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MICROBIOLOGICAL COLLECTION METHODS BY ANALYTE Resource Information may be available from individual laboratories offering these tests.			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Allergens	Air sampling: Impaction into liquid (Impingers) Filtration Bulk/Surface sampling: Bulk material collected directly Bulk material collected with a micro-vacuuming" cassette or other vacuuming technique. (This is often used for carpet sampling.) Bulk material or dust with sterile swab	Immunoassay – Fluorescent immunoassay Enzyme immunoassay (ELISA) Radio immunoassay (RAST) Multiplex ARray for Indoor Allergens (MARIA)	Analyzes viable & non-viable for available antigenic species. Antigen-antibody specific Cross-reactivity can occur. Typical sample allergens = dog, cat, rat, mouse, cockroach, dust mites, some fungi.

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Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Bacteria and Fungi (Genetic identification)	Air sampling: Filtration Bulk/Surface sampling: Bulk material collected directly Bulk material collected with a micro-vacuuming" cassette or other vacuuming technique. (This is often used for carpet sampling.) Bulk material or dust with sterile swab	Bioassay – Polymerase Chain Reaction (PCR)	Analyzes viable & non-viable for genetic identification using genus or species-specific DNA primers. DNA primers limited.

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Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Biological Contaminants/ Bioaerosols – Viable (Fungi, bacteria, etc…) (Identification and/or count)	Air/Bulk/Surface Sampling	Culture	Grow viable organisms into colonies on nutrient agar to better to identify and quantify the organisms. Impingers/Impactors/Cassettes/Filters/Vials/Containers require sterile collection. Reusable sampling equipment must be disinfected between uses to prevent cross contamination. Samples are collected on nutrient agar initially, or can be collected in/on inert media and prepared for culture at the laboratory. Must take into account special media/growth requirements for certain genus/species when culturing. For air samples, must ensure the sampling method has sufficient collection efficiency in the size range(s) of the bioaerosols present, or else you will not collect them. Requires several days for incubation. Of the viable organisms cultured, only a percentage of those will actually grow during incubation. Species cultured from a site may not be the most prevalent or important Reports only viable organisms, underestimating total. Large reference database. Identification can be made to the species level. Culturing can be done for all the viable sampling methods. Some of these sampling methods also allow you to choose other analysis methods – instead of or in addition to culturing. Some sampling equipment is suitable for personal air sampling.

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MICROBIOLOGICAL COLLECTION METHODS BY ANALYTE Resource Information may be available from individual laboratories offering these tests.			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Biological Contaminants/ Bioaerosols – Viable (Fungi, bacteria, etc…) (Identification and/or count) (continued)	Air sampling: Impaction into liquid (Impingers) Impaction onto agar Impaction onto water soluble collection media (e.g. - Via-Cell™ cassettes) Filtration (e.g. - Filter cassettes, Button sampler™, IOM sampler™. etc…) Collection into Eppendorf vials.	Culture	Impaction into liquid (Impingers): ♦ Liquid can evaporate or spill. Use mineral oil to eliminate evaporation (sterile mineral oil for fungus and bacterial culture) ♦ Water or water based impinger liquids are not recommended for collecting hydrophobic particles such as fungal spores. ♦ Glycerol impinger liquids are not recommended for viable microorganism collection because microorganism may die from osmotic pressure. Impaction onto agar: ♦ Multistage impactors allow size selective sampling. ♦ Must take into account special media/growth requirements for certain genus/species when choosing sampling media agar. ♦ Some impactors require positive hole correction Impaction onto water soluble collection media: ♦ Media can be used for direct microscopy. However, once media is diluted in water the solution can be transferred for culturing or bioassay/PCR or chemical analysis. Filtration: ♦ Sterile filters required for culturing. ♦ Standard filters can cause desiccation and limited viability. However, gelatin filters help to prevent desiccation and improve culturability. ♦ Filtration methods tend to concentrate particles at center of filter. ♦ Electrostatic charges may be collection problem for regular filter cassettes. ♦ Button samplers collect inhalable fraction. ♦ IOM samplers™ using Multidust™ foam discs allow size select into inhalable, thoracic, and respirable fractions. Collection into Eppendorf vial: ♦ Product uses a cyclone sampler. ♦ Gentle capture of sample and capable of sterile handling. ♦ Spores are collected and retained in a viable condition. The Eppendorf vial can be capped and refrigerated for subsequent evaluation.

