Bacterial Sampling and Data Interpretation

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Welcome to AEMTEK

- We are an environmental microbiology laboratory providing testing on mold, bacteria, lead and products.
- You are welcome to tour the lab and talk to the analytical personnel, but please stay on the carpeted areas
- Feel free to use the lunch room and the restrooms in the lab

Course Objectives

- Client communication program
- To understand the occurrence of bacteria in indoor environments
- To discuss practical methods for bacterial sampling
- To discuss how to interpret lab report
- To provide a networking opportunity for local IAQA chapter members

Course Outline

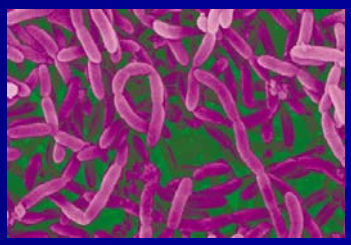
- Normal occurrence of bacteria in indoor environments
- Sewage contamination and verification testing
- *Legionella* sampling and risk assessment
- Sampling for pathogenic bacteria

I. Bacteria in Indoor Environments

- Biology and ecology of bacteria
- Gram-positive and Gram-negative bacteria
- Bacterial populations in indoor environments
- Bacterial sampling and media selection
- Laboratory analysis and data interpretation
- Rapid field assessment of bacterial load

What are bacteria?

- Single-celled prokaryotic organisms
- 0.5 – 1 µm in diameter, 0.5-5 µm long
- In nature, bacteria often exist in communities, rarely in isolated single cells



Biofilms

- In natural environments, bacteria often attach to the soil or plant surface and form dense aggregations, called biofilms.
- Biofilms may contain different species of bacteria
- Biofilms are complex structures that help to increase food supplies and protect the bacteria living in them

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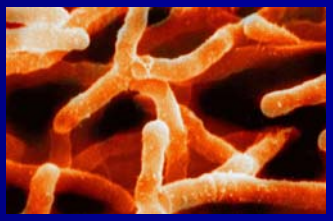
Natural Habitats for Bacteria

- Soil
- Water
- Air
- Acidic hot springs
- radioactive waste
- Organic matter
- Plants
- Animals
- Other microorganisms



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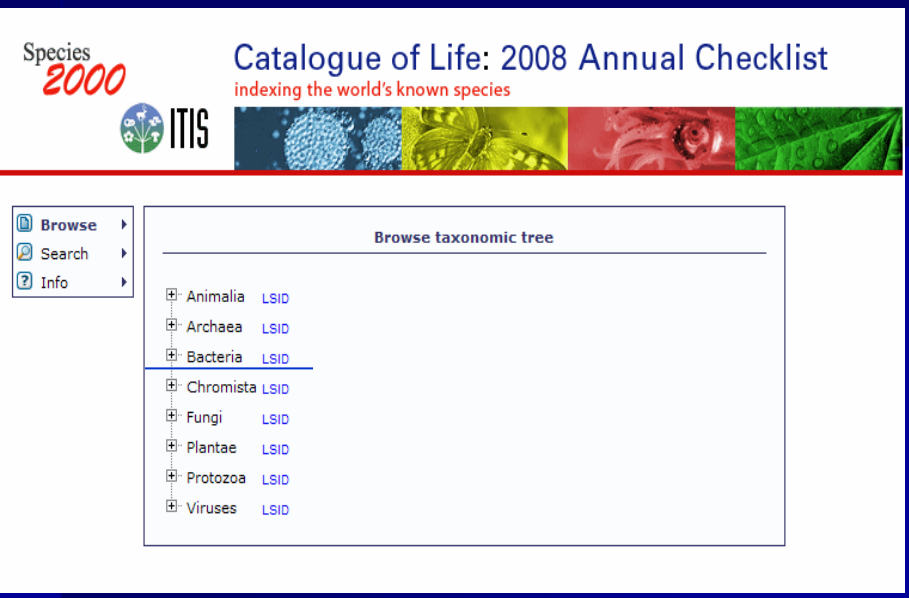
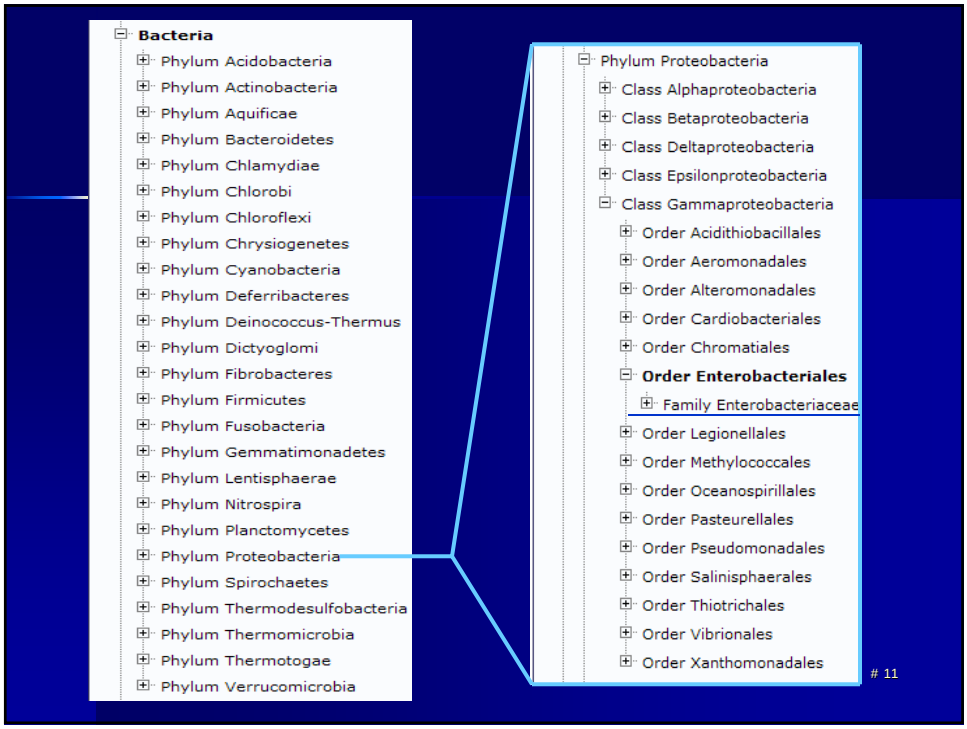
Bacteria are abundant and diverse

- One gram of soil: 6,400 – 38,000 species, 40 million bacterial cells.
- One milliliter of fresh water: one million bacterial cells
- One liter of seawater: 20,000 species of bacteria
- Estimated total numbers of bacterial species range from 10 million to one billion
- There are approximately five nonillion (5×10^{30}) bacteria on Earth




