

**Molecular Microbiology**  
*The Secrets of PCR Revealed*

Robert Liao, Ph.D., D(ABMM)  
 CHICA Eastern Ontario Conference  
 September 25, 2008




---

---

---

---

---


---

---

---

**Molecular Microbiology**  
 PCR: Arcane black-box testing to that  
 warm familiar sweater you like to  
 snuggle up in at the end of the day

Robert Liao, Ph.D., D(ABMM)  
 CHICA Eastern Ontario Conference  
 September 25, 2008




---

---

---

---

---


---

---


---

**Historical Perspective of Diagnostic Microbiology**


**Traditional Culture** in the first 70 years  
 - Slow answers  
 - Manual labour is highly intensive



Next 20 years **Immunological Detection & DNA/RNA probes**  
 - Faster with improved Sensitivity



Last 10 years **Molecular Amplification** methods (PCR)  
 - Faster with even higher Sensitivity and Specificity




---

---

---

---

---

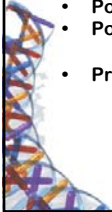
---

---

---

## Advantages of Molecular Microbiology

- Decreased **time** for detection of infectious agents
- Detection of agents that are **not culturable**
- Accurate **quantitation** of infectious agents
- Possibility for **multiplex testing**
- Potential for **automation**
- Provides additional information:
  - **Epidemiological** fingerprinting
  - **Genotype** for treatment decisions
  - **Sequence identification**




---

---

---

---

---

---

---

---

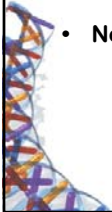
## Applications to Infection Control

- Faster Results and Higher Sensitivity



- Nosocomial Pathogens

**MRSA**  
**VRE**  
**Clostridium difficile**  
**Norovirus**  
**Influenza and RSV**




---

---

---

---

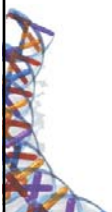
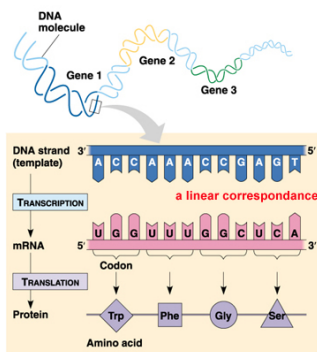
---

---

---

---

## The Genetic Code




---

---

---

---

---

---

---

---

## Polymerase Chain Reaction (PCR)

1. Amplification of target nucleic acid
2. Product detection
3. Confirmation of amplified products



---

---

---

---

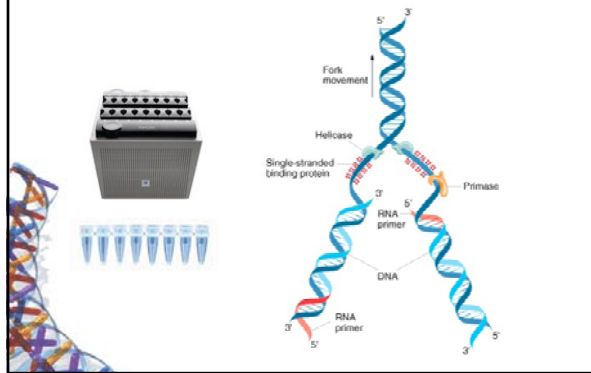
---

---

---

---

## PCR: DNA Replication in a Tube



---

---

---

---

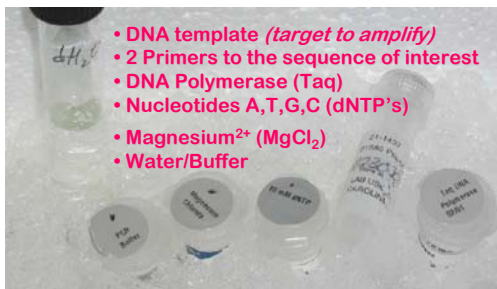
---

---

---

---

## The Recipe for PCR



---

---

---

---

---

---

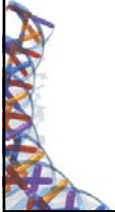
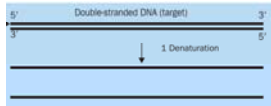
---

---

## PCR Amplification Cycle

3 different temperatures for different steps in 1 cycle

Denaturation



---

---

---

---

---

---

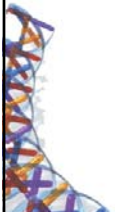
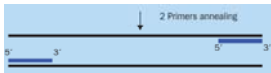
---

