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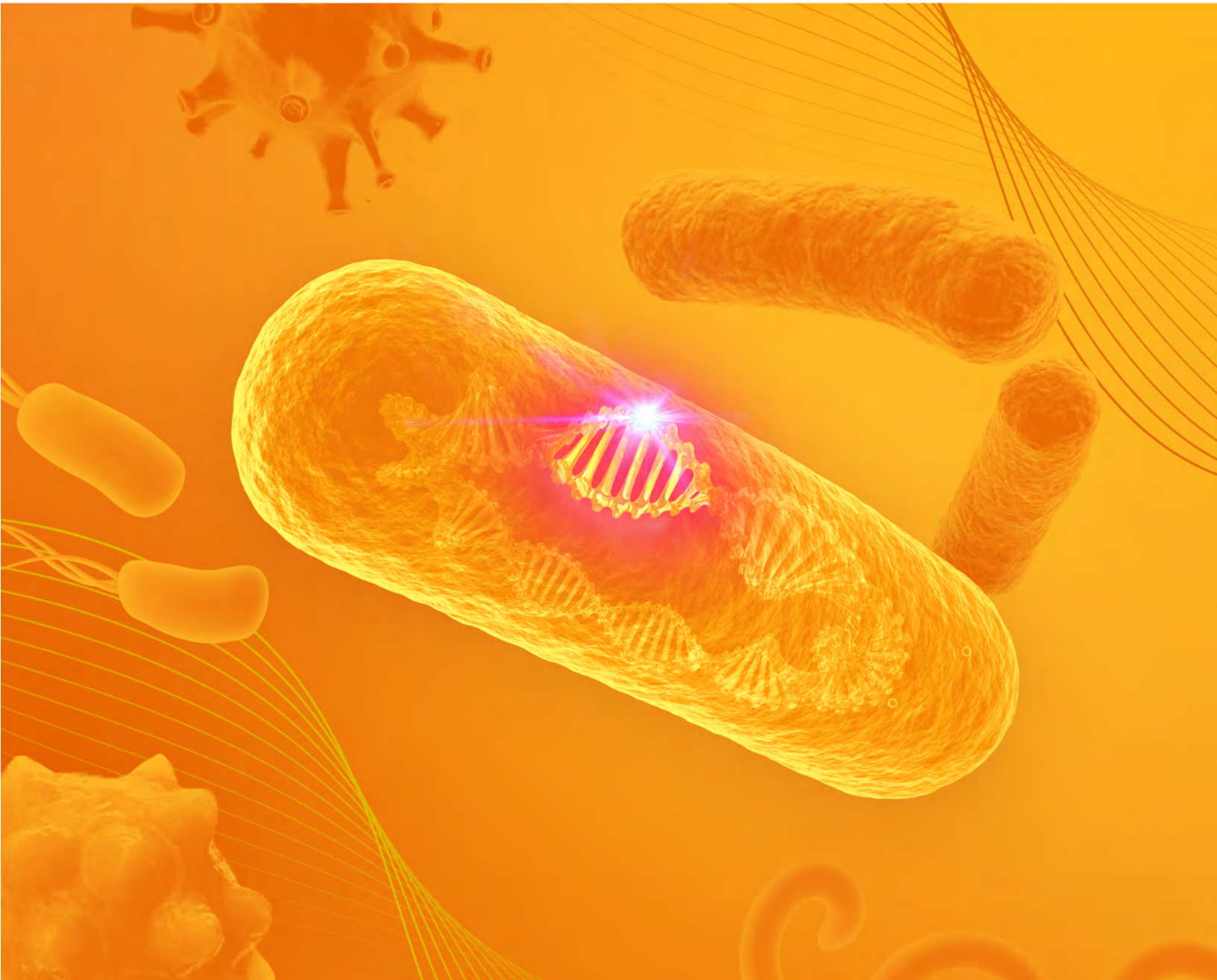
Precision metagenomics: a rapid solution for identifying and characterizing microbial agents of infectious disease

Precise targets. Enhanced detection. Comprehensive results.



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The spectrum of culture-dependent and culture-independent testing for pathogen detection and characterization

The availability of rapid testing for pathogenic microbial agents, such as bacteria, viruses, and fungi, can optimize clinical care and improve patient outcomes by guiding and accelerating actionable treatment.[1,2] Outside of the clinic, testing for microbial agents provides essential information for public health decision-makers regarding infectious disease surveillance and epidemiologic analyses.[3,4]

In order to explain new technological breakthroughs for pathogen detection and characterization, we must first discuss the existing methods in culture-dependent and culture-independent pathogen detection and characterization. Traditional, culture-based approaches have long been a mainstay of testing for bacterial and fungal infections (Figure 1). However, microbial culture techniques often rely on labor-intensive, and time-consuming protocols. Moreover, some pathogenic microbes are unable to grow within a laboratory setting and are therefore missed in culture-based approaches.[5] The adoption of molecular approaches, such as nucleic acid-based assays, represents a major paradigm shift that has the power to enhance both patient care and public health surveillance.[5,6] Many molecular approaches, which differ in complexity and depth of coverage, are available for the culture-independent elucidation of microbial samples.[7,8] Protein-based approaches, such as matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) and immunoassays can be used for the direct detection of pathogen-associated protein biomarkers.[7] On the other hand, nucleic acid amplification tests (NAATs), such as PCR and reverse transcription PCR (RT-PCR), rely on detecting genomic fragments of pathogens. These PCR-based tests utilize highly specific primer sets to amplify candidate microbial targets, thereby analyzing either a single microbial target or multiple microbial targets (multiplexed PCR) depending on the experimental design (Figure 1).[8]

In addition to PCR-based testing, next-generation sequencing (NGS) represents a versatile technology for microbial identification and characterization. NGS offers flexibility to scale methods based on the experimental interest. Widely available NGS methods include amplicon sequencing, hybrid capture, whole-genome sequencing (WGS), and shotgun metagenomics (MG) (Figure 1).[9-12]

Microbial culture[5,6]	Refers to the laboratory culture of microbial specimens on various media for pathogen identification (by microscopy or other methods) and often characterization of drug-resistance profiles
Molecular tests[7,8]	**Protein-based assays** A group of molecular tests, such as MALDI-MS or immunoassays, that are used to detect pathogen-specific proteins **NAATs** Uses single target or multiplexed gene-specific primers to genomic regions of pre-defined pathogens
Next-generation sequencing (NGS)[10-12]	**Amplicon** Uses target-specific, multiplexed primers and ultra-deep sequencing to amplify and characterize a set of candidate pathogen genomic regions **WGS** Uses NGS technology to analyze the full microbial genome **Hybrid capture** Uses target-specific biotinylated probes to isolate pathogen-associated genomic regions of interest for of interest for deep sequencing **Shotgun MG** Uses NGS technology and bioinformatics for the comprehensive analysis and interpretation of all genomic material within a complex microbial sample

Figure 1: Common testing methods used for pathogen identification and characterization[5-12]

Each of the common microbial testing methods described above have associated benefits and limitation that should be considered when choosing the best method for a specific application (Figure 2).