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MICROBIOLOGICAL COLLECTION METHODS BY ANALYTE Resource Information may be available from individual laboratories offering these tests.			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Biological Contaminants/ Bioaerosols – Viable (Fungi, bacteria, etc…) (Identification and/or count) (continued)	Bulk/Surface sampling: Bulk material collected directly Bulk material collected with a micro-vacuuming" cassette or other vacuuming technique. (This is often used for carpet sampling.) Bulk material or dust with sterile swab	Culture	Non-destructive. Templates and kits available. Desiccation of sample possible. Limited viability. No correlation to airborne results. Multiple samples should be taken.
Biological Contaminants/ Bioaerosols – Non-Viable (Fungi, pollen, mycelia, fiber, skin cells, fiberglass, etc…) (Identification and/or count)	Air/Bulk/Surface Sampling	Microscope	Direct microscopic examination to identify and quantify organisms. Impingers/Impactors/Cassettes/Filters/Vials/Containers require sterile collection. Reusable sampling equipment must be disinfected between uses to prevent cross contamination. For air samples, must ensure the sampling method has sufficient collection efficiency in the size range(s) of the bioaerosols present, or else you will not collect them. For some organisms, there can be limited identification ability. Cannot distinguish some species. Background debris can interfere with identification. Some sampling equipment is suitable for personal air sampling.

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MICROBIOLOGICAL COLLECTION METHODS BY ANALYTE Resource Information may be available from individual laboratories offering these tests.			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Biological Contaminants/ Bioaerosols – Non-Viable (Fungi, pollen, mycelia, fiber, skin cells, fiberglass, etc…) (Identification and/or count) (continued)	Air sampling: Impaction into liquid (Impingers) Impaction onto glass/plastic slides/cassettes or tape or metal (stainless) impactors Impaction onto water soluble collection media (e.g. - Via-Cell™ cassettes) Filtration (e.g. - Filter cassettes, Button sampler™, IOM sampler™. etc...) Collection into Eppendorf vials.	Microscope	Impingers: ♦ Liquid can evaporate or spill. Use mineral oil or glycerol to eliminate evaporation. ♦ Water or water based impinger liquids are not recommended for collecting hydrophobic particles such as fungal spores. Impaction onto glass/plastic slides/cassettes or tape or metal (stainless) impactors: ♦ Multistage impactors allow size selective sampling. ♦ Some impactors allow size selection by adjusting the air flow rate (e.g. - VersaTrap™ cassettes). ♦ Many impactors with glass/plastic slides also have a sticky substance on the slide to help capture particles. ♦ Wall cavity sampling adaptors available for some types of impactor cassettes. Impaction onto water soluble collection media: ♦ Media can be used for direct microscopy. However, once media is diluted in water the solution can be transferred for bioassay/PCR or chemical analysis. Filtration: ♦ Filters with grids can be purchased for counting. ♦ Filtration methods tend to concentrate particles at center of filter. ♦ Electrostatic charges may be collection problem for regular filter cassettes. ♦ Button samplers collect inhalable fraction. ♦ IOM samplers™ using Mutidust™ foam discs allow size select into inhalable, thoracic, and respirable fractions. Collection into Eppendorf vial: ♦ Product uses a cyclone sampler. ♦ Gentle capture of sample and capable of sterile handling. ♦ The Eppendorf vial can be capped and refrigerated for subsequent evaluation.

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MICROBIOLOGICAL COLLECTION METHODS BY ANALYTE Resource Information may be available from individual laboratories offering these tests.			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Biological Contaminants/ Bioaerosols – Non-Viable (Fungi, pollen, mycelia, fiber, skin cells, fiberglass, etc…) (Identification and/or count) (continued)	Bulk/Surface sampling: Bulk material collected directly Bulk material collected with a micro-vacuuming" cassette or other vacuuming technique. (This is often used for carpet sampling.) Bulk material or dust with sterile swab Clear sticky tape or a sticky prepared slide placed directly over the surface to be sampled (If tape is used, place tape onto slide for transport.)	Microscope	Non-destructive. Templates and kits available. No correlation to airborne results. Multiple samples should be taken
β glucan **(1→3)-β-D glucan** (Fungal cell wall component)	Air Sampling: Filtration	Bioassay – Limulus amoebocyte lysate assay (LAL) Immunoassay – ELISA	β glucan is a cell wall component of all fungi; sampling is used as a marker for the presence of fungal contamination. Detects dead and living spores. Does not identify which fungal species is present.

