Microbiologic Sampling of the Environment

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1

Microbiologic Sampling of the Environment

- Lecture Goals
  - Microbiologic Sampling
    - Indications
    - Methods

2

Microbiologic Sampling of the Environment

- History
  - Pre-1970, hospitals regularly cultured air and surfaces (random, undirected sampling)
  - By 1970, AHA advocated discontinuation because HAI not associated with levels of microbes in the air and surfaces; not cost-effective
  - In 1981, CDC recommended targeted sampling (eg, sterilizers and dialysis water)

3

MICROBIOLOGIC SAMPLING OF THE ENVIRONMENT

- Do not conduct random microbiological sampling of air, water, and surfaces (IB)
- When indicated, conduct microbiologic sampling as part of an epidemiologic investigation (IB)
- Limit microbiologic sampling for QA to: biological monitoring, dialysis water, or evaluation of infection control measures (IB)

4

Environmental Sampling

- The only routine microbiologic sampling recommended as part of quality assurance program is:
  - Biological monitoring of sterilization process by using bacterial spores (e.g., steam sterilizers should be monitored at least once per week with commercial preparation of *Gs* spores)
  - Monthly cultures of water used in hemodialysis applications (e.g., water <200mo/ml, and dialysate at the end of dialysis <2,000mo/ml)

5

Microbiologic Sampling of the Environment CDC Guidelines for EIC, 2003

- Targeted microbiological sampling. Indications for microbiologic sampling of air, water and inanimate surfaces
  - Support of an investigation of an outbreak when environmental reservoirs or fomites are implicated epidemiologically in disease transmission
  - Research
  - Monitor a potentially hazardous environmental condition, confirm presence of biological agent, and validate successful abatement
  - Quality assurance to evaluate the effects of a change in infection control practice or ensure equipment performs according to expected outcomes

6

MICROBIOLOGIC SAMPLING OF THE ENVIRONMENT

- Select a high-volume sampler if level of microbial contamination are expected to be low (II)
- When sampling water, choose media and incubation temperature to facilitate recovery (II)
- When conducting environmental sampling, document departures from standard methods (II)

7

Microbiologic Sampling of the Environment
Justification

- Will environmental sampling provide meaningful, interpretable, and actionable data that help identify actual or potential contamination problems associated with a specific procedure or instrument
- Should not be done if no plan for interpreting and acting on the results obtained
- Is it justified on epidemiological grounds
- No accepted criteria for defining surfaces or air as clean/safe in healthcare

8

Microbiologic Sampling of the Environment
Investigation of an Outbreak

- When?
  - Environmental reservoirs or fomites are implicated epidemiologically in disease transmission (e.g., bronchoscopy)
  - Plan for interpreting and acting on the results
  - Plan to link microorganisms from the environment with clinical isolates by molecular epidemiology

9

Microbiologic Sampling of the Environment
Investigation of an Outbreak

Rutala et al. J. Thorac. Cardiovasc. Surg. 96:157-161.

- Outbreak: two patients in CT-ICU with symptomatic *B. cepacia*
- Epidemiologic investigation: case-control study revealed that both patients required an intra-aortic balloon pump (IABP) for circulatory support
- Microbiological investigation: water reservoir of IABP contained >10^5 *B. cepacia*/ml

