

# Environmental Evaluation

**John M. Boyce, MD**  
**President, J.M. Boyce Consulting, LLC**

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# **Role of Contaminated Environment in Transmission of Healthcare-Associated Infections**

- **There is increasing evidence that contaminated environmental surfaces can contribute to the transmission of healthcare-associated pathogens**
- **Factors that support the role of the environment include:**
  - **Frequent contamination of surfaces by pathogens**
  - **Ability of pathogens to survive on surfaces and remain pathogenic**
  - **Transmission of pathogens from surfaces to hands of healthcare workers (HCWs) or directly to patients**
  - **Prior-room occupancy as a risk for acquisition**
  - **Improved cleaning/disinfection of surfaces can reduce transmission**

**Weber DJ et al. AJIC 2010;38:S25**

**Otter JA et al. Infect Control Hosp Epidemiol 2011;32:687**

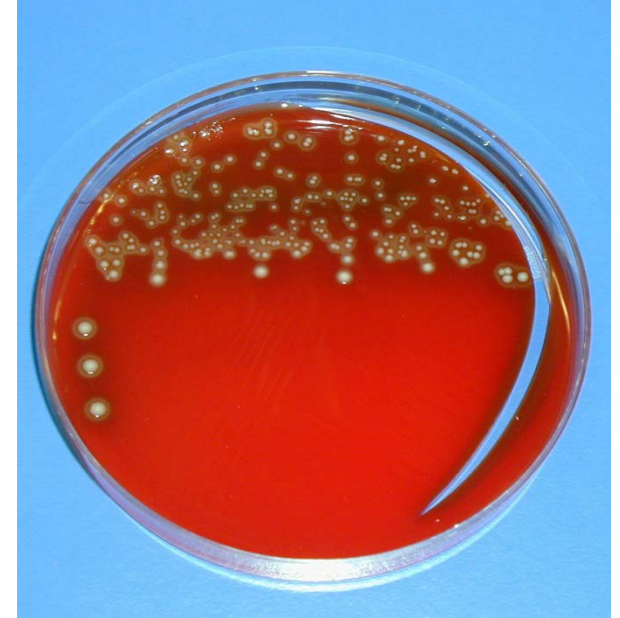
**Weber DJ et al. Curr Opin Infect Dis 2013;26:338**

**Mitchell BG et al. J Hosp Infect 2015;91:211**

**Hayden MK et al. Clin Infect Dis 2006;42:1552**

# Environmental Contamination by Vancomycin-Resistant Enterococci (VRE)

- **Vancomycin-resistant enterococci (VRE) are antibiotic-resistant bacteria that occur in the gastrointestinal tract of some patients**
- **VRE are also frequently present on the skin of patients who have VRE**
- **Patient with VRE shed the bacteria onto surfaces near them**
- **7% to 46% of environmental surfaces in the rooms of patients who have VRE are contaminated with VRE**



**VRE Cultured from  
a Bedside Rail**

**Boyce JM et al. J Clin Microbiol 1994;32:1148**

**Bonten MJM et al. Lancet 1996;348:1615**

**Weber DJ et al. Infect Control Hosp Epidemiol 1997;18:306**

**Sethi AK et al. Infect Control Hosp Epidemiol 2009;30:13**

# Frequency of MRSA Environmental Contamination in Hospital Settings

- **Percent of surfaces contaminated varies:**
  - 1% – 27% in MRSA patient rooms on regular wards
- **Frequency of contamination varies among patients with colonization/infection at different body sites**
  - 36% if MRSA in wound or urine vs 6% at other body sites
  - 59% with MRSA gastrointestinal colonization + diarrhea vs 23% if at other body sites, but not in stool
  - 19% of surfaces in an outpatient clinic were contaminated with community-acquired MRSA

Boyce JM et al. *Infect Control Hosp Epidemiol* 1997;18:622

Boyce JM et al. *Infect Control Hosp Epidemiol* 2007;28:1142

Johnston C et al. *Infect Control Hosp Epidemiol* 2006;27:1133

Chang S et al. *Clin Infect Dis* 2009;48:1423

# Frequency of *Clostridium difficile* Environmental Contamination

- Patients with colonization or diarrhea due to *Clostridium difficile* contaminate environmental surfaces in their vicinity
- Percent of environmental cultures positive varies
  - Rooms with no recent *C.difficile* patient: 2.6 – 8% (+)
  - Rooms of patients with *C.difficile* in their bowel, but do not have diarrhea: 7 – 29% (+)
  - Rooms of patients with *C.difficile* diarrhea: 20 – 90% (+)

Fekety R et al. Am J Med 1981;70:906

McFarland L et al. NEJM 1989;320:204

Struelens MJ et al. Am J Med 1991;91 (Suppl 3B):138S

Samore MH et al. Am J Med 1996;100:32

Sethi AK et al. Infect Control Hosp Epidemiol 2010;31:21

Weber DJ et al. AJIC 2010;38:S25

# Environmental Contamination by Gram-Negative Bacilli

- Multiple studies have shown that *Acinetobacter spp.* can survive on wet and dry surfaces and contribute to the spread of healthcare-associated infections (HAIs)
- Laboratory-based studies have given mixed results regarding survival of other Gram-negative pathogens on surfaces
- However, recent studies have documented widespread environmental contamination by carbapenem-resistant strains of *Klebsiella*