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MICROBIOLOGICAL COLLECTION METHODS BY ANALYTE Resource Information may be available from individual laboratories offering these tests.			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Endotoxins (Gram negative bacteria toxins)	Air Sampling: Filtration Bulk/Surface sampling: Bulk material collected directly Bulk material collected with a micro-vacuuming" cassette or other vacuuming technique. (This is often used for carpet sampling.)	Bioassay – LAL Kinetic chromogenic assay Turbidimetric assay	Cassettes/filters/vials must be sterile and endotoxin-free. Identifies toxins produced by gram negative bacteria. Can analyze air, dust, or bulk material samples (including water samples). May be useful as a post-remediation sample after a sewage spill or backup. Have shown correlation between occupant symptoms and endotoxin concentrations.
Ergosterol (Fungal cell wall component)	Air Sampling: Filtration	Chemical assay – High Performance Liquid Chromatography (HPLC) Gas Chromatography (GC) GC with Mass Spectrophotometry (MS)	Ergosterol is the main membrane sterol in most fungi; sampling is used as a marker for the presence of fungal contamination. Detects dead and living spores. Does not identify which fungal species is present. Long-term stability before analysis.
Extracellular Polysaccharides (EPS) (Fungal secreted carbohydrates)		Immunoassay - ELISA	EPSs are stable carbohydrates that are produced during fungal growth in the mycelial cell walls; sampling is used as biomarker for the presence of fungal contamination. EPSs have antigenic specificity, usually at the genus level.
Fatty Acids (Bacteria cell wall component)		Chemical assay - GC GC-MS	Fatty acids are cell wall components of bacteria. Analysis uses gas chromatography to determine the fatty acid profile, and then compares results to a reference database using statistical pattern recognition software.

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MICROBIOLOGICAL COLLECTION METHODS BY ANALYTE Resource Information may be available from individual laboratories offering these tests.			
Analyte	**Sample Information**	**Analysis Method(s)**	**Sampling Notes**
Microbial Volatile Organic Compounds (MVOCs) (Bacterial or fungal emissions)	Air Sampling: Sorbent tube Summa canisters	Chemical assay – GC GC-MS	MVOCs are produced by bacteria or fungi. Organisms must be actively growing for organic compound production. Little consensus on which MVOCs are "important" or medically significant.
Mycotoxins (Fungal secondary metabolites)	Air Sampling: Filtration Bulk/Surface sampling: Bulk material collected directly Bulk material collected with a micro-vacuuming" cassette or other vacuuming technique. (This is often used for carpet sampling.) Bulk material or dust with sterile swab	Chemical assay – Liquid Chromatography (LC) with MS HPLC GC/MS Solution fluorimetry Immunoassay – ELISA	Mycotoxins are secondary metabolites produced by some fungi. Mycotoxins are typically produced when the fungus is stressed. Sensitive analytical methods may not be available for all indoor air mycotoxins. Agriculturally important toxins methods widely available. Air sampling for mycotoxins has important limitations and requires high concentrations of a fungus to ensure accurate detection.

Analysis Methods:

Culture (viable) - Collect or transfer sample onto nutrient agar to grow colonies for counting and/or morphological identification.
Microscope (non-viable) – Examine under microscope for counting and/or morphological identification.
Bioassay – Analytical method in which the result is an observable effect on or in a biological system/organism. Includes: PCR (genetic identification using DNA primers), LAL, kinetic chromogenic assay and turbidimetric assay.
Chemical Assay – Analysis of chemical compounds produced by or contained in the organism. Includes: HPLC, GC, GC-MS, LC-MS and solution fluorimetry.
Immunoassay – Analysis based on the specificity of an antigen-antibody reaction. Includes: Fluorescent immunoassay, ELISA and RAST.