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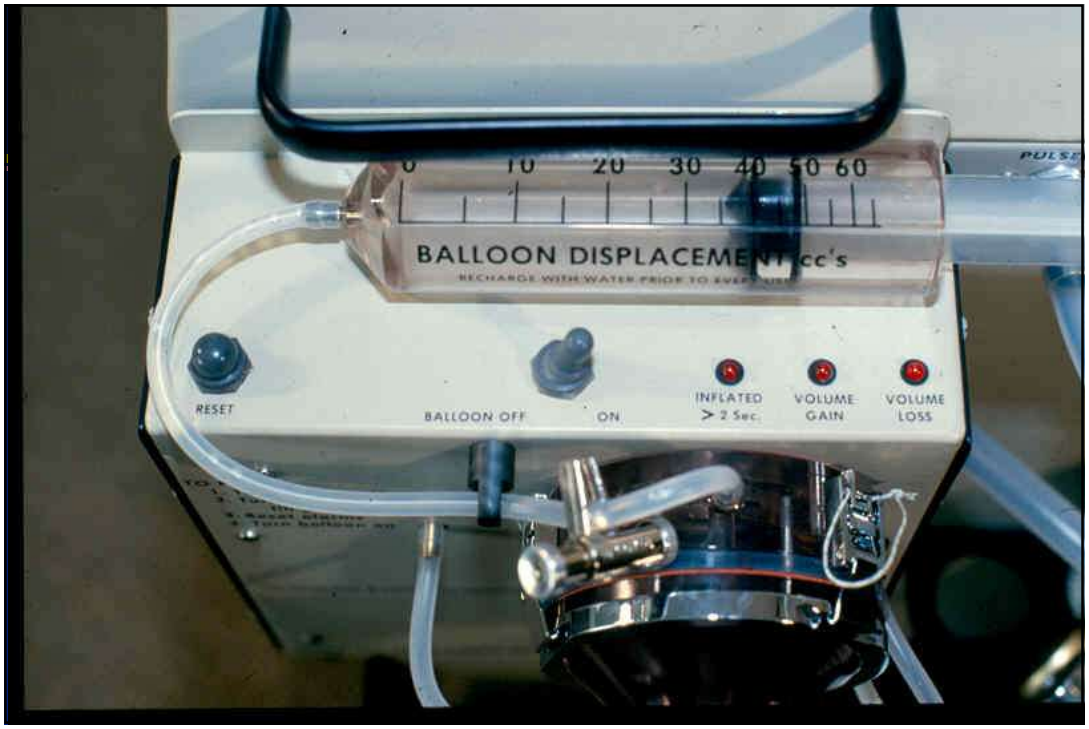
11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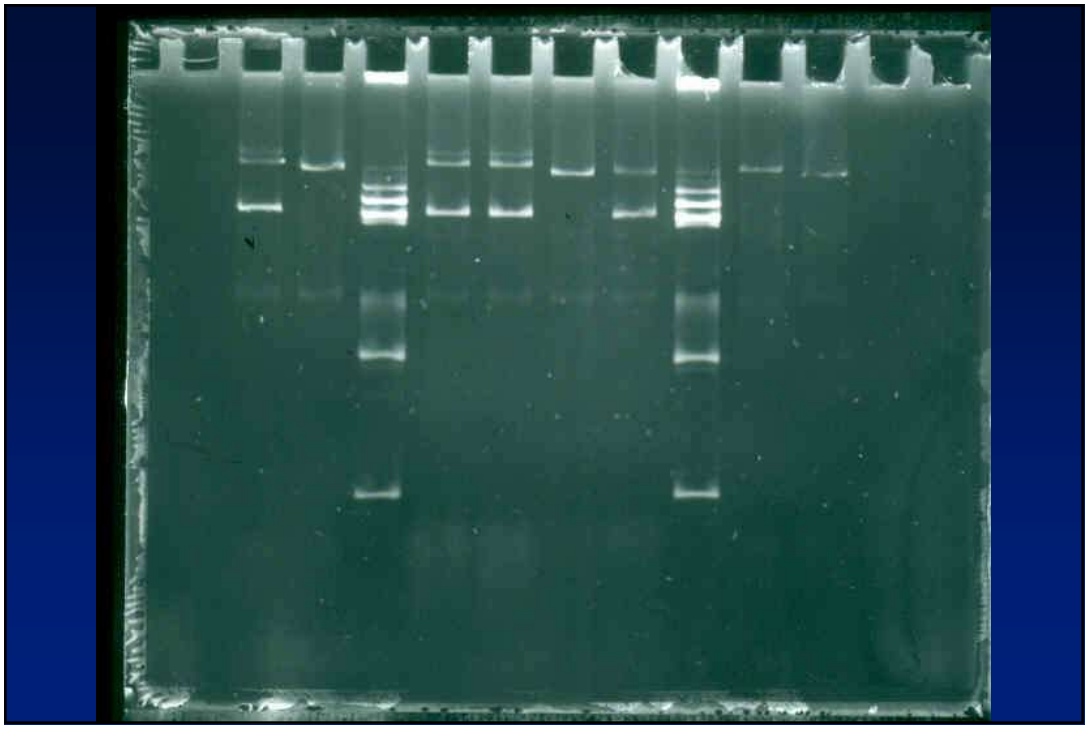
Microbiologic Sampling of the Environment Investigation of an Outbreak

Rutala et al. J. Thorac. Cardiovasc. Surg. 96:157-161

- Microbiologic investigation: causative organism isolated from several components of the IABP and the hands of a nurse who manipulated the IABP's buttons/switches.
- Molecular epidemiology: similar plasmid profile from strains from the patients and the IABP.
- Conclusion: transmission presumably occurred during manipulation of IV lines.

13



14

OVERVIEW OF *M. CHIMAERA* OUTBREAK

- July 2015: Invasive *M. chimaera* reported in 6 patients who underwent cardiac surgery with implants, 2008-2012, at one hospital in Zurich, Switzerland
- Investigations revealed *M. chimaera* in the water tanks of heater-cooler units (HCU); air sampling also positive for *M. chimaera* when the units were running
- Additional cases confirmed in several European countries and in US
- Studies suggest NTM from the HCU aerosolized from contaminated water in the device into the air
- Risk of disease not entirely clear
  - 0.39 cases per 10,000 person-years (5-year risk){Chand M, et al. CID)
  - If hospital has had 1 case, patient risk between 0.1% and 1% (CDC)
  - Risk higher if prosthetic material implanted
- Impact of outbreak: >250,000 cardiac bypass procedures done each year in US using HCU (CDC 2016).

