Rapid PCR Testing with MSSA/MRSA/C. *difficile* Toxin

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Objectives

- Review the mechanism of PCR
- Review Memorial's PCR machine
- Review hospital procedures with rapid PCR testing
- Discuss benefits of using in-house rapid PCR testing
- Discuss literature supporting use of targeted MRSA decolonization

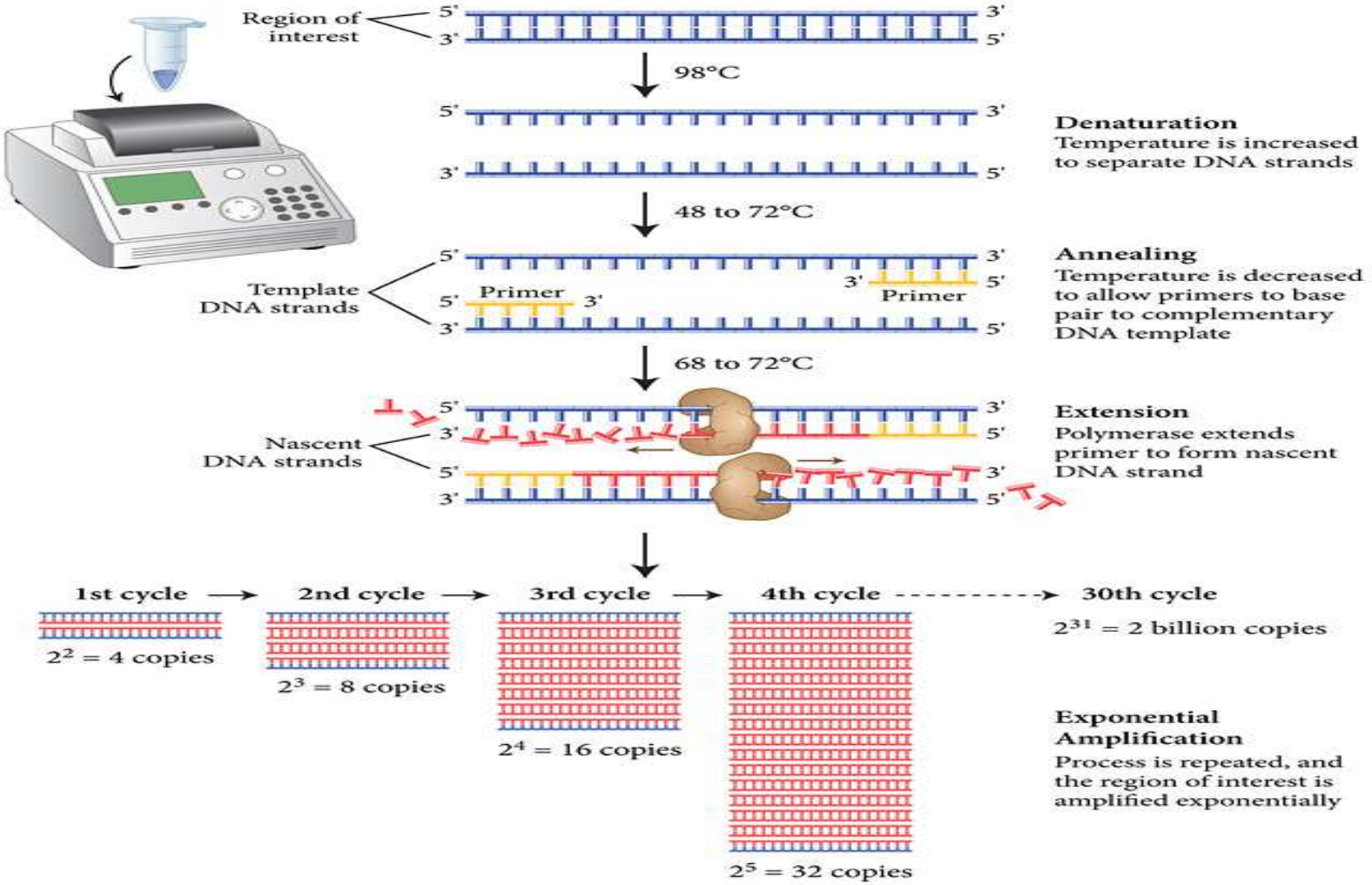
PCR

- What is PCR?
  - Polymerase Chain Reaction
  - Molecular analysis/diagnostics
  - Amplifying targeted DNA sequences
  - Produces millions of sequence copies
- PCR Uses
  - Once amplified, the DNA produced by PCR can be used in many different laboratory procedures
  - Allows us to detect the presence of a bacteria, virus, or toxin in a short period of time