Bacteria
Phylum Acidobacteria
Phylum Actinobacteria
Phylum Aquificae
Phylum Bacteroidetes
Phylum Chlamydiae
Phylum Chlorobi
Phylum Chloroflexi
Phylum Chrysiogenetes
Phylum Cyanobacteria
Phylum Deferribacteres
Phylum Deinococcus-Thermus
Phylum Dictyoglomi
Phylum Fibrobacteres
Phylum Firmicutes
Phylum Fusobacteria
Phylum Gemmatimonadetes
Phylum Lentisphaerae
Phylum Nitrospira
Phylum Planctomycetes
Phylum Proteobacteria
Phylum Spirochaetes
Phylum Thermodesulfobacteria
Phylum Thermomicrobia
Phylum Thermotogae
Phylum Verrucomicrobia
Phylum Proteobacteria
Class Alphaproteobacteria
Class Betaproteobacteria
Class Deltaproteobacteria
Class Epsilonproteobacteria
Class Gammaproteobacteria
Order Acidithiobacillales
Order Aeromonadales
Order Alteromonadales
Order Cardiobacteriales
Order Chromatiales
Order Enterobacteriales
Family Enterobacteriaceae
Order Legionellales
Order Methylococcales
Order Oceanospirillales
Order Pasteurellales
Order Pseudomonadales
Order Salinisphaerales
Order Thiotrichales
Order Vibrionales
Order Xanthomonadales
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Family Enterobacteriaceae
Genus Alterococcus
Genus Arsenophonus
Genus Brenneria
Genus Buchnera
Genus Budvicia
Genus Buttiauxella
Genus Cedecea
Genus Citrobacter
Genus Dickeya
Genus Edwardsiella
Genus Enterobacter
Genus Erwinia
Genus Escherichia
Genus Ewingella
Genus Hafnia
Genus Klebsiella
Genus Kluyvera
Genus Koserella
Genus Leclercia
Genus Leminorella
Genus Levinea
Genus Moellerella
Genus Morganella
Genus Obesumbacterium
Genus Pantoea
Genus Pectobacterium
Genus Phlomobacter
Genus Photorhabdus
Genus Plesiomonas
Genus Pragia
Genus Proteus
Genus Providencia
Genus Rahnella
Genus Raoultella
Genus Saccharobacter
Genus Salmonella
Genus Samsonia
Genus Serratia
Genus Shigella
Genus Sodalis
Genus Tatumella
Genus Thorsellia
Genus Trabulsiella
Genus Wigglesworthia
Genus Xenorhabdus
Genus Yersinia
Genus Yokenella
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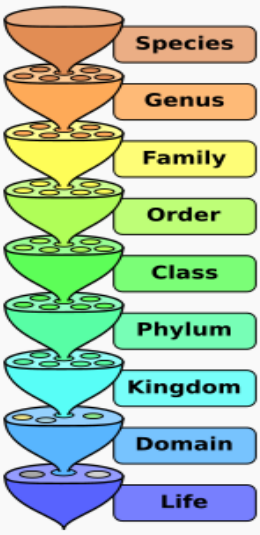
Scientific Classification



- *E. coli*
- *Escherichia*
- Enterobacteriaceae
- Enterobacteriales
- Gamma Proteobacteria
- Proteobacteria
- Bacteria
- Bacteria

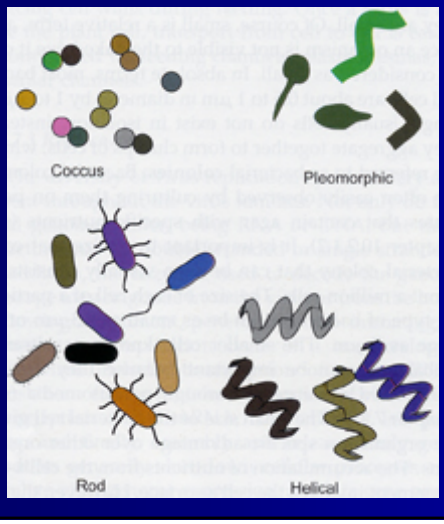
Bacteria Terminology

- Heterotrophic – carbon source is organic carbon compounds
- Autotrophic – carbon is obtained by fixing carbon dioxide
- Aerobes**:** need oxygen for metabolism
- Anaerobes: does not require oxygen for growth and may even die in its presence
- Facultative anaerobes can use oxygen, but also have anaerobic methods of energy production.
- Mesophilic – optimum growth temperature is between 15-40° C (e.g. 35 ± 2° C, majority of bacteria, pathogens)
- Thermophilic – optimum growth temperature is between 45-80 °C (e.g., 55 ± 2° C)

Quiz # 1

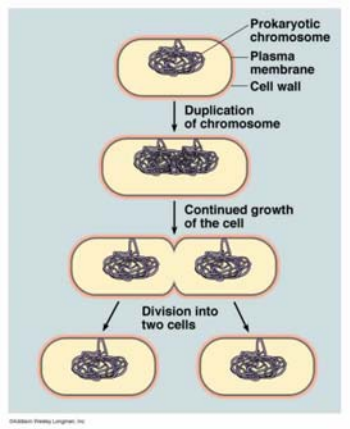
- Give an example of autotrophic bacterium
- Give an example of heterotrophic bacterium
- Give an example of Thermophilic bacterium

Shapes of Bacteria



Bacteria Reproduction

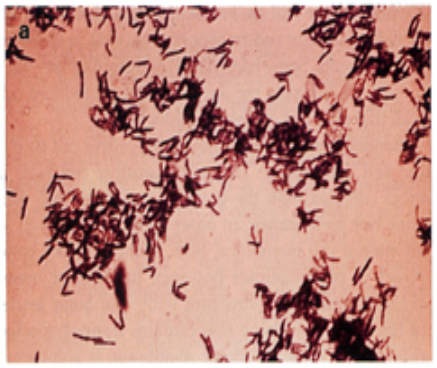
- Bacteria grow to a fixed size
- Reproduce by cell division (binary fission)
- Bacteria can grow and divide extremely rapidly
- Bacteria population can double as fast as every 10 minutes



Quiz #2

- If an *E. coli* cell can double its number in every 20 minutes, then how many *E. coli* cells will there be after 24 hours?

Gram-positive vs. Gram-negative



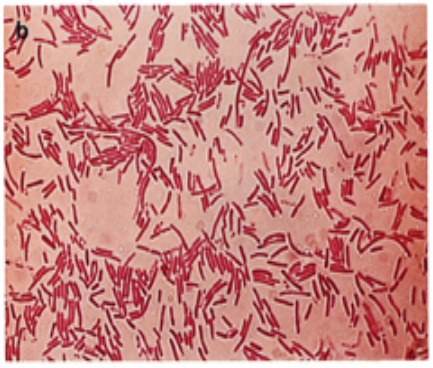


FIGURE 2.13 Typical gram-positive (blue) and gram-negative (red) bacteria identified by the Gram stain.

Gram-positive vs. Gram-negative

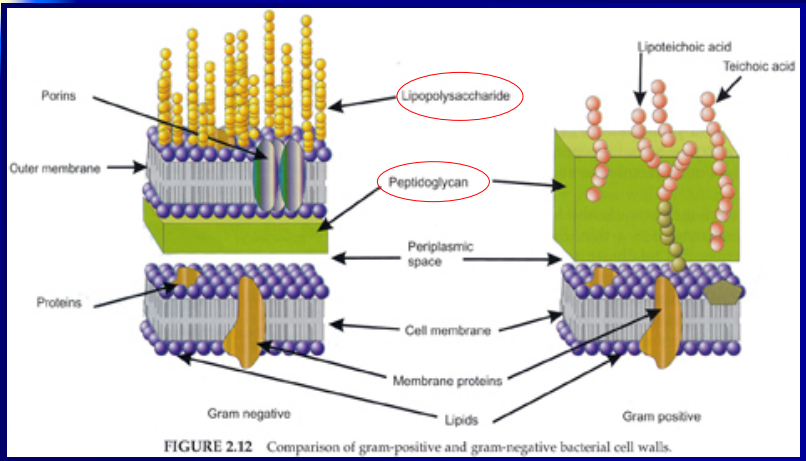
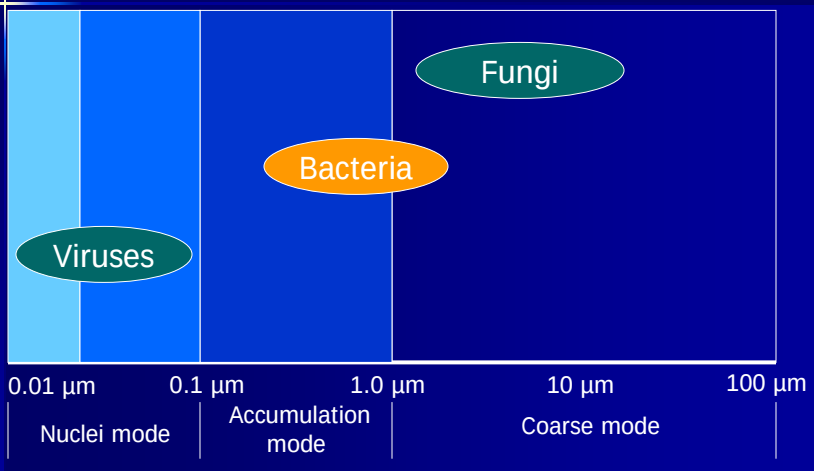


FIGURE 2.12 Comparison of gram-positive and gram-negative bacterial cell walls.