Maragakis LL et al. JAMA 2004;292:3006

Thom KA et al. AJIC 2011;39:711

Havill NL et al. Infect Control Hosp Epidemiol 2014;35:445

Weber DJ et al. Infect Control Hosp Epidemiol 2015;36:590

Weterings V et al. Eur J Clin Microbiol Infect Dis 2015;34:1647

Lerner A et al. J Clin Microbiol 2013;51:177

# Indications for Culturing the Environment

- Routine culturing of environmental surfaces, without input from infection preventionists, is not recommended
- Most frequently performed as part of quality assurance, monitoring of cleaning/disinfection practices, during assessment of hazardous situations
- Examples:
  - Biological monitoring of sterilization processes
  - Monthly cultures of water and dialysate in hemodialysis units
  - Evaluation of the adequacy of hospital housekeeping practices
  - As part of an epidemiological investigation of an outbreak
  - Some hospitals may culture duodenoscopes to assure disinfection

# Methods for Culturing the Environment

- Dozens of methods have been used by investigators for culturing the environment in hospital settings
- Relatively few standards for acceptable levels of microbial contamination exist in healthcare
  - Standards exist for hemodialysis water and dialysate
  - **No widely accepted criteria for defining surfaces as clean in healthcare**
  - **Level of contamination needed to prevent transmission is not known**
- Useful reviews of available methods are listed below

CDC Guidelines for Environmental Infection Control, 2003

Clinical Microbiology Procedures Handbook, eds:Garcia LS & Isenberg HD, ASM Press 2011

Moore G et al. J Appl Microbiol 2007;103:1090

Obee P et al. J Hosp Infect 2007;65:35

Dolan A et al. J Hosp Infect 2011;79:227

Galvin S et al. J Hosp Infect 2012;82:143

Claro T et al. AJIC 2015;43:1000



# Major Methods for Culturing Environmental Surfaces

Method	Type of Objects Sampled
Moistened swab	Irregular objects, instruments
Moistened swab & rinse (broth enrichment)	Irregular objects, instruments
Moistened wipe & rinse	Large, flat surfaces
Moistened sponge & rinse	Large flat surfaces
Direct immersion	Immerse small objects in broth Fluids, water, instruments
RODAC plates	Flat surfaces

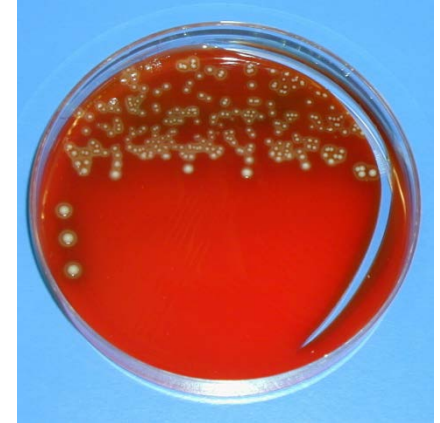
**RODAC: Replicate organism direct agar contact (or replicate organism detection and counting)**

**CDC Guidelines for Environmental Infection Control, 2003**

**Clinical Microbiology Procedures Handbook, eds: Garcia LS & Isenberg HD, ASM Press 2011**

# Moistened Swab with Direct Plating

- Use moistened swab to sample surfaces
  - If defined area not sampled; results are at best semi-quantitative
  - If a defined area is sampled using a template, results are quantitative (CFUs/cm<sup>2</sup>); preferable
- Moistening (wetting) agents include normal saline, broth media (most common), or broth containing disinfectant neutralizer(s)
- Swab is used to directly inoculate non-selective or selective media, followed by incubation x 48 hrs
- Useful for sampling irregularly shaped objects, medical equipment, hard to reach areas; HCP hands



VRE on Bedside Rail



Hand imprint culture

Lemmen SW et al. *Int J Hyg Environ Health* 2001;203:245

Duckro AN et al. *Arch Intern Med* 2005;165:302

Donskey CJ et al. *N Engl J Med* 2009;360:e3

# Moistened Swab with Direct Plating

- **Advantages:**
  - Easy to perform
  - Simple; can be used in many facilities with microbiology laboratory support, including those with limited resources
  - Can provide information about general level of contamination, or to look for specific pathogens
  - Can inoculate selective agar
- **Disadvantages:**
  - Least sensitive method for detecting organisms on surfaces
  - Non-standardized procedure makes comparison of studies difficult
  - Many factors can affect results

# Moistened Swab & Rinse Method (Broth Enrichment)

- Use moistened swab to sample surfaces
  - Swabbing defined area using template is preferred
- Swab is placed in broth (e.g., TSB or BHI), agitated, and incubated x 24 hrs; broth is plated onto non-selective or selective media, incubated x 48 hrs
  - Selective broth for *C. difficile* that does not require incubation in anaerobic conditions has been developed (Cadnum JL et al.)
- Can be used to sample irregularly shaped objects, medical equipment, hard to reach areas, HCP hands/gloves

Boyce JM et al. ICHE 1997;18:622

Mayer RA et al. Am J Infect Control 2003;31:221

Hayden MK et al. Clin Infect Dis 2006;42:1552

Morgan DJ et al. Crit Care Med 2012;40:1045

Cadnum JL et al. J Clin Microbiol 2014;52:3259

# Moistened Swab & Rinse Method (Broth Enrichment)

- **Advantages:**

- Simple, can be used in many facilities with laboratory support
- Not expensive, but requires both broth and solid media
- More sensitive than direct plating of swabs
- More sensitive than RODAC plates for detecting Gram-negative rods in some studies (Lemmen), but not others (Lerner)

- **Disadvantages:**

- Requires more laboratory processing & tech time than direct plating of swabs
- Results available 24 hr later than with direct plating
- Provides qualitative results unless broth inoculated onto agar immediately