PCR

- PCR mechanism
  - 1st – sample heated so DNA denatures into two pieces of single-stranded DNA
  - 2nd – Taq polymerase synthesizes two new strands of DNA, using the original strands as templates
    - Results in the duplication of the original DNA
    - Each of these strands now serve as templates
  - Cycle of denaturing and synthesizing new DNA is repeated 30-40 times
    - Temperature is changed every few minutes to allow DNA denaturing and synthesis

PCR Mechanism



GeneXpert

- In-house PCR machine
- On-demand results
  - Provides MSSA/MRSA results in approximately 1 hour with 86% sensitivity. Tests for *mec* (MRSA) and *spa (MSSA)* gene.
  - Provides C. *difficile* toxin results in approximately 30 minutes with 93.5% sensitivity and 94% specificity. Tests for toxin B gene.

GeneXpert Procedure

- Total hands-on time of approximately 1 minute for both MSSA/MRSA and C. *difficile* toxin tests
- Lab procedure
  - Insert swab into sample reagent vial and break
  - Vortex and dispense sample into specimen port
  - Insert cartridge and start assay

Hospital Procedures

- Nasal swabs will be taken of all patients admitted to the ICU for detection of MSSA/MRSA colonization
  - Patient's with positive results will receive decolonization treatment with mupirocin 2% ointment
  - 2% chlorhexidine baths?
- Positive gram stains with evidence of GPC will be analyzed with PCR
  - If PCR is positive, still have about 2 days for sensitivities
  - Current procedure – Lab calls stewardship with any positive PCR results

Benefits of Rapid PCR

- Timely and effective patient information
  - Optimize treatment earlier
    - Trial at Centennial Medical Center in Nashville, TN found a 28 hour improvement in MRSA detection with GeneXpert versus culture results
  - Improve MRSA infection rates
  - Improve bed management
  - Reduce isolation costs in the ICU

Targeted MRSA Decolonization

- "Targeted versus Universal Decolonization to Prevent ICU Infection"
- Pragmatic, cluster-randomized trial published on June 13th, 2013 in NEJM
- 43 hospitals including 74 ICUs and 74,256 patients
- Primary Outcome
  - ICU-attributable, MRSA positive clinical cultures
- Secondary Outcome
  - ICU-attributable bloodstream infections caused by MRSA and ICU-attributable bloodstream infections caused by any pathogen

Targeted MRSA Decolonization Continued

- Three strategy groups
  - Group 1 – screening and isolation
  - Group 2 – screening and targeted decolonization
  - Group 3 – universal decolonization
- Treatment
  - 5-day regimen of bid intranasal mupirocin 2% ointment plus daily 2% chlorhexidine bathing for entire ICU stay
- Trial Results
  - Hazard ratios for MRSA clinical isolates was 0.92 in group 1
  - Hazard ratios for MRSA clinical isolates was 0.75 in group 2
  - Hazard ratios for MRSA clinical isolates was 0.63 in group 3

Targeted MRSA Decolonization Continued

- Conclusion
  - Universal decolonization was most effective at reducing rates of MRSA clinical isolates and bloodstream infections from any pathogen
  - Targeted decolonization was intermediate between the effects of screening/isolation treatment and universal decolonization

Test Your PCR Knowledge

- Who developed the process of PCR gene amplification and in what year?
- Answer - Kary Mullis in 1983
  - Shared the 1993 Nobel Prize in Chemistry with Michael Smith who developed site-directed mutagenesis