Representatives of Gram-positive and Gram-negative bacteria

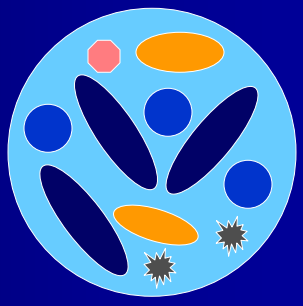
Gram-positive	Gram-negative
Micrococcus	Escherichia
Staphylococcus	*Pseudomonas*
Bacillus	*Salmonella*
Enterococcus	*Enterobacter*
Mycobacterium	*Legionella*
Streptomyces	*Shigella*

Bacteria are a component of bioaerosols



Composition of Bioaerosols

- Liquid or solid or mixture
- Mostly associated with airborne particles (dust or mist)



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Bioaerosol Pathways

- Launching
- Transport
- Deposition & adhesion

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Launching of Bioaerosols in Indoors

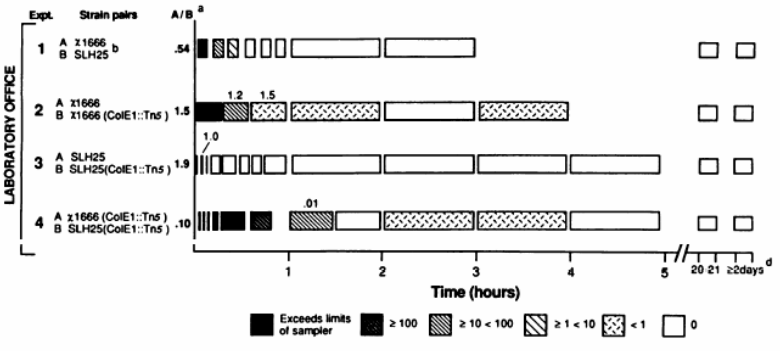
Deposition of Bioaerosols

- Gravitational settling
- Downward molecular diffusion
- Surface impaction
- Electrostatic deposition

Factors Influencing Microbial Viability

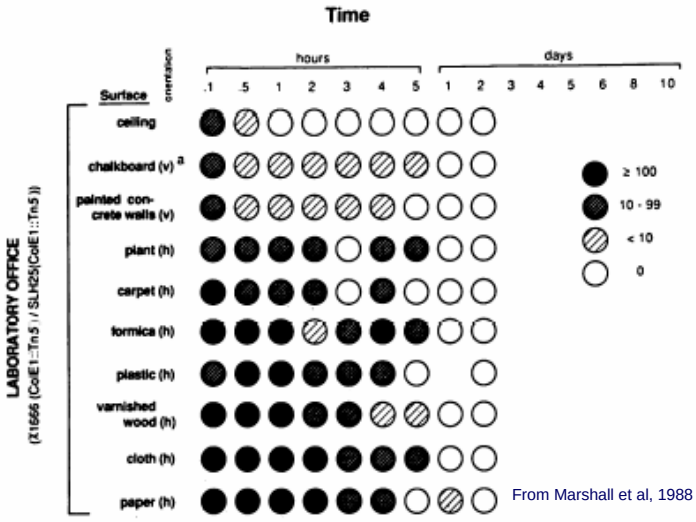
- Relative Humidity
  - Gram-negative bacteria survive longer at low RH
  - Gram-positive bacteria survive longer at high RH
- Temperature
- Radiation
- Oxygen, Ions, and Open Air Factor

Airborne *Escherichia coli* survival



From Marshall et al, 1988

Escherichia coli survival on surface



From Marshall et al, 1988

Bacteria Sampling – general consideration

- Have a clear and realistic objective
  - "I want to get an idea of the airborne bacteria load"
  - "I want to see what pathogens are in the air"
  - "I want to see what bacteria are in the air"
  - "I want to see if there is anything unusual"
- Try to define target organisms
- If an occupant is concerned about infection/disease, advise him to see a doctor first

Air Sampling Devices

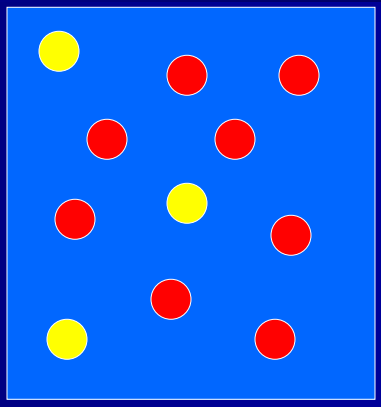
- Impingement
  - Trapping of bioaerosols in liquid
- Impaction
  - Forced deposition of bioaerosols on a solid surface
- Centrifugation
  - Forced deposition using gravity
- Filtration
  - Trapping of bioaerosols by size exclusion
- Deposition
  - Using natural deposition forces

Bacteria Air Sampling

- Andersen N-6 or equivalent
- At 28.3 lpm flow rate, sample 1 – 3 minutes
- Consider representativeness of samples
- For industrial clean rooms, sample 1 m^3 air (= 7 Andersen plates)

Bacteria Surface Sampling

- Swabs, wipes, contact plates
- Wet vacuum systems
- Define sampling area
- Randomly choose multiple sampling site to assess level of contamination



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Media for General Bacterial Sampling

- TSA or TSA w/ blood – bacterial load (count) and gram stain
- MacConkey – Gram-negative enteric bacteria
- Columbia CNA – Gram-positive bacteria
- PCA – Heterotrophic bacteria count

