Laboratory diagnosis of syphilis

What I’m going to cover

- History
- The Organism
- Epidemiology
- Tests available
- How to screen for syphilis
- Sensitivity of tests in early syphilis
- Confirmatory tests
- Serological Response to Treatment
- A few words on HIV, CNS & PCR
History

• First described in 16th Century – Brevi Helthe
• Imported from The New World - Christopher Columbus
• Established disease spread in Europe by urbanisation
• Great Pox – return of Christopher Columbus from America and mass movements of armies and populations in Europe

Aetiology

• T. pallidum subspecies pallidum
• Order: Spirochaetales
• Family: Spirochaetaceae
• Genus: Treponema
• Treponema pallidum subsp pertenue (yaws)
• Treponema pallidum subsp endemicum (bejel)
• Treponema pallidum subsp carateum (pinta)
The Organism

- Slender, tightly coiled, unicellular, undulating movement
- 5-15nm long, 0.09 to 0.18nm wide
- Cytoplasm surrounded by trilaminar membrane, peptidoglycan layer, periplast lipoprotein membrane and phospholipid OM with few surface exposed proteins
- Stealth organism – reducing the surface membrane bound targets for host immune system to recognise

T. pallidum subsp pallidum

- Genome- 1138 Kb pairs- one of the smallest prokaryotic genomes
- Flagella- endoflagellae- don’t protrude outside
- Pathogenic treponemes- not cultivable
  - Propagated in rabbit testicular tissue
- Nichol’s strain from 1912 still being propagated
Figure 1 Numbers of diagnoses of syphilis (primary, secondary and early latent) by sex, GUM clinics, England, Wales and Scotland, 1931-2005. Equivalent Scottish data are not available prior to 1945. Northern Ireland data from 1931 to 2000 are incomplete and have been excluded. Data source: KC60 statutory returns and ISD(D)/STISS data. GUM, genitourinary medicine.


Figure 1: Syphilis rate per 100,000 population by (PHEC) of residence: England 2017. Data source: GUMCAD

Dr Jayshree Dave
Map of syphilis rates per 100,000 residents by local authority in London, 2017. Data source: GUMCAD

Map of the number of syphilis diagnoses in MSM by local authority, London residents, 2017. Data source: GUMCAD
Number of syphilis (primary, secondary and early latent) diagnoses by gender: England, 2008 to 2017

![Graph showing number of syphilis diagnoses by gender from 2008 to 2017.](image)

- Data from specialist and non-specialist SHS GUMCAD returns
- Data type: service data

Rates of syphilis (primary, secondary & early latent) diagnoses by gender and age: England, 2017

![Graph showing rates of syphilis diagnoses by gender and age from 2017.](image)

- Data from specialist and non-specialist SHS GUMCAD returns
- ONS Census mid-year 2016 estimates used for denominators
- Data type: service data
Roles of syphilis serology

- Screen for infectious syphilis (ANY stage)
- Confirmatory tests
- Provide a guide to treatment status and monitor the efficacy of treatment
- Detect neurological involvement (CSF)
- Detect congenital infection
Lab tests for Syphilis diagnosis

- Direct detection of *Treponema pallidum*
  - dark ground microscopy (Sens 79-97; Spec 77-100)
  - fluorescent antibody test (Sens 73-100; Spec 100)
  - PCR (Sens ~ 89; Spec 99)

- Antibody detection
  - detects antibodies against all pathogenic treponemes

Antibody responses to Syphilis

- Antibodies to > 20 polypeptide antigens
- Variety and the intensity of reactivity changes
- Incubation period 9 – 90 days
- By the time clinical signs develop most patients have both IgG and IgM antibody
- the spectrum and intensity of reactivity decreases with duration of infection
- Treatment causes a loss of some antibodies
Antibody tests for Syphilis

1. **Non-Treponemal antigen**: VDRL, RPR (cardiolipin/lecithin/cholesterol)

2. **Treponemal antigen**: TPPA, TPHA, EIA, (extracts of whole *T. pallidum* OR synthetic proteins)

Detect a broad range of anti-treponemal antibodies

Are complex biomolecular systems; different formats
- consistent results difficult at low antibody levels
- one serum will react differently with different test
TPPA (or TPHA) test

EIA (T. pallidum enzyme immunoassay)
Time to Seropositivity

- **Treponemal tests**
  - EIA: 3 weeks
  - TPPA/TPHA: 4 to 6 weeks

- **Non-treponemal tests**
  - RPR: 4 weeks
  - VDRL: 4 weeks.