Increasing breadth of pathogen information provided ↓

		Benefits:	Limitations:
NAATs	**Single-target PCR**	• Sensitive, specific • Rapid, cheap	• Very limited scope • Low/no mismatch tolerance
NAATs	**Multiplex PCR**	• Sensitive, specific • Rapid, cheap • Easy to interpret, present, or absent results	• Limited number of targets in a single test • Low/no mismatch tolerance • Requires optimization of assay conditions (eg, annealing temperatures)
NGS	**Amplicon**	• Detects genetic variants • Affordable, easy workflow	• Limited scope • Low/no mismatch tolerance • Requires optimization of assay conditions (eg, annealing temperatures) • Amplification efficacy can decrease as the number of targets increase
NGS	**Hybrid capture**	• Can identify novel variants • High mutation resolution	• More hands-on processing steps • Limited test menu
NGS	**WGS**	• Does not require specific probes • High-resolution for SNP, CNV, and other variants	• Relies on culturable bacterial/fungal agents • Sequences single pathogen at a time
NGS	**Shotgun MG**	• No target enrichment required	• Assembling data using reference genomes requires bioinformatics expertise and computation • Variable sensitivity

Figure 2: Comparison of available molecular and NGS-based microbial testing methods[7]

What is precision metagenomics (PMG)?

PMG is an innovative solution for the rapid detection and characterization of pathogens within complex samples. PMG has a basis in shotgun MG, which uses NGS to sequence all of the genetic material within a biological sample. The large amount of data produced by shotgun MG requires complicated bioinformatics-based data analysis due to the sequencing depth necessary for adequate analytical sensitivity. During this analysis, bioinformatics is typically used to align the resulting sequences (called "reads") to reference genomes housed within reference databases, allowing for the assembly of the experimental microbial genome.[11]

Unlike shotgun MG, PMG is a focused approach that targets and interrogates specific, informative genomic regions within select microbes, thus potentially reducing sequencing costs and providing the user with results tailored to their specific interest. Therefore, PMG combines the breadth of MG with the sensitivity and actionability of conventional NAATs. Moreover, PMG is equipped with an integrated bioinformatics platform that uses proprietary search and alignment algorithms to leverage growing reference databases. These reference databases are curated with reference genomes for specific microbes and microbial variants that are associated with human disease. The alignment of target-enriched PMG reads to these curated reference genomes is carried out through robust algorithms that automatically interpret and report which microbes are present in the experimental sample, providing hands-off data analysis. The combination of smart assay design for the enrichment of relevant targets and integrated bioinformatics provides the user with actionable data that can be turned into valuable insights, as discussed further in sections 2-4.

Adding precision to the metagenomics workflow

The PMG workflow enriches for thousands of relevant microbial target genes while maintaining the specificity and high resolution of traditional molecular tests (eg, qPCR).[12] Additionally, high mismatch tolerance allows for the identification of variant strains and key genes or mutations that may confer higher pathogenicity or enhanced transmissibility of infectious agents.[12] In one study, PMG was able to produce 250-fold more sequencing reads with 10x lower sequencing depth compared to shotgun metagenomic analysis of the same sample.[13] Unlike MG, PMG uses an automated bioinformatics and data interpretation platform, providing expedited results without the need for highly trained personnel or advanced computation (Figure 3).

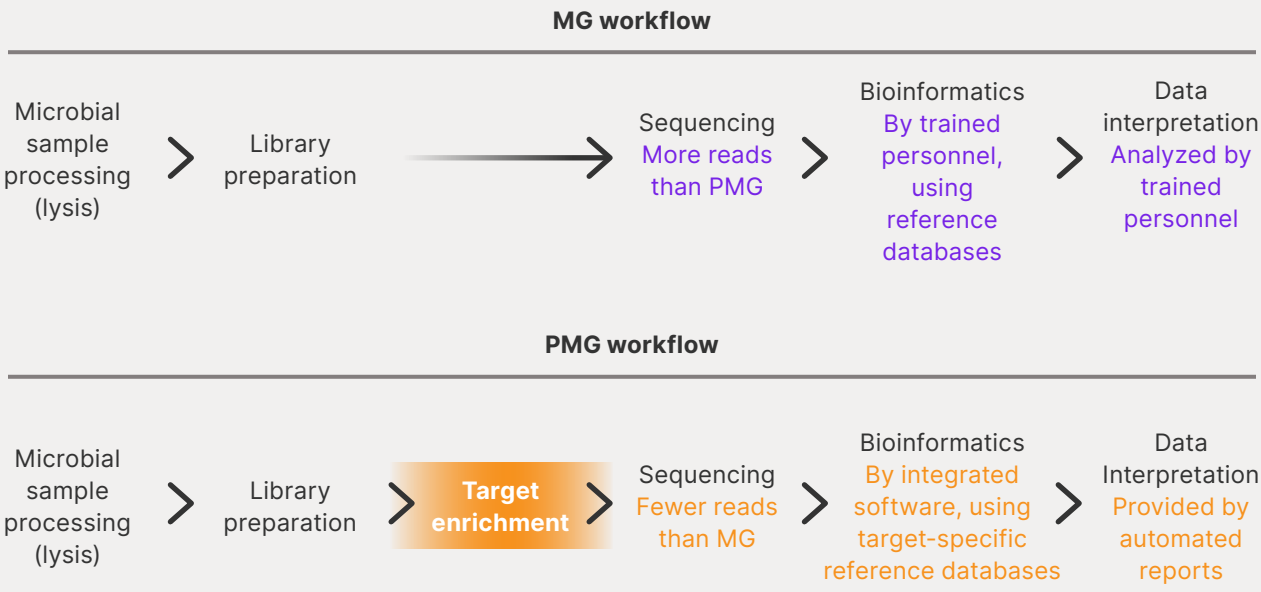


Figure 3: Comparison of the MG and PMG Workflow

Advantages of PMG over other NGS-based approaches

PMG offers a unique solution to certain limitations faced by other NGS-based approaches (Figure 4).

No. of microbes analyzed per run

compared to WGS

Required reads

compared to shotgun MG

Time to interpreted results

compared to shotgun MG and WGS

Figure 4: PMG analyzes more microbes per sample than WGS, requires fewer reads for analysis than shotgun MG, and takes less time to obtain interpreted results than shotgun MG and WGS.

