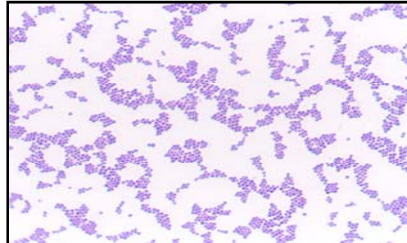


BACTERIA

Bacteria are unicelled prokaryotic organisms. Typical bacterial cells are about $1\mu\text{m}$ in width compared to a eukaryotic cell and are ubiquitous in nature. Depending on the environmental factors like moisture, temperature, oxygen level, they multiply rapidly in nature. Bacteria are unstable in the outdoor environment due the environmental stress. In the indoor environment, when conditions are appropriate they multiply rapidly resulting in emission of toxins into the surrounding environment.

Bacteria are of different shapes. The most common forms are the rods (bacillus) and round (cocci). For identification, these microorganisms are again classified on the basis of their staining ability. The common stain used is Gram's stain. They are classified as:

Gram positive – stains purple



Source: Shawn Becker; Soil microbiology; cell wall components.
http://filebox.vt.edu/users/chagedor/biol_4684/Methods/cellwalls.html

Gram negative – stains red



Source: Shawn Becker; Soil microbiology; cell wall components.
http://filebox.vt.edu/users/chagedor/biol_4684/Methods/cellwalls.html

COMMON ORGANISMS:

1. GRAM POSITIVE ORGANISMS:

Aerococcus viridans: This bacterium is found in air, meat brines, and processed vegetables. Commonly found as airborne organisms in hospitals.

Arthrobacter globiformis: This bacterium is widely distributed in the environment, particularly in soil.

Bacillus cereus: *Bacillus sp.* are ubiquitous in nature and occurring in soil, water and airborne dust. *B. cereus* can cause food poisoning associated with ingestion of meats, vegetables, pasta, desserts, cakes, sauces and milk. *B. cereus* spores can survive cooking and then germinate to produce viable bacteria that can divide on foodstuffs. It can also cause eye infections and wound infections following surgery, accidents, and burns.

Bacillus subtilis: *Bacillus sp.* is common in the indoor and outdoor environments. This bacterium inhabits soil, dust, water and materials of plant and animal origin.

Clostridium spp.: widespread in the environment. Many species produce potent exotoxins and are pathogenic.

Corynebacterium spp.: Widely distributed in nature. Normal skin flora and commonly found in soil and water. *Corynebacterium diphtheriae* causes diphtheria due to production of exotoxin. Also causes cutaneous infection .

Dermabacter hominis: This bacterium can be found on human skin.

Dermococcus nishinomiyaensis: widespread in nature and commonly found on human skin and other mammals.

Enterococcus faecalis: They are commonly found in soil and in water. *Enterococcus sp.* is also normal flora of the gastrointestinal tract of humans.

Enterococcus saccharolyticus: Occur widely in the environment.

Gemella bergi: Normal flora of oral cavity or upper respiratory tract.

Kocuria rosea: *Kocuria sp.* can be found in freshwater environments and the soil.

Kurthia: They are common in animal feces, meat products and widely distributed in the environment. Non-pathogenic.

Lactobacillus spp: Widely distributed in nature, especially in animals and vegetable products. They normally inhabit the gastrointestinal tract of birds and mammals. Rarely pathogenic.

Listeria spp.: Commonly found in nature and has been isolated from soil, water, sewage and decaying vegetable matter. Causes food poisoning and some species are pathogenic to humans and

animals.

Micrococcus luteus: Ubiquitous in the environment, although, it is primarily isolated from mammalian skin and soil.

Pediococcus acidilactici: Found naturally on plants. Commonly found in food and vegetation.

Rhodococcus equi: Widely distributed and commonly found in soil and herbivore dung.

Streptococcus pyogenes: Members of the *Streptococcus* genus may be isolated from the blood, alimentary, respiratory, and genital tract of humans. In the environment they have been found in milk and dairy products, soil, fecal-contaminated water and contaminated dust. This particular species is responsible for the classic strep throat. They are rarely transmitted by indirect contact through objects.

Staphylococcus epidermis: Is present as normal flora on the skin and mucous membranes of humans. The presence of “slime” results in the production of biofilm. This particular species is of low virulence, and an individual is usually immunocompromised in some way for an infection to develop.

Staphylococcus aureus: This organism is commonly shed from skin and mucous membranes humans or warm-blooded animals. It can cause opportunistic infection and also food poisoning. It may be isolated from the air and from indoor surfaces.

Staphylococcus saprophyticus: They are widespread in nature and commonly associated with skin and mucous membrane of humans and animals. This species shares many characteristics with *S. epidermidis* except that this species is important opportunistic pathogen in urinary tract infections in young women.

References:

Patrick .R. Murray (2003); Manual of Clinical Microbiology: 8th edition: pp 445

Diagonstics Microbiology 11th Edition: Betty A. Forbes, Daniel F. Sahm, and Alice.S. Weissfeld.

John.G.Holt, Noel R.Krieg, Peter H.A. Sneath, James T. Staley, Stanley T. Williams; Bergey’s Manual Of Determinative Bacteriology: 9th Edition.

