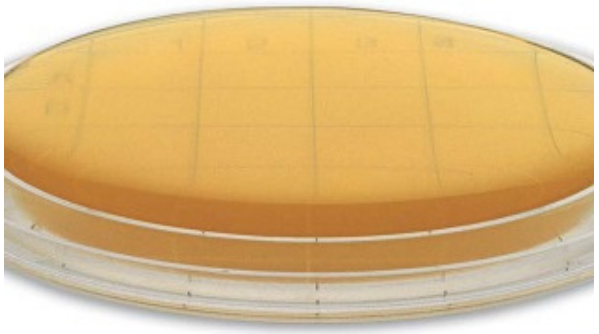
- Viruses do not contain or produce ATP molecules on their own
- Limited in ability to detect gram negative ATP
- Many field studies conclude very poor to no correlation between ATP and microbiological contamination.
- RLUs only provide an estimate of gross, general levels of cleanliness (presence of organic matter.)[1]

[1] Elaine L. Larson, et al., *Food Microbiology* (2003) 735–739

MICROBIAL SAMPLING

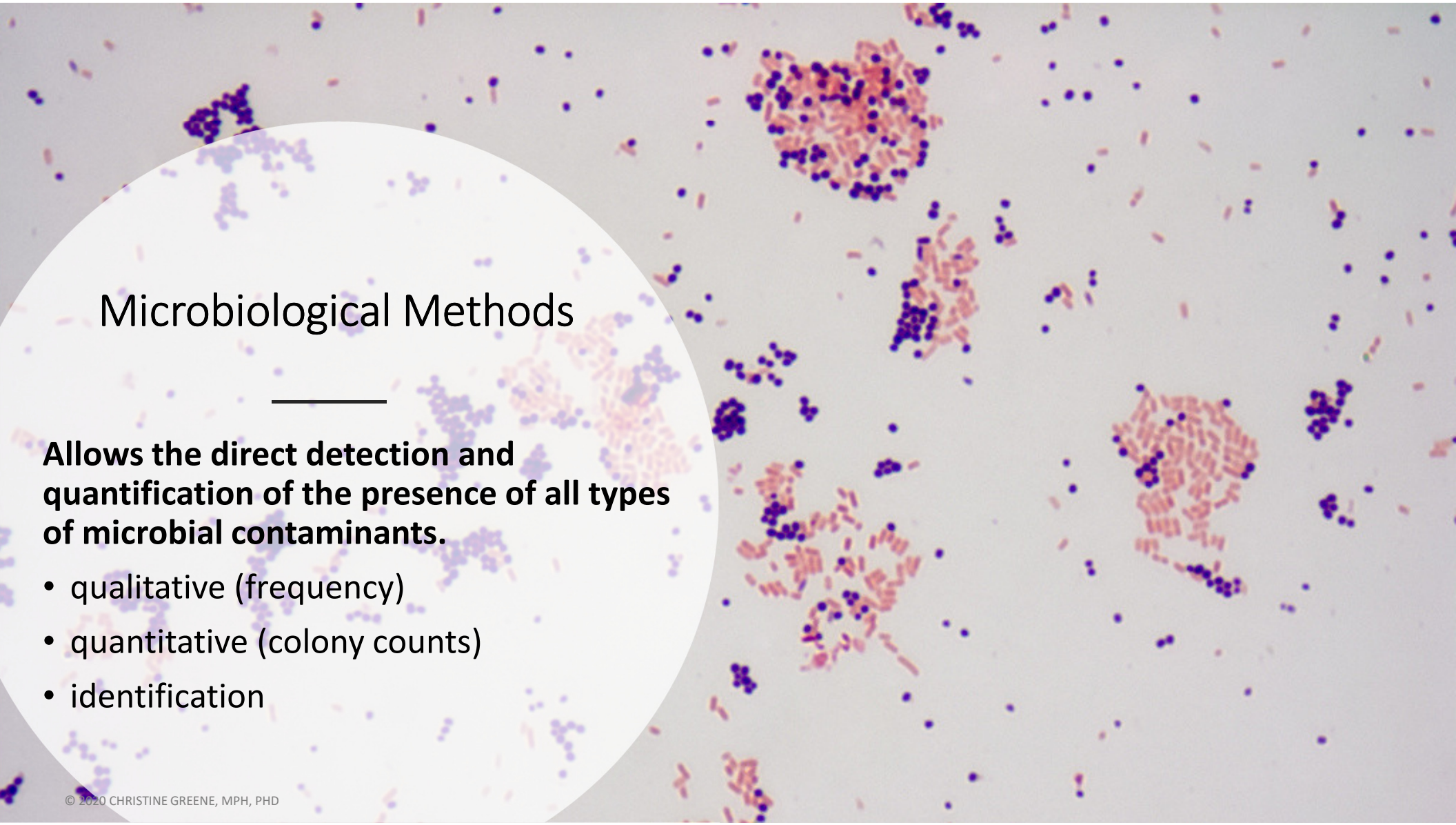
WHAT YOU NEED TO KNOW…





MICROBIOLOGICAL METHODS

- Microbial Swabbing
  - Flat + irregular shaped surfaces
- Contact Plates
  - Flat surfaces only



Microbiological Methods

Allows the direct detection and quantification of the presence of all types of microbial contaminants.

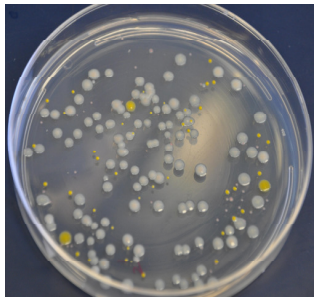
- qualitative (frequency)
- quantitative (colony counts)
- identification

WHAT DO I SAMPLE FOR?

- **Total aerobic colony counts**
  - Quantitative (CFU/mL)
  - Appropriate for most purposes including monitoring