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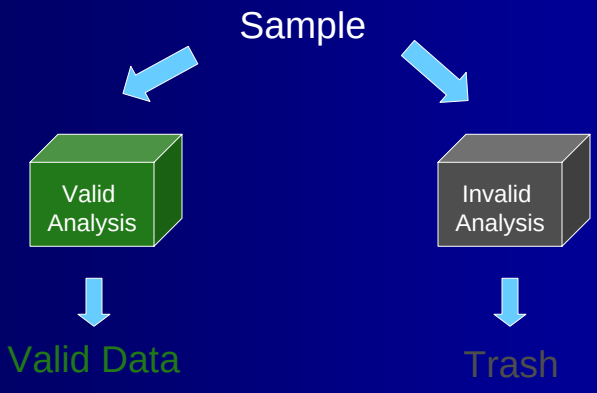
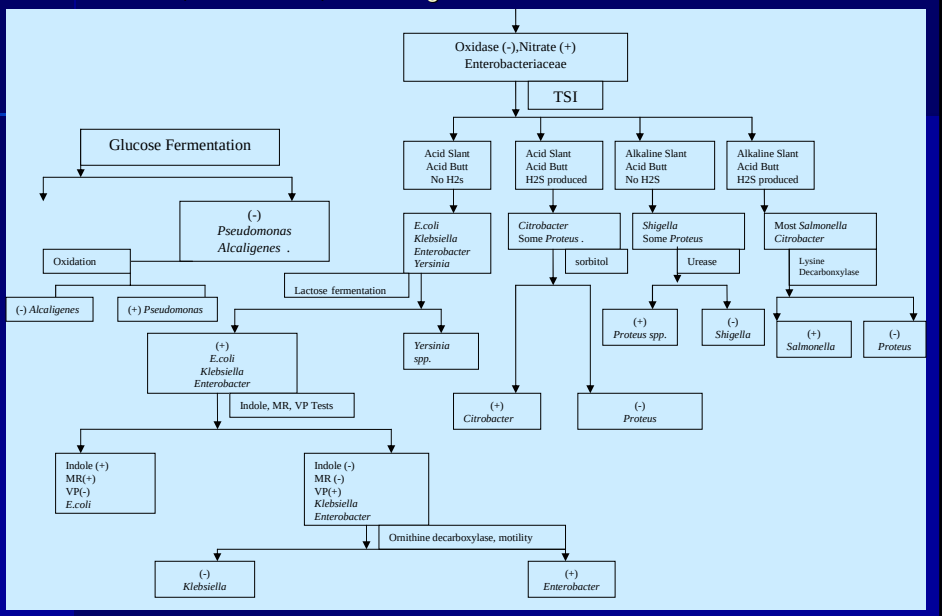
Laboratory analysis and data interpretation



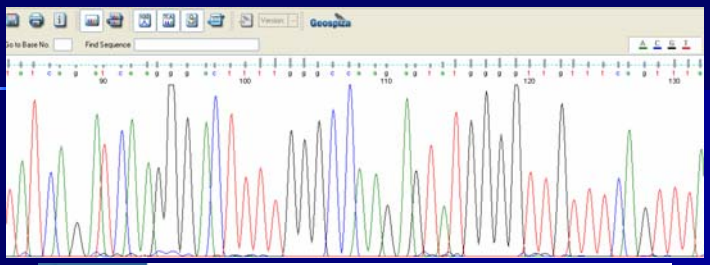
Analytical Methods for Bacteria

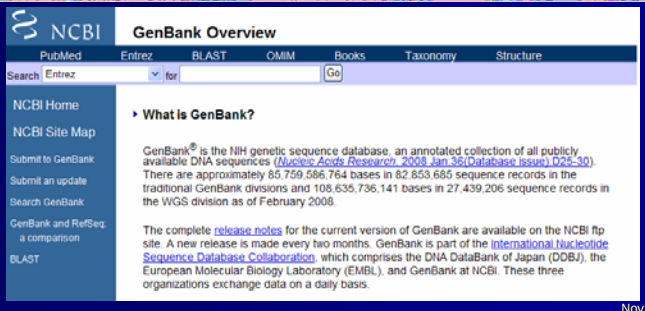
- Culture
- Biochemical assays
- Microscopic observation
- Automatic methods
- DNA sequencing
- Real-time PCR

Figure 1.1 Gram-negative Rod Bacteria ID Flow Chart
Oxidase Test, Nitrate Test, Gram-negative Bacteria



DNA sequencing method for bacteria identification





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What is CFU?

- Colony Forming Unit
- On direct impact (Andersen plates) or imprint samples (contact plates), one CFU may be from one bacterial community
- For prepared plates (swab, dust samples), one CFU may be resulted from one bacterial cell

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How to interpret CFU data

Area Sampled:	Counter Top	Counter Top
Sample Type:	Swab Sample	Contact Plate
Staphylococcus	1,000,000 CFU/cm^2	3 CFU/cm^2

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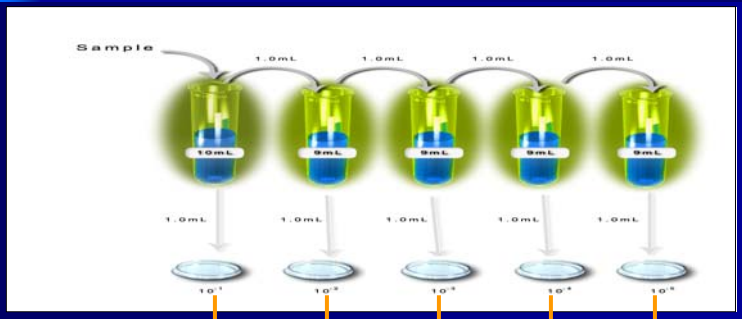
Serial Dilution (e.g., swab sample)



CFU:	TNTC	TNTC	>1,000	260	28

Results: 2.6 x 10^6 CFU/Sample

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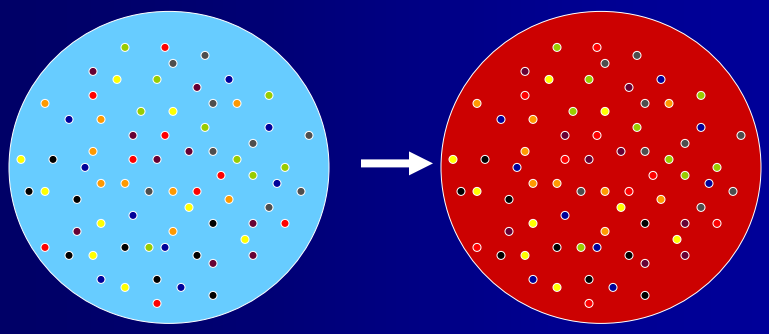
Contact Plates (25 cm^2)



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Put numbers into perspective

- Each visible bacteria colony consists of at least a million cells
- There may be significant changes in the bacteria population after sampling, depending on the shipping and storage condition
- Always ship bacteria samples cold

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What is the normal level of indoor bacteria?

- Tsai & Macher (2005), USEPA Building Assessment Survey and Evaluation (BASE) study
- 100 large US office buildings in 10 climate zones
- Building were selected randomly, excluding only buildings with highly publicized IEQ problems
- Andersen N-6 sampler, Tryptic soy agar (TSA)
- Conducted between 1994 – 1998, summer, winter, morning and afternoon
- Total 5201 samples (4782 air samples + 419 blank samples)

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Tsai & Macher (2005), BASE study
Average concentration of airborne culturable bacteria (CFU/m^3)

Bacterial Group	Summer		Winter	
	Indoor	Outdoor	Indoor	Outdoor
Gram+ rods	10.6	33.6	11.4	43.6
Gram+ cocci	48.3	26.2	28.7	21.8
Gram- rods	3.5	14.9	2.6	11.0
Gram- cocci	1.6	1.1	1.3	3.3
Unknown	51.8	89.1	42.6	114.7
Total bacteria	116.0	165.0	86.7	194.5

Common Gram-positive cocci:
Enterococcus, Micrococcus, Staphylococcus, Streptococcus

Quiz #3

- The BASE study indicates that the average indoor airborne Gram-positive cocci concentration is higher in summer than in winter. What might be the reasons for that?

