A P23 Labs White Paper

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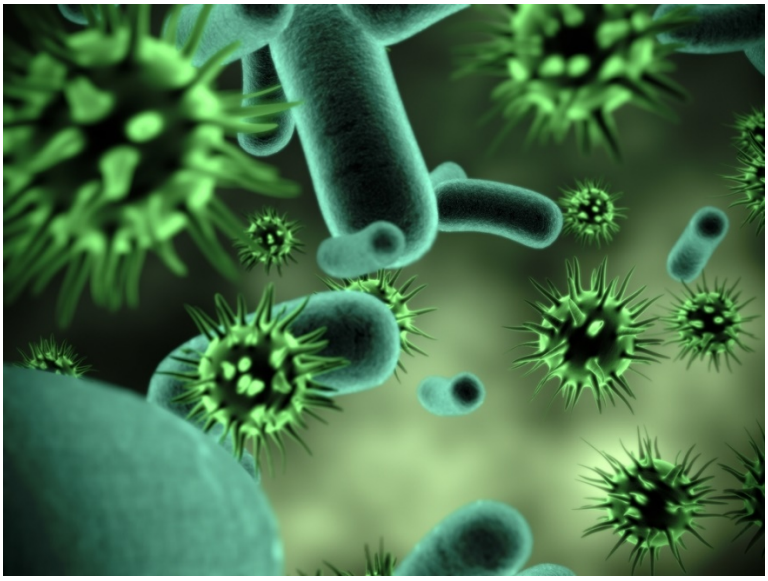
The Use of Molecular Detection of Microorganisms: Scientific Evidence to Support the PCR Testing to Replace Traditional Microbiology Cultures in the Clinical Setting



Executive Overview

P23 Labs is a privately-owned, independent reference laboratory focusing on screening, diagnostic testing and information utilized for molecular diagnostics in early cancer and cardiovascular testing and detection, genetic testing, and overall women's health. Due to our commitment to cancer prevention and early detection, we have remained current on scientific literature and have utilized the best available technologies at an affordable cost. As a result, P23 Labs offers innovative and proprietary high complexity polymerase chain reaction (PCR) genetic based assays with customized assays to detect and monitor a wide range of microorganisms use humans as hosts for survival. The microorganism DNA or RNA specific to over 40 species are identified using PCR as opposed to traditional microbiology culture methods.

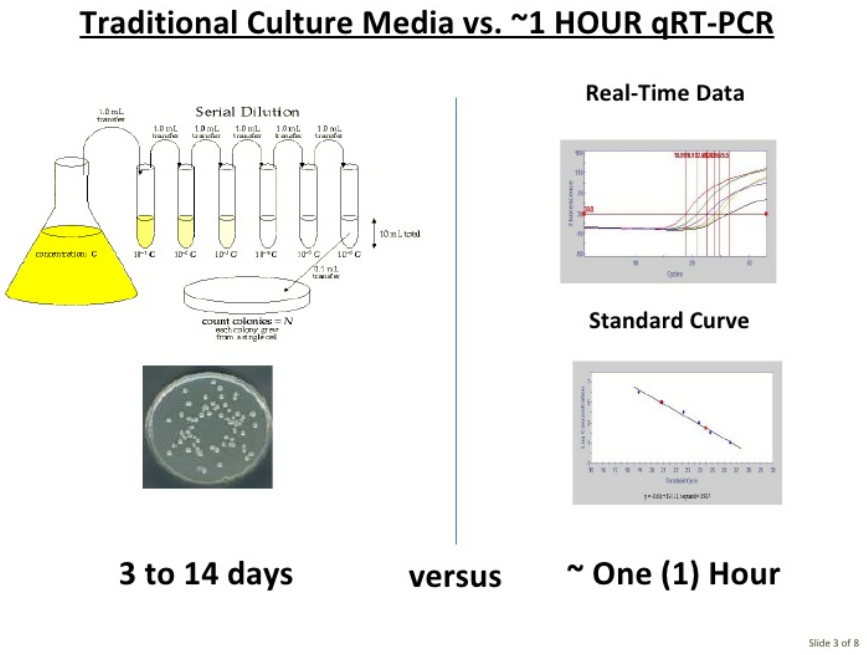


Figure 1. Traditional Culture Methods vs qRT-PCR

P23 Labs has designed the Microorganism Identification by PCR testing solution, a comprehensive test by adding both detection, susceptibility and typing in one easy to read report, eliminating days of culture time, decreasing TAT to 24 hours and providing highly accurate detection information.