Obee P et al. J Hosp Infect 2007;65:35

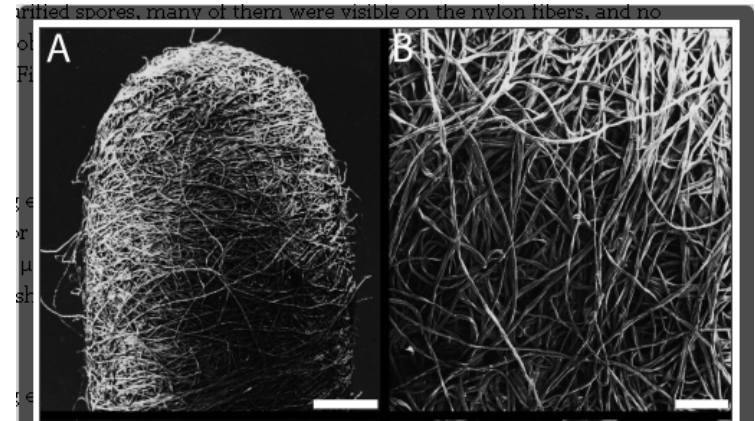
Lemmen SW et al. Int J Hyg Environ Health 2001;203:245

Lerner A et al. J Clin Microbiol 2013;51:177

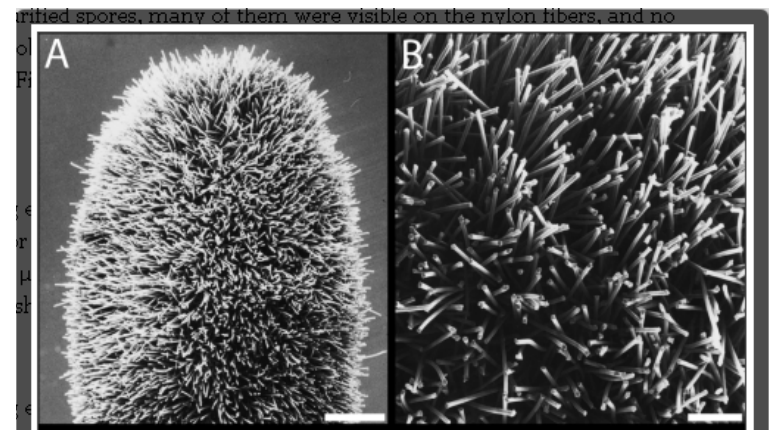
# Factors Affecting Results of Swab-Based Cultures

- **Type of swab used**
  - Cotton, rayon, dacron, flocked nylon
  - Flocked nylon picks up more than others
- **Wetting solution (presence of Tween 80 may increase yield)**
- **Is swab twirled during sampling?**
- **Swabbing pattern (swab at 90° angles)**
- **Surface area sampled**

Moore G et al. J Appl Microbiol 2007;103:1090  
Probst A et al. Appl Environ Microbiol 2010;76:5148  
Hedin G et al. J Hosp Infect 2010;75:112



Cotton Swab



Nylon-flocked swab

## Wipe-Rinse Method

- Useful for culturing large, flat, nonabsorbent surfaces
- Small (e.g., 2 cm x 2 cm) pre-moistened gauze pads or wipes are used to sample surface. Wipes are placed in broth (with or without vortexing) and incubated x 24-48 hrs, then subcultured to solid agar
- Wipes are most likely more sensitive than swab or RODAC cultures due to larger area sampled and use of broth enrichment.  
Can provide either qualitative or quantitative results
- Not used as frequently as swab, swab-rinse or RODAC methods

Al-Hamad A et al. J Hosp Infect 2008;70:328

Sethi AK et al. ICHE 2010;31:21

Attaway HH et al. Am J Infect Control 2012;40:907

Sitzlar B et al. ICHE 2013;34:459

# Moistened Cellulose Sponge – Rinse Method

- Useful for culturing relatively large surface areas (overbed tables, toilet seats, large bedside rails, floors)
- Sterile tongs or gloves are used to handle sponges without stick handle
- Sponges are put in bag with buffer, homogenized in Stomacher; effluent fluid is centrifuged, suspended in buffer, and inoculated onto agar plates, which are incubated and examined for growth



Dubberke ER et al. AJIC 2007;35:315

Boyce JM et al. ICHE 2008;29:723

Rose LJ et al. Appl Environ Microbiol 2011;77:8355

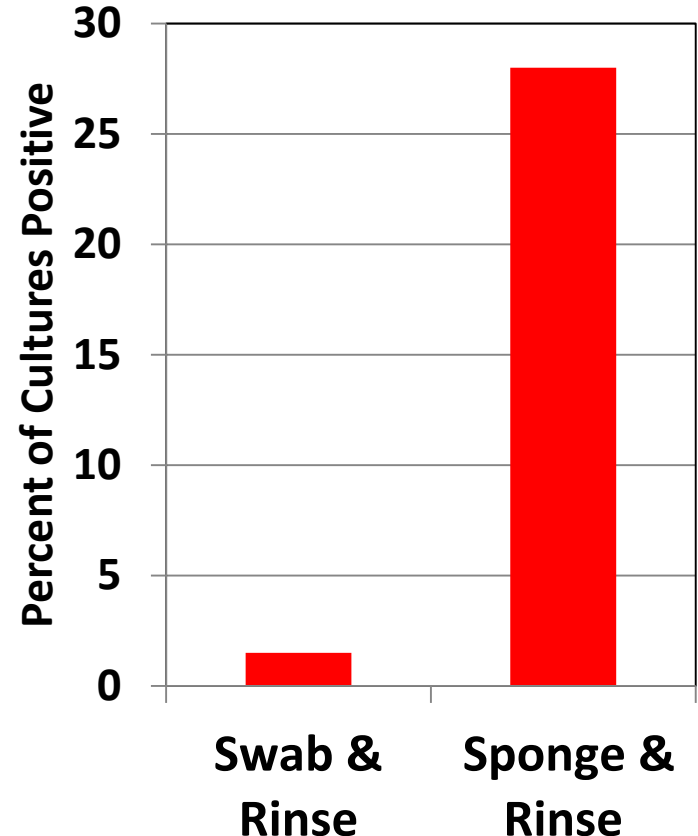
Llata E et al. Diag Microbiol Infect Dis 2011;71:72