Patrick R.Murray, Ken S.Rosenthal, George S.Kobayashi and Michael A.Pfailer; Medical Microbiology, 3rd Edition: pp209

Information provided by AIHA EMPAT program

MRSA (METHICILLIN RESISTANT STAPHYLOCOCCUS):

MRSA are a classification of staphylococcus bacterium that are resistant to common antibiotics like penicillin, oxacillin, vancomycin and methicillin. These are different from other Staph. spp. in that they are coagulase tests positive. These bacteria are commonly found in nose, skin and mucous membranes of humans. They are non-pathogenic unless entry into the body through wounds or cuts. Formerly they were considered to be nosocomial infection i.e. hospital acquired. Infections outside the hospital and health care facilities are becoming more prevalent.

MRSA is classified into two groups:

CA-MRSA (Community acquired): These are infections acquired outside the hospital or health care facilities. They are spread from person to person through contact or use of contaminated items. They are commonly seen in children (schools), athletes (school, gyms, locker rooms), offices, military recruits etc.

HA- MRSA (Hospital acquired): These are infections acquired after treatment from hospitals and health care facilities. They are commonly seen in immunocompromised patients.

Surface samples are the appropriate method for determining the presence of MRSA in an environment. The area of the sample is dependent upon if the sample is taken as an assessment sample or a post cleanup sample *and* the actual location, such as a locker room, wrestling room, surgical suites, or mechanical systems. Viable air samples are taken by an Andersen style sampler. Air samples are collected on a general lab media and a selective media. The total air volume is dependent upon the site and the desired sensitivity. When MRSA is the target organism it is best to discuss sampling strategies with your laboratory.

Analysis	Matrix	Test code	Requirements
MRSA & Total Bacterial culture W/ ID	Air	1121	BAP, Maconkey, MSA plates
	Wipe	1122	BAP, Maconkey, MSA plates
	Water	1123	BAP, Maconkey, MSA plates
	Bulk	1124	BAP, Maconkey, MSA plates

References and Informative websites:

Patrick .R. Murray (2003); Manual of Clinical Microbiology: 8th edition: pp 384

John.G.Holt, Noel R.Krieg, Peter H.A. Sneath, James T. Staley, Stanley T. Williams; Bergey's Manual Of Determinative Bacteriology: 9th Edition.

Bailey and Scott's Diagonstics Microbiology 11th Edition: Betty A. Forbes, Daniel F. Sahn, and Alice.S. Weissfeld.

Information provided by AIHA EMPAT program

- <http://www.oregon.gov/DHS/ph/acd/diseases/mrsa/mrsa.shtml>
- http://www.lapublichealth.org/acd/docs/MRSA/MRSA_Guideline_12_20_04.pdf
- http://www.cdc.gov/ncidod/dhqp/ar_mrsa.html
- [Management of antimicrobial-resistant organisms in long-term care facilities](#) (.pdf developed by Oregon ARM taskforce, June 1998; PDF 43k)
- CDC [guidelines on hand hygiene in healthcare settings](#) (MMWR article)
- Learning about MRSA: A guide For Patients: Minnesota Department For Health: <http://www.health.state.mn.us/divs/idepc/diseases/mrsa/book.html>

ANTHRAX

The organism involved is *Bacillus anthracis*. These are gram-positive rod and spore forming bacteria. They are commonly found in soil, where it is contracted by various herbivores and causes diseases in these animals. Not a part of normal human flora. Infections occur by inhalation of spores or the introduction through breaks in the skin or mucous membranes. Produces exotoxin and are causative organisms of cutaneous, pulmonary and gastrointestinal anthrax.



Source: Public health image library (PHIL)



Bacillus anthracis - positive gram stain

Source: Communicable disease control and prevention; San Francisco department of public health

Analysis	Matrix	Test code	Requirement
Anthrax analysis	Air	2098	Blood agar or 0.4μ cassette
	Wipe	2099	Swab
	Bulk	2003	Bulk

References and Informative websites:

Patrick .R. Murray (2003); Manual of Clinical Microbiology: 8th edition: pp 445

Diagnostics Microbiology 11th Edition: Betty A. Forbes, Daniel F. Sahm, and Alice.S. Weissfeld.

John.G.Holt, Noel R.Krieg, Peter H.A. Sneath, James T. Staley, Stanley T. Williams; Bergey’s Manual Of Determinative Bacteriology: 9th Edition.

Patrick R.Murray, Ken S.Rosenthal, George S.Kobayashi and Michael A.Pfailer; Medical Microbiology, 3rd Edition: pp209

Information provided by AIHA EMPAT program

Images from public health image library (PHIL)

- [Center for Infectious Disease Preparedness and Reponse \(CIDRAP\) - Anthrax site](#)
- [Centers for Disease Control \(CDC\) - Anthrax site](#)

2. GRAM NEGATIVE:

FERMENTATIVE GRAM NEGATIVE RODS (FGNR)

Campylobacter spp.: They microaerobic inhabitants of the gastrointestinal tract of various animals. They are known to contaminate drinking water and also associated with improperly pasteurized milk. Causes gastrointestinal and extra intestinal infections.