15

SOURCE OF *M. CHIMAERA* OUTBREAK

- Point-source contamination of 3T HCU suggested by 2 studies
  - Europe: *M. chimaera* isolates from 5 patients, 3T HCU from 3 different countries and from new 3T HCU and environment at manufacturer facility – identical by sequencing (typing unpublished – preliminary)
  - US: *M. chimaera* isolates from 11 patients and 5 3T HCU from PA and Iowa were the same by whole genome sequencing
- Manufacturing facility added disinfection and active drying procedures to production line in Sept 2014 due to *M. chimaera* contamination

Contamination during production of heater-cooler units by *Mycobacterium chimaera* potential cause for invasive cardiovascular infections: results of an outbreak investigation in Germany, April 2015 to February 2016

Mycobacterium chimaera Contamination of Heater-Cooler Devices Used in Cardiac Surgery — United States

Haller S, et al. Euro Surveill 2016;21(17), April 28 Perkins KM, et al. MMWR 2016;65:1117

16

Microbiologic Sampling of the Environment Research

- When? Experimental methods that provide new information about the spread of HAIs
- Example: Relation of the Inanimate Hospital Environment to Endemic Nosocomial Infection (NEJM 1982;302:1562).
- Cultured air, surfaces, and fomites in old/new hospital and despite major differences in contamination (17% positive vs 5%), incidence of NI remained unchanged.

17

Long-Term Care Facilities Environmental Surfaces

Rutala et al. ICHE. In press

	Resident Rooms			*Community Rooms*			*Overall Total*		
Pathogen Identified	Number of Positive Rodac with EIP	EIP Total Counts on Positive Rodacs	EIP Counts per Positive Rodac	Number of Positive Rodac with EIP	EIP Total Counts on Positive Rodacs	EIP Counts per Positive Rodac	Number of Positive Rodac with EIP	EIP Total Counts on Positive Rodacs	EIP Counts per Positive Rodac
C. difficile	34	856	25.18	5	7	1.40	39	863	22.13
MRSA	51	2998	58.78	15	101	6.73	66	3099	46.95
VRE	1	1	1.00	1	7	7.00	2	8	4.00
MDR GNR	10	43	4.30	7	144	20.57	17	187	11.00

18

Terminal Room Disinfection

	No UV-C	UV-C
Quat*	A	B
Bleach	C	D

**NOTE: Bleach always used in rooms of patients with suspected or confirmed C. difficile*

19

Definitions and Inclusion Criteria

Patient in "Seed Room"

Documented infection or colonization with
MRSA VRE
C. difficile
MDR-Acinetobacter

"TARGET MDROs"

Terminal Clean

"Exposed" Patient

In room ≥ 24 hours

Exposure days = Time spent in "seed room"

Potential "Incident Case"

Same organism as the patient in the "seed room" ***AND***

Positive culture while in room
OR

Positive culture after stay in room
- 90 days (MRSA, VRE, MDRAB)
- 28 days (*C. difficile*)

20

Enhanced Disinfection Leading to Reduction of Microbial Contamination and a Decrease in Patient Col/Infection

Anderson et al. Lancet 2017;289:805; Rutala et al. ICHE 2018

	Standard Method	Enhanced method		
	Quat	Quat/UV	Bleach	Bleach/UV
EIP (mean CFU per room)[a]	60.8	3.4	11.7	6.3
Reduction (%)		94	81	90
Colonization/Infection (rate)[a]	2.3	1.5	1.9	2.2
Reduction (%)		35	17	4

All enhanced disinfection technologies were significantly superior to Quat alone in reducing EIPs. Comparing the best strategy with the worst strategy (i.e., Quat vs Quat/UV) revealed that a reduction of 94% in EIP (60.8 vs 3.4) led to a 35% decrease in colonization/infection (2.3% vs 1.5%). Our data demonstrated that a decrease in room contamination was associated with a decrease in patient colonization/infection. First study which quantitatively described the entire pathway whereby improved disinfection decreases microbial contamination which in-turn reduced patient colonization/infection.

21

Objective and Design

- To determine if enhanced methods for terminal room disinfection decrease acquisition and infection due to multidrug-resistant organisms (MDROs)
- Prospective, multicenter, cluster-randomized, crossover trial to evaluate three strategies for enhanced terminal room disinfection
  - 9 hospitals
  - Randomization at level of hospital
  - 2x2 factorial design

22

Microbiologic Sampling of the Environment
Monitor a Potentially Hazardous Environmental Condition

