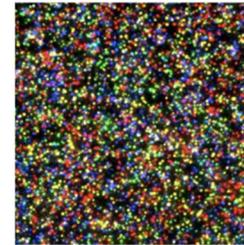
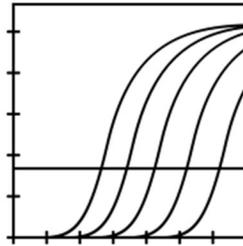


Applications for Molecular Diagnostics for Infectious Diseases



Increasing complexity

Conventional microbiology	Targeted molecular testing	Multiplexed molecular testing	Targeted metagenomics	“Shotgun” metagenomics
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Molecular technologies for pathogen detection

Molecular methods for detection of microbial genomic material may detect a single pathogen or pathogens (targeted approach) or take a syndromic approach in which a single test is capable of detecting many of the microorganisms most commonly associated with an infection. This is accomplished using multiplexed panels. These assays are highly sensitive and faster than culture-based methods. These panels also often include common resistance genes which can be used to target antimicrobial therapy before traditional bacterial culture-based susceptibilities can be determined. Several technologies can be employed on targeted or multiplexed molecular platforms, most frequently oligonucleotides/probe-based methods, or nucleic acid amplification tests such as polymerase chain reaction (PCR) or transcription-mediated amplification (TMA).

Technology	Oligo/probes	PCR	TMA
Application	Detection of pathogen and/or common resistance genes		
Common example	<ul style="list-style-type: none"> Gastrointestinal infections Sepsis (organism identification from positive blood culture) 	<ul style="list-style-type: none"> Respiratory infections Meningitis/encephalitis Gastrointestinal infections Sepsis (organism identification from positive blood culture) Sexually transmitted infections Bacterial vaginosis 	<ul style="list-style-type: none"> Sexually transmitted infections

Sequencing-based technologies to support infectious diseases diagnosis

For a broader approach, sequencing-based technologies can be harnessed for pathogens which may not be represented on targeted molecular panels. Targeted metagenomics utilize an amplification or enrichment step to select specific genes or regions.

Technology	Whole genome sequencing	Targeted metagenomics		“Shotgun” metagenomics
Application	Phylogenetic characterization from isolate	Organism identification from isolate	Organism identification direct-from-specimen	Organism detection direct-from-specimen
Details	<p>SNP analysis - Assesses and compares base changes at any position in the genome of a test strain with a closely related reference strain sequence</p> <p>Multilocus sequence typing - Discriminates bacteria based on sequence differences in select genes</p>	<p>Broad range PCR & sequencing</p> <p>Sequencing of 16S/18S rRNA gene to compare with known sequence databases for an organism class (i.e., bacterial, fungal)</p>		<p>Metagenomic sequencing</p> <p>Sequencing of all human and/or microbial nucleic acids (DNA, RNA, or both)</p>
Common application	<ul style="list-style-type: none"> Used for outbreak investigations to evaluate relatedness of isolates 	<ul style="list-style-type: none"> Used to provide atypical organism identification 	<ul style="list-style-type: none"> Pathogen detection from normally sterile tissue or fluids. Often following antibiotic administration (prosthetic joint infections, infective endocarditis, etc.) 	<ul style="list-style-type: none"> Pathogen detection from normally sterile fluid or tissue (cell-free DNA from plasma or CSF) Microbiome analysis



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