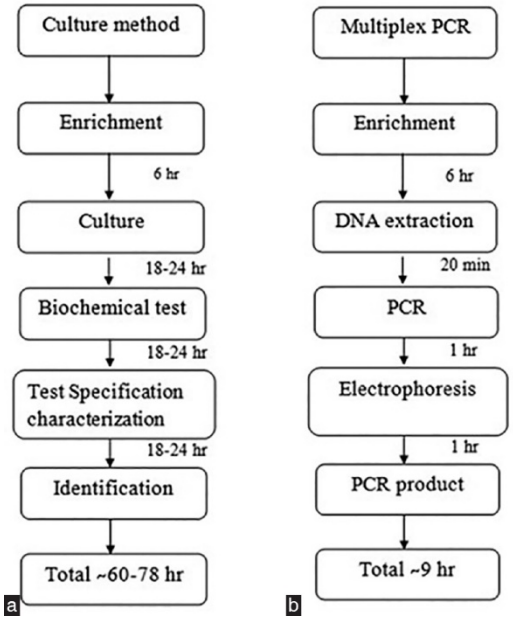


Figure 2. Comparison of Culture-Based Methods and Multiplex PCR

We are proud to have validated an innovative and proprietary assay with increased sensitivity and specificity to be used in early detection and effective treatment of microbial infections of the human body, specifically nail bed, skin (wounds), and urinary tract. We have designed several testing platforms and available sample types to deliver these needs. All available platforms are designed and validated to be tested from their respective specimen type, in this instance, we have validated the multiple types of specimen collection for the extraction of gDNA and mRNA to be used in molecular diagnostic and detection of disease. Our products are available both nationally and internationally.

Our vision is to be the domestic and international leader in actionable information for early cancer molecular diagnostic and infectious disease testing for all persons regardless of race, income, and geographical location. We are leaders in research and development of genetic molecular testing that can detect early DNA abnormalities and infectious disease, while educating all people and healthcare providers of our non-invasive, preventive testing that ensures longer life spans by detecting disease, infection, and illness before it is life-threatening. We understand treating infectious disease before causing cellular mutations of epidemiological level outbreaks in otherwise healthy communities.

Our mission is to accomplish this vision by providing tests that are easily accessible by the patient with or without the involvement of the physician and point of care testing to produce accurate molecular diagnostic results that are actionable.

Introduction

For many years, culture techniques have been the standard for detecting microorganisms. As of recent years, it has been shown that these culture techniques have low sensitivity and specificity and often result in false negatives. Gene sequencing has been shown to be a more accurate and reproducible method to detect microorganisms.[1] This novel technique has been able to detect new and unusual microorganisms not previously seen in culture. Prior to the use of PCR, it is thought that microorganisms were being wrongly classified into categories that they did not belong in. For example, *Helcococcus* species was previously classified into the *Streptococcus* species when using conventional culture methods. Sequence-based identification has been very reliable for classifying unusual microorganisms that had not been previously classified or correctly classified. This has improved our ability to recognize new, emerging pathogens as well as better define previously detected microorganisms in correct taxonomies as to further our understanding of microbial pathogenesis.

Culture techniques used to diagnose bacterial colonization and infection are time-consuming and due to low sensitivity, especially in polymicrobial environments, cannot determine the correct microorganism. PCR-based methods allow for reliable and consistent identification of all pathogen types, even anaerobic organisms, which proves to be very desirable in improvement of patient care. Real-time PCR is faster and 16S rRNA gene probes can be designed to target multiple pathogens to speed up the process of identification.[2] When culture results come up negative, yet clinical suspicion of infection remains high, PCR is a very useful technique for determining if there truly is an infection. Gene amplification and sequencing is proving helpful to improving our understanding of microorganism pathogenesis and is imperative for better prediction of patient responses to therapy and clinical outcome.

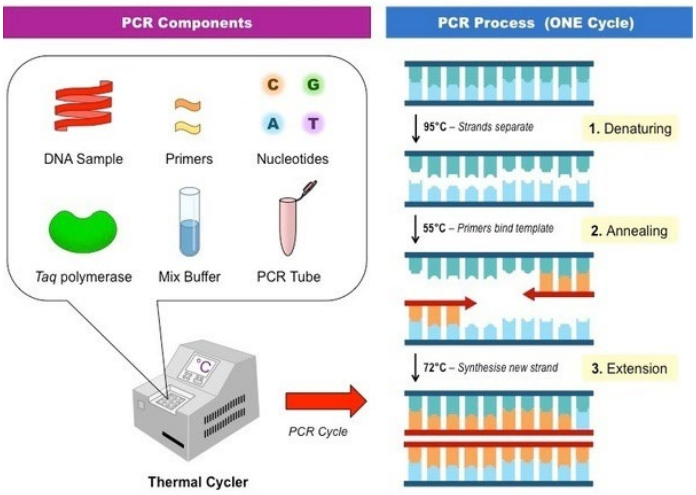


Figure 3. PCR components and process

What is Polymerase Chain Reaction (PCR)?