One Health approach and emerging pathogens

One Health is an interconnective approach to promoting human health by ensuring the health of animals and plants within our shared environment. This initiative has become increasingly important as the overlap between human and animal populations continues to grow due to increasing urbanization, international travel, and global temperatures.[14]

A key element of the One Health movement is public health surveillance through the monitoring of threats to human and animal health alike, including mobile genetic elements encoding AMR; respiratory, food-borne, and emerging infectious disease outbreaks; and surveillance of environmental samples, including wastewater. This multi-pronged approach aims to prevent and contain endemic, emerging, and novel human infections. Three core guidelines of the One Health Approach—coordinating, communicating, and collaborating—strive to ensure the best public health outcomes (Figure 5).[14]

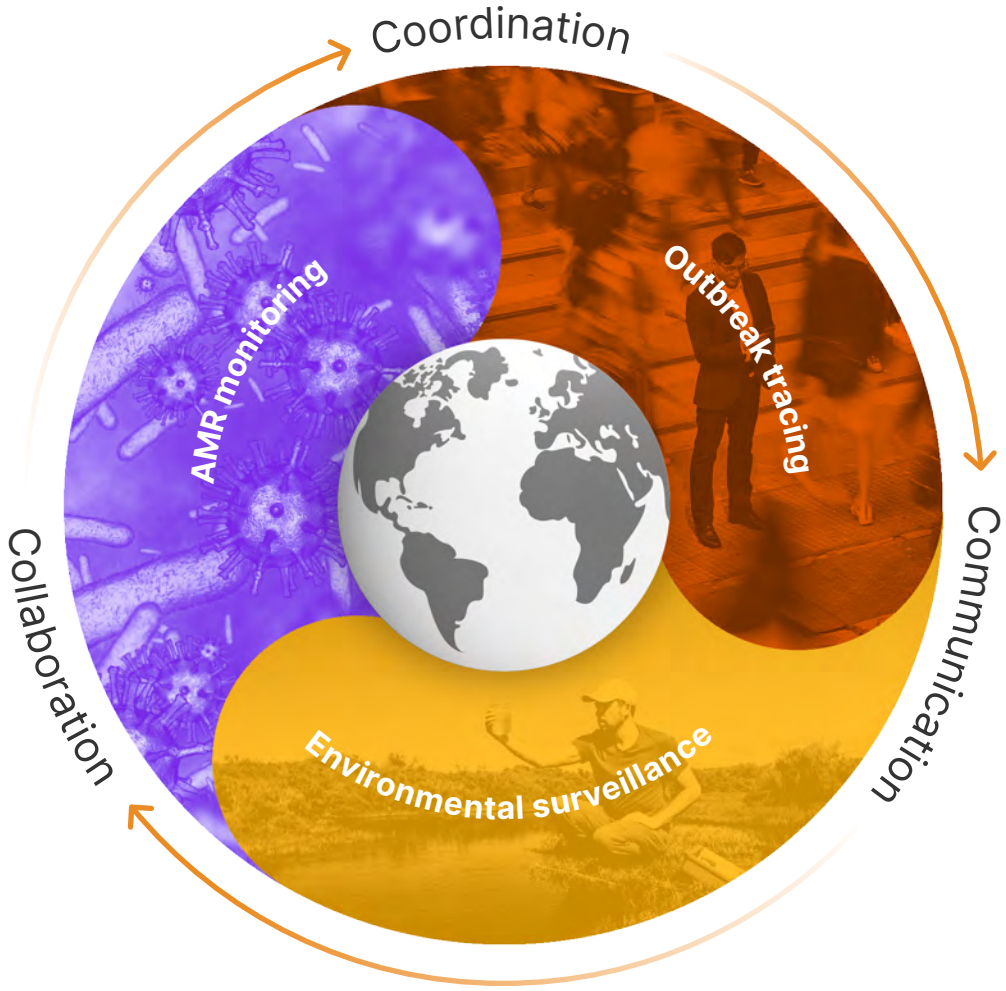


Figure 5: One Health surveillance approach for infectious diseases

Antimicrobial resistance (AMR) monitoring

AMR occurs when microorganisms, including bacteria, viruses, fungi, and parasites, evolve the ability to resist the effects of antimicrobial drugs.[15] AMR is a major public health burden, causing ~1.2 million deaths annually, and can be influenced by human behavior, including the routine use of antibiotics in animal and human populations.[15,16] Infections that no longer respond to treatment result in prolonged infections, which in turn increase the risk of disease spread and severe illness.[15] Moreover, these infections are associated with longer hospital stays, higher medical bills, and increased mortality for patients.[15,16]
A variety of microorganisms have gained the ability to circumvent once-effective treatments and now pose an urgent threat to human health[15,17] (Figure 6). The growing threat of AMR and its associated public health implications are predicted to have serious impacts on global healthcare fees (Figure 7).[17]

Urgent/serious bacterial threats	Estimated annual cases[17]:	Estimated annual deaths[17]:
Carbapenem-resistant *Acinetobacter* (CRAsp)	~8,500	~700
Carbapenem-resistant *Enterobacterales* (CRE)	~13,100	~1,100
Vancomycin-resistant *Enterococcus* (VRE)	~54,000	~5,400
Methicillin-Resistant *Staphylococcus aureus* (MRSA)	~323,700	~10,600
ESBL-producing *Enterobacterales*	~197,400	~9,100
Multidrug-resistant (MDR) *Pseudomonas aeruginosa*	~32,600	~2,700
Urgent/serious fungal threats		
Drug-resistant (DR) *Candida* species	~34,800	~1,700
DR *Candida auris*	323 clinical cases in 2018[17]	

Figure 6: Public health and economic burden of a select subset of urgent AMR infections[17]

Treating the bacterial threats listed above costs **$4.6 billion** in health care fees annually[18]

AMR will cost up to an estimated **$1 trillion** USD by 2050[19]

Figure 7: Global economic burden of AMR infections

Challenges in surveillance of AMR mechanisms

AMR can develop through *de novo* mutations in the genomes of microorganisms under the selective pressure of an antimicrobial agent or through horizontal gene transfer of plasmids containing AMR-conferring genes. Horizontal gene transfer can occur through the conjugation of resistant bacteria and susceptible bacteria, by the transformation of free DNA in the environment, and through bacteriophage-mediated transduction. Measures to control the spread of AMR rely on understanding the interplay between pathogens, the prevalence of AMR-encoding genes, and the presence of mobile genetic elements in the environment.[21] AMR surveillance has traditionally relied on phenotypic, culture-based antimicrobial susceptibility testing (AST), which assesses microbial growth in the presence of antimicrobial drugs.[21,22]

While AST can be carried out for virtually any antimicrobial drug, this method can only be used on cultivatable microorganisms. Further, many microorganisms require culturing for extended timeframes prior to phenotypic detection, thereby delaying actionable results for clinical decision-making. Moreover, many microbes require culture within high biosafety level settings, thus reducing accessibility to this testing method. Finally, AST uses specific culture protocols for specific microorganisms, preventing the applicability of this method for detecting polymicrobial infections in a single culture.[21, 22]

> Ultimately, though AST can detect the presence of AMR, it provides little information regarding mechanisms of resistance, epidemiological origins, or the chain of transmission.[21]

More modern methods of AMR surveillance rely on the genotypic characterization of resistance via a variety of molecular and NGS-based tests, which provide a platform for genomic surveillance at the public health level. Molecular tests, such as PCR and qPCR, work by rapidly amplifying and detecting target genes associated with resistance; however, the number of resistance-associated genes detected by these assays is limited to pre-defined, relatively small panels. Even with the option of multiplexed qPCR, optimizing primer annealing temperatures for various genetic targets can limit the breadth of these tests.[8, 22]

Moreover, given the continuous evolution and identification of antimicrobial resistance genes, genetic panels must be routinely updated in order to detect newly evolved or identified mutations conferring AMR.[22]