Citrobacter freundii: *Citrobacter sp.* are enteric organisms that inhabit the intestinal tract of humans and animals. They can be found in soil, water, sewage and food.

Edwardseilla tarda: Occurs in the intestine of cold-blooded animals and their environment especially in fresh water. Occasionally found in humans and warm-blooded animals.

Enterobacter agglomerans (Pantoea agglomerans): This organism is found in water, soil and other parts of the environment. It is occasionally isolated from humans and has been implicated in neonatal meningitis and sepsis.

Enterobacter cloacae: Part of the normal flora of human intestinal tract. Can cause opportunistic infection of urinary tract as well as other parts of the body.

Erwinia spp.: Rarely isolated from humans. Associated with plants as pathogens, saprophytes or part of the epiphytic flora.

Escherichia coli: *E.coli* is an enteric organism found in the gastrointestinal tract of humans and animals. It can also be found environmentally in soil, sewage, and in water.

Hafnia alvei: This genus contains only this one species. *Hafnia alvei* is found in animal feces, sewage, soil, water and dairy products.

Klebsiella oxytoca/pneumoniae: Organisms in the genus *Klebsiella* can be found in human feces, soil, water, grain, fruits and vegetables. These organisms are responsible for a variety of nosocomial infections.

Leclercia adecarboxylata: Isolated from food, water and the environment. An occasional human pathogen.

Morganella morganii: Occurs in the faeces of human, dogs and other mammals and reptiles. Opportunistic human pathogen.

Proteus vulgaris: *Proteus sp.* are enteric organisms found in human and animal intestines, manure, soil, and polluted waters. They can cause a myriad of extra-intestinal infections.

Providencia stuartii: Widely distributed in plants and soil, water and in the intestine of humans and animals.

Serratia marcescens: Commonly found in water, soil, food, plant surfaces and contaminated indoor environments. Prominent opportunistic pathogen for hospital patients causing septicemia and urinary tract infection.

Shigella : Commonly found in overcrowded areas. Causes shigellosis and not present in the common human bowel flora. Isolated from areas of poor sanitary conditions.

Salmonella choleraesuis: *Salmonella sp.* occurs in humans, warm and cold-blooded animals, and in the environment. Bacteria in this genus are pathogenic for humans. They can cause typhoid fever, enteric infections and sepsis.

Yersinia spp.: Primarily environmental organisms but more adapted to aquatic environment. May colonize warm or cold-blooded host in a transient manner.

NON FERMENTATIVE GRAM NEGATIVE RODS (NFGNR)

Aeromonas hydrophila: Aquatic environments worldwide. It is also recovered from a variety of grocery store produce and seafood.

Acaligenes: Occur in soil and water. some are saprophytic inhabitants of intestinal tract of vertebrates. Are opportunistic human pathogens.

Acinetobacter baumannii : *Acinetobacter sp.* are widely distributed in nature including soil, water, and sewage. Human infections usually follow colonization of hospitalized patients and may involve the urinary tract, respiratory tract, wounds, soft tissue infections and bacteremia.

Brucella: They are intracellular parasites. Causes brucellosis. Infection by ingestion of contaminated food, inhalation of the spores and consumption of unpasteurized milk.

Burkholderia cepacia: *Burkholderia* bacterium is an environmental organism found commonly in water, soil, and plants. They are often isolated from hospital environments due to their ability to survive in aqueous environments. *B. cepacia* causes opportunistic infections associated with hospital equipment, medication and disinfectants. It is often isolated from patients with chronic granulomatous disease and cystic fibrosis.

Budvicia aquatica: Commonly found in fresh water.

Brevundimonas diminuta: Widely distributed. Commonly found in water, soil and plants mainly on fruits and vegetables.

Chromobacterium violaceum: *C. violaceum* is common in temperate climates where it is present in soil and surface waters. It can cause sepsis, lymphadenitis and other infections in humans.

Chryseomonas meningoseptica: Widely distributed in the environment. Commonly saprophytic and rarely pathogenic.

Delfia acidovorans: Have been isolated from soil, sediment activated sludge crude oil, brine, water and various clinical specimens.

Flavobacterium: Widely distributed in soil and water; also found in raw meat, milk and in hospital environment.

Francisella tularensis: Causative agent of tularemia in humans and animals.

Moraxella spp: Widely spread in nature. Rarely infectious. Commonly infects the urogenital tracts, mucous membranes covering the nose, throat and other parts of the respiratory tracts. May colonize the skin. Can cause bacteremia, sepsis and endocarditis.

Myroides odoratus/Myroides odoratimimus: These bacteria used to be known as *Flavobacterium odoratum*. Human infections are rare with these organisms, however, they have been isolated from urine, sputum and wounds. They occur environmentally in soil, water, plants and foodstuffs.

Oligella urethralis: Widely distributed in nature.

Pleisiomonas shigelloides: Occurs in fresh surface water, fish, shellfish and other aquatic animals. It is associated with opportunistic human infections

Pseudomonas aeruginosa: Widely distributed in soil, water and plants. It survives in hot tubs, whirlpools, contact lens solution, sinks and showers. It can cause a number of opportunistic infections including infections of the skin, external ear canal and of the eye.