# Moistened Cellulose Sponge – Rinse Method

- **Advantages:**
  - Can sample large areas with single sponge
  - Easier than RODAC plate for sampling irregular surfaces
  - More sensitive and yields higher colony counts than swabs due to greater surface area sampled
- **Disadvantage:**
  - Cost and availability of sponges
  - Significantly greater laboratory equipment and time required for processing

**Yield of *C. difficile*:  
Swab & Rinse vs  
Sponge & Rinse Methods**



Boyce JM et al. ICHE 2008;29:723

Otter JA et al. Am J Infect Control 2009;37:517

Rose LJ et al. Appl Environ Microbiol 2011;77:8355

## Direct Immersion in Broth

- **Direct immersion of small items or small amounts of liquid into broth media can be useful in some circumstances**
- **Examples:**
  - **Immersion of potentially contaminated disposable medical supplies into bags of broth to look for contamination of the outer surface of supply packaging**
  - **Small quantities of disinfectants or other liquid specimens have been immersed in neutralizer broth to look for contamination**
- **Sensitive, but level of contamination cannot be determined**

Otter JA et al. ICHE 2013;34:472

Gillespie JL et al. Urology 2007;69:912

# RODAC Plates (Direct Agar Contact Method)

- Small petri plate filled with agar in order to provide convex surface
- Agar surface is pressed against a flat surface, plate is incubated
- Advantages:
  - Very easy to perform; more standardized approach than others
  - Results can be expressed as CFUs/cm<sup>2</sup> (quantitative result)
  - May be preferable for detecting Gram-positive bacteria (e.g., MRSA)
  - Neutralizer – containing media (Dey-Engley) are available
- Disadvantages:
  - Greater cost; limited media available; sample small area per plate

Obee P et al. J Hosp Infect 2007;65:35

Rutala WA et al. ICHE 2010;31:1025

Galvin S et al. J Hosp Infect 2012;82:143

Anderson DJ et al. ICHE 2013;34:466

Lerner A et al. J Clin Microbiol 2013;51:177

# RODAC Plates


## Cultures of Overbed Table

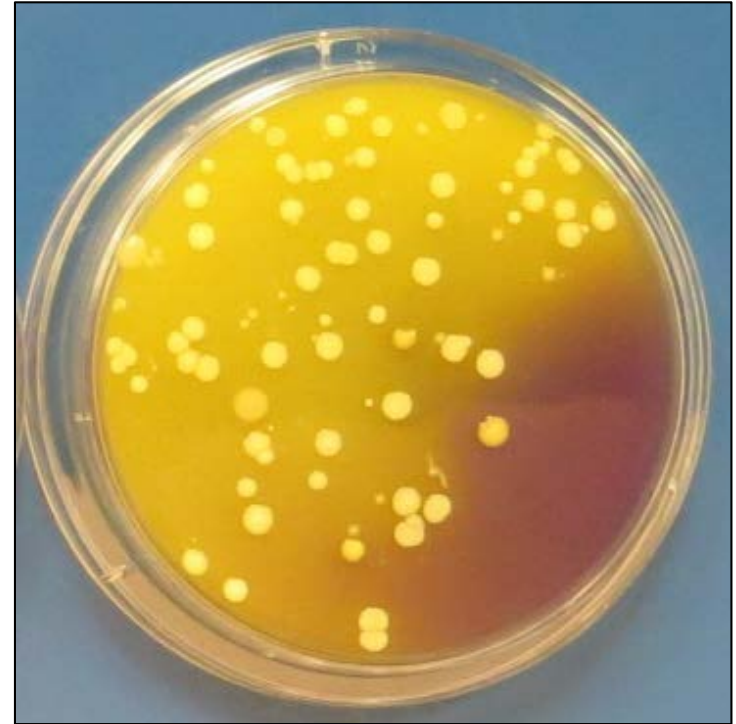


Before Cleaning

After Cleaning

# What Level of Contamination is Considered Acceptable “Clean”?

- Several authors have suggested that an aerobic colony count (ACC) < 2.5 CFUs/cm<sup>2</sup> is considered clean
  - Equivalent to < 65 CFU/plate
- Does this culture plate reflect a clean surface? 
- Should 10 CFUs/plate be given consideration as a new breakpoint?
- Further studies correlating level of surface contamination with pathogen transmission are needed