The most comprehensive platforms for genotypic characterization of AMR are the NGS-based methods WGS and shotgun MG.[9, 12, 21, 22] However, like AST, WGS relies on cultivatable microorganisms and often requires long culture times and high biosafety level labs.[21] Shotgun MG, on the other hand, does not require microbial culture prior to analysis and is not limited by specific genetic test menus. However, analysis and interpretation of MG data requires prior expertise in bioinformatics and often high-cost instrumentation and software. Moreover, given the complexity of assessing full microbial communities, MG often fails to provide the genome coverage and resolution needed to track AMR-associated genes.[12, 21] Despite their shortcomings, both WGS and MG provide data regarding mechanisms of resistance, epidemiological origins, and transmission/contact tracing.[21, 22]

PMG as a solution for AMR surveillance

PMG provides a solution to current challenges associated with genotypic characterization of AMR. Like molecular-based tests and shotgun MG, PMG removes the need for culture, thus reducing the time to result and extending surveillance to non-cultivable microbes. Unlike shotgun MG, PMG first enriches a sample for targeted regions, which include AMR-conferring genomic regions of many pathogenic microbes. Targeted enrichment results in high resolution and target specificity, allowing for reliable AMR-detection and information regarding mechanisms of resistance, epidemiological origins, and transmission/contact tracing. Moreover, integrated, hands-off data processing removes the need for complicated bioinformatics-based analysis. In sum, PMG provides a rapid genomic surveillance platform for AMR detection and characterization (Figure 8).

	Culture	NAAT	NGS-based methods		
	Antimicrobial susceptibility testing (AST) [21,22]	**PCR and qPCR** [8, 22]	**Whole genome sequencing (WGS)** [21,22]	**Shotgun metagenomics (MG)** [12, 22]	**Precision metagenomics (PMG)**
Description	Culturing microbes in the presence of antimicrobial drugs to phenotypically determine AMR	Amplifying and detecting specific AMR-associated genes using gene-specific primers and probes	Comprehensive sequencing of full microbial genome from culture	Comprehensive sequencing of full microbial community from primary sample	Targeted sequencing of AMR-associated genes from primary sample
Insights	Susceptibility or resistance to antimicrobial drugs	Presence (PCR) and relative quantification (qPCR) of AMR-associated genes	Presence and sequence of AMR-associated genes Data for determining mechanisms of resistance, epidemiology, and tracing	Presence and sequence of AMR-associated genes Data for determining mechanisms of resistance, epidemiology, and tracing	Presence and sequence of AMR-associated genes Data for determining mechanisms of resistance, epidemiology, and tracing
Benefits	Knowledge of genetic origin of resistance is not required Tests for expression of (not just presence of) AMR genes	Does not require cultured microbes Rapid and easy-to-use	Provides full genetic information of sample	Does not require cultured microbes Not limited by specific genetic test menus	Does not require cultured microbes Data analysis and interpretation is automated and user-friendly
Limitations	Requires microbial culture Can require extended culture times Can require BSL3-4 labs	Test panels are spatially limited and require updates as new AMR-associated mutations arise May fail to detect evolution of new strains or variants	Requires microbial culture Can require extended culture times Can require high BSL3-4 labs	Some facilities may lack the capability to achieve the sequencing depth required for some surveillance applications Requires bioinformatics expertise	Detection limited to enriched targets AMR detection is correlative, not proven

Figure 8: Comparison of phenotypic and genotypic characterization of AMR

Outbreak investigation

Outbreak investigation refers to the public health principle of assessing the identity, origin, and transmission of an ongoing infectious disease outbreak. Infectious disease outbreaks come in many forms, but the most common are respiratory outbreaks, such as influenza, COVID-19, and respiratory syncytial virus (RSV);[23] foodborne outbreaks, such as those caused by *Salmonella, Shigella, E. coli*, hepatitis A, norovirus, rotavirus, adenovirus, and astrovirus;[24] and emerging infections, including emerging or zoonotic infections such as avian influenza, COVID-19, Ebola, and mpox.[25]

Traditionally, passive surveillance (often through syndromic testing) has been used for outbreak detection, which relies on hospital patient data and medical provider reporting to track the origin, prevalence, and transmission of an outbreak.[26] However, in the case of (often seasonal) respiratory pathogen outbreaks, symptoms aren't specific enough for syndromic surveillance to distinguish between potential causative agents.[23] Therefore, the popularity of and need for genomic surveillance, which provides molecular-level discrimination for tracking the identity, prevalence, and spread of seasonal outbreaks, is growing.

Moreover, during a foodborne outbreak, tracing the origin of an outbreak is important for subsequent public health action, including widespread recalls. Traditionally, these outbreaks have been characterized using complex, time-consuming reconstruction of the chain of transmission based on specific food transportation routes and distribution information, ultimately delaying response times.[27] Using genetic tools to trace the epidemiology of an outbreak can help pinpoint the origin of the microbial contaminant and provide clues about its onward transmission that may inform changes in practices and procedures to prevent future outbreaks of the same nature.

Finally, while emerging infections, including novel infections, are typically identified first via passive syndromic surveillance systems, the use of genetic sequencing in actively characterizing, tracking, and responding to these infections has become critical to their identification and control.[28]

PMG can enhance outbreak investigation and support molecular epidemiology

Genomic surveillance can expedite the determination of prevalence, spread, and origin of an infectious disease. Moreover, for emerging infections, pathogen genomics can aid in the characterization of pathogens to detect known determinants of enhanced transmission and virulence and can aid in the discovery of novel mutations associated with these features.[28] Genomic surveillance was especially applicable and beneficial during the COVID-19 pandemic, where it was used to estimate the transmissibility of emerging strains,[29] determine epidemiological lineages,[30] and monitor local outbreaks.[31]

The most common methods used for COVID-19 genomic surveillance were amplicon sequencing, hybrid capture, and shotgun MG.[29-31] However, each of these common testing methods come with their associated limitations (Figure 8).

With the growing popularity and widespread use of genomic surveillance for outbreak investigation, PMG can fill the gap between limited targets and expedited data analysis by providing panels with a broad range of targets and automated data interpretation and reporting.

Moreover, the high resolution of targets and high mismatch tolerance provided by PMG can allow for more detailed variant identification for origin determination, contact tracing, and emergence of seasonal, food-borne, and emerging outbreaks.

Environmental surveillance and wastewater monitoring

Environmental surveillance of pathogens is a public health effort involving the collection and analysis of air, water, food, and other environmental samples to track the transmission and understand the burden of infectious agents. Several studies have positioned wastewater surveillance as an accurate, population-level view of disease, with trends in microbial abundance in wastewater strongly correlative with the number of clinical cases within a shared region.[32]

Wastewater monitoring is a major facet of environmental surveillance for characterizing the abundance of, relationships between, and genetic features (including AMR) of infectious agents, especially those shed in fecal matter or urine, such as poliovirus, *Vibrio cholera, Leptospira* and enterohemorraghic *E. coli*.[33-35] Recent evidence suggests that environmental monitoring may also be informative for a broader range of pathogens, notably SARS-CoV-2.[34] Moreover, diseases that are under-reported, such as those with nonspecific symptoms or acute symptoms that increase in severity if untreated, are good candidates for wastewater monitoring programs.[26, 32, 33]

