



Objectives for Today's Presentation Introduction to environmental sampling principles and practices for indoor healthcare settings CDC's "EIC" Guideline Air - General methods 2003 - Microbiology vs. particle counts Surfaces - General methods Environmental Services - Environmental surfaces, instruments and devices,

- hands
- Water
- General methods
- Ensuring accurate and meaningful results



Where Can I Find the EIC **Guidelines?**

- Part II Recommendations:
 - MMWR 2003; 52 (RR-10): 1-44
 - Errata: MMWR 2003; 52 (42): 1025-6
- Full text version:
 - www.cdc.gov/ncidod/dhqp/gl environinfection.html
- Print version (ASHE):
 - www.hospitalconnect.com/ashe/resources/ Importantresources.html

Environmental Sampling Section

Environmental Sampling

- Environmental microbiology is not clinical microbiology
- Sampling is supported by epidemiologic assessment
- Random, undirected sampling is not recommended
- Sampling requires a protocol for sampling and culturing, analysis of results, and action based on the interpretation of results

Environmental Sampling

- Suggested uses:
- Support for outbreak investigation when epi suggests environmental reservoirs or fomites are implicated in disease transmission
- Research in environmental infection control
- Monitor a potentially hazardous situation
- Evaluate a change in environmental infection control for quality assurance purposes
 Parform periodic regular maintenance of equipment (i.e. HVAC
- Perform periodic regular maintenance of equipment (i.e., HVAC systems)
 Legal issues
- Legal issues
 Current routine monitoring:
- Biological monitoring of sterilization processes
 Monthly culturing of water used in hemodialysis applications and for final dialysate use dilution

Environmental Sampling

- Expensive and time-consuming; subject to many variables in protocol, analysis, and interpretation
- "Sampling is a public exercise and is always subject to disclosure; therefore, the investigator is required to minimize false negatives and, more rarely, false positives."
- Quotation source: Chapter 10, Sampling Design Strategy, in Recognition, Evaluation, and Control of Indoor Mold, Prezant B, Weekes DM, Miller JD, eds. AIHA, Fairfax VA; 2008

Air Sampling

- Primarily to determine bacteria and fungi identities and concentration in biological aerosols
- Major methods:
 - Impingement in liquids
 - Impaction on solid surfaces
 - Sedimentation (e.g., settle plates)
- Requires an understanding of what is being measured and a full description of the circumstances during sampling

Factors to Consider Prior to Conducting Microbiological Air Sampling

- Conditions of the aerosol: particle size, amount of inert material, microorganism concentration, environmental factors
- Sampling process: type of samplers and method, numbers of samples, duration of collection/sampling
- Assay method to optimize microorganism recovery
- Proper transport conditions to ensure sample quality and viability



Compare and Contrast the Main Air Sampling Methods				
Method	Principle	Suitable for Measuring	Collection Media or Surface	Points to Consider
Impingement in liquids	Air drawn in through small jet, directed against liquid surface	Viable microorganisms, water aerosols	Buffered gelatin, peptone, nutrient broth, tryptose saline	Used for Legionella spp. sampling
Impaction on solid surface	Air drawn into sampler, particles deposited on dry surface	Viable particles, viable microorganisms	Dry surfaces, coated surfaces, agar	Used for bacteria and fungal agent sampling; high volumes can be sampled
Sedimentation (settle plates)	Particles and microorganisms settle via gravity	Viable particles, viable microorganisms	Nutrient agars in plates or slides	Simple, best suited for qualitative sampling; not used for fungal spores

Before You Do Microbiological Air Sampling...

- Define your objective and analytical approach
 Qualitative vs. quantitative
 - Concentration over time? Particle size?
- Compare to counts from outdoor air
- Understand that results will not reflect "real time"
- Fully describe the circumstances in the area where sampling is occurring
- High volume sampling most efficient

Unresolved Issues and Microbiologic Air Sampling

- Unknown incubation period for IPA
- Infectious dose for Aspergillus spp. is unknown
- Lack of standard sampling protocols
- No standards or action levels for results

Unresolved Issues and Microbiologic Air Sampling

- Variability and sensitivity of sampling devices
- Lack of details re: sampling makes comparison of results with other outbreaks difficult
- Lack of correlation between fungal strains in clinical specimens and those found in the environment

Particle Sampling

- Simple to perform, immediate results
- Verify HVAC system performance:
 - Filtration efficiency
 - Rank order from "dirty" to "clean"
- Verify infection control measures during construction:
 - Construction barrier and dust containment