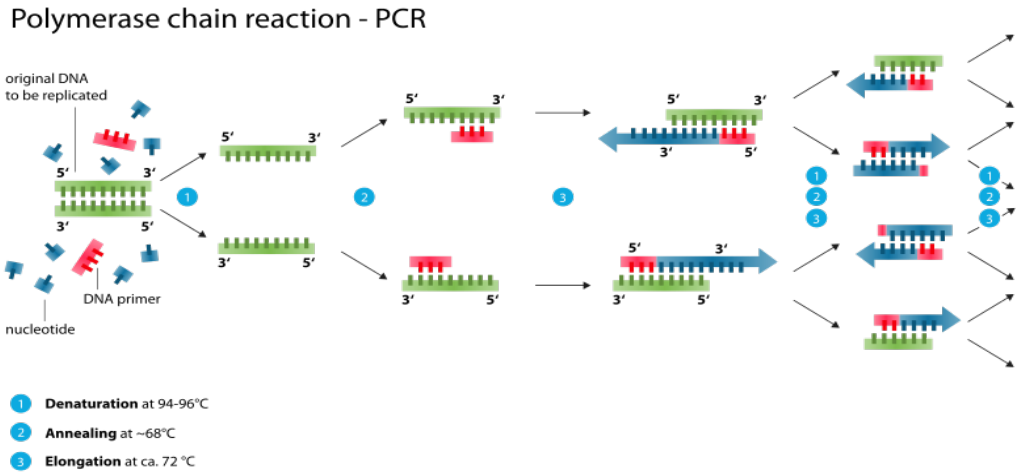


Figure 4. Elaboration of Steps of Multiplex PCR Reaction

PCR relies on the ability of DNA polymerase to synthesize new strands of DNA that are complementary to the template strand (original DNA to be replicated). A DNA primer is needed because DNA polymerase can only add a new nucleotide onto a preexisting 3'-OH (hydroxyl) group. This requirement is how this technique can delineate to a specific sequence of the template strand that researchers or clinicians want to amplify. There are five ingredients to a PCR reaction and these are the DNA template, primers, DNA nucleotide bases (dNTPs), Taq polymerase, and a buffer to ensure proper conditions for the reaction.

PCR involves a process of heating and cooling called thermal cycling which is carried out by a machine. There are three steps to PCR: denaturation, annealing, and elongation. Denaturing is when the double-stranded template DNA is heated to separate it into two single strands. Annealing is when the temperature is lowered to enable the DNA primers to attach to the template DNA. Elongation is when the temperature is raised, and the new strand of DNA is made by the Taq polymerase enzyme. These three steps are repeated, and this doubles the number of DNA copies each time. The result is a new double-stranded molecule of DNA and many copies of the specific DNA segment created in a short period of time.

Comparing and Contrasting Culture Methods and PCR

Identification of the pathogen causing illness in patients as well as early initiation of the appropriate treatment is a very critical stage in treatment. Even short delays in diagnosis and treatment increase the rate of morbidity and mortality. Culture-dependent methods were previously the gold standard for detection and diagnosis, but as seen in many studies, the sensitivity and specificity is relatively low. Delays in diagnosis and treatment can be avoided by the routine use of PCR-based molecular methods in patients. Through these techniques, the high sensitivity and specificity lead to better treatment for patients.

Culture-dependent techniques also take much longer to perform while PCR-based techniques can be done in a matter of hours. Time can be a huge factor in cost reduction as well as in treatment and so using culture-independent techniques are more advantageous. Culture-dependent techniques also produce a lot of false negatives while PCR is often used to confirm when the suspicion is high that there is still an infection in patients. Other techniques like ELISA have been used, but these were not as successful, and this is thought to be because of poor binding to the antibodies of interest. PCR-based methods can detect microorganisms in samples and when they do, reflex testing is available to further identify the pathogen causing illness.