Tsai & Macher (2005), BASE study
The indoor/outdoor ratio

- Temporal variation influences the assessment
- A portion of the airborne bacteria in the indoors could be attributed to the entry of bacteria from outdoors
- The indoor/outdoor ratios ranged 0.1 – 16.4
- Whether the indoor/outdoor ratio >1 or $\leqslant$ 1, indoor sources of bacteria were present
  - Indoor/outdoor >1, average indoor concentration = 68 + 0.91 (outdoor concentration)
  - Indoor/outdoor $\leqslant$ 1, average indoor concentration = 54 + 0.14 (outdoor concentration)

Tsai & Macher (2005), BASE study
Conclusions and Implications

- Seasonal pattern across ten climate zones
- Gram-positive rods were more abundant in outdoor air nationwide
- Airborne Gram-positive cocci concentration was higher indoors than outdoors
- Typical indoor bacterial air concentration in offices and similar non-manufacturing workplace:
  - 75th percentiles: 124 CFU/m^3
  - 90th percentiles: 175 CFU/m^3

What type of indoor environment is this?

- *Chryseobacterium* – plant materials, raw milk, water, and animals.
- *Klebsiella* –soil, water, grain, fruits, and vegetables
- *Kluyvera* –food, soil, sewage and human clinical specimens
- *Kocuria* – commonly found on human skin.
- *Lactococcus* – lactic acid bacteria, used in the dairy industry
- *Leuconostoc* –plants and in dairy and other food products.
- *Micrococcus* – occur primarily on mammalian skin and in soil
- *Pseudomonas* – often found in moist area.
- *Psychrobacter* – Associated with fish, processed meat and poultry products.
- *Rothia* – Normally inhabit the human mouth and throat.
- *Staphylococcus* – skin and mucous membranes of warm-blooded vertebrates
- *Vibrio* – Found in aquatic habitats with a wide range of salinities.

Field Assessment of Bacterial Load

- ATP monitor
  - Measures adenosine triphosphate (ATP)
  - Used on food preparation surface
  - Measures general cleanness
  - No significant correlation between the ATP monitor readings and the microbial load
- QuikAlert (Vista Enterprise)
  - For food contamination testing

II. Sewage Contamination and Testing

- Sewage Constituents
- Health Risk of Sewage Contamination
- Sewage Indicator Organisms
- Verification Test Methods
- Air sampling issue

Major Constituents in Sewage (1)

- Water (> 95%)
- Pathogens: bacteria, viruses, and parasites.
- Non-pathogenic bacteria (>100,000 CFU/ml) & fungi
- Organic particles: feces, hairs, food, vomit, paper fibres, plant material, humus, etc.
- Soluble organic material: urea, fruit sugars, soluble proteins, drugs, pharmaceuticals, etc.

Major Constituents in Sewage (2)

- Inorganic particles: sand, grit, metal particles, ceramics, etc.
- Soluble inorganic material: ammonia, road-salt, sea-salt, cyanide, hydrogen sulphide, thiocyanates, thiosulphates, etc.
- Animals: protozoa, insects, arthropods, fish, etc.
- Macro-solids: sanitary towels, nappies/ diapers, condoms, needles, toys, dead pets, body parts, etc.

Major Constituents in Sewage (3)

- Gases: hydrogen sulphide, carbon dioxide, methane, etc.
- Emulsions: paints, adhesives, mayonnaise, hair colourants, emulsified oils, etc.
- Toxins: pesticides, poisons, herbicides, etc.

(From: Wikopedia – wastewater)

Health Risk of Sewage Contamination

- Pathogens
- Secondary metabolites
- Abiotic Constituents:
  - Heavy metals such as lead and mercury
  - Insecticides, herbicides, pesticides
  - Industrial wastes, radioactive and toxic compounds

Bacterial Pathogens in Human Sewage

- *Aeromonas hydrophila*
- *Camphylobacter jejuni*
- *Clostridium difficile* and other species of *Clostridium*
- *Escherichia coli*
- *Helicobacter pylori*
- *Klebsiella pneumoniae*
- *Listeria monocytogenes* and other species of *Listeria*
- *Salmonella typhi* and other species of *Salmonella*
- *Shigella dysenteriae* and other species of *Shigella*
- *Vibrio cholera*
- *Yersinia enterocolitica* and other species of *Yersinia*

Virus Pathogens in Human Sewage

- *Adenovirus*
- *Calicivirus*
- *Coxsachievirus* (some strains)
- *Echovirus*
- *Hepatitis*
- *Norwalk virus*
- *Poliovirus*
- *Reovirus*
- *Rotavirus*

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Parasites in Human Sewage

- *Ascaris* species
- *Balantidium coli*
- *Cryptosporidium*
- *Cyclospora*
- *Entamoeba histolytica*
- *Giardia lambia*
- *Isospora balli* and *I. hominis*
- *Necator americanus*
- *Taenia* species
- *Toxoplasma gondii*
- *Trichuris trichuria*

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Secondary Metabolites in Sewage Contamination

- Endotoxin – cell-membrane constituents of Gram-negative bacteria
- Enterotoxins – proteinaceous toxins produced by Gram-negative bacteria on their outer membranes
- Volatile Compounds – MVOCs, Ammonia, Hydrogen sulfide (rotten egg gas).

Why Test for Sewage Indicator Organisms

- Testing for the enteric pathogens is often
  - Difficult
  - Lab intensive
  - Time-consuming
  - Expensive

Criteria for Sewage Indicator Organisms

- Naturally occurs in the digestive systems of human and warm-blooded animals, but not grow in the environment
- Be present whenever enteric pathogens are present
- Survive longer than all the enteric pathogens
- Their quantity correlates to the degree of pollution
- The testing method for the indicator organism should reliable, fast, inexpensive and easy to perform

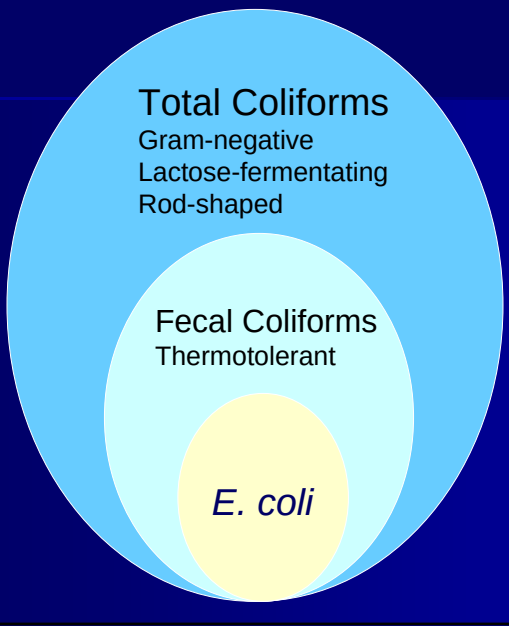
Sewage Indicator Bacteria

- Total coliforms
- Fecal coliforms
- *E. coli*
- Fecal streptococci and enterococci
- Developed and validated for ground, drinking, recreational waters and wastewater
- Used in IAQ practices

Coliforms as Indicator Organisms

- They do not persist for extended periods of time, such indicate recent contamination
- Abundant in the digestive systems of human and warm blooded animal
- They occur in greater number than the major pathogens
- They can be readily tested
- But, they are sensitive to desiccation and biocides

Total coliforms, fecal coliforms and *E. coli*

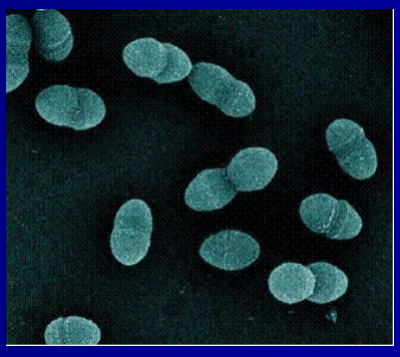


Escherichia coli

- Gram-negative bacilli
- Spread by fecal-oral route or by contaminated food, water, milk, and improperly cooked beef
- Four strains
- *E. coli* O157:H7