Example of Air Particle Analysis Using Condensate Particle Counter				
Location	Particles/cc	Pressure (Pascales)	Filter Efficiency	
Outside	8500	na		
Lobby	1500	na	90 %	
BMT Area Corridor	450	(+) 4 - 10	99.97 %	
21 of 32 Rooms (BMT)	< 10	(+) 6.8 - 30	99.97 %	
9 of 32 Rooms (BMT)	10 – 80	(+) 8.6 - 17	99.97 %	
2 of 32 Rooms (BMT)	> 500	(+) 11 & 12	99.97 %	
Radiation Therapy	1000	na	90 %	
Adjacent Building	5300	na	50 %	
Source: A. Streifel & J. Widem	an, AIHAce 2004	Demonstrate: pressure	s rank order and	

Breacours to Sample Air Preoccupancy verification of ventilation and cleanliness Establish baseline data (based on particle removal) BMTU, OR's, NICU, other critical areas BMTU, OR's, NICU, other critical areas Post infection evaluation (outbreak?) Verification of baseline data Rule out ventilation as a source Discover source of infectious fungi (reservoir?) Boutine surveillance CDC: not recommended without purpose Some methods provide assurance of status quo May be useful for finding deviations in baseline data

Baseline Data Development in Healthcare Air Sampling

- Provides verification of filtration efficiency
- Should show relative drop of viable/non-culture particles Should show $\geq 90\%$ drop of particles for 90% efficient filters
- Provides micro-flora verification in affected space
 Air is not sterile; should reflect isolates similar to outside
 Baseline should compare data from indoor space and outdoors
- Baseline data is best established pre-occupancy
 - Ventilation systems should be working according to specs
 Testing should be finished and specified ventilation parameters assured
 - Ideally sampling should be conducted before occupancy to avoid variables.

Source: A. Streifel and J. Wideman, AIHAce 2004

Environmental Surface Sampling

- Decision to sample should be driven by epidemiology, infection control
- Disinfectant neutralizers may be needed
- Major methods include:
 - Sample/rinse using sponges, wipes or swabs
 - Direct immersion
 - Containment (interior surfaces of a container)
 - RODAC plate (direct surface sampling)

Things to Consider Prior to Surface Sampling

- Background literature and present activities
 Preliminary results from epidemiological investigation
- Locations to sample
- Collection method and equipment

Source: A. Streifel and J. Wideman, AlHAce 2004

- Number of replicate samples needed
- Are controls or comparisons needed?
- Parameters for assay; qualitative, quantitative, or both?
- Estimate of maximum allowable microbial numbers or types on surface(s) sampled
- Some anticipation of a plan of action based on results

Compare and Contrast Surface Sampling Methods

Sample Type	Description	Target	Uses	Biological Agents
Wipe	Sterile 2 x 2 non- cotton gauze, moistened; wipe area of known size	Nonporous surfaces, usually small in area	Screening small nonporous surfaces extent of contamination decontamination effectiveness	Bacteria, viruses, fungi, biological toxins
Swab	Sterile non-cotton swab, individually wrapped, then moistened with sterile solution; wipe area of known size	Nonporous surfaces, usually very small in area, complex surfaces with crevices, corners	Screening small nonporous surfaces extent of contamination decontamination effectiveness	Bacteria, viruses, fungi, biological toxins
RODAC	Convex agar surface in culture dish, press onto surface, incubate	Nonporous surfaces, relatively small area	Screening small nonporous surfaces extent of contamination decontamination effectiveness	Bacteria, fungi

Source: Busher A, Noble-Wang J, Rose L. Surface Sampling, in Sampling for Biological Agents in the Environment

Things to Consider Before Conducting Surface Sampling

- Asepsis is critical
 - Sterilized sampling materials
 - Aseptic technique
- Document the circumstances of sampling
- State of the surface and its preparation, if any, prior to sampling
- Prepare a sampling strategy or plan that ensures the validity of the results and is appropriate for the organism(s) being sampled

Wipe Method

- Materials used:
 - Sterile gloves, sterile sample containers, sterile wrapped 2x2 gauze sponge pads, disposable sterile sampling template, sterile water or other appropriate fluid, plastic bags, identification tags
- Affix the template
- Aseptically wet the gauze with fluid and thoroughly wipe the area within the template
- Fold the gauze so the exposed side is inward and place in sample container; label
- Repeat with new template and new gauze if another surface is to be sampled

Source: Busher A, Noble-Wang J, Rose L. Surface Sampling.

Swab Sampling Procedure

- Materials used:
 - Sterile items: gloves, sample containers (e.g., large cfg tubes), wrapped non-cotton swabs, wetting solution, scissors, disposable template
 - Sealable plastic bags, identifying markers, tags
- Affix the template to the surface
- Wet the swab and wipe using an S-shaped pattern (vertically & horizontally), rolling the swab over the surface
- Place the swab aseptically in a sample tube; label
- Change gloves and use a new template if sampling another surface

Source: Busher A, Noble-Wang J, Rose L. Surface Sampling.