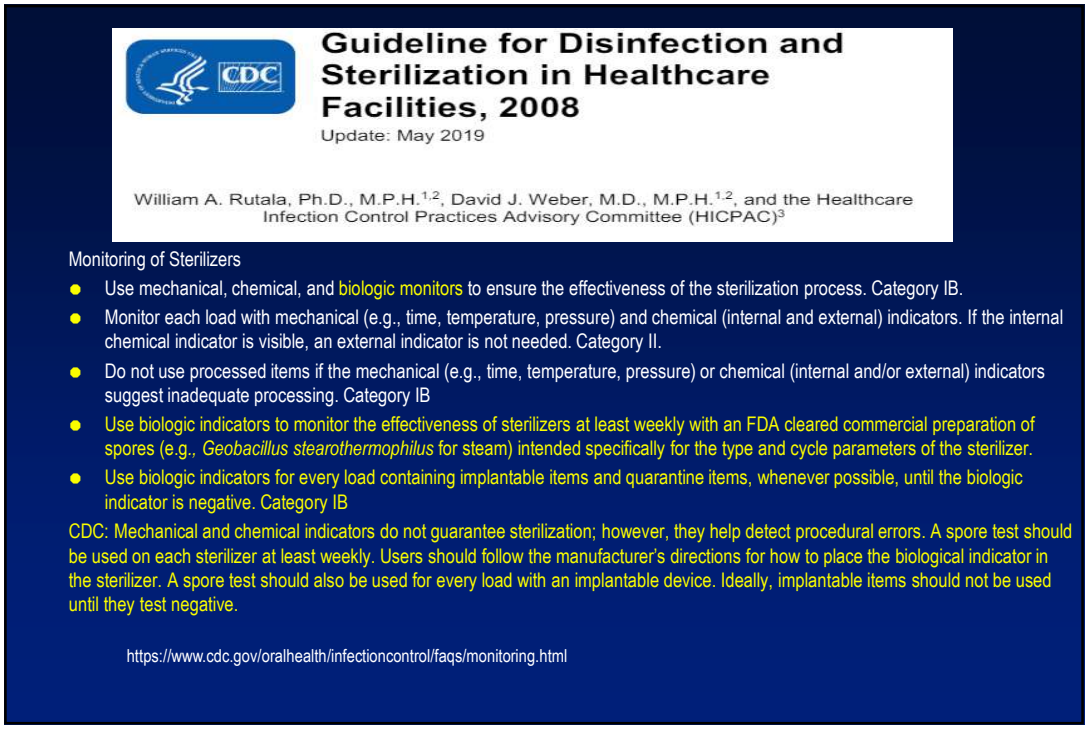
- When? Confirm the presence of a hazardous chemical/biological agent, and validate abatement of the hazard
  - Examples
    - Detect bioaerosols (eg, ultrasonic cleaner, water fountain-*Legionella*)
    - Detect agent of bioterrorism
    - Sample for industrial hygiene (eg, sick building)

23

Microbiologic Sampling of the Environment
Quality Assurance

- When? To evaluate the effects of a change in infection control practice or ensure equipment/systems perform as expected
  - Air sampling during construction/renovation to qualitatively detect breaks in infection control measures (e.g., OR)
  - Only routine sampling recommended: biological monitoring of sterilizers, monthly cultures of water used in hemodialysis
  - Endoscopes

24



25

Microbiologic Sampling of the Environment
Air Sampling

- General comments
  - Particles in a biological aerosol usually vary from <1 to >50 µm.
  - Particles consist of a single, unattached organism or clumps
  - Vegetative cells do not ordinarily survive long in air
  - Pathogens may settle on surfaces and become airborne again with sweeping, etc

26

Microbiologic Sampling of the Environment
Air Sampling

- Air sampling for QA is problematic due to the lack of uniform air quality standards
- The critical number of *Aspergillus* that poses a risk for neutropenic patients is not known
- Results affected by factors (traffic, time of year)
- Results need to be compared to other defined areas

27

Air Sampler

28

Methods for Culturing Air
J Boyce 2012

- 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

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


Settle plate


Hand-held
Air sampler


Cyclone air sampler

Anderson sieve
volumetric air sampler

29

Air Sampling
J Boyce 2012

- Settle plates can be expressed as number of bacteria in an area (e.g., patient room) for a specified time (e.g., 1 hour)
- Liquid impingers can provide data on the number of particles/microbes per volume of air sampled (e.g., 100 CFU/20 ft^3)
- Volumetric Sieve samplers (e.g., Anderson-stage 1 8µm and above, stage 2-0.8 to 8.0µm) can size particles and sample specific volume (e.g., 20 CFU of respirable particles/20 ft^3)

30

Microbiologic Sampling of the Environment
Air Sampling

- Factors in Selecting an Air Sampling Device
  - Viability and type of organism
  - Skill required to operate sampler
  - Availability and cost of sampler
  - Availability of auxiliary equipment (vacuum pump)
  - Assumed concentration and particle size
  - Sensitivity of microorganisms to sampling
  - Compatibility with the selected method of analysis

31

Microbiologic Sampling of the Environment
Air Sampling

- **Impingement in liquids**
- **Impaction on solid surfaces**
- **Sedimentation**
- Filtration
- Centrifugation
- Electrostatic precipitation
- Thermal precipitation

32

Microbiologic Sampling of the Environment
Air Sampling

- Impingement in liquids-collects (mo directed against a liquid [nutrient broth], conc over time)

  Ex. Water aerosols for *Legionella*
- Impaction on solid surfaces (sieve)-collects (mo deposited on agar), sizes, conc per unit volume of air (CFU/ft^3). Ex. *Aspergillus*
- Sedimentation (settle plates)-mo settle on agar via gravity, conc over time (CFU/time)