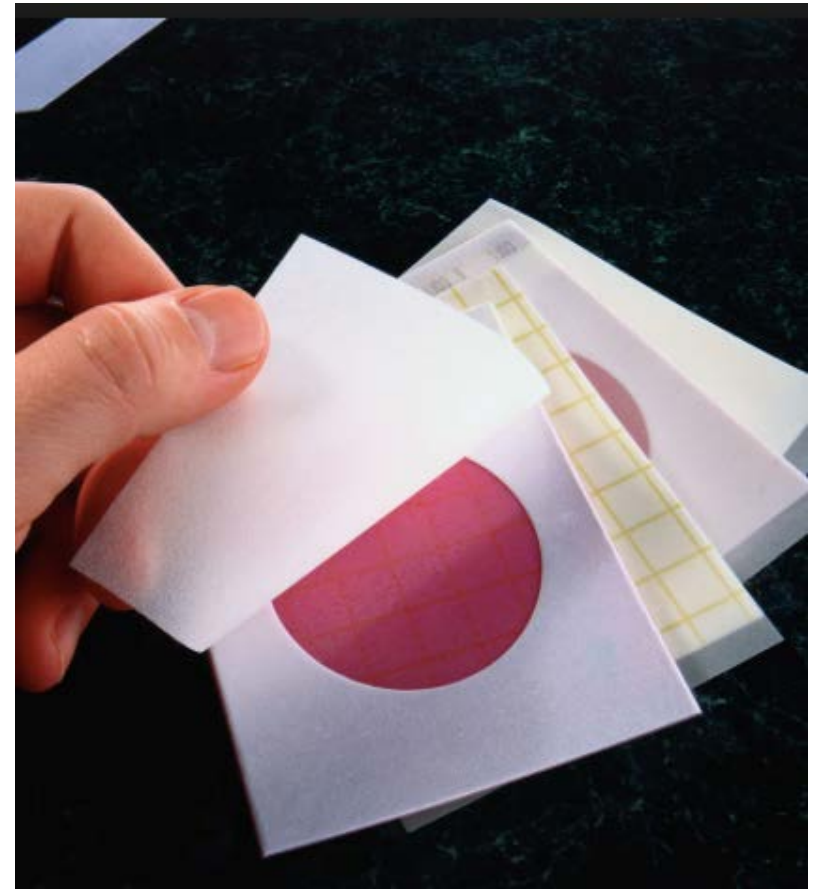
Malik RE et al. AJIC 2003;31:181

Dancer SJ J Hosp Infect 2004;56:10

Boyce JM et al. ICHE 2011;32:1187

# New Approaches to Environmental Cultures

- **RODAC contact agar plates are useful for quantifying the level of contamination, but are expensive**
- **Recent studies have utilized a method used in food industry**
- **Thin bottom film with foam barrier, a round plating surface, and thin top film to cover the agar**
- **Different agars can be used, plates are less expensive, and have been used to culture hospital surfaces**



Claro T et al. *J Clin Microbiol* 2014;52:3426

Claro T et al. *Infect Control Hosp Epidemiol* 2014;35:869

Claro T et al. *AJIC* 2015;43:1000

# Membrane Filtration Cultures for Water or Other Liquid Samples

- Moderate to large volumes of water, liquid medications, or rinses from equipment channels should be cultured using membrane filtration methods
  - Especially important if low-level bacterial contamination is likely
- Fluids are put through sterile funnel with 0.22  $\mu\text{m}$  or 0.45  $\mu\text{m}$  filter using vacuum apparatus; filters are placed on agar plates and incubated x 48 hr

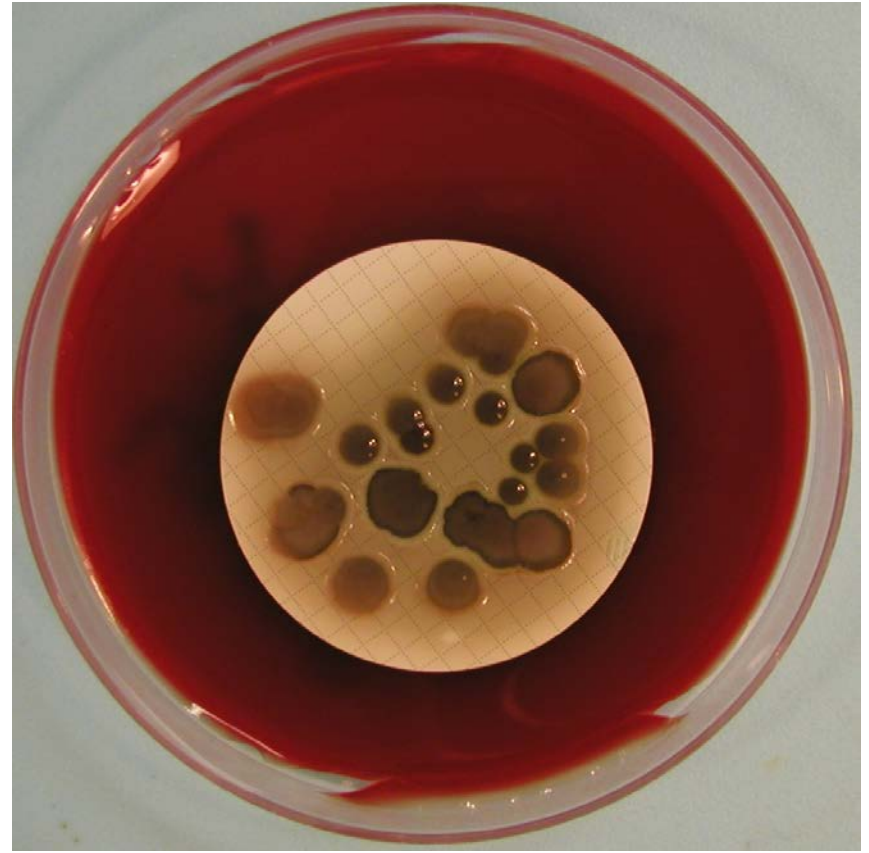
Weber DJ et al. *Am J Infect Control* 1999;27:59  
Srinivasan A et al. *N Engl J Med* 2003;348:221  
Blossom DB et al. *Arch Intern Med* 2009;169:1705  
Palamore TN et al. *ICHE* 2009;30:764  
Haupt TE et al. *ICHE* 2012;33:185  
Sax H et al. *Clin Infect Dis* 2015;61:67



## Membrane Filtration Cultures for Water or Other Liquid Samples



**Water culture from wall fountain**



**Fluid culture from bronchoscope**



# Molecular Methods

- **RT-PCR has been useful in detecting viruses (Norovirus, Rotavirus, SARS, MERS-CoV, Ebola) on surfaces**
  - Less useful for bacterial contamination
- **Advantages:**
  - Rapid turnaround time
  - Can be very sensitive
- **Disadvantages:**
  - Does not differentiate between viable and non-viable organisms
  - Cost and need for advanced laboratory resources will limit use