  - Non-specific

- **Specific indicator organism**
- **MRSA, VRE, *C. difficile*, etc**
  - Quantitative
  - Qualitative
  - Requires special media/equipment
  - Make sure the lab can test for it!
  - Have an action plan of what you will do if you find it

- **Identification of all bacteria in the sample**
  - Frequency measurement
  - Genus / Species level ID
  - MALDI-TOF (most rapid and affordable method available).



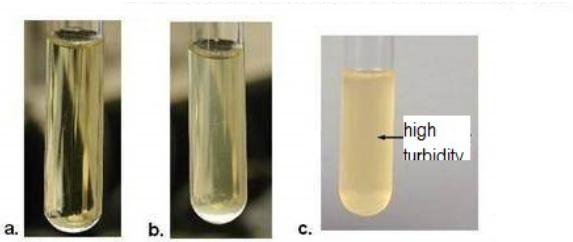
QUALITATIVE VS QUANTITATIVE ANALYSIS

Quantitative

- Colony Counts (ACC)
- Reported as: **colony forming units** (CFU) per sample or per cm^2
- Log Reduction and % Reduction in bioburden

Qualitative

- Growth or no growth of bacteria
- Reported as: present or absent
- % reduction in positive samples



a. Sterile (uninoculated broth) - note how clear the media is
b. Broth showing slight turbidity (some bacterial growth)
c. Broth showing significant turbidity (a lot of bacterial growth)

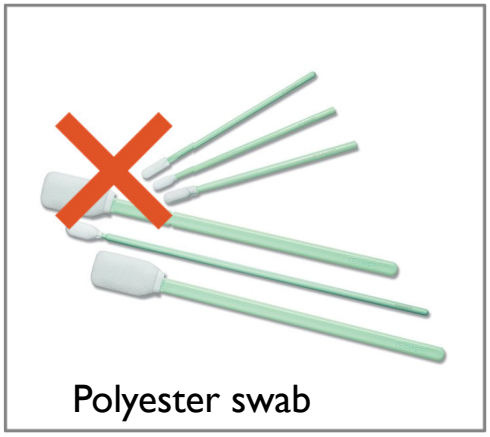
BENCHMARKING (Pass/fail criteria)