Pseudomonas alcaligenes/orizyhabitans/stutzeri: All commonly found in the environment.

Ralstonia pickettii: Widely distributed in nature. Found in soil, water and plants.

Sphingobacterium: Commonly isolated from water, soil and plants. Not a human pathogen.

Stenotromonas maltophila: Found in soil, water and food.

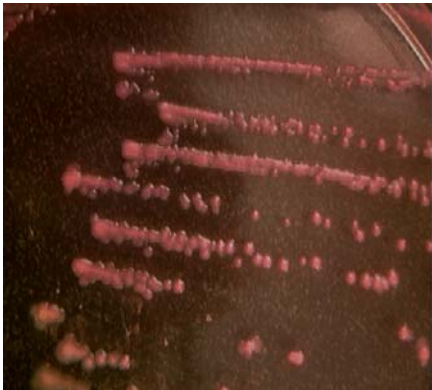
Analysis	Matrix	Test code	Requirement
Total bacterial count w/ID	Air	1005	TSA
	Bulk	1008	Sterile container
	Water	1007	Sterile bottle w/ $\text{Na}_2\text{S}_2\text{O}_3$
	Wipe /surface	1006	Sterile swab
Total bacterial count	Air	1001	TSA
	Bulk	1004	Sterile container
	Wipe/surface	1003	Sterile swab
Heterotrophic plate count	Water	2056	Sterile bottle w/ $\text{Na}_2\text{S}_2\text{O}_3$

COLIFORMS:

These are gram-negative bacteria that come under Enterobacteriaceae. These are commonly found in the environment and also in the feces of human and warm-blooded animals. Coliforms are used as an indicator for contamination of drinking water by fecal and environmental sources. They enter the water sources from untreated human sewage, failing septic tanks, agricultural runoff and animal waste run off. Naturally in the environment, they are not pathogens but when present in drinking water can cause health problems.

Coliforms include bacteria like:

- E.coli



- Serratia



Source: Public health image library (PHIL)

- Klebsiella



Source: Public health image library (PHIL)

- Citrobacter
- Yersinia
- Hafnia

- Enterobacter



Enterobacter cloacae

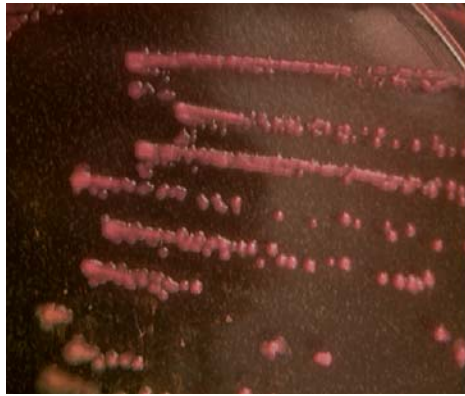
Source: Public health image library (PHIL)

Coli forms are divided into:

Total coli forms: These coliforms are commonly found in the environment (soil and vegetation) and are harmless. They are opportunistic pathogens and include fecal coliforms. Presence of these coliforms shows contamination of water from environmental sources and also indicates a probable contamination by fecal coli forms.

Fecal coli forms: These are coliforms found in the intestine of warm-blooded animals and in humans. These are released to the environment by fecal contamination of the drinking water. These are indicator of recent sewage or animal waste contamination. The most common genera are E.coli, Klebsiella, Citrobacter and Serratia.

E.coli (Escherichia coli):



E.coli on Macconkey agar (pink color)

This group of bacteria comes under fecal coliforms. The presence of these bacteria in water is an indicator of water contamination with human and animal waste. They are commonly found in the intestines of humans and animals. They enter the water sources after run off by rain or snow. When these water sources are not treated and used as drinking water, results in E.coli infection. Not all types of E.coli are pathogenic and cause gastrointestinal diseases. The main groups of E.coli that are pathogenic are:

- Enteroinvasive E.coli (EIEC): Commonly found in humans only. They cause diarrhea similar to shigellosis. They do not produce any toxins.
- Enterotoxigenic E.coli (ETEC): Causes diarrhea in humans and animals. they produce enterotoxins.
- Enteropathogenic E.coli (EPEC): Causes diarrhea in humans and animals. Causes the change in intestinal cell structure. Symptoms similar to shigellosis.
- Enteroaggregative E.coli (EAaggEC): Causes aggregation of tissues in humans only.
- Enterohaemorrhagic E.coli (EHEC): Found in humans and animals. Includes the virulent type O 157: H7. Causes diarrhea in humans.

Analysis	Matrix	Test code	Requirement
Total coliforms /E.coli	Air	1074	TSA & Maconkey plates
	Bulk	1013	Bulk
	Wipe /surface	1012	Sterile swab
Potable, Total coliforms/E.coli*	Water	1010	Sterile bottle w/ Na ₂ S ₂ O ₃
Non-potable, Total coliforms /E.coli*	Water	1011	Sterile bottle w/ Na ₂ S ₂ O ₃

* Potable and non-potable test gives only qualitative analysis of the sample i.e. it shows only the presence/absence of coliforms. Number of organisms not available.