- *Delayed seroreactivity or false-negative non-treponemal serology may occur if there is HIV co-infection*
Two other tests for Syphilis antibodies

- FTA-Abs (Fluorescent Treponemal Antibody-Absorbed)
  Long seen as the “Gold Standard”; now little used.

- Immunoblot (Inolia)
  Reaction with protein fixed on nitrocellulose strips.
  Developed as better confirmatory tests.

What we want in an ideal screening test?

- Sensitive (100%)
- Specific (100%)
- Simple to perform (automation)
- Consistent quality of reagents
- Objective reading
- Reproducible
- Cheap

You don’t always get what you want!
**Screening tests for syphilis**  
**What is available?**

- **Non-treponemal tests**
  - VDRL slide test (read microscopically)
  - Rapid plasma reagin or carbon antigen test (RPR or VDRL/RPR)

- **Treponemal tests**
  - TPHA (erythrocytes as carrier)
  - TPPA (gelatin particles as carrier)
  - EIA (native and recombinant antigen)

**What should we use as a primary screening test?**

1. VDRL / RPR ?
2. TPPA ?
3. Treponemal EIA ?
Screening with RPR/VDRL

- Specificity ≥ 99%
  - Problem of Biological False Positives

- Sensitivity varies by stage
  - 70-85% in primary
  - ~100% in secondary
  - 60-80% in late stage infection

  (Prozone phenomenon: false negative due to lack of agglutination with high antibody levels)

- Cheap reagents and simple to perform
- Labour intensive - not suited for automation
- Subjective

Screening with TPPA

- Specificity ≥ 99.5%

- Sensitivity
  - 90-95% in primary syphilis
  - 100% in all other stages (untreated and treated)
  - Antibody persists after treatment (may go neg in HIV)

- Not suited to automation
Screening with EIA

- Specificity ≥ 99.5%
- Sensitivity
  - 80-85% in primary and 100% in all other stages
  - Antibody persists after treatment (may become neg in HIV)
- Suited to automated testing/electronic reporting
- Can test for other blood borne infections using same sample

What to use as a primary screening test?

- Analysis of performance criteria of tests as outlined above
  - Risk of missing infectious syphilis
- Choice influenced by
  - Available resources (equipment, money, personnel)
  - Volume and frequency of testing
What to use as a primary screening test

- EIA (first choice)
- TPPA (second choice)
- TPPA/TPHA plus VDRL (third choice)

- Maximum detection of primary syphilis depends on high index of clinical suspicion
  - Window of 1-2 weeks when screening tests may be negative

Sensitivity of EIA kits in untreated primary syphilis (n=33)

<table>
<thead>
<tr>
<th>Kit</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>93.9%</td>
</tr>
<tr>
<td>BioKit</td>
<td>97%</td>
</tr>
<tr>
<td>bioMerieux</td>
<td>97%</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>97%</td>
</tr>
<tr>
<td>Dade</td>
<td>97%</td>
</tr>
<tr>
<td>Dijesee</td>
<td>97%</td>
</tr>
<tr>
<td>Microgen</td>
<td>97%</td>
</tr>
<tr>
<td>Newmarket</td>
<td>97%</td>
</tr>
<tr>
<td>Omega</td>
<td>97%</td>
</tr>
<tr>
<td>Trinity</td>
<td>97%</td>
</tr>
</tbody>
</table>