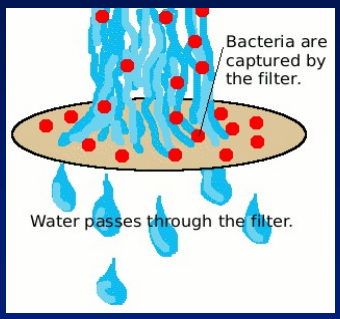
33

Microbiologic Sampling of the Environment
Water Sampling

- When? Routine testing of water not indicated (except dialysis) but sampling in support of outbreak investigation can help determine infection control measures
- Use established methods (eg, sample water ASAP after collection, 100ml minimum, sterile collection equipment, neutralizers, recovery media and incubation temp [diluted peptone, 30^oC], pour plates [high counts], membrane filtration-0.2µ [low counts, larger volumes])
- Filters are placed on agar plates and incubated for 48h

34

Filtering



35

Microbiologic Sampling of the Environment
Surface Sampling Methods

- Sample/Rinse-use sterile wipe/sponge/swab, media, qualitative/quantitative assays
- Direct Immersion-immerse in media, then assay
- Containment-interior surfaces of containers
- RODAC (replicate organism detection and counting)-sampling flat, nonabsorbent surfaces, direct assay

36

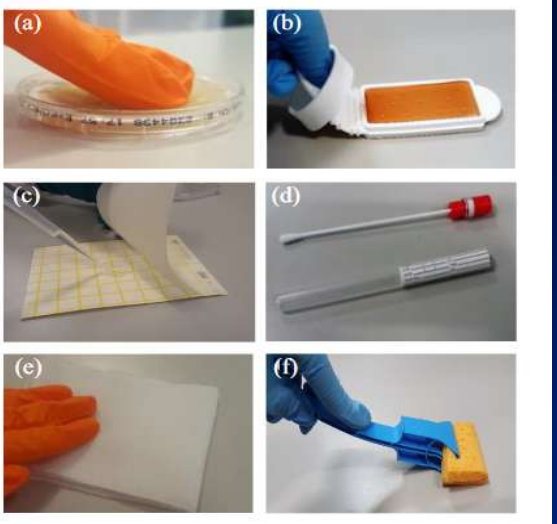


Figure 2. Devices most commonly used for the collection of microbiological samples from surfaces in the publications included in this review: (a) contact plate, 24%; (b) dipslide, 6%; (c) petrifilm, 3%; (d) swab, 53%; (e) sponge, 9%; and (f) wipe/gauze, 5%.

37

Rawlinson
JHI 2019;103:363

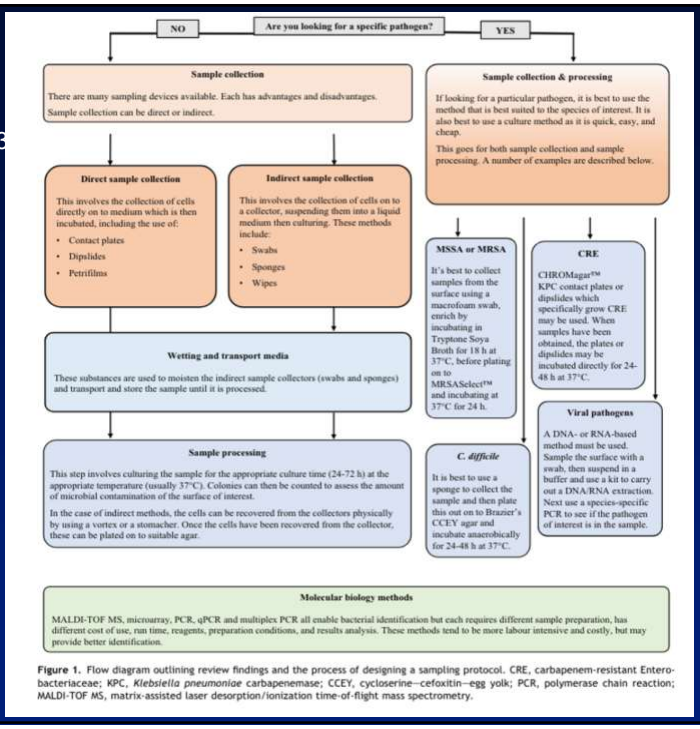
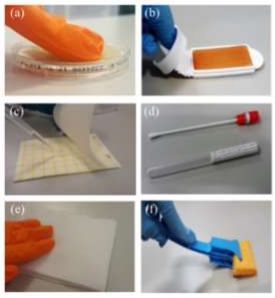


Figure 1. Flow diagram outlining review findings and the process of designing a sampling protocol. CRE, carbapenem-resistant Enterobacteriaceae; KPC, *Klebsiella pneumoniae* carbapenemase; CCEY, cycloserine–cefoxitin–egg yolk; PCR, polymerase chain reaction; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

38



How to carry out microbiological sampling of healthcare environment surfaces? A review of current evidence

Rawlinson S, et al.
JHI 2019;103:363-374

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Suitability of sampling method for different surface condition and target organism

	Contact plate	Dipslide	Petrifilm	Swab	Sponge
Wet surface			+	+[a]	
Dry surface	+		+		
Flat surface	+	+			+
Uneven surface	–	+	+	+	+
High bioburden	–			+	
Low bioburden	+	+	+		+
Injured cells				+	+
S. aureus and MRSA	+		+		
C. difficile					+
Gram negative bacteria				+	
Viruses	–	–	–	+	–

MRSA, meticillin-resistant *Staphylococcus aureus*.
[a] Cotton, rayon, polyester or macrofoam. Brush-textured swabs perform poorly on wet surfaces. Empty cells indicate lack of data.