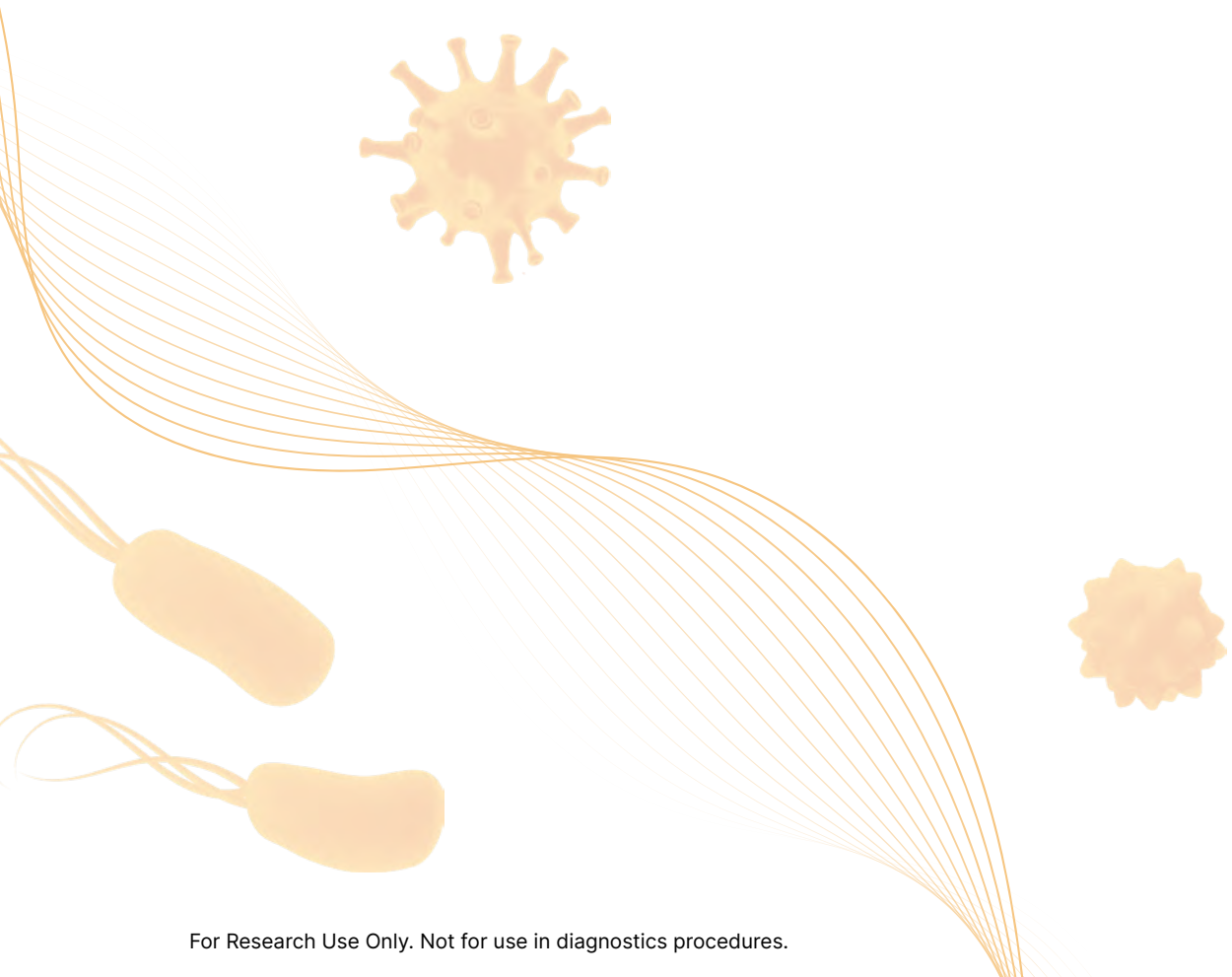
Steps for wastewater monitoring include:[32]

1. Collection of samples from community- or institution-level sewage systems
2. Laboratory-based amplification of genetic material
3. Data interpretation for pathogen identification and characterization
4. Public health reporting

Several challenges have limited the widespread adoption of wastewater surveillance. Namely, the vast quantity and complexity of human and animal microbes that end up in wastewater can reduce sequence resolution and complicate data analysis by shotgun MG. Moreover, molecular tests lack the breadth for the assessment of more than one to a few microbial agents. Finally, the geographical reach of wastewater surveillance is inadequate in areas with limited public health capacities, such as rural and unsewered settings.[32]

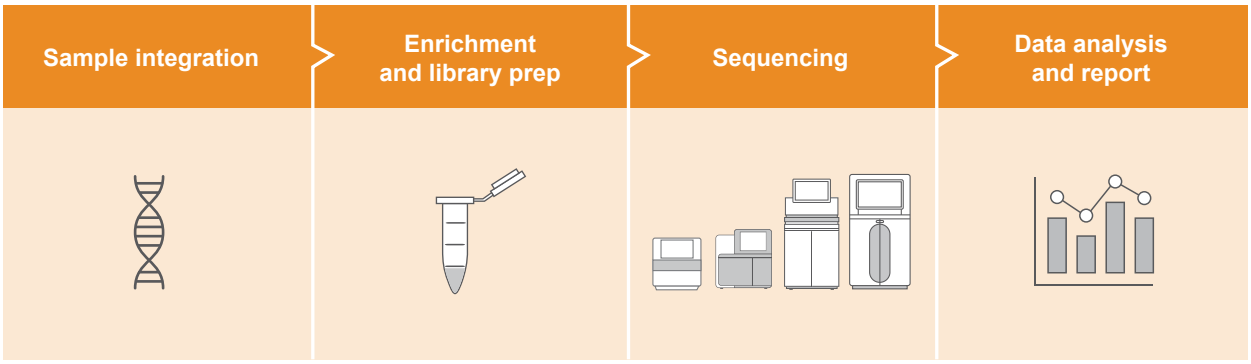
PMG can monitor a broader range of pathogens

Environmental monitoring and wastewater surveillance significantly boomed with the onset of the COVID-19 pandemic, and many studies were carried out to monitor transmission and variant evolution using RT-PCR or NGS technology.[32-35] However, these techniques were limited to the amplification primers chosen for testing (RT-PCR and amplicon sequencing), the need for updating primers every time a new variant of concern emerged (RT-PCR and amplicon sequencing), and the feasibility of rapid data analysis (shotgun MG).[8-12] On the other hand, the broader portfolio of microbial probes, higher mismatch tolerance of probes compared to primers, and integrated data analysis provided by PMG can mitigate the limitations of other methods to expand the scope of tested pathogens. Moreover, PMG's ease of use and automated report generation can increase the accessibility of environmental surveillance to broader geographical and economic regions while expediting the time to results and subsequent public health reporting.



Illumina PMG workflow with the Explify™ platform

A standard workflow for Illumina NGS involves the isolation of genetic material, preparation of sequence-ready libraries, processing with sequencing instrumentation, and data analysis and interpretation.



PMG incorporates a unique enrichment step at the library preparation stage which leverages a curated database for target selection and design of probe-based panels to focus on microbes of interest and to simplify the complexity of microbial samples. Moreover, the integrated Explify platform powers rapid and automated data analysis and report generation.