**Test sensitivity in untreated primary infection:**  
**Edinburgh Jan 2004 - March 2005 (n=50)**

Manavi, Young, McMillan Int J STD AIDS. 2006 Nov;17(11):768-71

<table>
<thead>
<tr>
<th>Method</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA</td>
<td>84%</td>
</tr>
<tr>
<td>VDRL</td>
<td>70%</td>
</tr>
<tr>
<td>TPPA</td>
<td>96%</td>
</tr>
<tr>
<td>Blot</td>
<td>94%</td>
</tr>
<tr>
<td>EIA-IgM</td>
<td>88%</td>
</tr>
</tbody>
</table>

*TPPA relative to: EIA  P=0.046; VDRL  P=<0.0005  
EIA relative to EIA-IgM  P>0.56


Manavi, Young, McMillan

<table>
<thead>
<tr>
<th>Method</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIA</td>
<td>89%</td>
</tr>
<tr>
<td>VDRL</td>
<td>71%</td>
</tr>
<tr>
<td>TPPA</td>
<td>98%</td>
</tr>
<tr>
<td>Blot</td>
<td>97%</td>
</tr>
<tr>
<td>EIA-IgM</td>
<td>83%</td>
</tr>
</tbody>
</table>

There was no significant difference in sensitivity of the screening EIA & EIA-IgM in all early infection (P=0.6). The screening EIA was more sensitive than EIA-IgM in detecting syphilis of unknown duration (P=0.0003)
Summary of primaries missed by screening EIA, TPPA or EIA-IgM

- **Screening EIA missed 16% (8/50) primaries**
  - Seven detected by EIA-IgM
  - Six by TPPA (all 8 by IgM + TPPA)
  - Five by immunoblot
  - Two by VDRL
- **TPPA was negative in 4% (2/50)**
  - Positive only in EIA-IgM
- **EIA-IgM was negative in 12% (6/50) primaries**
  - Six detected by TPPA
  - Five by screening EIA
  - Six by immunoblot
  - Three by VDRL

The problem with screening

In low prevalence populations, even tests with high specificity turn up a lot of false positives.

- e.g.
  - If Prevalence 0.1%, and Test Specificity 99%,
    Positive Predictive Value is 10%

So you must confirm
**What to use as a confirmatory test?**

- **Depends on**
  - Resources and test volume
  - Screening test used

- **Confirmatory test should be**
  - a treponemal test of a different type
  - equivalent sensitivity and specificity

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**What to use as a confirmatory test**

- TPPA when EIA is used to screen
- EIA when TPPA is used to screen

Optimal profile to help stage disease; monitor treatment; and detect re-infection should include

- quantitative VDRL
- quantitative TPPA
- EIA for anti-treponemal IgM
Can we use the immunoblot as a confirmatory test?

- Line immunoassays using recombinant antigens

  - Sensitivity 100%
  - Specificity 99.3%

Line immunoblots using recombinant antigens are useful to resolve discrepancies between screening and confirmatory tests.
VDRL / RPR and Response to therapy

- Seroreversal rates vary depending on:
  - Pre-treatment titre
  - Stage of disease
  - Previous episode of syphilis
  - Treatment regimen

- Decrease in VDRL/RPR titre
  - Brown et al JAMA 1985; 253: 1296 - 9
    - times 4 at 3 months; times 8 at 6 months
    - times 4 at 6 months; times 8 at 12 months
    - Early latent - times 4 at 12 months

Repeat screening

- 6 & 12 weeks after a single ‘high risk’ exposure
- 3 months - individuals at ongoing risk due to frequent ‘high risk’ exposures, screening as part of routine sexual health check-up
- 2 weeks after presentation - dark field or PCR negative ulcers
False negative syphilis serology

- Screening tests can be negative before a chancre and two weeks after chancre
- False negative VDRL/RPR can occur in secondary or early latent syphilis. More likely to occur in HIV pos individuals
- RPR/VDRL may be negative in late syphilis

False positive serology

- Can occur with any of the tests
- Autoimmune, older age and IV drug users
- When symptoms are absent, no previous history or positive IgM test
- Transient reactivity with a single antigen test
**Treponemal Serology**