39

Methods for Culturing Surfaces

CDC, 2003; Boyce, 2012

- Moistened swab (with template ideal)
- Moistened swab and rinse (broth enrichment)
- Moistened sponge and rinse
- Moistened wipe and rinse
- Direct Immersion
- RODAC plates

- Irregular objects
- Irregular objects
- Large, flat surfaces
- Large, flat surfaces
- Immerse in broth
- Flat surfaces

40

Table II
Factors causing variation in sampling efficiencies and recoveries

Factors affecting organism recovery	Details	References
Target organism and strain	Different sampling techniques recover different species with varying success. Different strains of the same organism can recover differently, even with the same technique.	[13,16], [19] [a], [25,26,45], [49] [a], [51,52]
Level of contamination	Some sampling techniques are not appropriate for surfaces with a high bioburden. For highly contaminated surfaces, sponges were significantly better for recovering *C. difficile* ($P < 0.05$) than contact plates. Contact plates may also show confluent growth leading to inaccurate counts.	[23], [30] [a], [44], [46] [a], [51]
Wet/dry surface	Cotton swabs recovered significantly more colonies than other swabs from a wet surface. Brush textured swabs performed poorly. 3M Enviroswabs gave better recovery on some surface types.	[21,44,53]
Adsorption of cells	Adsorbed cells are best recovered with direct contact methods such as contact plates and dipslides.	[13,15,24,27,44,54]
Pressure and contact time	Insufficient pressure will not recover all organisms from the surface, and contact time of 10 s must be adhered to for maximum recovery.	[13,23,28], [46] [a], [53]
Surface material and topography	Smoother surfaces are generally easiest to recover from. Some sampling devices are inappropriate for uneven or rough surfaces, such as contact plates. Some methods are more suitable for smaller and uneven areas such as swabs.	[13,14,16,18,22], [30] [a], [51,53,54]
Media	Different types of media recover different organisms and can inhibit growth of others. Target organism and potential surface bioburden must be considered before selection.	[15], [19] [a]
Pre-wetting, enrichment, transport medium and post-test processing	Wetting solutions and diluents can either aid or hinder recovery, depending on the target organism. Choice of transport medium is important [73] and the choice should vary between the target organism, time taken to transport to the lab, and post-test storage conditions and storage time. Most losses occur during processing, such as vortexing.	[17,21,22,24,26], [28–30[a]], [44,48], [49] [a]
Brand	Cherwell contact plates were shown to give better recoveries than Oxoid or bioMérieux, with significantly better recovery for *S. epidermidis*	[13]
Cell injury and environmental stressors	Uninjured cells recover better than injured or stressed cells. Sponges were shown to potentially recover injured *L. monocytogenes* from a steel surface, though to no statistical significance.	[15,17,45,54,55]
Size of surface sampled	If a large surface area is to be sampled, the method choice should reflect this. Sponges and roller-devices can easily sample large surface areas.	[24,25], [30] [a], [46] [a], [49] [a]
No. of samples	Time of processing may make some methods less suitable.	[56] [a], [57] [a]
Technician time and skill	Some methods, such as contact plates, allow fast sampling and easy interpretation, and require less training. Other techniques, such as swabs, can have variability in method between technician and require some skill to allow proper sample recovery.	[26]
Cost	Some sampling techniques, while giving better recoveries, may not be used in favour for sampling equipment that is cheaper or more readily available in the clinical environment.	[17], [30] [a], [45], [47] [a], [58]
Sensitivity	More sensitive methods will give truer results. Macrofoam swabs gave the best sensitivity for MRSA over contact plates and swabs, needing the lowest concentration to give a positive result. Dipslides were the most sensitive for adsorbed cells.	[14,15], [30] [a], [44], [46] [a], [51,52]
Hospital or ward speciality	There is a difference in contamination found between wards and ward type (general or specialist). Rooms with infected or colonized patients show increased recovery of the same organism.	[49] [a], [56] [a], [59], [60] [a]

41

Factors Affecting Organism Recovery

Rawlinson et al. J Hosp Infect 2019;103:363

- Target organism and strain
- Level of contamination
- Wet/dry surface
- Adsorption of cells
- Pressure and contact time
- Media
- Pre-wetting, enrichment
- Brand on contact plates
- Cell injury/stressors
- Size of surface sampled
- Number of samples
- Cost
- Sensitivity
- Difference in contamination