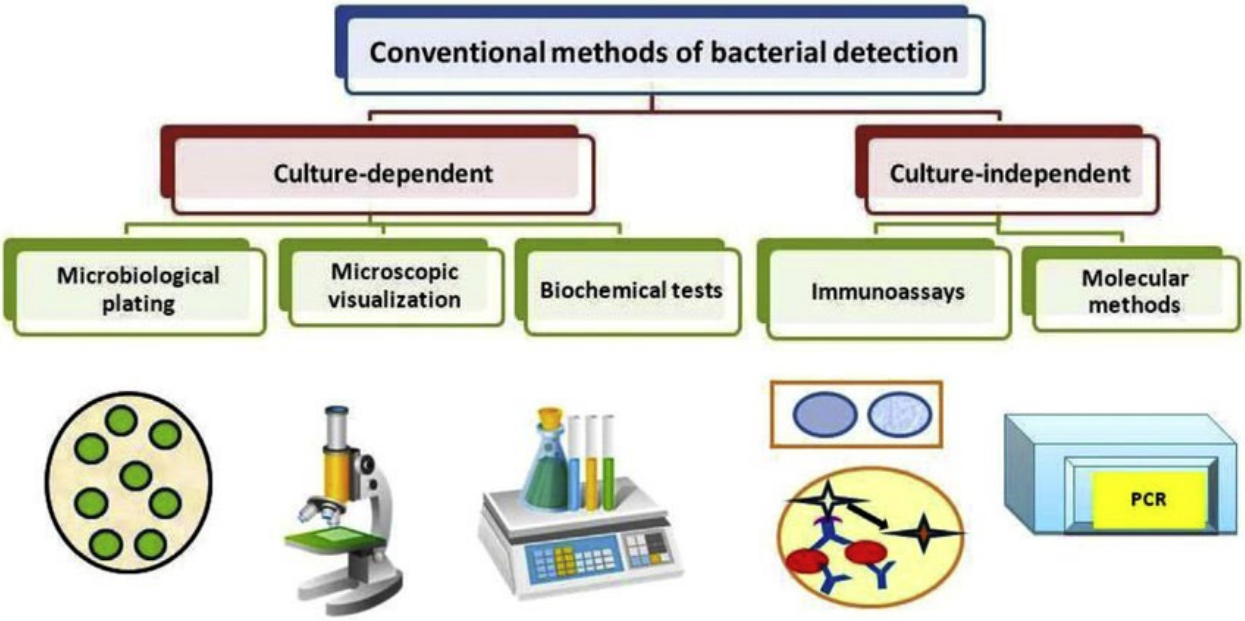


Figure 5. Comparison of Conventional Methods of Bacterial Detection

There are two conventional methods of bacterial detection: culture-dependent and culture-independent methods. Culture-dependent methods include microbiological plating, microscopic visualization, and biochemical assays. Culture-independent methods include immunoassays like ELISA and molecular methods such as PCR and next-generation sequencing (NGS).

Extracting DNA for Different Types of Samples

DNA isolation is an extraction process of DNA from various sources. The type of method used for isolation is dependent on the source, age, and size of the sample. In general, extraction methods aim to separate DNA present in the nucleus of the cell from other cellular components. Extraction of DNA is often an early step in many diagnostic processes used to detect bacteria and viruses in the environment as well as diagnosing disease and genetic disorders. The basic outline of the process includes three basic steps: lysing (breaking it open) the cell that contains DNA of interest, separating the DNA from other cellular components, and isolating the DNA. After DNA is isolated, analysis via PCR can be performed.

Here at P23 Labs, a variety of different sample types can be used for analysis with PCR. This includes buccal swab, oral rinse sample, blood, urine, vaginal or anal swab, stool sample, nail sample, and nasopharyngeal swab.

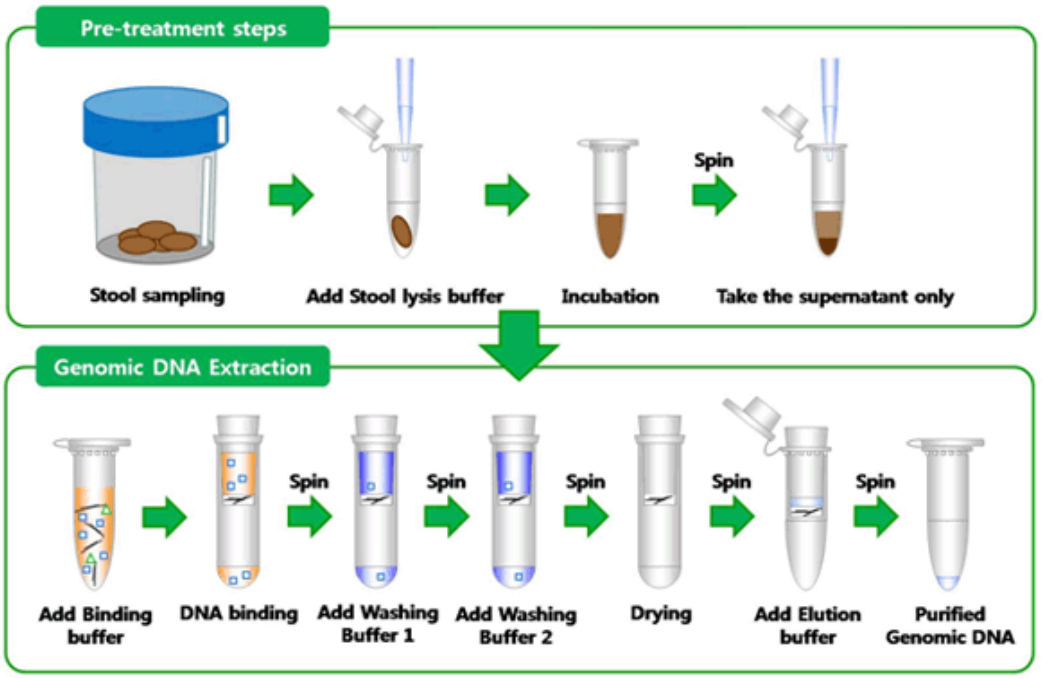


Figure 6. Example of DNA Extraction process

This image outlines the steps needed to obtain purified genomic DNA for PCR. The sample must be pre-treated with lysis buffer then incubated so that the supernatant can be taken and used for extraction. Then, the sample is ready for the desired extraction process for specific sample type.

Detecting Common Microorganisms using PCR

Respiratory (RP) Profile

The Respiratory Profile tests for a comprehensive set of 20 respiratory viral and bacterial pathogens in about an hour. This Profile identifies the most common viral and bacterial pathogens that cause respiratory tract infections that present with nearly indistinguishable symptoms. The rapid and accurate identification of the causative agent helps determine how a healthcare provider chooses to treat a respiratory tract infection. The RP Profile has been shown

to significantly reduce ICU days and duration of antibiotic use. It also optimizes patient management with clinically actionable results. The RP Profile also dramatically reduces time to results compared to traditional testing methods. It enables clinicians to diagnose patients faster and get them on the road to recovery more quickly, freeing up valuable healthcare resources faster. The RP Profile has also been shown to reduce overall healthcare costs. Significant savings were demonstrated in an adult ICU population in both patients that tested positive for a respiratory pathogen as well as those who tested negative.