References and Informative websites:

Patrick .R. Murray (2003); Manual of Clinical Microbiology: 8th edition:

Diagnosis Microbiology 11th Edition: Betty A. Forbes, Daniel F. Sahm, and Alice.S. Weissfeld.

John.G.Holt, Noel R.Krieg, Peter H.A. Sneath, James T. Staley, Stanley T. Williams; Bergey's Manual Of Determinative Bacteriology: 9th Edition.

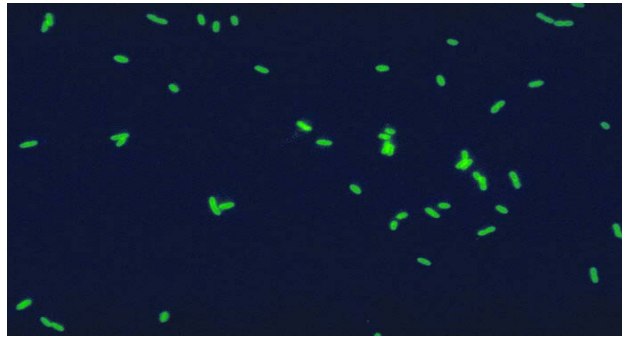
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Information provided by AIHA EMPAT program

- <http://www.doh.wa.gov/ehp/DW/Programs/coliform.htm>
- <http://www.epa.gov/safewater/contaminants/ecoli.html>
- CDC: E.coli: <http://www.cdc.gov/ecoli/>

LEGIONELLA:

They are widely distributed in nature mainly in lakes and ponds. These are gram-negative rods and require selective media (BCYE) for their isolation and growth. Commonly found in natural and man-made environment. Natural rubber, plastics and wood influence the growth of legionella while copper inhibits the growth. *Legionella sp.* can be isolated from surface water, mud and from thermally polluted lakes and streams, this organism can also be found in water-cooling towers, hot water tanks, evaporative condensers, spas, respiratory therapy equipment, showers, water faucets, decorative fountains, ultrasonic mist machines, technical-medical equipments and damp potting soil. They can survive extreme range of environments for long period of time.



Source: Public health image library(PHIL)

Causative agent of Legionnaire's Disease and Pontiac Fever. Legionella infection is mainly from environmental sources. Disease transmission occurs mainly by inhalation of the infectious aerosols from work place, hospitals or industrial settings. Water and wipe samples are more viable than air samples. Air samples can indicate the level of Legionella contamination from water into air. Disinfection methods include chlorination, ozonation, use of ultra violet, heat-flush (heat shock) method and copper silver ion systems.

Analysis	Matrix	Test code	Requirements
Legionella	Water	1015	Sterile bottle w/ $\text{Na}_2\text{S}_2\text{O}_3$
	Air	1016	BCYE, DGVP & BAP plates
	Wipe	1017	Sterile swab
	Bulk	1063	Bulk sample

References and Informative websites:

Bioaerosols: Assessment And Control: ACGIH; 1999: CHAPTER 18.

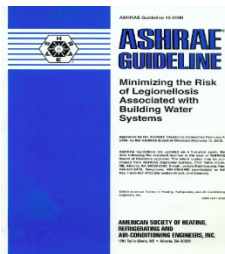
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John.G.Holt, Noel R.Krieg, Peter H.A. Sneath, James T. Staley, Stanley T. Williams; Bergey's Manual Of Determinative Bacteriology: 9th Edition.

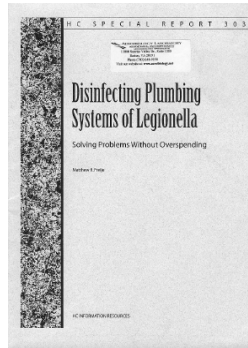
Patrick R.Murray, Ken S.Rosenthal, George S.Kobayashi and Michael A.Pfailer; Medical Microbiology, 3rd Edition.

- ASHRAE GUIDELINE: Minimizing the Risk of Legionellosis associated with building water systems.

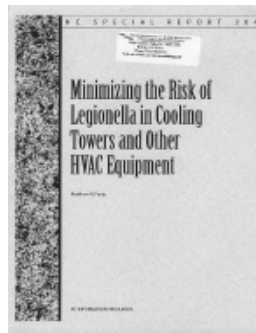


- Environmental sampling: <http://www.cdc.gov/legionella/files/LegionellaProcedures.pdf>
- Legionella: Current Status And Emerging Perspectives: James. M.Barbaree, Robert F.Briman and Alfred P. Dufour; 1993

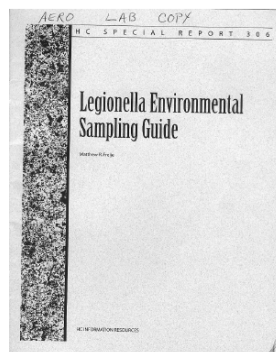
- Disinfecting plumbing systems of Legionella.



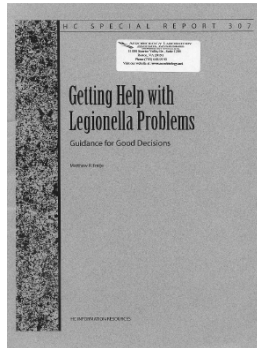
- Minimizing the risk of legionella in cooling towers and other HVAC equipment.