Enterococci as Indicator Organisms

- Mainly *E. faecalis* and *E. faecium*
- Colonize the gastrointestinal and genital tracts of humans
- More tolerant to salt water, chlorination, and other stress
- Fit for recreational water testing



Sewage Cleanup Verification Criteria

- Disinfection or elimination of potential health hazards
- Testing for indicator bacteria
- Parasites, Enteroviruses, endotoxins, allergens, irritants
- Endotoxin

Verification Test Methods

- Coliform culture-based methods
- Other culture-based methods
- Chemical-based methods
  - Caffeine
  - Fecal sterols (coprastanol is unique to human)
  - Endotoxin
- Molecular-based methods
  - Bacteria, viruses

Comparison of Sewage Cleanup Verification Testing Methods

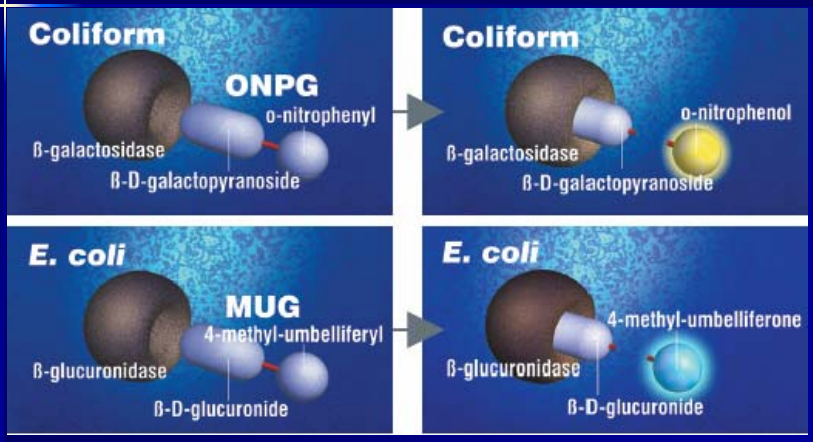
Method	Sensitivity	Viability	False -	False +	TAT	Cost
E. coli Culture	High	Yes	Rare	Rare	2 days	$
Enterococci Culture	High	Yes	Rare	Possible	2 days	$
Bifidobacterium Culture	Low	Yes	Possible	Rare	3 days	$$
Phage & Viruses Culture	Low	Yes	Possible	Rare	3 days	$$$
Bacteria Molecular	High	No	Rare	Rare	1 day	$$$
Phage & Viruses Molecular	High/Low	No	Possible	Rare	1 day	$$$
Caffeine, Pharmaceuticals	High/Low	No	Possible	Rare	1 day	$$$
Endotoxin	High	No	Rare	Rare	1-2 days	$$
Facal sterols	High/Low	No	Possible	Rare	1 day	$$$

Coliform Testing - ONPG & MUG Method

- Colilert™
  - Total coliform
  - *E. coli*
- Incubate at 35±0.5˚C for 24 hours
- When total coliforms metabolize nutrient-indicator, ONPG, the sample turns yellow
- When *E. coli* metabolize nutrient-indicator, MUG, the sample fluoresces

Coliform Testing – ONPG & MUG Method

(IDEXX Literature)



Coliform Testing – ONPG & MUG Method

(IDEXX Literature)

1 Presence/Absence

Add reagent to sample and incubate 24 hours at 35°C.

Read results. Colorless – negative
Yellow – total coliforms
Yellow/Fluorescent – *E. coli*

Accurate

- Detects a single viable coliform or *E. coli* per sample
- Suppresses up to 2 million heterotrophs per 100 mL
- Eliminates the subjective interpretation found in traditional methods

Cost-Effective

- 20–50% less expensive than traditional methods[1]
- 95% lower equipment costs compared to membrane filtration
- Minimizes evening and weekend work
- Up to 12-month shelf life at room temperature from manufacture date

Flexible

- A Colilert Snap Pack can be used for P/A or quantification testing
- Quanti-Tray® provides counts to 200 per 100 mL without dilutions
- Quanti-Tray®/2000 provides counts to 2,419 per 100 mL without dilutions
- Colilert is also available predispensed in 10-mL MPN tubes

[1]*AWWARF Research Applications*; May, 1999.

2 Quantification

Add reagent to sample and mix well.

Pour into Quanti-Tray® (counts from 1 to 200) or Quanti-Tray®/2000 (counts from 1 to 2,419).

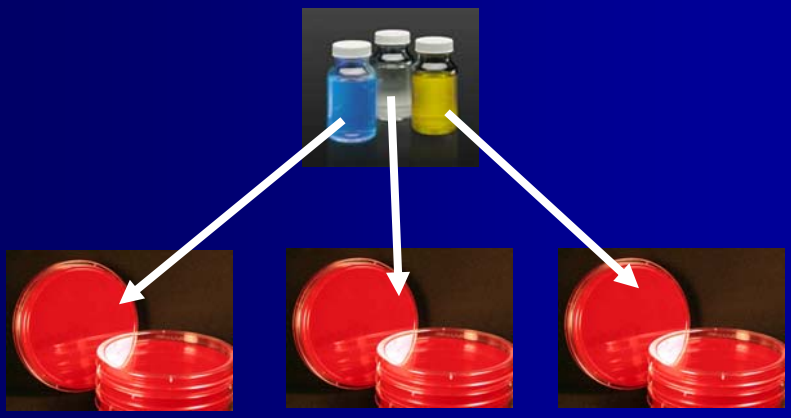
Seal in Quanti-Tray Sealer and place in incubator for 24 hours at 35°C.

Read Quanti-Tray results.
Yellow wells – total coliforms
Yellow/Fluorescent wells – *E. coli*
Refer to MPN table.

or

Read Quanti-Tray/2000 results.
Yellow wells – total coliforms
Yellow/Fluorescent wells – *E. coli*
Refer to MPN table.

Coliform Testing – Aemtek Study
What is really there?



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Coliform Testing – Aemtek Study
What is really there?

Colilert Results		What we have found	Note
Total Coliforms	*E. coli*		
+	+	*E. coli* *Citrobacter* *Enterobacter*	Fecal-origin coliform Coliform Coliform
+	-	*Citrobacter* *Enterobacter* *Pseudomonas*	Coliform Coliform Gram-negative
-	-	*Brevundimonas* *Pantoea* *Pseudomonas* *Bacillius*	Gram-negative Gram-negative Gram-negative Gram-positive

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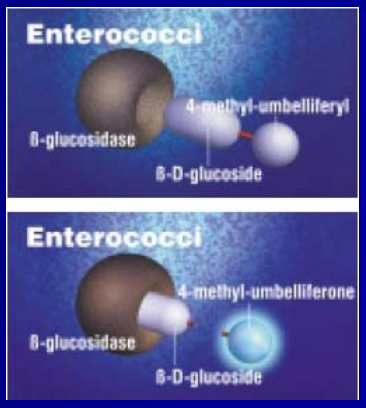
Enterococci Testing
(IDEXX Literature)

- Enterolert™
  - *Enterococcus faecalis*
  - *E. faecium*
- Incubate at 41±0.5˚C for 24 hours
- When enterococci metabolizes the nutrient-indicator, the sample fluoresces



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Enterococci Testing
(IDEXX Literature)

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Enterococci Testing – Aemtek Study
What is really there?

