MAYO CLINIC
Mayo Medical Laboratories

THE MML DIFFERENCE:
RAPID PCR TECHNOLOGY

BACKGROUND

Polymerase chain reaction (PCR) is a technique used to amplify (massively replicate) segments of DNA. This technique targets specific DNA sequences, and through a chain reaction, uses the newly amplified DNA (amplified product or amplicon) as a template for subsequent cycles of DNA synthesis. PCR enables fast, sensitive detection, even when very small amounts of targeted DNA were present in the specimen.

PCR is a multistep process that includes separate procedures for extraction, amplification (thermocycling), and detection of amplified product. Rapid PCR uses real-time PCR, an automated rapid thermocycling process that incorporates amplification and detection in a single procedure inside a closed reaction vessel. This process significantly reduces the risk of contamination by nontarget DNA.

Rapid PCR technology medical applications can include:

- Identifying infectious organisms.*
- Determining antibiotic resistance (eg, *vanA* and *vanB* genes for vancomycin-resistant enterococci (VRE), *mecA* gene for methicillin-resistant *Staphylococcus aureus* (MRSA), *katG* gene of *Mycobacterium* for isoniazid resistance).
- Detecting genes and gene mutations.**
- Screening for substances in bioterrorism threats (eg, anthrax, smallpox).

PATIENT CARE INFORMATION

Impact on the Patient

- Accurate diagnosis.
- Rapid diagnosis.
- Prompt and appropriate treatment.

HEALTH CARE ECONOMICS

Rapid PCR technology is fast and highly accurate. By providing more accurate test results in a faster timeframe, Rapid PCR technology saves health care dollars. It also is cost-effective compared to other detection methods. For example, comparing our previous method for detection of group A streptococcus (GAS) from throat swabs (rapid antigen immunoassay with culture for immunoassay negative specimens) with rapid PCR, sensitivity improved 55% over immunoassay and 7% over culture. Final results were available the same day for rapid PCR, compared to up to 48 hours for most patients using the previous approach (most patients tested negative by immunoassay and culture was performed). Personnel time was 3 minutes/specimen for rapid PCR, compared to 7 minutes/specimen for the combined rapid antigen-culture method. Based on the performance of rapid PCR

*A growing list of organisms are detectable by Rapid PCR including viruses (eg, EBV, CMV, HSV, VZV, JC/BK virus, influenza virus, rotavirus, enterovirus), bacteria (*Streptococcus, Bordetella, Legionella*), *Babesia, Ehrlichia/Anaplasma, Borrelia, Trophermyma whippleii*, fungi, and mycobacteria.

**A growing list of genes/mutations are detectable by Rapid PCR. MML tests include genes for alpha 1 antitrypsin, hemochromatosis, hereditary pancreatitis, Canavan disease, protein S Heerlen mutation, galactosemia, and short-chain acyl-coA dehydrogenase (SCAD).

for GAS, we eliminated the need for back-up throat cultures on patients with negative antigen-based strep test results and decreased laboratory costs.

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) now approaches 60% of selected in-patient populations and nearly a 30% community acquired incidence in certain regions. MRSA patients had an average increased cost of $68,000 per hospital encounter and a greater frequency of repeat hospitalization[4,5] The ability to quickly identify, isolate, and treat these patients leads to significantly decreased cost, reduced spread of infection, and avoidance of repeat hospitalization. Studies have shown that MRSA patients had an average increase of 4.5 days length of stay, and a increased cost of $3,805 per day.[4,5]

TECHNOLOGICAL COMPARISON

Compared to organism culture and conventional susceptibility testing:

- Direct detection in primary specimen; growth in culture media not required.
- Viable organisms not required for successful identification:
  - Less rigorous specimen transportation requirements.
  - Detects organisms that do not grow or grow poorly in culture.
- Faster turnaround time.
- Sensitivity equals and frequently exceed culture, enabling detection of <10 copies of target nucleic acid per reaction.***
- Target specific.
- Based on genotype (vs phenotypic expression under "artificial" laboratory conditions).

Compared to conventional PCR:

- Much faster turnaround time (1 day versus 2-3 days or more).
- Less labor-intensive.
- Same or better sensitivity.
- Eliminates contamination problems seen with PCR.

How Does Mayo's Test Differ?

- Mayo is the leader in developing these assays and disseminating procedural knowledge.
- For example, Mayo rapidly developed an anthrax screening test during a national emergency.
- Mayo's performance is documented in peer-reviewed literature.
- Mayo has replaced >90% of virus tests with Rapid PCR and more tests have been identified for conversion.
- Mayo is expanding LC technology across the various laboratory disciplines.

The application of Rapid PCR technology is changing the face of laboratory medicine, and its greatest benefit will be realized when deployed as near to the patient as possible. MML prides itself in a demonstrated ability to support hundreds of clients each year with technology transfer. Because of our position at the leading edge in development of Rapid PCR, we offer clients the guidance and hands on training necessary for tests that can be brought into your laboratory.

***Increased sensitivity compared to culture: VRE 219%, VZV 91%, CMV 88%, HSV 23% (Mayo data).

REFERENCES

1. Cockerill FR 3rd: Application of rapid-cycle real-time polymerase chain reaction for diagnostic testing in the clinical microbiology laboratory. Arch Pathol Lab Med 2003 Sep;127(9):1112-20
2. Cockerill FR 3rd: Genetic methods for assessing antimicrobial resistance. Antimicrobial Agents Chemother 1999 Feb;43(2):199-212
3. Cockerill FR 3rd: Conventional and genetic laboratory tests used to guide antimicrobial therapy. Mayo Clin Proc 1998 Oct;73(10):1007-21
4. McHugh CG, Riley LW: Risk factors and costs associated with methicillin-resistant *Staphylococcus aureus* bloodstream infections. Infect Control Hosp Epidemiol 2004 May;25(5):425-30
5. Kopp BJ, Nix DE, Armstrong EP: Clinical and economic analysis of methicillin-susceptible and -resistant *Staphylococcus aureus* infections. Ann Pharmacother. Epub 2004 Sep;38(9):1377-82