Sample preparation

If the microbial sample was taken as part of a respiratory or genitourinary syndromic surveillance analysis for pathogen identification or AMR profiling, the Illumina Respiratory Pathogen ID/AMR Panel (RPIP) or the Urinary Pathogen ID/AMR Panel (UPIP) can be used for targeted enrichment. RPIP has been leveraged for use with bronchoalverolar lavage, tracheal aspirate, sputum, and nasopharyngeal swab sample types, while UPIP can be applied to urine samples. Moreover, these panels offer the potential for the re-sequencing of historical isolates initially prepared for alternative downstream analyses.

When processing a sample using PMG, its important to consider and control preanalytical variables, such as environmental factors (eg, temperature, humidity, light exposure) and specimen handling (eg, sterility, collection tubes, transport media) during sample collection, processing, storage, and transport.[36]

Target capture and enrichment

The first step of the PMG workflow involves enriching the microbial sample with the appropriate probe-based RPIP or UPIP panels. These panels enable accurate detection of known and emerging pathogens and are easily integrated into the automated Explify data analysis platform.

RPIP Panel

Comprehensive detection and quantification of >280 respiratory–associated organisms and >2,000 AMR markers

Detects:

- Bacteria: 187
- Viruses: 42
- Fungi: 53
- AMR markers: 2,097
- Includes WGS of SARS-CoV-2 and influenza A and B for strain detection

UPIP Panel

Comprehensive detection and quantification of >174 urinary tract–associated organisms and >3,700 AMR markers from urine sample

Detects:

- Bacteria: 121
- Viruses: 35
- Fungi: 14
- AMR markers: 3,728
- Parasites: 4

Library preparation

After target enrichment, the enriched sample continues to the library preparation workflow, resulting in thousands of fragments ready to be sequenced in one of Illumina's sequencing platforms.

Sequencing

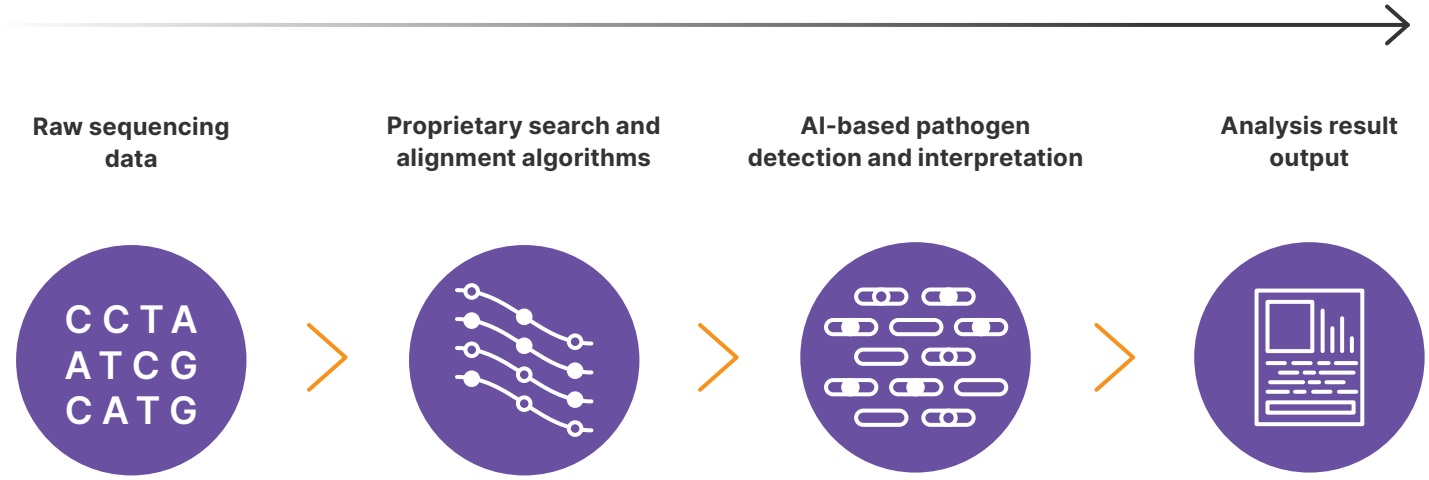
A variety of Illumina sequencing instrumentation options with flexible throughput can be used for PMG applications:

Sequencer	Single reads per run	Maximum read length	Maximum output
iSeq™ 100	4 million	2 ×150 bp	1.2 Gb
MiniSeq™	7 - 25 million	2 × 150 bp	7.5 Gb
MiSeq™	1 – 25 million	3 × 300 bp	15 Gb
NextSeq™ 550	130 - 400 million	2 × 150 bp	120 Gb
NextSeq™ 1000/2000	100 million - 1.2 billion	2 × 300 bp	360 Gb
NovaSeq™ 6000	650 million - 10 billion	2 × 250 bp	325 - 400 Gb
NovaSeq™ X	1.6 billion - 26 billion	2 × 150 bp	165 Gb - 16 Tb

Data analysis

A major benefit of the PMG platform is the integration of hands-off data analysis, interpretation, and report generation powered by Explify platform. This platform incorporates rapid, automated search and alignment to reference databases, robust pathogen detection and interpretation, and integrated output for easy reporting.

Explify data analysis and interpretation workflow



Sequencing data aligned to proprietary, curated reference genomes comprised of:

Microbial reference databases
- 50,000+ microorganisms
- 34,600+ viruses
- 13,300+ bacteria
- 4400+ fungi
- 150+ parasites
- 10M+ sequences

AMR reference databases
- >5000 AMR detection models
- >2000 acquired resistance genes
- >1000 mutation-based genotypes

Medical relevance databases
- >30M publications mined
- >37,000 reportable organisms identified
- >1000 disease profiles with established, relevant pathogens

Algorithm trained on microorganisms enables pathogen detection and AMR profile with high resolution and accuracy

Automated identification of closely-related organisms, drug resistance prediction, and disease-pathogen association profiling for prioritized and actionable reporting

Additional pathogen surveillance workflows from Illumina

If the microbial sample has a suspected viral origin, several Illumina enrichment panels exist for target enrichment and subsequent whole-genome sequencing of viral pathogens. These workflows flow seamlessly into sequencing on Illumina instruments and data analysis with the DRAGEN Microbial Enrichment App.

Library prep	Sequencing	Data analysis
 Illumina RNA Prep Kit with Enrichment (Tagmentation) **Enrichment panels:** • **Viral Surveillance Panel (VSP):** Hybrid-capture target enrichment of 66 viruses that are of high risk to public health, including SARS-CoV-2, influenza, monkeypoxvirus (mpox), and poliovirus • **Respiratory Virus Oligo panel (RVOP):** Hybrid-capture target enrichment of ~40 common respiratory viruses, including various strains od SARS-CoV-2 • **Pan-corona virus panel:** Hybrid-capture target enrichment of ~200 known coronavirus genera for surveillance of animal reservoirs and sequencing of known coronaviruses	 NextSeq™ 550  NextSeq™ 1000 NextSeq™ 2000  NextSeq™ 6000  iSeq™ 1000  MiniSeq™  MiSeq™	 DRAGEN Microbial Enrichment App

The Illumina RPIP and UPIP enrichment panels power PMG applications in both clinical research and basic research settings. For clinical research, PMG can be used to detect and characterize lower respiratory tract infections (LRTIs), urinary tract infections (UTIs), sexually-transmitted infections (STIs), and other common infections caused by microbial agents detected by the RPIP and UPIP enrichment panels.