Ganime AC et al. *Am J Infect Control* 2012;40:544

Tuladhar E et al. *Appl Environ Microbiol* 2012;78:7769

Galvin S et al. *J Hosp Infect* 2012;82:143

Youkee D et al. *PLoS One* 2015;10:e0145167

Bin SY et L. *Clin Infect Dis* 2016;62:755

# Methods for Culturing Air

- Culturing air is often performed as part of an outbreak investigation, during construction or for research purposes
- Common methods include:
  - Use of agar “settle” plates (open lid)
  - Impaction on solid agar plates
  - Impingement of air in liquids
- Settle plates are easiest to use, and useful for culturing air for bacteria
  - Not recommended for fungal cultures
- With the exception of agar settle plates, special equipment and expertise are needed



Settle plate



Hand-held  
Air sampler



Cyclone air sampler



Anderson sieve  
volumetric air sampler

Sherertz RJ et al. *Ann Intern Med* 1996;124:539

Boswell TC et al. *J Hosp Infect* 2006;63:47

Roberts K et al. *BMC Infect Dis* 2008;8:7

Sax H et al. *Clin Infect Dis* 2015;61:67

# Methods for Culturing Air

- Results of settle plates can be expressed as number of viable bacteria/area of agar exposed/time (CFU/area/time)
- Liquid impinger or solid impactor samplers can provide data on number of particles or number of microorganisms per volume of air sampled (particles or CFU/m<sup>3</sup>)
- Volumetric sieve samplers (e.g., Anderson sampler) can differentiate respirable particles (< 5 µm) from larger particles
- Caveats:
  - Currently no uniform air quality standards for healthcare facilities
    - Lack of standards linking fungal spore levels to infection rates
  - Results may be affected by number and activity of personnel, temperature, humidity, time of day or year, and equipment used

# Methods for Assessing Cleaning Practices

- Visual inspection of surfaces
  - Check lists sometimes used
- Observation of housekeeper technique
- Fluorescent marker system
- Aerobic colony counts
- ATP bioluminescence assays

Griffith CJ et al. J Hosp Infect 2000;45:19

Cooper RA et al. Am J Infect Control 2007;35:338

Dancer SJ J Hosp Infect 2009;73:378

Luick L et al. Am J Infect Control 2013;41:751

# Visual Inspection of Surfaces

- **Simple, can be conducted in any facility**
- **Usually performed by housekeeping managers**
- **Assess surfaces to detect visible dirt/stains**
- **Problem: Surfaces that appeared clean by visual inspection often failed to pass criteria for cleanliness when tested by objective measures: aerobic colony counts or ATP bioluminescence**

**Griffith CJ et al. J Hosp Infect 2000;45:19**

**Cooper RA et al. AJIC 2007;35:338**

**Luick L et al. AJIC 2013;41:751**

# Observation of Housekeeper Technique

- **Covert or overt observation of housekeepers during routine cleaning/disinfection activities**
  - Establish variations in amount of time spent cleaning or disinfecting high-touch objects
  - Determine number of disinfectant wipes used/room
  - Detect which surfaces are not wiped adequately
  - Establish if housekeepers are allowing disinfectant to remain on surfaces for appropriate contact time

Hayden MK et al. Clin Infect Dis 2006;42:1552

Boyce JM et al. Infect Control Hosp Epidemiol 2010;31:99

Guerrero D et al. Infect Control Hosp Epidemiol 2013;34:524

# Aerobic Colony Counts Using RODAC Plates

- **Can be useful in assessing adequacy of cleaning practices**
  - Generally record aerobic colony counts, without identification
  - Agar (e.g., Dey-Engley) should contain neutralizers
  - May be most informative when looking for specific pathogens, such as *C. difficile*, VRE, MRSA, or CPE Gram-negatives
    - Selective agar, if available, facilitates pathogen identification
- **Has been used to determine the relative effectiveness of different surface disinfectants, if cultures are obtained both before and after cleaning was performed**
- **Currently, expense is a limiting factor for frequent use**

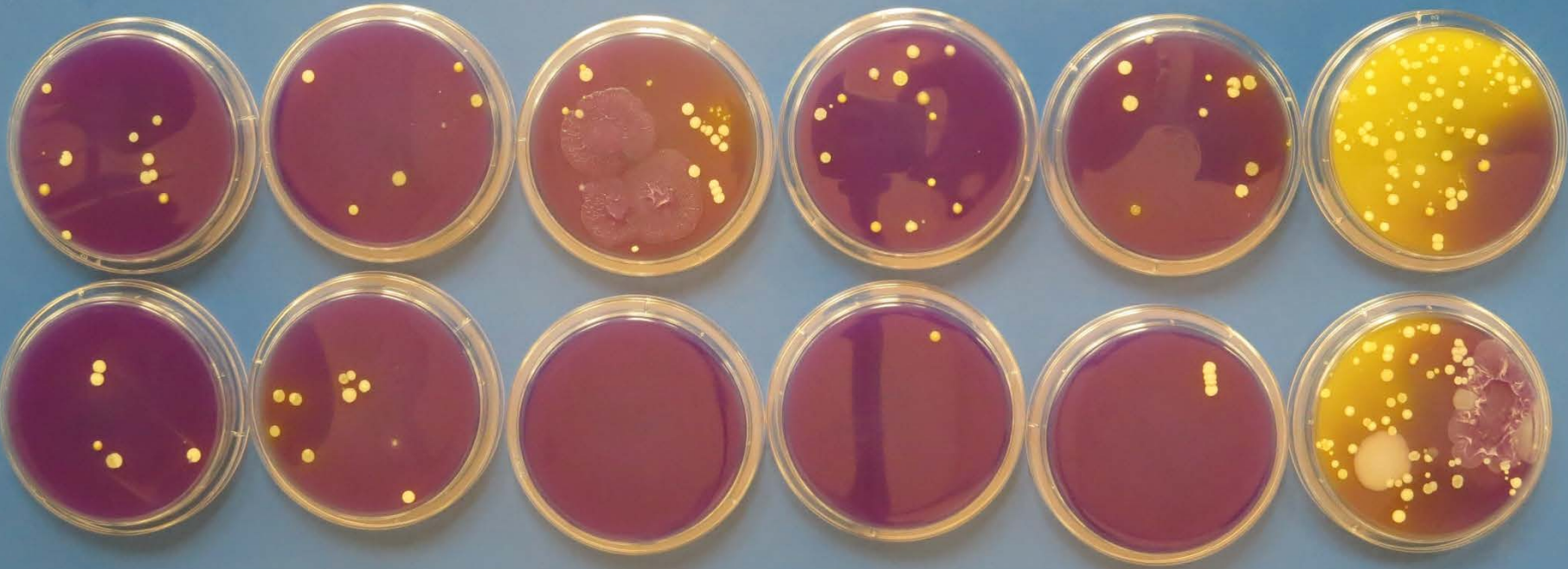
Rutala WA et al. *Infect Control Hosp Epidemiol* 2010;31:1025