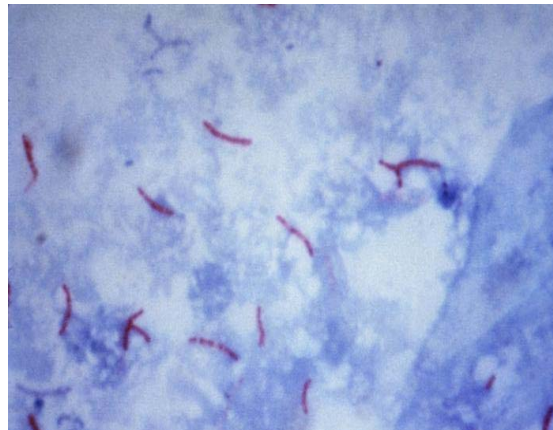
- Legionella environmental sampling guide



- Getting help with legionella problems: guidance for good decisions.



MYCOBACTERIUM:



Source: Public health image library(PHIL)

These are rod shaped, slow growing bacteria that are widely distributed in soil and water. They may be branched or filamentous or mycelium like. These organisms are acid fast (stain) due the presence of waxy coating on cell wall (mycolic acid). The two human pathogenic strains are: *Mycobacterium tuberculosis* and *Mycobacterium leprae*. Some common species of environmental mycobacterium are:

***Mycobacterium fortuitum*:** This *Mycobacterium sp.* can be isolated from water, soil and dust. They can be linked to hypersensitivity pneumonitis reactions in health spa whirlpools. They are the most common species to be associated with nosocomial outbreaks due to rapid growth. Commonly found in water, soil and dust.

Mycobacterium avium: This species of *Mycobacterium* is ubiquitous in nature and has been isolated from water, soil, plants, house dust and a number of other environmental sources.

Mycobacterium chelonae: Commonly found in soil, dust. Linked to hypersensitivity pneumonitis in metal working fluids.

Mycobacterium smegmatis: Commonly found in soil and are non pathogenic.

Analysis	Matrix	Test code	Requirements
Mycobacterium	Water	1096	Sterile bottle
	Air	1067	Middlebrook agar
	Wipe	1068	Sterile swab

References and Informative websites:

Bioaerosols: Assessment And Control: ACGIH; 1999: CHAPTER 18.

Patrick .R. Murray (2003); Manual of Clinical Microbiology: 8th edition.

Diagnosis Microbiology 11th Edition: Betty A. Forbes, Daniel F. Sahm, and Alice.S. Weissfeld.

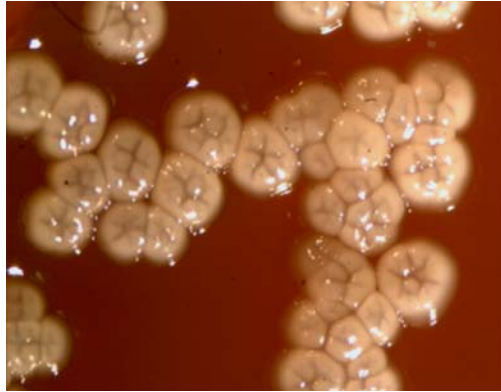
John.G.Holt, Noel R.Krieg, Peter H.A. Sneath, James T. Staley, Stanley T. Williams; Bergey's Manual Of Determinative Bacteriology: 9th Edition.

Patrick R.Murray, Ken S.Rosenthal, George S.Kobayashi and Michael A.Pfaler; Medical Microbiology, 3rd Edition.

Information provided by AIHA EMPAT program.

4. ACTINOMYCETES:

These are gram-positive organisms but are filamentous and highly branched. They are slow growers and widely distributed in nature.



Streptomyces griseus colonies

Streptomyces spp.: They are found in the soil, decaying vegetation, agricultural work environment. May also be found in carpets, HVAC and other indoor sources.

Nocardia spp.: They help in decomposition of organic plant material. They are opportunistic human pathogens and also infect animals. Causes damage in wastewater and sewage plants due to bulking (floating of sludge).

Rhodococcus spp.: Commonly isolated from soil, and farm animals and also from fresh and salt waters. Rhodococcus equi is only species associated with human infection mainly immunocompromised individuals.

Analysis	Matrix	Test code	Requirement
Total Thermophilic Actinomycete Count	Air	1045	TSA & nutrient agar
	Bulk	1048	Sterile container
	Wipe /surface	1046	Sterile swab
	Water	1047	Sterile container

References

Bioaerosols: Assessment And Control: ACGIH; 1999.

Patrick .R. Murray (2003); Manual of Clinical Microbiology: 8th edition.

Betty A. Forbes, Daniel F. Sahm, and Alice.S. Weissfeld: Diagnostics Microbiology :11th Edition:

John.G.Holt, Noel R.Krieg, Peter H.A. Sneath, James T. Staley, Stanley T. Williams; Bergey's Manual Of Determinative Bacteriology: 9th Edition.

Information provided by AIHA EMPAT program.