Lower respiratory tract infections

Lower respiratory tract infections are infections that affect the airways below the level of the larynx, including the trachea and alveolar sacs. These infections, which typically present as acute bronchitis and pneumonia, represent a major cause of morbidity and mortality worldwide.

LRTIs are notoriously difficult to distinguish and identify due to:

1. Lack of specific symptoms that can be attributed to a single etiological agent[38]
2. Variability in sampling methodology across institutions and healthcare provider safety considerations[38]
3. Lack of sampling access to distal bodily sites where LRTIs occur[37]
4. Widespread clinical presentations that can range from asymptomatic to respiratory failure[37]

Despite these difficulties, early diagnosis of LRTIs is extremely important, as these infections can spread easily from person to person via droplets and aerosols.[37]

PMG can address the challenges associated with LRTI detection and diagnosis by (1) genetically distinguishing etiological agents, (2) standardizing laboratory and analysis workflows, and (3) removing the need for invasive sampling.The quantitative reporting feature of PMG with RPIP provides supporting evidence for distinguishing pathogenic and commensal microflora when analyzing biologically complex samples. The RPIP panel includes several common causative agents of LRTIs, including RSV, influenza viruses, coronaviruses, *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, Aspergillus* fungal species, and many more.[38-40] PMG solutions use standardized protocols, test menus, and reporting parameters to ensure lab-to-lab reproducibility.[12] Moreover, PMG with RPIP can be applied directly to noninvasive sample types in addition to enabling the targeted detection of difficult-to-culture microbes that require multiple weeks of culture, enhanced biosafety level facilities, or specific culture conditions, such as *Mycobacterium tuberculosis, Coccidioides, and Brucella*.[41-43]

Urinary tract infections

Urinary tract infections are common infections of the urinary system, which includes the kidneys, ureters, bladder, and urethra. UTIs can be caused by a variety of bacteria and fungi, though they are predominantly caused by uropathogenic *E. coli*. Though UTIs typically respond well to antimicrobial drugs, in some cases, UTIs fail to respond to standard treatment regimens. Treatment failure can be due to underlying medical conditions of the patient or due to antimicrobial resistance. In “complicated” UTI cases, infection can lead to more extreme clinical manifestations, including permanent kidney damage, sepsis, and death. The determination of UTI causes is complicated by the lack of specific signs and symptoms to distinguish potential etiological agents, the subjective thresholds required for a positive diagnosis by culture-based urine tests, and the frequent contamination of urine samples taken for further analysis.[44]

PMG can illuminate the causative agents of UTIs, determine the profiles of AMR genes in samples, and monitor evolutionary variants that may arise. Ultimately, PMG analysis of UTIs can lead to faster, more specific treatment of individual patients and reduce the overall prevalence of AMR uropathogens

Sexually transmitted infections

More than 30 pathogens, including bacteria, viruses, and parasites, can be transmitted through sexual contact.[45] While many STIs are curable with effective treatment, some can be associated with severe complications if left untreated. Moreover, several STIs are associated with an increased risk for contracting HIV.[46] Currently, more than 1 million STIs are acquired every day, and many challenges prevent the timely detection of these infections, including the lack of accurate, inexpensive tests and the high prevalence of asymptomatic cases.[46, 47]

The UPIP panel currently identifies 27 sexually transmitted pathogens, including *Chlamydia trachomatis, Haemophilus ducreyi*, herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), *Neisseria gonorrhoeae, Treponema pallidum, and Trichomonas vaginalis*. Moreover, several difficult-to-culture, difficult-to-distinguish, or non-cultivatable pathogens detected by UPIP, such as *Mycoplasma genitalium*, *Trichomonas vaginalis, and Haemophilus ducreyi* can currently only be detected by molecular methods.[48-50]

Other common infectious syndromes for PMG applications

Other common infectious syndromes that may benefit from PMG-mediated detection include neurological infections and sterile site infections. Neurological infections can be caused by a range of microbial pathogens, which can lead to life-threatening encephalitis or meningitis.[51] A variety of microbial infections associated with neurological complications and symptoms are covered by the UPIP and RPIP panels, including *Mycobacterium tuberculosis* (RPIP), *Neisseria meningitidis* (RPIP and UPIP), *Streptococcus pneumoniae* (RPIP), and HSV-1 (UPIP).

Sterile site infections are invasive microbial infections in normally sterile sites, such as cerebrospinal fluid (CSF), other body fluids, and internal body sites such as the brain, heart, liver, and more.[52-54] These infections can cause severe, life-threatening illnesses such as toxic shock syndrome, sepsis, meningitis, and pneumonia. Rapid detection and characterization of these pathogens is critical for therapeutic responses. PMG represents a robust platform for detection and AMR profiling of a variety of sterile site-associated pathogens, such as *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Staphylococcus aureus*.[55]

Research applications of PMG

PMG can be used to power many research studies, especially those that begin with large-scale comparative analyses of complex samples that require high-throughput processing and high-resolution results. In particular, the rich field of human microbiome research has advanced our understanding of complex commensal and pathogenic communities and their underlying roles in various diseases, such as pneumonia.[55] Additionally, PMG can power the evolving understanding of the urinary microbiome (the “urobiome”).[57]