The microorganisms that are detectable via the RP Profile:

- Bacteria:
  - *Bordetella parapertussis*
  - *Bordetella pertussis*
  - *Chlamydia pneumoniae*
  - *Mycoplasma pneumoniae*
- Viruses:
  - Adenovirus
  - Coronavirus HKU1
  - Coronavirus NL63
  - Coronavirus 229E
  - Coronavirus OC43
  - Human Metapneumovirus
  - Human Rhinovirus/Enterovirus
  - Influenza A
  - Influenza A/H1
  - Influenza A/H3
  - Influenza A/H1-2009
  - Influenza B
  - Parainfluenza Virus 1
  - Parainfluenza Virus 2
  - Parainfluenza Virus 3
  - Parainfluenza Virus 4
  - Respiratory Syncytial Virus

Gastrointestinal (GI) Profile

The Gastrointestinal Profile tests for a comprehensive set of 22 gastrointestinal pathogens. This Profile tests stool specimens for common pathogens associated with gastroenteritis. Quickly identifying the correct pathogen can ensure appropriate treatment, patient management, and help detect infectious gastroenteritis which can lead to severe illness or death. Clinicians rarely receive accurate or timely answers with traditional stool testing methods and often have to make patient management decisions without a laboratory result. With the GI Profile, timely and accurate results are provided that have been demonstrated to lead to more targeted therapy as well as reduced length of stay, reduced downstream radiologic tests such as CT scans, X-rays, and ultrasounds, and reduction in time from sample collection to antimicrobial therapy.

The microorganisms that are detectable via the GI Profile:

- Bacteria:
  - *Campylobacter (jejuni, coli, and upsaliensis)*
  - *Clostridium difficile* (toxin A/B)
  - *Plesiomonas shigelloides*
  - *Salmonella*
  - *Yersinia enterocolitica*
  - *Vibrio (parahaemolyticus, vulnificus, and cholerae)*
    - *Vibrio cholerae*
- Viruses:
  - Adenovirus F40/41
  - Astrovirus
  - Norovirus GI/GII
  - Rotavirus A
  - Sapovirus (I, II, IV, and V)
- Diarrheagenic E. coli/Shigella:
  - *Enteroaggregative E. coli* (EAEC)
  - *Enteropathogenic E. coli* (EPEC)
  - *Enterotoxigenic E. coli* (ETEC) *lt/st*
  - *Shiga-like toxin-producing E. coli* (STEC) stx1/stx2
    - *E coli O157*
  - *Shigella/Enteroinvasive E. coli* (EIEC)
- Parasites:
  - *Cryptosporidium*
  - *Cyclospora cayetanensis*

- *Entamoeba histolytica*
- *Giardia lamblia*

Bacterial Vaginosis (BV) Profile

The Bacterial Vaginosis Profile is the first FDA market-associated, microbiome-based, polymerase chain reaction assay that directly detects the 3 most common infectious causes of vaginitis: Bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis. Vaginal infections are among the most common reasons for which women in the US seek medical care—resulting in approximately 10 million visits to physicians' offices annually. Traditional diagnostic techniques tend to be subjective with variable sensitivity and specificity. This potentially leads to continued symptoms, repeat visits, inappropriate treatment, poor antimicrobial stewardship, and unnecessary associated healthcare system costs. With the BV Profile, efficiency can be maximized with 1 collection and 1 test for the 3 most common infectious causes of vaginitis. This assay also supports antimicrobial resistance initiatives by reporting *Candida krusei* and *C. glabrata.*

The microorganisms that are detectable via the BV Profile are:

- Lactobacillus species (L. crispatus and L. jensenii)
- Garnerella vaginalis
- Atopobium vaginae
- Bacterial vaginosis associated bacteria-2 (BVAB-2)
- Megasphaera-1

Urinary Tract Infection (UTI) Profile

The Urinary Tract Infection Profile tests for a comprehensive set of 12 bacterial pathogens, designed to target the 16S rRNA gene. The assay uses PCR amplification primers and hydrolysis-probe detection, which increases the specificity of each assay. This Profile allows for a convenient way to quickly detect the presence of pathogenic microorganisms from urine or urogenital swabs.

The microorganisms that are detectable via the UTI Profile are:

- *Acinetobacter baumanii*
- *Bacillus atrophaeus*

- *Bacterial vaginosis*
- *Candida species*
- *Chlaymydia trachomatis*
- *Citrobacter freundii*
- *Citrobacter koseri*
- *Enterobacter aerogenes*
- *Enterobacter cloacae*
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *Escherichia coli*
- *Gardnerella vaginalis*
- *Klebsiella oxytoca*
- *Klebsiella pneumoniae*
- *Megasphera1*
- *Morganella morganii*
- *Mycoplasma genitalium*
- *Mycoplasma hominis*
- *Neisseria gonorrhoeae*
- *Proteus mirabilis*
- *Psuedomonas aeruginosa*
- *Serratia marcescens*
- *Streptococcus agalactiae*
- *Staphylococcus aureus*
- *Staphylococcus saprophyticus*
- *Streptococcus pneumoniae*
- *Trichomonas vaginalis*
- *Ureaplasma urealyticum*

Wound Profile

The Wound Profile is a comprehensive test for 8 of the most common bacterial and fungal pathogens present in wounds. This Profile also includes a section that tests for antibiotic resistance genes and a complete antibiotic profile so that patients receive the most appropriate treatment possible and numbers of antibiotic resistance cases can be reduced.