---

## PCR Amplification Cycle

3 different temperatures for different steps in 1 cycle

Annealing



---

---

---

---

---

---

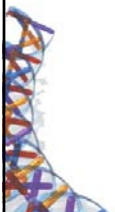
---

---

## PCR Amplification Cycle

3 different temperatures for different steps in 1 cycle

Extension



---

---

---

---

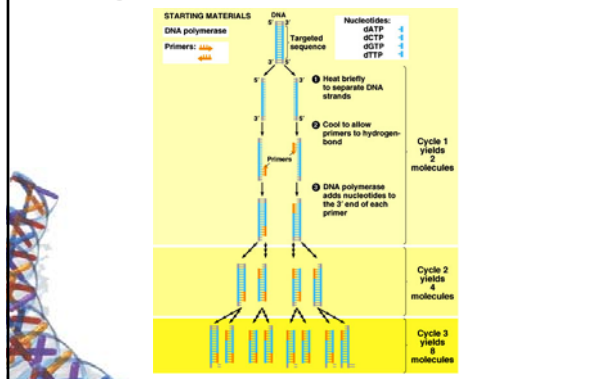
---

---

---

---

## Polymerase Chain Reaction




---

---

---

---

---

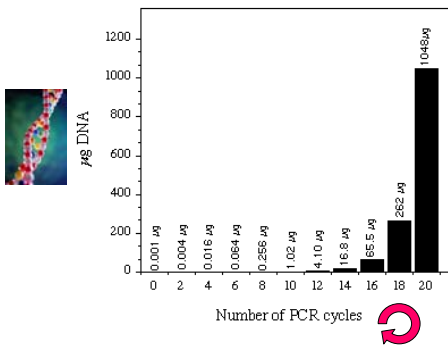
---

---

---

## 2 Million Copies in 20 Cycles

DNA amplification of 0.001 mg DNA template




---

---

---

---

---

---

---

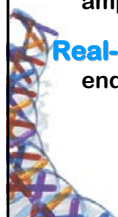
---

## Different PCR Methods

**Standard (Endpoint) PCR**  
measures endpoint after a fixed cycle number

**Reverse-Transcriptase (RT)-PCR**  
amplification of RNA target

**Real-time PCR**  
endpoint occurs during amplification




---

---

---

---

---

---

---

---

## PCR Limitations

- false negatives
- false positives



- A limited ability to detect **multiple targets**
- An Inability to detect **antibiotic resistance**
- PCR remains highly **technical**



---

---

---

---

---

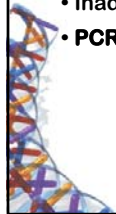
---

---

---

## PCR false Negatives

- **Small sample** volumes for PCR reactions
- **Sample processing** problems
- Ineffective **release** of microbial DNA from cells
- Inadequate removal of PCR **inhibitors**
- **PCR reaction** is poorly designed



---

---

---

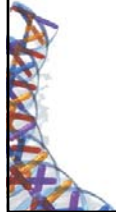
---

---

---

---

---



---

---

---

---

---

---

---

---

## PCR false Positives

- Erroneous **annealing** of primers
- Error rate of **DNA polymerase** (*base pair mismatch*)
- Background **DNA contamination**
- Detection sensitivity exceeding **clinical significance**  
*Subclinical colonization vs latent or active disease???*



---

---

---

---

---

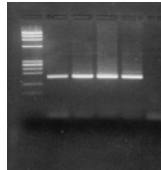
---

---

---

## Conventional Endpoint PCR

1. Amplification of target nucleic acid
2. Product detection by visualizing with agarose gel electrophoresis
3. Confirmation of amplified product



---

---

---

---

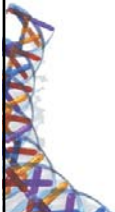
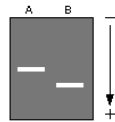
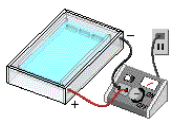
---

---

---

---

## Conventional PCR



---

---

---

---

---

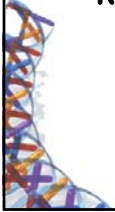
---

---

---

## Limitations of Endpoint PCR

- **Time-Consuming**
- **Precision** is poor
- **Endpoint** is variable between samples
- **Resolution** of agarose gel is poor



---

---

---

---

---

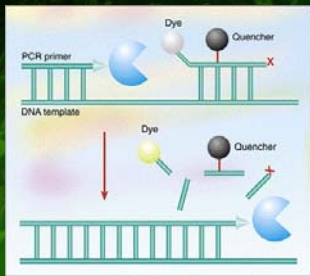
---

---

---

## Real Time PCR

- **measurement of amplification product during each cycle with fluorescent probes**