1. Treponemal test 1 (EIA or CLIA detecting treponemal IgG and IgM)
   - Positive or equivalent C:
     - Treponemal test 2 (TPPA/TPHA/TPPLA) c.f. p. 8
     - RPR
     - Treponemal IgM (only if primary infection suspected clinically)
     - See note for interpretation of results and comments

2. Negative
   - If suspected early primary infection, consider TPPA and treponemal IgM

**Report Comments for Treponemal Serology**

Note that the table of comments is a guide, and that clinical details and previous serological results should always be considered when interpreting treponemal serology results. If possible, compare antibody titres (particularly RPR) by testing in parallel. The table cannot cover all serological profiles but should cover most of those encountered in clinical practice. A full repertoire of tests for final interpretation may include referal tests, depending on the local laboratory test repertoire.

<table>
<thead>
<tr>
<th>Immun assay 1 (EIA, CLIA)</th>
<th>Immun assay 2 (TPLA, TPMA, TPPA)</th>
<th>RPR 2A (RPR tests should always be reported)</th>
<th>RPR 2B (RPR tests should always be reported)</th>
<th>IgM</th>
<th>Comment</th>
</tr>
</thead>
</table>
| a' | Positive | Positive | Positive | Positive | | 'Consistent with treponemal infection at some time: If first sample add: Advise repeat to confirm, if clinically indicated
If treated and this is follow up sample, review previous results and report changes in RPR titre.
This would be consistent with a recent infection if seroconversion, or a four-fold rise in RPR titre on parallel testing, were seen in comparison to an earlier sample.
RPR titre x16 does not exclude active infection especially if there are signs suggesting syphilis or where adequate treatment of previously diagnosed syphilis has not been documented. |
| a'' | Positive | Positive | Positive | | | 'Consistent with recent or active treponemal infection: If first sample add: Advise repeat to confirm, if clinically indicated
Monitor following treatment.
If treated and this is follow up sample, review previous results and report changes in RPR titre.' |
## Epidemiology of STIs and Bacterial Infections

### Laboratory Testing

<table>
<thead>
<tr>
<th>IA1</th>
<th>IA2</th>
<th>RPR&lt;16</th>
<th>RPR&gt;16</th>
<th>IgM</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>c</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Reactive (at least 2 times or more) <em>b</em></td>
<td>As previous (b) Consider possibility of misinterpretation if increase in RPR and new IgM reactivity.</td>
</tr>
<tr>
<td><strong>d</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Reactive (at or near or equal to)</td>
<td>As previous (b). Add comment if low level. Low level treponemal IgM reactivity is often false and nonspecific, so is of doubtful significance. Consider IgM immunoblot to check IgM specificity.</td>
</tr>
<tr>
<td><strong>e</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Consistent with relatively recent or active treponemal infection.</td>
</tr>
<tr>
<td><strong>f</strong></td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td>Confirm specific reactivity using a second EIA or treponemal IgM immunoblot. If confirmed, report as “Consistent with recent or active treponemal infection at some time”. If not confirmed, request further sample for repeat testing.</td>
</tr>
<tr>
<td><strong>g</strong></td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
<td>Evaluate clinical details and level of activity in immunobase. In low risk patient, eg tested as part of antenatal screen, consider reporting as: No serological evidence of treponemal infection. Initial EIA reactivity not confirmed and is probably non-specific. Please repeat if clinically indicated. In high risk patients eg tested at GU Clinic consider IgG immunoblot and IgM testing before reporting. Request second specimen. If this is a follow up sample in a treated patient, review previous results before reporting.</td>
</tr>
</tbody>
</table>