5. MISCELLANEOUS BACTERIAL ANALYSIS:

➤ *Nitrifying bacteria:*

These bacteria convert organic nitrogenous materials from ammonium to nitrates. In water, aggressive nitrifiers can result in production of high concentration of nitrogen. They are of potential health risk especially to infants. The presence of these bacteria indicates degradation of nitrogen rich organic matter, pollution of water by these organisms from septic tanks, sewage systems and industrial wastes.

➤ *Sulphur reducing bacteria*

These bacteria generate hydrogen sulphide. They clog water wells and pipelines, corrode pumps, catalyze reactions soils and cause built structures to decay. They cause a number of significant problems with water ranging from “rotten egg” odor to the blackening of equipment, slime formation and initiation of corrosion. They are difficult to detect and are found deep in the biofilm.

➤ *Iron filamentous bacteria*

These bacteria have outer coating called “sheath” which contain the cells. They use iron for their metabolism. Taste, odor problems and “red water” are the common symptoms related to iron bacteria. They commonly grow in ferrous iron containing natural waters; fresh water & marine habitats. They are frequently found in ferruginous mineral springs, wells, drain pipes & storage basins in water works. They cause problems in pipelines.

Eg: Gallionella , Leptospirillum ,Crenothrix, Sphaerotilus, Siderocapsa and Thiobacillus ferroxidans

Analysis	Test code	Requirement
Nitrifying bacteria	1070	Sterile bottle
Sulphur reducing bacteria	1072	Sterile bottle
Filamentous iron bacteria	1071	Sterile bottle

References:

- a. Product insert from BartTM Droycon bioconcepts, Inc. 2004.
- b. Bergey’s manual of systematic bacteriology, volume 20.section 20, “iron and manganese oxidizing and /or depositing bacteria”. 1989

HEALTH CONDITIONS ASSOCIATED WITH BACTERIA:

Several health conditions are related to exposure to bacteria, bacterial antigens, endotoxins and by-products. Bacterial antigens can be found in the outdoor air, in settled dust, active indoor reservoirs and humidifiers or other water related sources.

Some of the conditions related to exposure to bacteria are asthma, building-related symptom (BRS), humidifier/inhalation fever, hypersensitivity diseases (allergy), infectious diseases, Legionnaires' disease and pneumonia.

Building related asthma is characterized by complaints of chest tightness, wheezing, cough, and shortness of breath that is worse on workdays and improves on weekends or vacations. Symptoms may occur within an hour of exposure, onset may be delayed for 4 to 12 hours, or both. A work-related pattern of reduced peak airflows often indicates that asthma is due to a building-related exposure. Exposure to bacterial antigens from office cool-mist humidifiers has been established. Asthma has also been seen in the industrial setting in high prevalence among workers with elevated daily endotoxin exposures from contaminated water sprays.

Building-related symptoms (BRS) has been correlated with humidification and cooling systems due to microbial contamination. The chief candidates for relationships between BRSs and bioaerosols are endotoxins from gram-negative bacterial cell walls and microbial volatile organic compounds (MVOCs).

Humidifier/inhalation fever is a flu-like illness, characterized by fever, chills, muscle aches, malaise, and check symptoms. These symptoms usually arise within 4 to 8 hours of exposure and subside within 24 hours, usually with long-term effects. People with humidifier fever rarely consult physicians for this short-lived, flu-like illness. Recent work suggests that intermittent, high exposure to endotoxin may cause humidifier fever, whereas daily endotoxin exposure may result in asthma. Repeated, severe episodes of inhalation fever due to endotoxin exposure may lead to emphysema.

Hypersensitivity diseases result from exposure to bacterial antigens, which stimulate a specific immunological response. It is a disease for which a subsequent exposure to an antigen produces a greater effect than that produced on initial exposure. Hypersensitivity pneumonitis (HP) is an inflammatory disease of the lung due to cell-mediated immunological reactions in pulmonary tissues. It is associated with antigen-specific immunoglobulin G (IgG), elevated T lymphocytes in bronchoalveolar lavage fluid, and granuloma on lung biopsy.

Other antigen-mediated diseases, allergic rhinitis, sinus responses and most allergic asthma occur in persons with a genetic makeup that allows production of antigen-specific IgE. HP and elevated asthma risk can co-exist in problem buildings. Most building-related antigens are assumed to be bacterial or fungal origin, but protozoa have also been implicated.

HP is characterized by (a) acute, recurrent pneumonia with fever, cough, chest tightness and lung infiltrates, (b) progression of cough, shortness of breath, fatigue, and chronic lung fibrosis, or (c) an intermediate pattern of acute and chronic lung disease. Often the specific microorganism(s) involved in an HP outbreak or its source is not known. Indoor environment that may harbor bacteria known to cause HP are mists in industrial settings, indoor swimming pools, indoor humidifiers, contaminated air-handling units and bioaerosols from water damaged materials. Typical bacterial agents of HP are mycobacterium species, thermophilic actinomycetes, *Bacillus subtilis*, *Sphingobacterium* in addition to others.

Legionnaire's Disease is a bacterial pneumonia caused by *Legionella pneumophila*. It is typically caused by inhalation of aerosols from cooling towers, evaporative condensers, misters and other water associated devices. It is also been documented to cause the infection by drinking contaminated water followed by aspiration. After exposure the incubation period for onset of symptoms is 2 to 10 days.