Enterolert Results	What we have found	Note
+	*Enterococcus* (2 species)	Fecal-origin, Gram-positive
	Serratia	Coliform
	Flavobacterium	Gram-negative
	Sphingobacterium	Gram-negative
-	*Bacillius*	Gram-positive

Note: after Enterolert testing and incubation at 41±0.5˚C

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What you should know about sewage screen testing

- The Colilert™ & Enterolert™ methods are EPA approved for water testing
- It was adapted for surface swab testing
- Soil samples may require plating method or need confirmation testing because presence of other bacteria may result in false positive or false negative
- Absence of Coliforms or Enterococci do not indicate absence of other bacteria

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Air sampling after sewage contamination

Not recommended, but if you have to:

- Use Andersen or similar sampler
- Sample meaningful volume, e.g., 1 m^3
- Target on Gram-negative bacteria
- Use selective media, e.g., MacConky
- Incubate at 35˚C
- Consider sample endotoxin

III. *Legionella* Sampling and Analysis

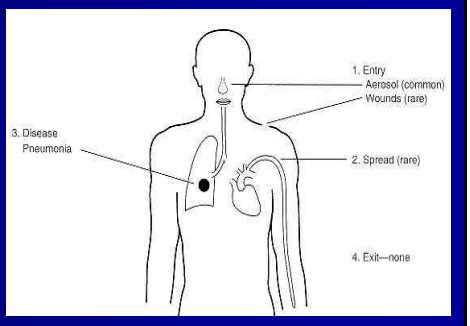
- *Legionella* growth conditions
- *Legionella* sampling and analysis strategies

Legionnaire's Disease

- First reported at American Legion Conference in 1976 (221 infected, 34 deaths)
- The causative agent was named *Legionella pneumophila*
- It is the most common airborne bacterial infection associated with contamination in building water systems

Legionnaire's Disease - Route of Entry

- Entry by inhalation of aerosols containing *Legionella* or aspiration
- Affect the respiratory tract
- Cause pneumonia



Pontiac Fever – a case study

- Hotel's whirl pool spa and swimming pool
- Symptoms: headache, fever, chills, shortness of breath and fatigue.
- *Legionella micdadei* was isolated
- High heterotrophic plate count (68,000,000 CFU/mL)
- High endotoxin level (14,400 endotoxin units/mL)

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Legionella

- Gram-negative bacilli
- Ubiquitous natural inhabitants of fresh water
- >50 species
- *Legionella pneumophila* Serogroup 1
- Optimum growth temperature: 35°C – 45°C (20°C – 50°C)
- Growth in biofilms and get nutrients from algae, protozoa, and other bacteria

Nov. 12, 2008 # 84

Possible *Legionella* Growth Sites

- Air Washers
- Architectural Fountains and Waterfalls
- Cooling Towers
- Direct Evaporative Air Coolers
- Emergency Water Systems (Fire Protection)
- Evaporative Condensers
- Fluid Coolers (Closed Circuit Cooling Towers)
- Hot and cold water faucets
- Humidifiers
- Indirect Evaporative Air Coolers
- Metal Working Systems
- Misters and Atomizers
- Municipal Water Supplies
- Pools, whirlpools and spas
- Potable Water Systems
- Storage tanks and hot water heaters
- Any other source of aerosolized water

Nov. 12, 2008 # 85

Legionella Sampling

- Water samples
- Swab sample for faucet, pipeline, etc.
- Use sterile container/swab
- Water sample volume: 100 mL – 1L
- Add potassium thiosulphate or sodium thiosulphate to inactivate chlorine and other oxidizing biocides
- Deliver the sample to the lab within 2 days
- Ship/store the sample at 5 ± 3˚C

Nov. 12, 2008 # 86

Quiz #4

- What is the preferred sample volume for water *Legionella* testing?

Legionella Detection – Culture Method

- Standard Methods 9260J - for analyzing water samples with high bacterial count
- ISO-11731– Part 2: Direct membrane filtration method for waters with low bacterial counts

Legionella Detection – Culture Method

- Membrane filtration of water sample to concentrate the sample
- Recovery on buffered charcoal yeast extract (BCYE) agar (*Legionellae* do not grow on TSA agar)
- Heat or acid treatment to reduce other microbes
- Incubation at 36 ± 2˚C for up to 10 days, suspect colonies are confirmed as *Legionellae* if they grow on BCYE containing cysteine, but not on agar without cysteine added.
- Use immunological assay to identify the species and/or serogroups present in the sample.

Legionella Detection – QPCR Method

- Quantitative Polymerase Chain Reaction (QPCR)
- QPCR employs the DNA amplification technology using target-specific DNA primers and the fluorescence detection technology, allowing amplification to be read in real time.
- Quantification is performed by calculating starting number of DNA copies
- Reported as DNA-equivalent number of cells
- Pro: quick turn around time
- Con: does not differentiate live or dead cells

Data Interpretation

- The EPA has suggested an acceptable level of *Legionella* in drinking water to be 0 CFU/ml.
- Regulatory agencies and scientific community agree that any species or serotype of *Legionella* that is detected in a building water distribution system above 1 CFU/ml is unacceptable and that some measure of response is required.

Nov. 12, 2008 # 91

OSHA Guidelines:

- Use the following suggested guidelines seen in Table 1. to assess the effectiveness of water system maintenance and to interpret sampling results. These guidelines are based on limited data and are subject to change. They are intended to apply only to water systems being used by healthy individuals and are not necessarily protective for people who are immuno-compromised.

Table 1. Colony forming units (CFU) of LDB per milliliter

Action	Cooling tower/Evaporative Condenser	Potable water	Humidifiers and Misters
1	100	10	1
2	1,000	100	10

The levels requiring action vary for the source of exposure, based on the assumption that some routes of exposure result in a greater dose to the lung. For this reason humidifiers and similar devices (such as misters and evaporative condensers) produce aerosol mists and, therefore, need to be controlled to lower levels than cooling towers and domestic water supplies to minimize the risk of inhalation. Levels of LDB equal to or greater than the values in the table constitute a need for action, as described below:

Action 1

- Cleaning followed by biocide treatment of the system, if appropriate.

Action 2

- Cleaning and or biocide treatment.
- Take immediate steps to prevent employee exposure.

**Remember that these numbers are only suggested guidelines, and the goal is zero detectable LDB in a water source.

Legionella Data Reporting

- "Make the results of all water monitoring tests available to building occupants when excessive levels of LDB [Legionnaires' disease bacteria] are found. Visitors and employees have rights to sampling results as per OSHA Standard."

  OSHA website

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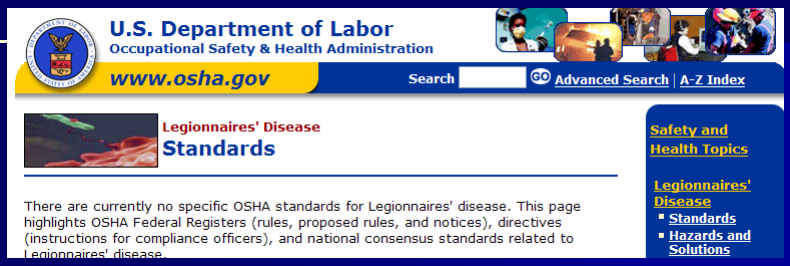
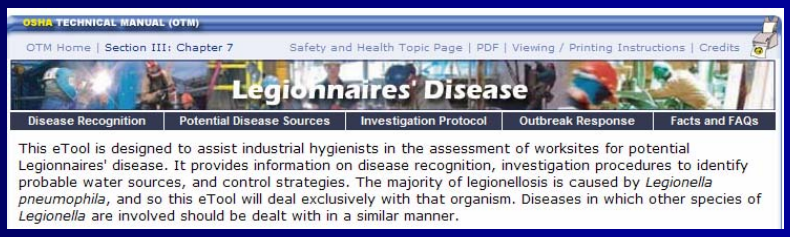
Internet Resources for *Legionella* Sampling