Boyce JM et al. *Infect Control Hosp Epidemiol* 2011;32:1187

Lerner A et al. *J Clin Microbiol* 2013;51:177

# Post-Cleaning Cultures of Five Sites in Two Patient Rooms Cleaned with Different Disinfectants

Top Row - room cleaned with Disinfectant A



Bottom Row – room cleaned with Disinfectant B



# Improving Cleaning Practices by Using Fluorescent Marker System

- 1404 objects were evaluated before the intervention
- 744 objects were evaluated after the intervention
- Proportion of objects cleaned
  - Before intervention: 47%
  - After interventions: 76 - 92%
- Technique improved in all 3 hospitals ( $p < 0.001$ )
- This method has been used to improve cleaning practices in several larger studies



Carling PC et al. Clin Infect Dis 2006;42:385

Carling PC et al. Infect Control Hosp Epidemiol 2008;29:1

Carling PC et al. Crit Care Med 2010;38:1054

## Evaluating Cleaning Measures in an ICU Using Fluorescent Marker System

- **Prospective study of the impact of cleaning interventions on environmental contamination by MRSA and VRE**
- **Intervention consisted of**
  - Change from use of pour bottles to bucket immersion of cleaning cloths
  - Educational campaign for housekeepers
  - Feedback regarding adequacy of terminal room cleaning
- **15 surfaces in rooms were marked with a fluorescent dye, and 6 surfaces in patient rooms were cultured for MRSA and VRE**
- **Results:**
  - Removal of fluorescent dye occurred on
    - 44% of surfaces during baseline period
    - 71% of surfaces during intervention period
  - Cultures (+) for MRSA or VRE decreased from 45% at baseline to 27%

# **Monitoring Hospital Cleanliness Using ATP Bioluminescence Assays**

- **ATP bioluminescence assays have been used to monitor cleanliness of surfaces in hospitals**
  - **Daily cleaning or terminal cleaning**
  - **Assess variations in housekeeper performance**