The microorganisms that are detectable via the Wound Profile are:

- *Psuedomonas aeruginosa*
- *Staphylococcus aureus*
- Acinetobacter baumannii
- Citrobacter freundii
- Enterobacter aerogenes
- Enterobacter cloacae
- Escherichia coli
- Enterococcus faecium
- Enterococcus faecalis
- Morganella morganii
- Coagulase-negative staphylocci
- Candida organisms
- Gonorrhea organisms
- Chlamydia organisms

Nail Profile

The Nail Profile is a comprehensive test for 16 bacterial and fungal pathogens present in nail samples. These pathogens are the most common causes for onychomycosis otherwise known as a nail infection. Four types of onychomycosis are recognized based on the site and pattern of fungal invasion. Dermatophyte fungi are the predominant pathogens, but yeasts (especially Candida albicans) and nondermatophyte molds may also be implicated. Onychomycosis is more difficult to treat than most dermatophytoses because of the inherent slow growth of the nail. Older antifungal agents (ketoconazole and griseofulvin) are unsuitable for onychomycosis because of their relatively poor efficacy and potential adverse effects. Three recently developed antimycotic agents (fluconazole, itraconazole, and terbinafine) offer high cure rates and good safety profiles. In addition, the short treatment times (<3 months) and intermittent dosing schedules are likely to enhance compliance and reduce the costs of therapy

The microorganisms that are detectable via the Nail Profile are:

- Dermatophyte fungi
- Epidermophyton floccosum
- Trichophyton mentagrophytes
- Trichophyton rubrum
- Nondermatophyte fungi
- Acremonium
- Alternaria species
- Aspergillus species

- Botryodiplodia theobromae
- Fusarium species
- Onycochola canadensis
- Yeast/Candida species

Antibiotic Resistance and Profiling

Resistance to drugs that target pathogens is a serious public health concern. Resistance occurs when there are specific genes harbored by microorganisms that reduce the impact of drug molecules. These gene products have mechanisms that are employed that include covalent modification of the drug or target, removal of the drug from the cells, or activation of an alternative pathway. To understand an organism's reaction to a drug or family of drugs, researchers must know which genes act on which set of drug compounds. Since the discovery and production of antibiotics, there has been a reduction in mortality and morbidity of humans and a subsequent increase in the life span as well. Antibiotics can work through several different mechanisms such as inhibition of protein synthesis, like with tetracycline, aminoglycoside, and macrolide. Other mechanisms include interacting with the synthesis of DNA and RNA like with rifampin and quinolone. Another mechanism is through inhibition of synthesis or damage to bacterial cell wall like with B-lactam. It was originally thought that antibiotic resistance in bacteria was unlikely, but since the initiation of antibiotics, it has been shown that this is not true. Bacteria can exhibit horizontal gene transfer (HGT) to acquire antibiotic resistance genes with ease.

Over time, it has been shown that the use of antibiotics contributes to antibiotic resistance in humans and livestock. There has also been a rise of methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) since the development of antibiotics. Bacterial cells can achieve antibiotic resistance via chromosomal DNA mutations that alter existing bacterial proteins. It has been found that single nucleotide polymorphisms (SNPs) can also be a cause for resistance against synthetic drugs like quinolones, sulfonamides, and trimethoprim[2]. Genetic components known as mobile genetic elements also contribute to the spread of antibiotic resistance genes and are influenced by pressure in the environment, host factors, and intrinsic properties of the genetic elements themselves.

PCR-based methods allow for rapid detection of antibiotic resistance and profiling for the best medication based on personal genetics. Antibiotics and antibiotic resistance genes have a relationship that can be categorized by antimicrobial type. There are several categories including: Aminoglycosides, Beta-lactams, Erythromycin, Quinolones and Fluoroquinolones, Macrolides, Lincosamides, Streptogamins, Tetracyclines, and Vancomycin. The resistance genes for

aminoglycosides encode for acetyltransferases, adenyltransferases, and phosphotransferases. The transferases and phosphatases act enzymatically on the drug compounds, leading to inactivation of the drug. The beta-lactam resistance genes protect microorganisms by hydrolyzing the beta-lactam ring of various compounds, including penicillins, cephalosporins, and more. These genes are classified as groups based on their function and classes based on sequence similarity. The erythromycin resistance gene ereB encodes for an erythromycin esterase, which hydrolyzes the macrolactone ring, thus enzymatically inactivating the drug compound. The genes conferring resistance to quinolones and fluoroquinolones have diverse molecular mechanisms, including altering DNA topology, performing enzymatic modifications, and acting as drug efflux pumps. The molecular mechanism of the genes conferring resistance to macrolides, lincosamides, and streptogamins include enzymatic modifications of the drug and their targets, as well as acting as drug efflux pumps. The tetracycline resistance genes tetA and tetB encode for proteins that act as tetracycline efflux proteins. The efflux pump proteins prevent the accumulation of tetracycline in the microbial cell, resulting in sub-inhibitory concentrations. The vancomycin resistance genes vanB and vanC encode for D-alanine-D-lactate ligases, which alter the terminal amino acid residues of the vancomycin targets, NAM/NAG-peptide subunits.