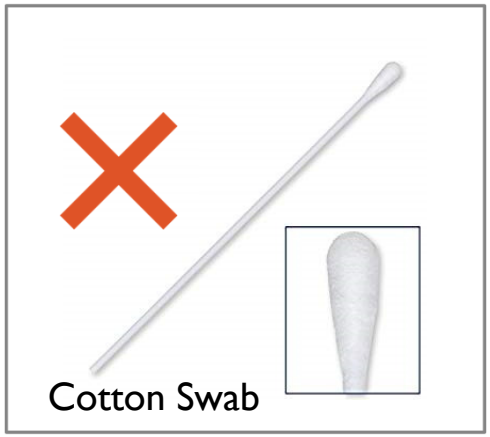
- Microbiological
  - Cleaning/disinfecting quality standards[1,2]
    - Aerobic Colony Counts: <2.5 cfu/cm^2
    - Specific indicator organisms: <1 cfu/cm^2)
  - Pharma requirements depend on ISO Classification
    - "Clean rooms"

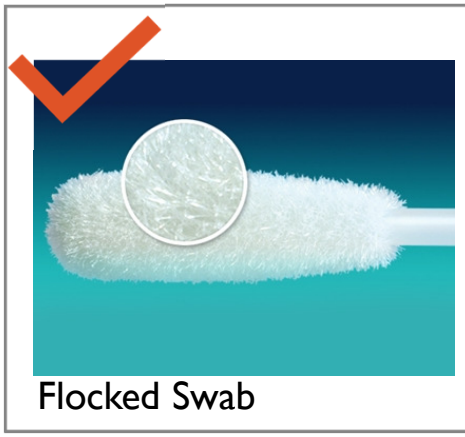
1 Mulvey *et al. J Hosp Infect* 2011;77:25-30.
2 Malik *et al. Am J Infect Control* 2003;31:181-187.

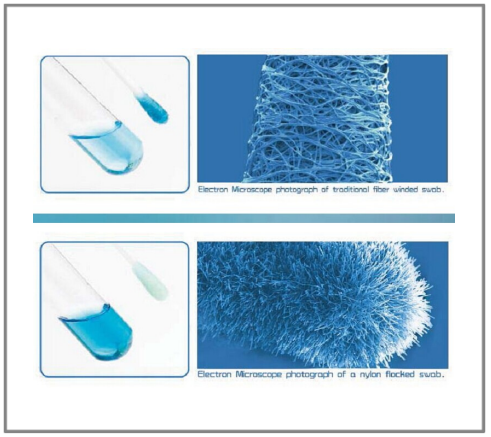
Important considerations before collecting microbial swab samples …

~ swab selection
~ transport media
~ sampling technique


Polyester swab


Cotton Swab


Flocked Swab



SELECTING A SWAB

- Many swab types available
  - Avoid cotton, fiber or polyester swabs
    - sample stays entrapped in the fiber wad
  - Flocked Swabs have no "insides" so the sample is instantly and entirely released for analysis
- Ensure swab is made for environmental surface sampling

TRANSPORT MEDIA

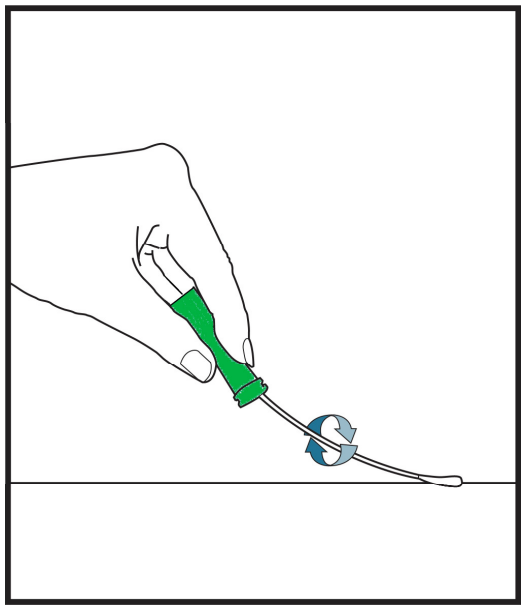
Transport media is needed to preserve samples during transport to the analysis lab

- Must contain a buffer to prevent cell death
- Must contain broad-spectrum neutralizer to prevent continued action of residual disinfectants.