42

Moistened Swab with Direct Plating
Boyce, 2012

- Use moistened swab to sample surfaces
  - If defined area not sampled, results are semi-quantitative
  - If defined area sampled using a template, results are quantitative ($CFUs/cm^2$); preferable
- Moistening (wetting) agents include normal saline, broth media (neutralizers)
- Swab is used to directly inoculate non-selective or selective media, followed by incubation x 48h
- Use for sampling irregular-shaped objects

43

Moistened Swab with Direct Plating
Boyce, 2012

- Advantages
  - Easy to perform
  - Simple; can be used in many facilities with microbiology laboratory support
  - Provides information about general level of contamination or for specific pathogens
- Disadvantages
  - Least sensitive method for detecting or organisms on surfaces
  - Non-standardized procedure makes comparison of studies difficult

44

RODAC Plates

Boyce, 2012

- Small petri plate filled with agar to provide convex surfaces
- Agar plate is pressed against a flat surface, plate is incubated
- Advantages: very easy to perform and standardized; results expressed as CFU/cm^2 (suggested clean 2.5 CFU/cm^2 or 65CFU/Rodac); neutralizer available
- Disadvantages: greater cost; sample small area per plate

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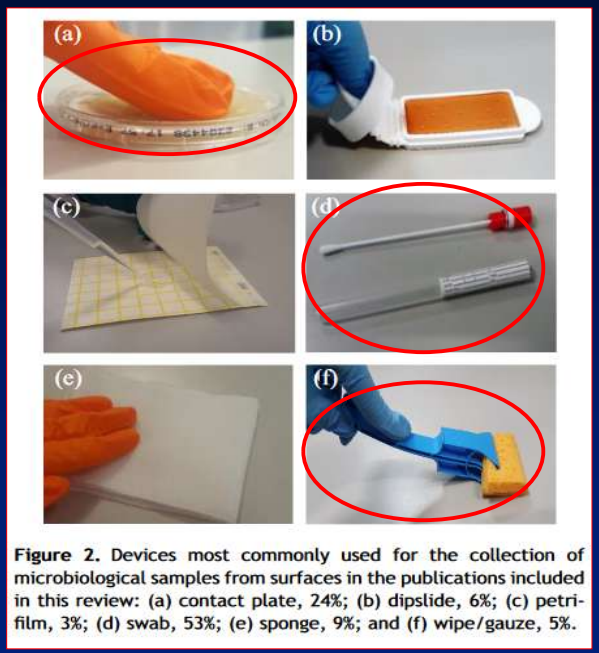

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46

A Comparison of Four Different Sampling Methods Used to Recover Bacterial Contamination from Environmental Surfaces

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Introduction

- We hypothesized that tools for sampling environmental surfaces with the largest surface area would be the most efficient at recovering bacteria.
- To test this hypothesis, we evaluated four different sampling methods to determine which was most effective at recovering bacteria from common environmental surfaces.