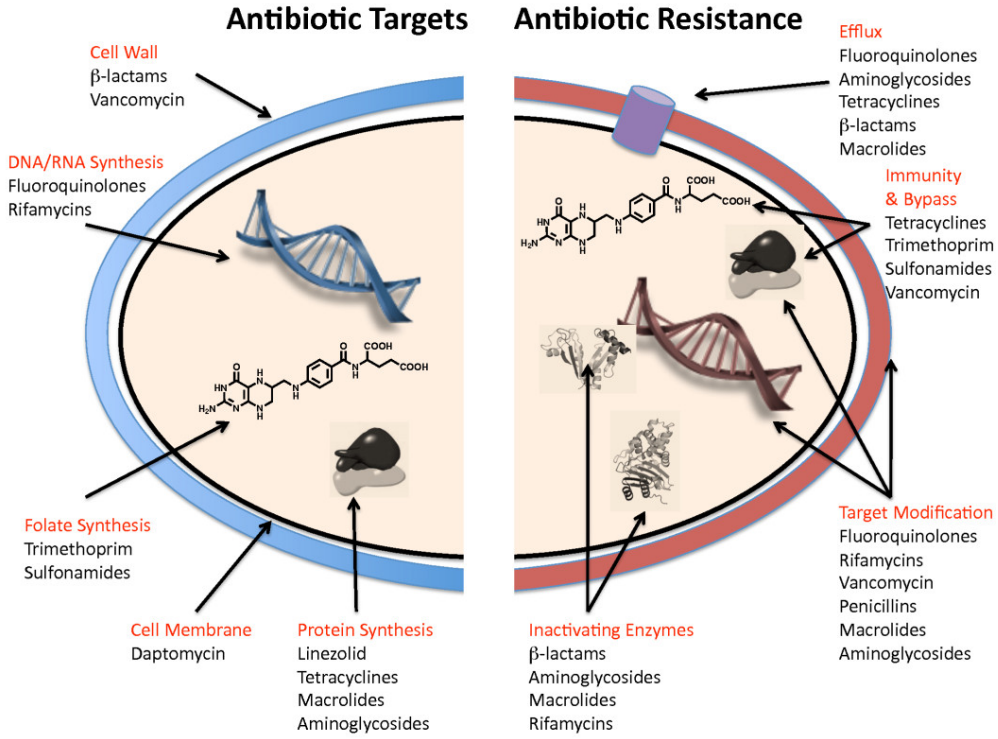


Figure 7. Antibiotic Targets and Resistance Pathways

Resistance Genes

aacC1
AmpC
blaKPC
blaNDM
ccrA
CTX-M-Group 1
ermA
mecA
QnrS
tetM
vanC

Antibiotic Stewardship

Antibiotic stewardship is any activity that helps promote the appropriate dose, type, and duration of antibiotics. Since the discovery and use of antibiotics, the practice of medicine has been transformed. Infections that at one time were fatal to patients are now treatable, and procedures like chemotherapy and organ transplants are possible. Even though rapid initiation of antibiotics to treat infectious disease has shown to save lives, 20-50% of all antibiotics prescribed in the US are either unnecessary or inappropriate. Antibiotics have shown to cause serious side effects, including adverse drug reactions and Clostridium difficile infection. When patients are unnecessarily prescribed antibiotics, it puts them at risk for serious adverse events and does not provide any clinical benefit. When antibiotics are misused, this contributes to antibiotic resistance, which is a very serious public health concern. The CDC estimates that there are over 2 million people infected with organisms that are antibiotic-resistant which results in 23,000 deaths annually. Antibiotic stewardship is based on the "three Ds": the right drug, the right dose, and the right duration. When detecting microorganisms via PCR, it is not only important to properly identify the microorganism, but also consider the appropriate treatment, dosage, and duration.

List of Microorganisms able to be detected by P23 Labs

Bacteria:	Beta-hemolytic streptococci
Acinetobacter baumanii	Bordatella parapertussis
Acinetobacter spp.	Bordatella pertussis
Aerococcus urinae	Burkholderia cenocepacia
Atopobium vaginate	Burkholderia cepacian

Burkholderia pyrrocinia

Burkholderia vietnamiensis

Campylobacter (jejuni, coli, and upsaliensis)

Candida species

Chlamydia organisms

Chlamydia pneumoniae

Chlamydia trachomatis

Clostridium difficle (toxin A/B)

Coagulase-negative staphylococci

Coliform bacteria, including E. coli, Enterobacter species, and Klebsiella pneumoniae

Enteroaggregative E. coli (EAEC)

Enterococcus faecalis

Enterococcus faecium

Enterococcus, vancomycin-resistant (eg. Enterococcus van A, van B)

