What does PCR add to the diagnosis of syphilis

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Syphilis is (of course) still with us

**NSW 2007-9:** 800-900 cases per year

**Australia 2002-7:** all categories
Notifications – 11-14/100,000

*Comm Dis Intell; 33: June 2009*
Rates: infectious syphilis by age & sex 2007

Comm Dis Intell; 33: June 2009
Diagnosis of syphilis

• Dark ground microscopy
  – Not very sensitive (10^5 orgs/mL)
  – Specific and fast if (and only if):
    • Skilled microscopist; on-site (specimen warm);
    • Correct specimen – commensal treponemes

• Histopathology
• Rabbit infectivity
• Serology
• PCR – rapid, sensitive, specific (?)
Treponema pallidum
dark ground microscopy
DGM unavailable:
• Colposcopy clinic
• Paediatric ward
• General practice
Diagnosis of syphilis

- Dark ground microscopy
- Histopathology
  - silver & *(Tp*-specific) immuno-histochemistry or immuno-fluorescence stains
  - Not rapid; special circumstances
    - stored tissues/stored smears from 1° lesions
- Serology
- PCR – rapid, sensitive, specific (?)
Diagnosis of syphilis

- Dark ground microscopy
- Histopathology; silver & IF stains

- **Serology** - “non-specific” RPR + anti *T.p*
  - Still the one(s) for:
    - Screening
    - Monitoring Rx response
    - 2° syphilis
  - Drawbacks - delay; interpretation
    - Early primary, neurosyphilis, congenital syphilis
  - *T.p*-specific IgM; immunoblot can help

- PCR – rapid, sensitive, specific (?)
Diagnosis of syphilis

- Dark ground microscopy
- Histopathology; silver & IF stains
- Serology
- PCR – 1st described 1990s
  - Most common targets used:
    - 47 kD protein gene;
    - DNA polymerase 1 gene (polA);
  - Rapid, robust, sensitive, specific
  - What does it add – if anything?
Syphilis PCR – m/c lesions

• 100 episodes in 98 patients
  – ano-genital or oral lesions
• PCR result corresponded with clinical Δ - 95
• 26 PCR+ve (18=1°; 8=2°)
• 70 PCR-ve – clinically not active syphilis
• 2 patients HIV+ve;
  – PCR+ve 12 & 21 days before serology
• 1 PCR+ve; serol –ve; Rx

**Conclusion: sensitive and specific**

*Palmer et al, Sex Trans Infect 2003;79:479-83*
Syphilis PCR - latent syphilis

- PCR: 47kD - blood, serum + plasma
- 18 cases treated syphilis – all negative
- 69 latent syphilis: 235 specimens
- PCR+ve:
  - 39% blood; 45% plasma; 26% sera
  - 57% ear lobe scrapings

Syphilis PCR – congenital syphilis

- 148 infants of women with syphilis
- “Gold standard” – rabbit infectivity test
- 76 infants – no prior Rx
- 17/76 +ve CSF RIT = CNS infection
- Best predictors of +ve CSF RIT:
  - +ve PCR blood: 94% sens.; 90% spec.
  - +ve IgM blot blood: 100% sens; spec. 66%
  - vs CSF VDRL; 53% sens; 90% spec

Clinical utility of syphilis RT-PCR

Primary syphilis: 716 anogenital ulcers; STI clinic setting

<table>
<thead>
<tr>
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<tr>
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<td>-ve</td>
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Clinical utility of syphilis RT-PCR

Primary syphilis: 716 patients; anogenital ulcers; GP setting

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Practical application in a reference lab (CIDM)

- Target 47 kD protein (in-house)
- In use since 2000 – gel-based
  - Change to RT-PCR – 2007 (more sensitive)
- ~ 60 tests per month (717 in 2009-10)
  - Batched x 2 per week
- Overall 13% +ve (92 in 2009-10)
  - 14% males +ve
  - 2% females (1/53 +ve 2009-10)
- 95% swabs; 1-2% CSF; vitreous; tissue
Molecular typing of *T. pallidum*

Why type?

- Epidemiology
  - Geographical distribution
  - Transmission events/clusters
  - ??virulent and/or azithromycin Rx strains
  - Tissue tropism e.g. CNS
  - Distinguish relapse from reinfection
Molecular typing of *T. pallidum*

- **How?** - various targets:
  - **CDC method:**
    - No. of repeats in *arp* gene
    - RFLP of *tpr* subfamily II genes
      subtype 14d, common = >50%
  - **Other methods** (*Marra et al* JID 2010;202:1380-7)
    - Incl. sequence typing of 84bp in *tp0548*
Molecular typing of *T. pallidum*

- *T. pallidum* DNA
  - 158 patient specimens (USA – 3 cities, China, Ireland, Madagascar) & 15 isolates
- 6 targets – 3 most discriminatory
  - CDC subtypes + *tp058* sequence type
- 14 CDC subtypes/ 25 strains
  - Most common 14d/f (n=70); 14d/g (n=32)

*Marra et al* JID 2010;202:1380-7
Molecular typing of *T. pallidum*

• 84 patients from Seattle
  – 70 HIV+ve (trend - different strains; p=0.08)
  – 3 patients >1 episode
    • 1 same strain (despite Rx); 2 different strains
  – Changes in strain distribution over 10 yrs
    • New strain coincided with increased incidence
  – Neurosyphilis in:
    • 21/42 (50%) with 14d/f vs
    • 10 /41 (24%) with 7 other strains (p=0.02)

*Marra et al JID 2010;202:1380-7*
Conclusions

• *T. pallidum* PCR
  – rapid, robust, sensitive & specific
• Significant benefits over DGM
• Complementary to serology
  – Early primary, congenital & neurosyphilis
• Allows further investigation
  – Epidemiological typing
  – Azithromycin resistance
Is Fracastoro impressed with PCR?