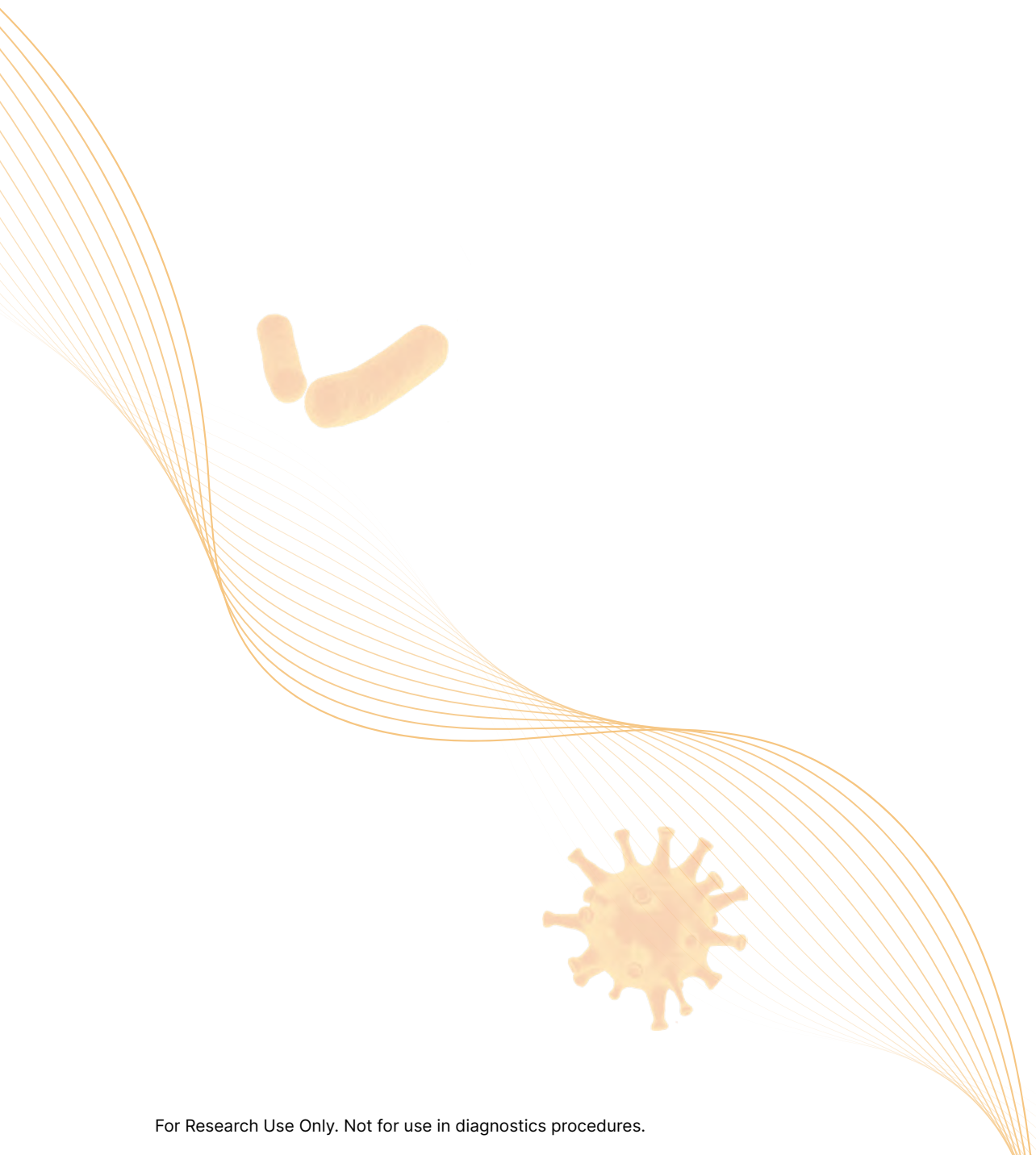
Disease monitoring use cases of PMG

PMG has already been leveraged as a tool to support clinical research, accelerate variant analysis, and bolster AMR surveillance.[58-61]

Clinical research	**UPIP:** A study comparing retrospective clinical decisions regarding treatment of UTI patients identified and characterized uropathogens by either culture-based AST or PMG (UPIP). Four physicians reviewed both the AST and PMG results independently and provided hypothetical clinical decisions for treatment based on the results. Consensus on whether to hypothetically treat patients was reached for 24 out of 25 cases for both AST and PMG and treatment decisions were concordant between methods for 88% of cases. Importantly, UPIP detected additional organisms and AMR markers than did AST alone (in 7/25 samples).[58] **RPIP:** A study of 104 clinical samples from 56 unique patients who were intubated due to COVID-19 were assessed by culture-based AST and PMG (RPIP). PMG detected bacterial and fungal pathogens in 74% of clinical samples compared with 63.4% detected by culture, while a combination of both methods detected bacteria in 77.9% of samples. This study provided a precedent for combining RPIP and culture to enable selective reflexing to AST while minimizing time to result.[59]
Variant analysis	**RPIP:** >1800 publicly available SARS-CoV-2 genomes were processed with the Explify RPIP algorithm to assess the accuracy of variant detection and lineage (strain) designation. The variant detection and lineage designation provided by Explify RPIP matched 99.7% of reference data sets. After independent confirmation, Explify RPIP accurately assigned all SARS-Cov-2 variants (12 total) present in the publicly available data sets used for analysis. This study positions Explify RPIP as a useful public health tool for identifying and classifying pathogen variants via a rapid, automated data analysis solution.[60]
AMR surveillance	**UPIP:** A study assessing antimicrobial resistance to Trimethoprim-sulfamethoxazole (TMP-SMX), a widely used first-line antimicrobial agent for management of UTIs in Europe and the United States, compared the detection capacity of associated resistance markers by PMG (UPIP) to culture-based AST. This study found a concordance of 97% for resistance prediction by PMG and AST for TMP-SMX in 37 samples, positioning PMG as a culture-independent alternative for detection of AMR markers in uropathogens.[61]

Summary

- Current common microbial testing methodologies are limited in scope, speed, or accessibility.
- PMG represents an easy-to-use tool that combines microbial target enrichment with automated bioinformatics and reporting.
- PMG supports public health applications such as outbreak tracing, AMR monitoring, and environmental surveillance.
- Illumina UPIP and RPIP solutions enrich for a broad range or urinary and respiratory pathogens, respectively.
- Illumina PMG can be leveraged for both clinical research and basic research applications.



Glossary of terms

AI-based algorithms: computational programs that provide instructions to enable the automated alignment of experimental sequences to reference genomic databases

Amplicon sequencing: a next-generation sequencing approach for ultra-deep sequencing of PCR products of targeted genomic regions

Antibiotic Susceptibility Testing: a subset of experimental assays that measure the ability of certain microorganisms (eg, bacteria and fungi) to grow in the presence of candidate antibiotics

Antimicrobial resistance (AMR): the development of a microbes ability to survive exposure to an antimicrobial agent, through mutation or horizontal gene transfer

Bioinformatics: the application of computational tools to analyze and interpret biological data

Data interpretation: the process of assigning experimental sequencing data to a reference genome for determination of species within the tested sample/specimen

Emerging infection: an infectious disease that has newly appeared in a population or has recently increased in incidence or geographic range

Environmental Surveillance: the collection of samples from environments involved in pathogen transmission, such as water, sewage, air, and more

Genomic Surveillance: the use of genomic sequencing to monitor infectious diseases in representative populations

Immunoassay: an experimental assay that uses antibodies to detect specific proteins or peptides in a sample

Library Preparation: converting a genomic DNA sample into a library of fragments that are compatible with a next-generation sequence platform

Matrix-assisted laser desorption/ionization Mass Spectrometry (MALDI-MS): an ionization and mass spectrometry technique for identifying proteins within an experimental sample

Multiplexed PCR: the use of PCR to amplify several different DNA sequences simultaneously

Next-generation sequencing (NGS): a massively parallel sequencing technology that offers ultra-high throughput, scalability, and speed

Novel infection: an infectious disease that hasn't been found in humans in the past

Outbreak investigation: a public health initiative to identify the source of ongoing outbreaks and prevent additional cases

Polymerase Chain Reaction (PCR): a molecular technique to amplify and detect specific regions of DNA

Real-time PCR (RT-PCR): a molecular technique based that uses labeled probes to measure and quantify RNA or DNA during PCR amplification

Read depth: the number of times that a given nucleotide in the genome has been read in an NGS experiment.

Reference databases: a database of template genomes that experimental genomes can be aligned to in order to determine which organisms/genetic regions are within a sample/specimen

Shotgun metagenomics: a next-generation sequencing approach that allows researchers to comprehensively sample all genes in all organisms present in a complex sample

Target Enrichment: the use of hybridized probes to capture and enrich specific genomic regions prior to next-generation sequencing

Targeted NGS: any next-generation sequencing method that focuses on the sequencing of specific regions of interest in the genome

Whole-Genome sequencing (WGS): a comprehensive next-generation sequencing method for analyzing entire genomes of experimental samples

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