Enteropathic E. coli (EPEC)

Enterotoxigenic E. coli (ETEC) lt/st

Enterococci

Enterovirus

Escherichia coli

Escherichia fergusonii

Shiga-like toxin producing E. coli (STEC)

E. coli O157

Shigella/Enteroinvasive E. coli (EIEC)

Shigella boydii

Shigella sonnei

Shigella dysenteriae

Shigella flexneri

Gardnerella vaginalis

Gonorrhea organisms

Heliobacter pylori

Klebsiella pneumoniae

Lactobacillus sp. 1

Lactobacillus sp. 2

Legionella pneumophila

Morganella morganii

Mycobacterium sp. 1

Mycobacterium sp. 2

Mycobacterium avium intracellulare

Mycoplasma pneumoniae

Neisseria gonorrhea

Neisseria pneumoniae

Pan Bacteria 1

Pan Bacteria 3

Plesiomonas shigelloides

PPC

Pseudomonas aeruginosa

Salmonella

Staphylococcus aureas

Staphylococcus aureas (methicillin resistant)

Staphylococcus epidermis

Staphylococcus sp. 1

Staphylococcus sp. 2

Streptococcus agalactiae

Streptococcus, group A

Streptococcus, group B

Streptococcus sp. 1

Streptococcus sp. 2

Streptococcus sp. 3

Treponema pallidum

Trichomonas vaginalis

Vibrio (parahaemolyticus, vulnificus, and cholerae)

Vibrio cholerae

Yersinia enterocolitica

Viruses:

Adenovirus

Adenovirus F40/41

Astrovirus

Coronavirus HKU1

Coronavirus NL63

Coronavirus 229E

Coronavirus OC43

Cytomegalovirus

Hepatitis B virus

Hepatitis C virus

Herpes Simplex virus

HIV-1

HIV-2

Human herpesvirus 6

Human Metapneumovirus

Human papillomavirus

Human Rhinovirus/Enterovirus

Influenza A

Influenza A/H1

Influenza A/H3

Influenza B

Norovirus GI/GII

Parainfluenza Virus 1

Parainfluenza Virus 2

Parainfluenza Virus 3

Parainfluenza Virus 4

Rotavirus A

Respiratory Syncytial Virus

Sapovirus (I, II, IV, and V)

Parasites:

Cryptosporidium

Cyclospora cayetanesis

Entamoeba histolytica

Giardia lamblia

Fungal:

Acremonium

Alternaria species

Aspergillus flavus

Aspergillus species

Aspergillus fumigatus

Aspergillus niger

Botryodiplodia theobromae

Candida albicans

Candida glabrata

Candida krusei

Candida parapsilosis

Dermatophyte fungi

Fusarium species

Epidermophyton floccsum

Mucor/Rhizopus spp.

Nondermatophyte fungi

Onycochola canadensis	Scytalidium hyalimum
Pan Aspergillus/Penicillium	Trichophyton mentagrophytes
Pneumocystis jirovecii	Trichophyton rubrum
Pyrenochaeta unguis-hominis	Yeast/Candida albicans
Scytalidium dimidiatum scopulariopsis species	

Conclusion

Overall, PCR-based methods are superior to culture-dependent methods for detection and diagnosis of microorganisms.

SUMMARY

Points of agreement

1. Highly multiplexed molecular tests have clinical value; they provide a syndromic approach to diagnostics, which is particularly useful for infections in which it is not possible to determine the etiologic agent based only on symptoms.

2. The use of highly multiplexed tests is more closely aligned with traditional culture methods, where clinicians do not need to identify a specific pathogen for testing but rather think in broad terms about whether there is a bacterial infection in the respiratory tract or the blood.

3. Rapid sensitive diagnostic tests have the potential to transform the medical management of patients with infectious diseases.

4. Multiplex tests should be developed in consultation with clinical microbiologists and clinicians so that the Profile members reflect clinical reality.

5. Implementation of Profile tests should be done in consultation with clinicians, so there is a clear understanding of the appropriate use and interpretation of test results.

Issues to be resolved

1. A rapid, accurate diagnosis of viral respiratory infection will likely decrease the use of antibiotics and allow for a more targeted approach to using antivirals, although outcome studies are needed in this area.

2. The value of using highly multiplexed tests as front-line diagnostics will depend on the clinical situation: while it is easier to justify Profile testing for respiratory viruses, it is more difficult when the Profile includes pathogens that are very rare, when all pathogens in the Profile do not cause overlapping clinical syndromes, or when some pathogens are found only in specific patient populations (immunocompromised patients).

3. Understanding the performance characteristics of all members of the Profile is essential, as the sensitivity and specificity for the detection of each pathogen may vary. The prevalence of the pathogen will greatly affect the positive and/or negative predictive value of the test.

4. Cost assessments of Profile tests need to consider the overall cost or cost savings to the health care system, not just the cost of the test to the microbiology laboratory. Factors to consider include decreased use of antibiotics, decreased ancillary testing, decreased length of stay in the hospital or emergency department, and time off work.

Angela M. Caliendo, Editor, Journal of Clinical Microbiology Point-Counterpoint

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