### Syphilis Serology

<table>
<thead>
<tr>
<th>IA1</th>
<th>IA2</th>
<th>RPR&lt;16</th>
<th>RPR&gt;16</th>
<th>IgM</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>h</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td></td>
<td>Report as “RPR reactivity is likely to be biological false positive. Treponemal infection unlikely but please repeat to confirm.”</td>
</tr>
<tr>
<td><strong>i</strong></td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
<td>Evaluate clinical details if available: Low risk patient: TPPA/TPHA reactivity is likely to be false unless early infection is suspected. Please repeat to confirm if clinically indicated. No serological evidence of treponemal infection. High risk patient: Test using a second EIA. Consider immunoblot and IgM to investigate for early infection. Report as indicated by further test results. Consider performing second EIA or immunoblot to clarify. If specific reactivity confirmed, report as “Consistent with treponemal infection at some time. Alternatively, request repeat sample for immunoblot.”</td>
</tr>
<tr>
<td><strong>j</strong></td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td></td>
<td>Perform second EIA or immunoblot. If specific reactivity confirmed, report as “Consistent with treponemal infection at some time.” Alternatively, request repeat sample for immunoblot.</td>
</tr>
</tbody>
</table>
Syphilis serology in HIV infection

- Very high levels of antibody often produced
  - Increased risk of prozone phenomenon
    - "Delayed seropositivity" rather than “Seronegative”

- Titres may not fall as expected after treatment
  - Conflicting reports, response dependent on:
    - Previous syphilis; stage; pre-Rx titre; regimen

- TPPA and EIA may become negative

- Neurological signs and symptoms (including ophthalmic involvement) require CSF to exclude neurosyphilis

Neurosyphilis

- Neurological examination

- Consider CT/MRI in presence of signs and symptoms prior to LP

- Routine CSF examination for latent syphilis pts not recommended

- LP indicated when clinical suspicion of neurosyphilis or treatment failure in late syphilis
Diagnosis of Neurosyphilis

- Needs a full clinical assessment and careful thought
- Serum RPR/VDRL - negative, can exclude neurosyphilis
- RPR≥32 predict CSF abnormalities compatible with neurosyphilis
- Positive RPR/VDRL in CSF confirms neurosyphilis, negative does not exclude
- Negative TPPA in CSF excludes neurosyphilis, positive does not confirm

CSF criteria supporting Neurosyphilis

<table>
<thead>
<tr>
<th>CSF</th>
<th>HIV Negative</th>
<th>HIV Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>&gt;5 cells/μL</td>
<td>&gt;20 cells/μL OR 6–20 cells/μL (on ART/plasma HIV VL undetectable, or blood CD4 &lt;200)</td>
</tr>
<tr>
<td>Protein</td>
<td>&gt;0.45 g/l</td>
<td>&gt;0.45 g/l</td>
</tr>
<tr>
<td>VDRL/RPR</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TPPA</td>
<td>&gt;1:320</td>
<td>&gt;1:320</td>
</tr>
</tbody>
</table>
Exposed infant should be evaluated for active syphilis

- Symptomatic infant
- Maternal non-treponemal titre (VDRL, RPR) increased 4 fold
- Infant VDRL/RPR is 4 fold greater than maternal titre
- Maternal syphilis was untreated or inadequately treated during pregnancy
- Maternal syphilis treated non penicillin regimen
- Treatment for maternal syphilis was commenced less than 1 month before delivery
- Can rpt maternal and neonatal blood at birth
- LFTS, Long bone XRays, ophthalmic assessment, CSF for VDRL, risk for HIV
- A negative CSF VDRL does not exclude syphilis

- Treated infants followed up 3, 6 and 12 months of age until serologic non-treponemal tests become non reactive or titre decreased four fold.

Current/future diagnostic developments?

- Molecular detection
- Near Patient Tests
- Syphilis specific serological tests
Any Questions

Guide to stage and treatment status

- RPR/VDRL detect IgG and IgM and are positive 10-14 days after appearance of chancre (4-5 weeks after infection).
- Titre of $\geq 16$ diagnostic of acute infection
- 2ry stage – titres of 16-128,
- 30% of patients with late stage disease- titres negative
- TPHA titres low (80-320 in acute infection but rise to 5120 in 2ry infection, latent infection titres of 80-1280).
Treated syphilis

- Serological pattern varies in treated infection depending on stage of infection at time of treatment, duration since treatment
- VDRL/ RPR tend to become negative, particularly if infection treated at early stage
- Treponemal test positive for life except in 10-20% of HIV positive patients – TPHA, EIA are negative.