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IV. Sampling for Pathogenic Bacteria

- Bacterial pathogens
- Important bacterial toxins
- Bacterial recovery
- Sampling efficiency
- Novel or high volume sampling

IE significant: *Enterobacteriaceae*

- Include many genera of gram negative bacilli
- Some colonize the intestinal tracts of humans
- Grow well on blood agar and MacConkey at 35°C

- *Enterobacter*
- *Escherichia*
- *Klebsiella*
- *Proteus*
- *Salmonella*
- *Shigella*
- *Yersinia*

Pseudomonas

- Aerobic, gram negative bacilli
- Widely distributed in the environment and in moist areas
- Nosocomial infections in hospitals
- Infection sources include hydrotherapy baths, respiratory therapy equipment
- *Pseudomonas aeruginosa*

Nov. 12, 2008 # 97

Important Airborne Bacterial Pathogens

Pathogens	Diseases
Bacillus anthracis	Pulmonary anthrax
Bordetella pertussis	Whooping cough
Clamydia psittaci	Pneumonia
Corynebacterium diptheriae	Diptheria
Klebsiella pneumoniae	Pneumonia
Legionella pneumophila	Legionellosis

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Important Airborne Bacterial Pathogens

Pathogens	Diseases
Mycobacterium tuberculosis	Pulmonary tuberculosis
Neisseria meningitidis	Meningococcal infection
Salmonella typhi	Typhoid fever
Staphylococcus aureus	Staph, respiratory infection
Streptococcus pyogenes	Strep., respiratory infection
Yesinia pestis	Pneumonic plague

Staphylococcus

- Gram positive, catalase-positive cocci in pairs and clusters
- Commonly colonize the surface of skin and mucosal membranes of mammals and birds
- *Staphylococcus aureus* is frequently encountered human pathogen, causing skin to systemic infections

Methicillin Resistant *Staphylococcus aureus* (MRSA)

- *Staphylococcus aureus* that is resistant to methicillin and other more common antibiotics such as oxacillin, penicillin and amoxicillin.
- Most common skin infection
- Invasive MRSA infections occur in health care facility
- MRSA is an important pathogen
  - Pathogenicity
  - Fewer antibiotic treatment options
  - MRSA are transmissible

Nov. 12, 2008 # 101

Detection of MRSA

- Culture: the cefoxitin disk screen test, the latex agglutination test for PBP2a, or a plate containing 6 µg/ml of oxacillin in Mueller-Hinton agar supplemented with NaCl (4% w/v; 0.68 mol/L)
- PCR: detect the *mec*A gene, which mediates oxacillin resistance in staphylococci.

Nov. 12, 2008 # 102

Mycobacterium tuberculosis

- Causes tuberculosis in humans
- The leading cause of death in the world from a bacterial infection
- Affects 1.8 billion people/year (i.e., 1/3 of the world's population)
- Has an important place in history and masterpiece literature

Nov. 12, 2008 # 103

Sampling for Airborne *Mycobacterium*

- Get public health professionals involved
- Culturable bioaerosol sampling
  - Liquid impinger
  - Filter cassettes
- The bacterium is 1-10 x 0.2-0.7 µm, with characteristic mycolic acids in cell wall

Nov. 12, 2008 # 104

Analysis of *Mycobacterium*

- Requires special media (agar-based, egg-based or liquid)
- Incubation at 35-37ºC
- Genus-specific and species-specific DNA probes
- It was estimated that the indoor airborne *Mycobacterium tuberculosis* droplet nuclei concentration is as low as 1 infectious unit per 310 m^3
- Infectious dose is less than 10

Nov. 12, 2008 # 105

Important Airborne Bacterial Toxins

- Botulinal A toxin (*Clostridium botulinum*)
  - Lethal dose by inhalation is 0.3 µg, neurotoxin
- Staphylococcal enterotoxin
  - Lethal dose by inhalation is 25 µg
- Lipopolysaccharides (endotoxin)
  - Dose for toxic effect by inhalation is <10 ng

Nov. 12, 2008 # 106

Endotoxin

- The outer membrane of gram-negative bacteria: lipopolysaccharide
- Stable: e.g., it requires 250°C and a duration of 30min to break down endotoxin
- Release to the environment during active bacterial cell growth or after bacterial cell lysis
- Can be found in office buildings & homes where there are humidification systems, sewage treatment facilities, and agriculture product processing facilities

Health Effects of Endotoxin Exposures

- Allergen
- Cause respiratory distress syndromes
- Acute lung effects – reported zero-change threshold as low as 90 EU/m^3
- Chronic lung effects – exposure of 10 – 200 EU/m^3 may lead to adverse respiratory health effect

Environmental Endotoxin Sampling

- Settled dust or air can be sampled by using filter cassettes (e.g., polycarbonate membrane filters or Teflon filters)
- Use endotoxin-free sampling cassettes
- Seal the cassettes well after sampling
- Ship the samples cool to avoid significant changes in gram-negative bacterial population

Nov. 12, 2008 # 109

Endotoxin Analysis

- Chromogenic Limulus Amebocyte Lysate (LAL) assay, utilizing blood from the Horseshoe crab.
- Very sensitive
- The results are read by a microplate reader

Nov. 12, 2008 # 110

Bacterial Recovery

- Know your target bacteria
- Select analytical method first
- General purpose vs. selective media
- Know method detection limit

Quiz #5

- What should we be concerned about when sampling for a pathogen?

Sampling Efficiency

- Efficiency of locating randomly distributed bacteria
- Efficiency of collecting bacteria on selected location
- Efficiency of extracting bacteria from sampling device

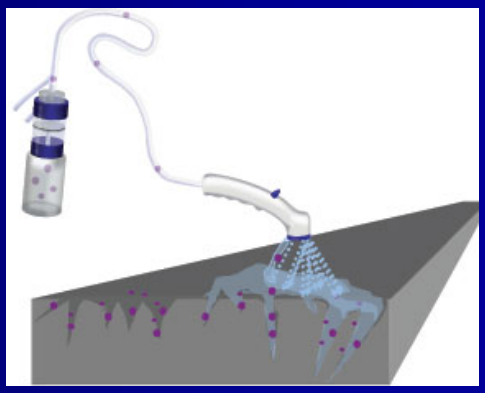
Nov. 12, 2008 # 113

Novel or High Volume Sampling Methods (examples)

- Surface: M-Vac Sampling System
- Air: High volume sampler
- Air: Long-term sampling devices

Nov. 12, 2008 # 114

M-Vac System



Nov. 12, 2008 # 115

Liquid Impinger

e.g., BioSampler (SKC, Inc.)

- 8 hours or less
- Dilute buffer solution
- Typical airflow rate: 12.5 lpm
- Culture, PCR, biochemical analysis, and immunoassay

Nov. 12, 2008 # 116

High Volume Air Sampler

- Collection media: Liquid
- 5 minutes to 6 hours
- Airflow rate: 450 lpm
- Culture, PCR, biochemical analysis, and immunoassay
- For fungi, bacteria, viruses

Nov. 12, 2008 # 117

Personal Protection when sampling for pathogenic bacteria

- Consult with public health experts
- Investigators should use appropriate personal protective equipment
  - Respiratory protection gear
  - Microorganism-resistant clothing
- Avoid cross contamination
- Practice good personal hygiene

Nov. 12, 2008 # 118

Internet Resources for Pathogen Information

Thank you!

- Questions?