**Griffith CL et al. J Hosp Infect 2000;45:19**

**Malik RE et al. AJIC 2003;31:181**

**Cooper RA et al. AJIC 2007;35:338**

**Lewis T et al. J Hosp Infect 2008;69:156**

**Boyce JM et al. Infect Control Hosp Epidemiol 2009;30:678**

**Boyce JM et al. Infect Control Hosp Epidemiol 2010;31:99**

**Moore G et al. AJIC 2010;38:617**

**Havill NL et al. AJIC 2011;39:602**

**Anderson RE et al. J Hosp Infect 2011;78:178**

# ATP Bioluminescence Method



**Step 1**

**Use special swab  
to sample surface**



**Step 2**

**Place swab in  
reaction tube**



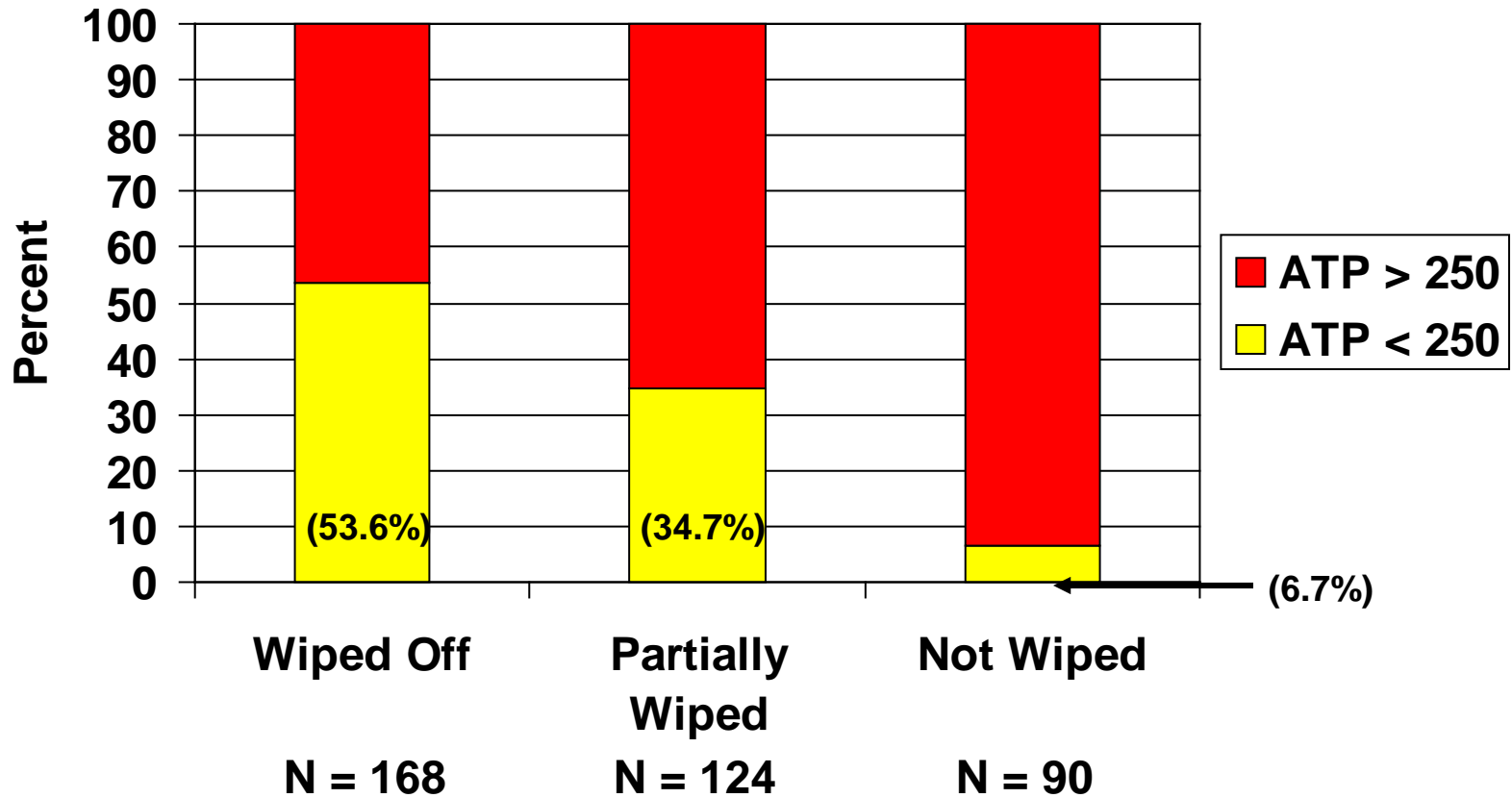
**Step 3**

**Place tube in luminometer  
Results: Relative Light Units**

# Assessing Terminal Cleaning Practices Using 3 Methods

- **Prospective study to compare how many surfaces would be considered clean, based on**
  - **Aerobic colony counts obtained by agar contact plates**
  - **Fluorescent marker method**
  - **ATP bioluminescence assay system**
- **5 high-touch surfaces were sampled in a convenience sample of 100 hospital rooms**
- **Adjacent surfaces on 5 high-touch surfaces were sampled before and after terminal cleaning**

# 382 High-Touch Surfaces Classified as Not Clean Before Terminal Cleaning, Results for Fluorescent Marker and ATP



## Summary

- Cultures of the environmental surfaces in hospitals should be coordinated by infection preventionists, as part of outbreak investigation or monitoring of cleaning/disinfection practices
- Using moistened swabs with direct plating of solid agar is easy to perform, yields useful semi-quantitative results, but is the least sensitive method for detecting microorganisms
- Moistened swabs & rinse (broth enrichment) method is more sensitive than direct plating
  - Will detect lower levels of bacterial contamination
  - Yields qualitative results due to incubation of broth before plating

## Summary

- **Wipe-rinse and sponge-rinse methods are useful for sampling larger areas, and are more sensitive than swab-based methods due to larger area sampled**
  - **Require more laboratory equipment and processing than swabs**
- **Culturing flat surfaces using RODAC plates is easy to perform, samples a defined area, and provides quantitative results**
  - **Currently the more standardized approach to quantifying levels of bacterial contamination of surfaces**
  - **Preferable to use neutralizer-containing (D/E) plates if residual disinfectant is likely to be on surfaces**



# Summary

- **Moderate to large volumes of water or other liquid samples should be cultured using membrane filtration methods**
  - Also true for smaller volumes when low-level bacterial contamination is suspected
- **Culturing of air samples in healthcare is somewhat controversial given the lack of standards for indoor air quality in hospitals, and the special expertise and equipment required**
  - Useful for investigation of suspected airborne transmission (especially of fungal disease), during construction, and perhaps monitoring air quality during surgical procedures (implant surgery)

# Summary

- **Monitoring the effectiveness of cleaning/disinfection practices in healthcare settings is recommended**
- **Useful approaches include:**
  - **Fluorescent marker methods**
  - **ATP bioluminescence assays**
  - **Aerobic colony counts or culture for specific pathogens**
- **Some facilities have found used a combination of these methods**

# Credits

**Thanks to Nancy L Havill, MT who performed the environmental cultures reported by our group**

## **Correlation Between Aerobic Colony Counts and ATP Bioluminescence Assays**

<b>Author</b>	<b># Samples Taken</b>	<b>Statistical Method</b>	<b>Correlation</b>
Poulis JA	378	Linear regression	R < 0.4
Aycicek H	280	Coeff of Kappa	K = 0.249, p <.001
Willis C	108	Correlation (?method)	R = 0.15
Boyce JM	100	Spearman correlation	R = 0.36-0.65, p <.001 - .024
Boyce JM	1000	Mixed model ANCOVA	R = 0.03, p = .76
Shama G	Not stated	Coeff. of variation	R <sup>2</sup> = 0.078
Sciortino CV	Not clear	Pearson correlation	R = - 0.036 – 0.218

**Note: studies were conducted in different settings and with different ATP assays**

**Poulis JA et al. Int J Food Microbiol 1993;20:109**

**Aycicek H et al. Int J Hyg Environ Health 2006;209:203**

**Willis C et al. Br J Infect Control 2007;8:17**

**Boyce JM et al. Infect Control Hosp Epidemiol 2009;30:678**

**Boyce JM et al. Infect Control Hosp Epidemiol 2011;32:1187**

**Shama G et al. Int J Hyg Environ Health 2013;216:115**

**Sciortino CV et al. AM J Infect Control 2012;40:e233**