Recent infections caused by Methicillin-Resistant *Staphylococcus aureus* (MRSA) in non-healthcare setting, designated CA-MRSA, community acquired MRSA, have increased significantly since the 1990's. Healthcare-acquired MRSA, HA-MRSA has been a significant agent of nosocomial infections since the 70's. Approximately 25 % to 30% of the population is colonized with *S. aureus* in the nasal passages. *Staph aureus* is one of the most common causes of skin infections in the US but can also cause serious infections following surgical procedures and may cause sepsis. About 0.8%, or 2.4 million people in the US, is thought to be colonized with MRSA.

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TERMS ASSOCIATED WITH BACTERIA:

Aerobic bacteria: These are bacteria that require oxygen for growth.

Anaerobic bacteria: These are bacteria not dependent on oxygen for growth.

α-Hemolytic streptococcus: These are commonly found in the mouth and nasal flora. These are gram-positive chaining bacteria.

Bacteremia: Presence of bacteria in blood.

Bacillus: Rod shaped bacteria.

Bactericidal: Toxin produced from bacteria capable of killing other bacteria.

Coliforms: Gram-negative bacteria that can ferment carbohydrates like lactose with acid and gas formation at 35°C within 48 hrs.

CONS (coagulase negative staphylococcus): These are commonly staphylococcus spp. that are coagulase test negative. They are present as normal flora on the skin and mucous membranes of humans. They are of low virulence, and an individual is usually immunocompromised in some way for an infection to develop. Eg: Staphylococcus epidermis.

Enterics: These bacteria belong to the group Enterobacteriaceae.

Endocarditis: Inflammation of the lining of heart.

Endotoxin: Are “toxins” found in the outer membrane of gram-negative rods. They are comprised of lipopolysaccharides. Endotoxins are encountered in the environment as part of the whole cell or fragments of the membrane. Endotoxin is a powerful, non-specific stimulant to the immune system and exposure to elevated concentrations can cause fever and malaise, changes in white blood cell counts and respiratory distress.

Endospore: Thick walled spore formed inside the bacterial cell

Exotoxin: These are protein toxins released from the bacterial cells into the surrounding medium.

Fermentation: Ability of the bacterial cells to degrade carbohydrates enzymatically.

Fermentative gram- negative rods: These bacteria can ferment carbohydrates like lactose, cellulose, sucrose etc.

Multiple gram-negative rods: Represents the presence of different types of gram-negative bacteria in the given sample.

Non-fermentative rods: These bacteria can not ferment carbohydrates like lactose, sucrose and cellulose.

Nosocomial infection: Infection acquired from hospitals and health care facilities.

Opportunistic pathogen: Organisms that originally do not cause diseases but become pathogenic under certain circumstances.

Saprophytes: An organism that lives on dead organic matter.

Septicemia: Condition due to the multiplication of bacterial cell in blood.

Zoonosis: Diseases transmitted from animals to humans

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Information provided by AIHA EMPAT program.

Images from: Public health image library (PHIL)

<http://phil.cdc.gov/phil/quicksearch.asp>

SAMPLING MEDIA FOR BACTERIA:

Organisms	Sampling Media			
	TSA	BA	Maconkey	Supplemental
CONS	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Micrococcus	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Staphylococcus	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Staphylococcus aureus (MRSA)	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	Mannitol
Bacillus	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Bacillus anthracis	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	PLET
Corynebacterium	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Mycobacterium	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	Middlebrook
E.coli	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Coliforms	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Legionella	NA	<input checked="" type="checkbox"/>	NA	BCYE, DGVP
Pseudomonas	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Acinetobacter	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Gram negative rods	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Klebsiella	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Listeria	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Yersinia	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Streptococcus	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Salmonella	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Citrobacter	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Enterococcus	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Gram positive bacteria	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	NA	
Enterobacter	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Serratia	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Proteus	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	
Streptomyces	<input checked="" type="checkbox"/>	NA	NA	Nutrient agar
Aeromonas	<input checked="" type="checkbox"/>	NA	<input checked="" type="checkbox"/>	

TSA contains trypticase soy broth. For isolation and cultivation of fastidious and non-fastidious bacteria.

BA (blood agar) contains trypticase soy broth and 5% sheep's blood. Used for the cultivation of fastidious microorganisms & determination of hemolysis.

Maconkey used for isolation and differentiation of fermenting and non-fermenting gram-negative bacteria

MUELLER HINTON agar used for the antisusceptability disc diffusion test for MRSA.

MIDDLE BROOK agar used for isolation and cultivation of mycobacteria.

PLET used for selective isolation of Bacillus anthracis from environmental samples, animal products and non-sterile sites.

BCYE (buffered charcoal yeast agar) used for the cultivation and isolation of legionella spp.

DGVP contains dye, glycine, vancomycin and polymyxin B for the isolation and differentiation of legionella spp.

MANNITOL agar used for selective isolation and identification of staphylococcus spp.

NUTRIENT AGAR
Contains nutrients required for the growth of actinomycte.