COLLECTING MICROBIAL SWAB SAMPLES

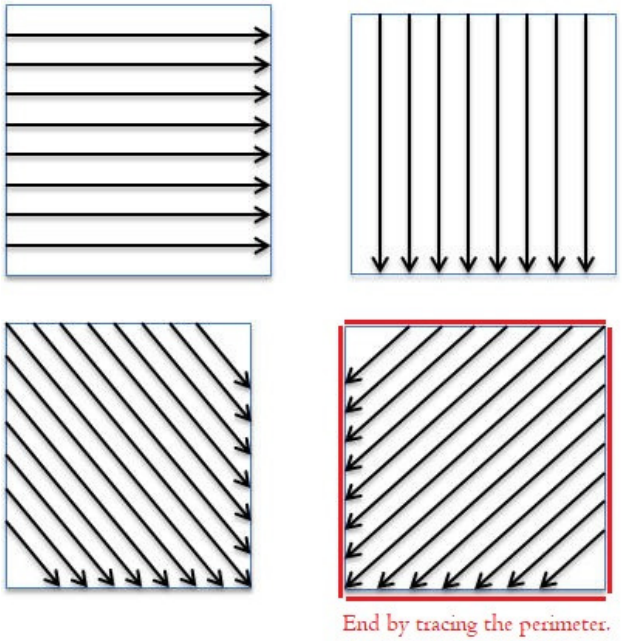
1 Apply consistent pressure!

Rotate or flip over the swab throughout the process.



2 Using a template, ensure full coverage of the surface area.

Keep sampling area size consistent between samples

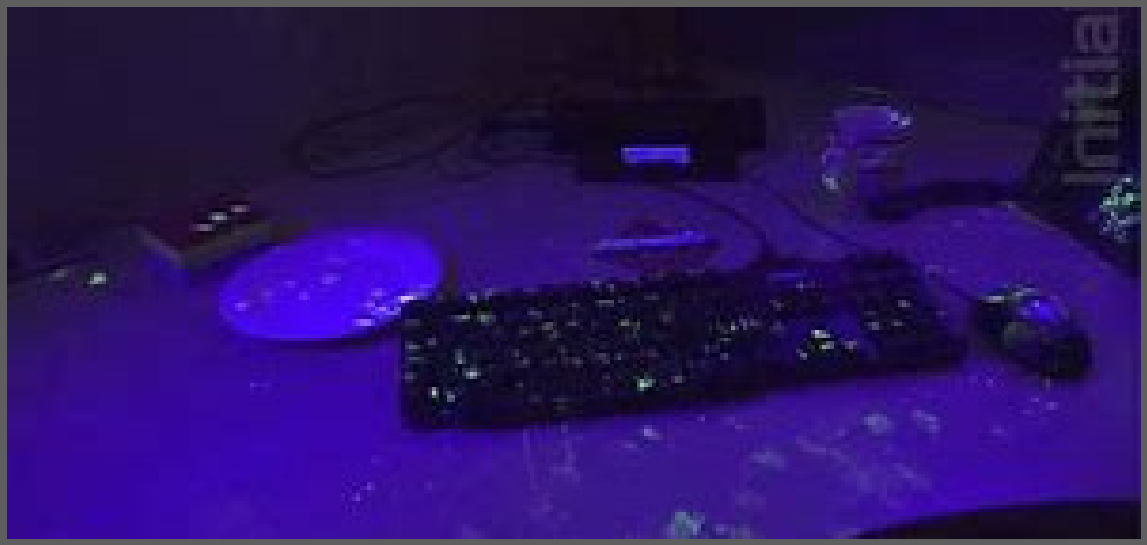


SAMPLE TRANSPORTATION

- Ship using freezer packs in insulated shipping box.
- Do NOT use dry ice!
- DO NOT use ice in a ziplock baggie!
- Don't forget to include documentation!!