RODAC Plate Sampling Method

- Materials used:
 - RODAC plate (agar medium is overfilled to give a convex surface)
- Used to sample cleaned surfaces; not suitable for visibly dirty or irregular surfaces
- Neutralizers can be incorporated into the medium if surface disinfectant residuals are present
- Press the convex medium onto the surface; do not twist or move the plate around

Source: Busher A, Noble-Wang J, Rose L. Surface Sampling.

Neutralizir	ng Agents
Disinfectant	Neutralizer or Neutralizing Media
Sodium hypochlorite, chlorine dioxide, iodine	Sodium thiosulfate, Dey Engley (D/E) broth or agar
Formaldehyde, glutaraldehyde	Glycine, D/E broth or agar
Hydrogen peroxide	Catalase
Phenolics	Tween 80, D/E broth or agar
Quaternary ammonium compounds	Lecithin + Lubrol W, Letheen broth or agar, D/E broth or agar
Vaporized hydrogen peroxide	None needed – end products are H_2O and O_2

Adapted from Russell AD. Principles of antimicrobial activity and resistance, p. 31-56. in Block SS (ed). Disinfection, Sterilization, and Preservation. 5th Ed., Philadelphia PA, LWW: 2001

Visual Methods Currently Used to Evaluate Cleaning

- Application of clear chemicals that fluoresce under UV light
 - Glo Germ
 - Gluten-derived "glues" + detergent + fluorescent dye
- Qualitative: Yes / No
- ATP
 - Proprietary swabs and solution
 - Luminometer reads presence of organic matter -
 - expressed in relative light units (RLU)
 - Can be quantitative



ATP Method to Evaluate Cleaning

	ATP Bioluminescence (RLU)			
Medical Ward	Standard Clea Mean	aning Protocol Range	Modified Clea Mean	aning Protocol Range
Commode	590	(320 – 1200)	14	(6 – 29)
Drugs Trolley	460	(260 – 1100)	12	(5 - 60)
Bedside Locker	140	(31 – 300)	34	(12 – 76)
Bedside Table	340	(130 – 550)	180	(27 – 280)
Tap Handle	450	(95 – 750)	130	(17 – 490)
Toilet Handle	340	(27 – 3100)	19	(11 – 80)

Adapted from: Lewis T, Griffith C, Gallo M, Weinbren M. J Hosp Infect 2008; 69: 156-63.

Water Sampling

- Sampling "finished" water often requires the use of a chlorine neutralizer (i.e., sodium thiosulfate)
- Use media and incubation temperatures appropriate for culture of "stressed" organisms (R2A, diluted peptone; ambient temperature)
- Specific methods are used for Legionella and other waterborne microorganisms

Considerations When Sampling for *Legionella* Spp.

- Point-of-use devices and system surfaces
- Faucet aerators and showerheads:
 Swab surfaces of these fixtures first
 Water samples are collected after the aerators and
- shower heads are removedCollect 1 L water samples in sterile containers
- Collected swabs should be immersed in 5 10 mL of water from the same device
- Suitable media for Legionella culture: BCYE (buffered charcoal yeast extract)

From: CDC EIC guideline, Appendix C. Water

For More Details...

- CDC's EIC Guideline
- Emanuel P, Roos JW, Niyogi K. Sampling for Biological Agents in the Environment. Washington DC, ASM Press: 2008.
- Hung LL, Miller JD, Dillon HK. Field Guide for the Determination of Biological Contaminants in Environmental Samples. Fairfax VA, AIHA Press: 2005
- Bond WW, Schulster LM. Microbiological Assay of Environmental and Medical Device Surfaces. Chap 13-10 *in* Clinical Microbiology Procedures Handbook, 2nd Ed, Isenberg HD, ed. Washington DC, ASM Press: 2004

Thank You!

Division of Healthcare Quality Promotion Centers for Disease Control and Prevention

"Protect patients, protect health-care personnel, and promote safety, quality, and value in the health-care delivery system"

THE NEX	T FEW TELECLASSES
25 Jun. 08	(South Pacific Teleclass) Peripheral Line Sepsis Speaker: Dr. Steve McBride, Aukland District Health Board
26 Jun. 08	CBIC Teleclass 3 - The CIC Examination Process: Computer Based Testing Speaker: CBIC Board Members & Guests
17 Jul. 08	(Free Teleclass) Community-Associated MRSA - What's Up & What's Next Speaker: Dr. Rachel Gorwitz, CDC
22 Jul. 08	(Free British Teleclass) Progress Report from the Chief Nursing Officer Speaker: Christine Beasley, British Department of Health
7 Aug. 08	(Free Teleclass) Disinfection & Sterilization - Current Issues & New Research Speaker: Dr. William Rutala, University of North Carolina
14 Aug. 08	Frae Teleclass) Extended Spectrum Beita Lactmass and Infection Control Speaker: Prof. David Patterson Broadcast live from New Zsaland infection control conference
www.web	obertraining.com.schedulep1.php