Materials & Methods

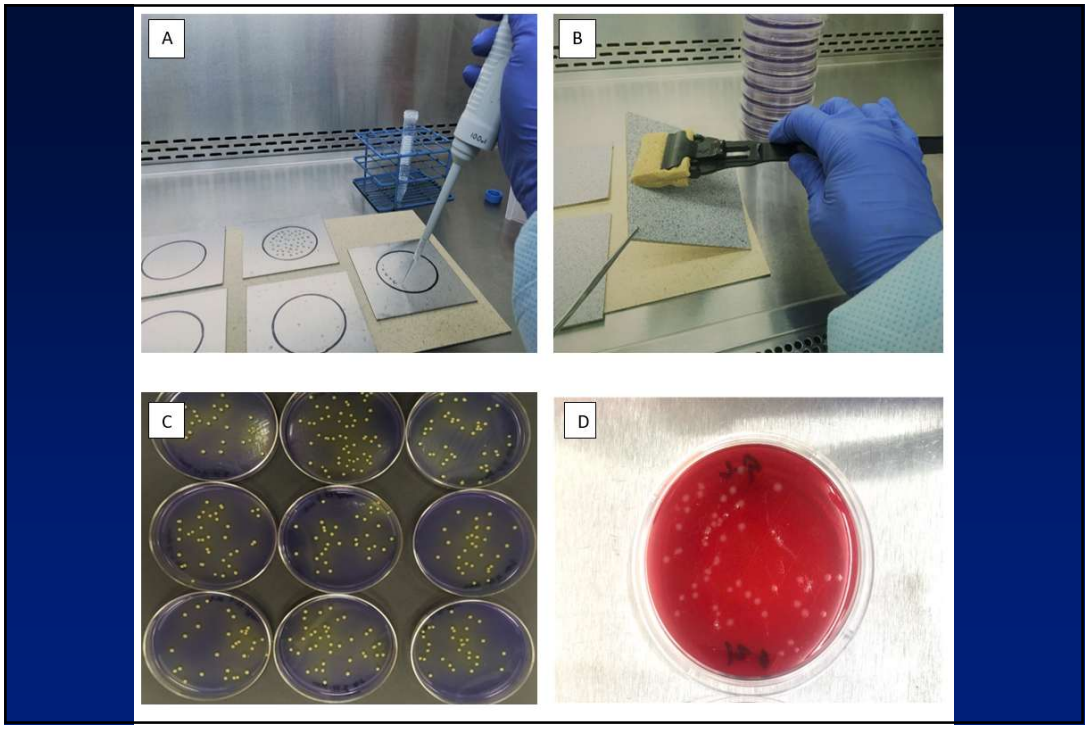
Materials & Methods

- Sponge Stick samples were collected by rubbing a pre-moistened sponge over the test surface. Each sponge head was ejected into a bag containing saline. For manual agitation, each bag was kneaded by hand for 1 minute. For Stomacher method, bags were processed for 1 minute. The contents of each bag were poured into tubes, then centrifuged. The supernatant was removed from each tube. An aliquot of each was inoculated to agar plates.
- Plates were incubated at 35°C, then colonies were counted for each plate.

Conclusion

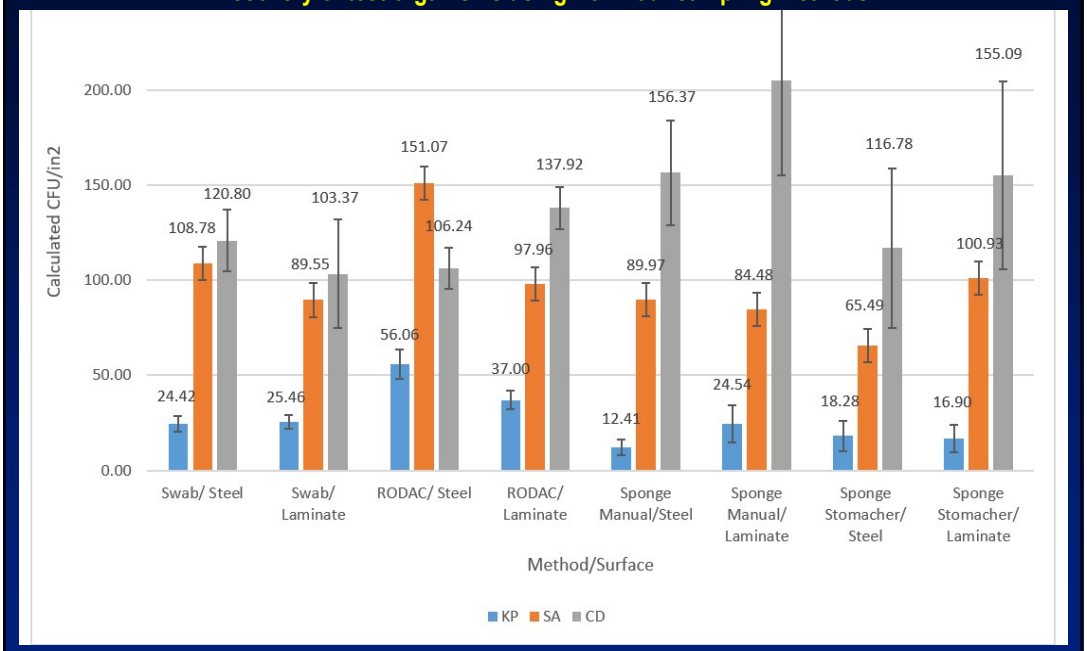
- Organism type, not sampling method, was the most important factor in bacterial recovery. Recovery of SA was significantly higher than KP, likely because it was able to better withstand manipulation and the physical stress of drying on test surfaces.
- The sampling tool appeared to have the second largest impact. RODAC yielded the highest recovery, followed by swabs, then sponges.

47



48

Recovery of test organisms using from four sampling methods



49

A Comparison of Methods for Microbiologic Environmental Sampling

Thompson SC, Rutala WA, et al. ICHE, 2022

- Organism type was the most important factor in bacterial recovery from contaminated surfaces
- *Klebsiella* had the lowest tolerance to the effects of drying on test surfaces
- Processing a swab of RODAC sample takes less time than processing a sponge stick
- Readily available tools and methods are able to detect viable bacteria on environmental surfaces

50

Microbiologic Sampling of the Environment Surface Sampling

- Used for research (potential reservoirs of pathogens, survival of mo on surfaces, source of contamination), as part of an epidemiologic investigation, or QA purposes
- Media (nutrient-rich such as TSA or BHI), reagents, and equipment required for surface sampling available in micro lab
- Effective sampling requires moisture

51

Other Microbiologic Sampling

- **Biological indicators**
- **Hemodialysis water**-200/ml, 2000/ml
- Infant formula-hospital prepared
- Pharmacy-hospital prepared
- Respiratory therapy
- Blood bank water bank-used to thaw plasma
- Endoscopes

52

Microbiologic Sampling of the Environment Conclusions

- Do not conduct random microbiological sampling of air, water, and surfaces
- When indicated, conduct microbiologic sampling as part of an epidemiologic investigation
- Limit microbiologic sampling for QA to: biological monitoring, dialysis water, or evaluation of IC measures

53

Microbiologic Sampling of the Environment

- Lecture Goals
  - Microbiologic Sampling
    - Indications
    - Methods

54

Reference

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55