Amplification of DNA Polymerase I gene of *T. pallidum* from whole blood of persons with syphilis
Amplification of DNA Polymerase I gene of *T. pallidum* from whole blood of persons with syphilis

Data obtained during a study of

Molecular subtyping of *Treponema pallidum* during an outbreak investigation of syphilis in Maricopa County, Arizona 1997-1999
Syphilis Cases in Maricopa County and US, 1988-1999

- No. Cases in Maricopa County
- US cases/1,000,000
- Year 2000 objective

Year

Number of Cases
Amplification of DNA Polymerase I gene of *T. pallidum* from whole blood of persons with syphilis

DSTDP/NCHSTP
DASTLR/NCID
Maricopa County STD Clinic
Methods

Study population

- Persons attending Maricopa County STD clinic
- Signs or symptoms of syphilis, or
- Sex partner with syphilis
Methods - Case Definitions

**Incubating** - no signs or symptoms; significant sexual exposure to infectious syphilis; nonreactive RPR and MHA-TP

**Primary** - genital ulcer with positive dark field

**Secondary** - rash and/or lymphadenopathy with reactive RPR and MHA-TP

**Latent** - no signs or symptoms with reactive serology
Methods

Data Collection
• Review of medical records at STD clinic
• RPR and MHA-TP reactivity abstracted

Specimen Collection
• 5-10 ml blood collected in EDTA tubes
• stored at 4°C
• Shipped to CDC for analysis
Laboratory Methods

- Samples screened by PCR to amplify DNA polymerase I gene (*polA*)
- Analyzed by agarose gel electrophoresis
- The validity of *polA* PCR was reconfirmed with two additional targets (*arp, tpr*)
Use of *PolA* as Diagnostic PCR for *T. Pallidum*

- House keeping gene - highly conserved
- Primers selected based on 2 unique features
  - Four additional inserts in sequence
  - High in cystein content
Additional Targets used for Molecular Typing

- Acidic repeat protein (arp) gene
  - multiple repeats
  - can be used to distinguish among clinical strains

- Tpr gene
  - multiple gene family
  - can be used to distinguish among clinical strains
RESULTS

PCR attempted on 32 blood specimens

13 (41%) pol A positive

7 (22%) positive by at least one additional target (arp, tpr)
## PCR Results in Whole Blood, by Syphilis Disease Stage

<table>
<thead>
<tr>
<th>Stage</th>
<th>No.</th>
<th>polA +</th>
<th>≥2 targets +</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubating</td>
<td>8</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>primary</td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>secondary</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>latent</td>
<td>12</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>non-syphilis ulcer</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Conclusions

- *T. pallidum* DNA amplified from whole blood samples from persons known to have untreated syphilis or exposure to syphilis

- The viability of *T. pallidum* that yielded amplified DNA is unknown

- Data suggest that potentially infectious spirochetes were present in the blood during incubating, primary, secondary and latent stages
Possible Reasons for Differences between CDC and ARC Study

• Differences in populations: Patients with untreated early syphilis seen at STD clinics vs patients with late latent or treated syphilis

• Differences in blood component: Treponemes may not be present in platelets but are present in other components
PCR Positivity in Sera of Patients in Different Stages of Syphilis
Microbiologica 1999;22