---

---

---

---

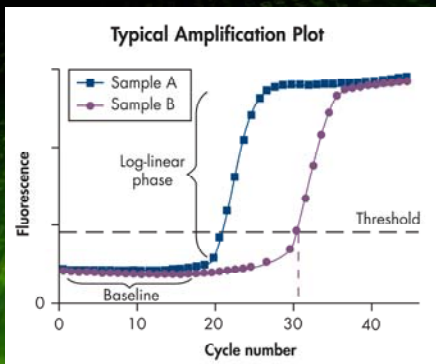
---

---

---

---

## Real Time Amplification Plots



---

---

---

---

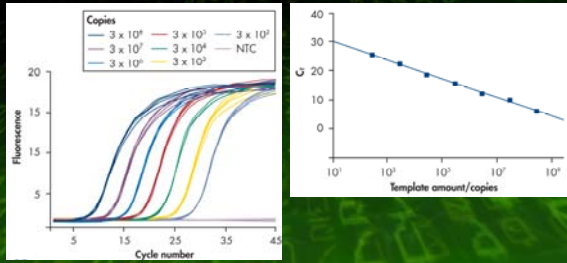
---

---

---

---

# Real Time PCR Quantification




---

---

---

---

---

---

---

---

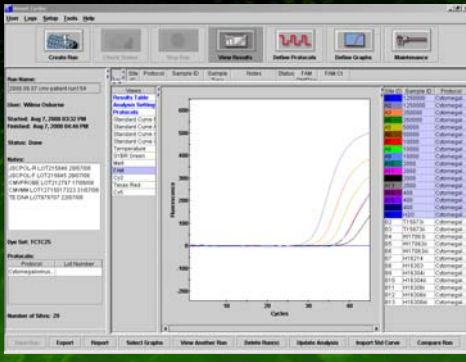
---

---

---

---

# Real Time PCR Quantification




---

---

---

---

---

---

---

---

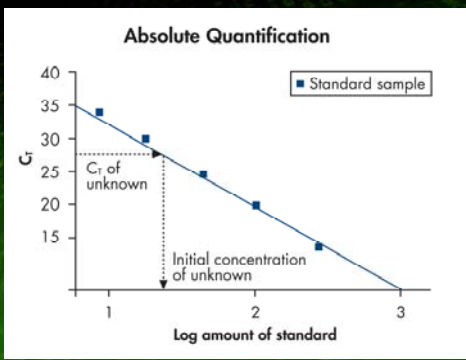
---

---

---

---

# Real Time PCR Quantification




---

---

---

---

---

---

---

---

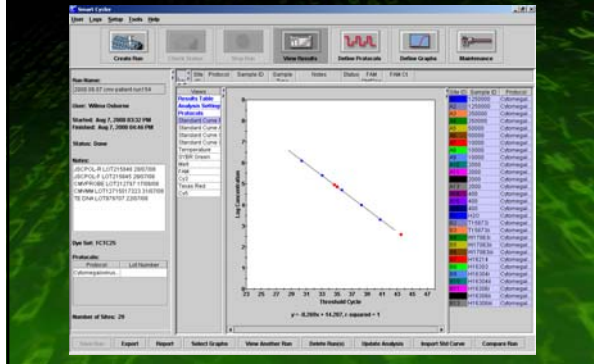
---

---

---

---

## Real Time PCR Quantification




---

---

---

---

---

---

---

---

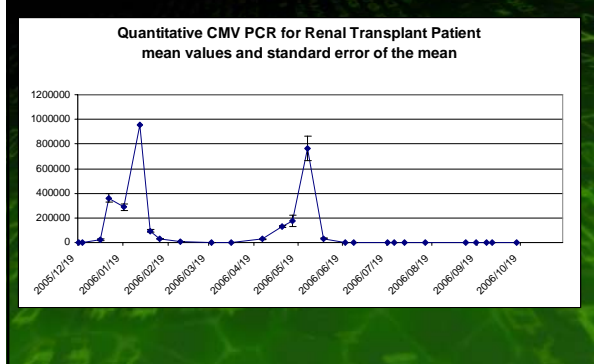
---

---

---

---

## Real Time PCR Quantification




---

---

---

---

---

---

---

---

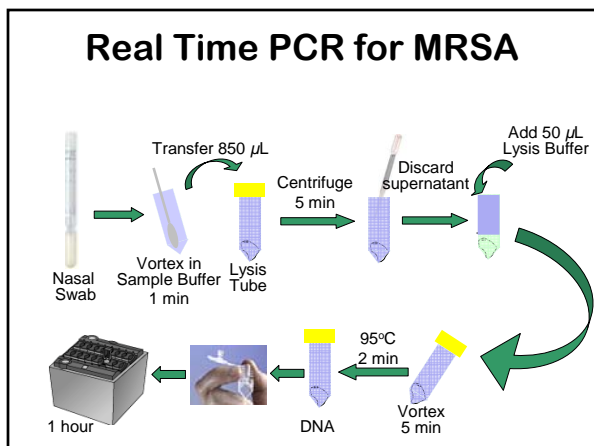
---

---

---

---

## Real Time PCR for MRSA




---

---

---

---

---

---

---

---

---

---

---

---