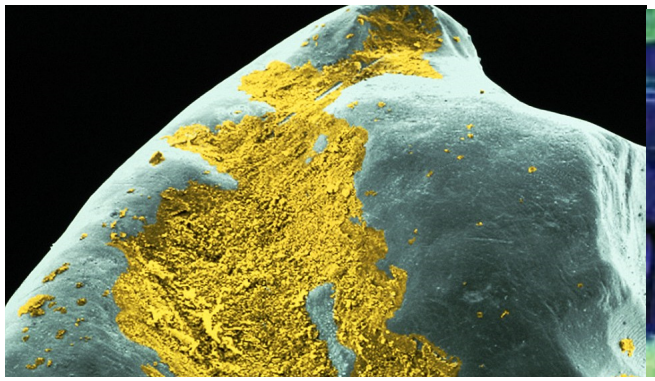
- Ship overnight to the lab!

POLLING QUESTION:



THE VARIABILITY OF SOIL & BACTERIA DISTRIBUTION IS A CONFOUNDER, *REGARDLESS OF TYPE OF TESTING.*

- Technique matters!!!
  - Bacteria love to hide in grooves and crevice's
- Sample frequency matters!
  - Increasing the number of samples helps to account for variability.



DEVELOPING A SURFACE SAMPLING PLAN:
THE BASICS

- ✓ WHERE TO COLLECT SAMPLES?
- ✓ WHEN & HOW OFTEN TO COLLECT SAMPLES?
- ✓ ACTION PLAN
- ✓ WHEN TO USE ATP OR MICROBIAL METHODS?

WHERE – INDUSTRY SPECIFIC

SAMPLING PRIORITY (EXAMPLES)

Hospital Facility

Zone 1	• **High touch surfaces in patient area.** Patient areas with potential for direct patient contact. • CDC listed high-touch surfaces
Zone 2	• **Surfaces adjacent to patient areas -** potential for direct or indirect patient contact. • Patient bathrooms, mobile equipment, nursing stations
Zone 3	• **Surface areas in non-patient areas** i.e. public areas, waiting rooms, elevators, etc.

Food Manufacturer / Food Prep

Zone 1	• **Food Contact Surfaces** • Slicers, peelers, hoppers, screens, conveyor belts, air blowers, knives, racks, worktables, etc.
Zone 2	• **Non-Food (Near) Contact Surfaces** • Directly adjacent to Zone 1, exterior and under equipment framework, refrigeration units, switches, etc.
Zone 3	• **Non-Food (Distant) Contact Surfaces** • Adjacent/surrounding zone 2, phones, air return covers, drains, equip wheels, etc.

Validation

To compare 2+ products/processes

- Before and after cleaning process
- Before and after product usage

Provides log reduction or % reduction efficacy data.

Only microbial methods can reliably measure efficacy and provide reduction calculations.

Monitoring

To monitor for microbial control

- After Cleaning/Disinfection
- During aseptic processing/operation

Provides trends analysis over time and is compared to baseline

WHEN DEPENDS ON WHY

HOW OFTEN

Monitoring and validation should be performed on a regular, periodic basis.

- Annually or Semi-Annually
  - Baseline/background levels
  - Validation of cleaning protocols
- Monthly or quarterly
  - General monitoring of critical areas
- Weekly or daily
  - Outbreaks or contamination issues

YOU HAVE YOUR RESULTS … *NOW WHAT?*

- Passed Test
  - You're doing great! No actionable items.
  - Use the data to be proactive!
- Failed Test
  - Thoroughly disinfect the area and resample
  - Re-evaluate cleaning protocols
  - Validate the cleaning protocols
- Specific action plan depends on the facility

WHEN SHOULD I USE ATP VERSUS MICROBIAL SWABBING?

- 1st consider any applicable regulations.
- Then ask …

ATP TESTING

Cleaned?

Low Risk Areas

"Zones" 2 and 3

Ex:

- Daily cleaning
- Public Areas
- Low Touch Surfaces

MICROBIAL TESTING

Disinfected?

Validating Efficacy?

High Risk Areas

"Zones" 1 and 2

Ex:

- Room Discharge
- Patient Areas
- High touch areas

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QUESTIONS?