Laboratory diagnosis of syphilis
(Treponema pallidum subspecies pallidum)

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Etiological agent – 
*Treponema pallidum subspecies pallidum*

Family – *Spirochetaceae*
Order – *Spirochetales*
Genus – *Treponema*
Species – *T. pallidum*

- *T. pallidum ssp. pallidum*: venereal syphilis
- *T. pallidum ssp. pertenue*: yaws
- *T. pallidum ssp. endemicum*: bejel, end. syp.
- *T. pallidum ssp. carateum*: pinta

Not differentiated in serology!

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a Central Africa, Central America, S-E Asia; children (inoculation), bone deformities
b Middle East; around the mouth, spread within families
c Central & South America; hyperkeratosis of extensor aspects of joints
## Pathogenic treponememes of humans

<table>
<thead>
<tr>
<th>Organism</th>
<th><strong>Syphilis</strong></th>
<th><strong>Yaws</strong></th>
<th><strong>Bejel</strong></th>
<th><strong>Pinta</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
<td><em>T. pallidum</em> subspecies <em>pallidum</em></td>
<td><em>T. pallidum</em> subspecies <em>pertenue</em></td>
<td><em>T. pallidum</em> subspecies <em>endemicum</em></td>
<td><em>T. carateum</em></td>
</tr>
<tr>
<td><strong>Transmission</strong></td>
<td><strong>Sexual contact</strong></td>
<td>Skin contact</td>
<td>Skin contact Oral</td>
<td>Skin contact</td>
</tr>
<tr>
<td><strong>Lesion type</strong></td>
<td><strong>Primary</strong></td>
<td><strong>Secondary</strong></td>
<td><strong>Late</strong></td>
<td><strong>Secondary</strong></td>
</tr>
<tr>
<td><strong>Primary</strong></td>
<td>Chancre</td>
<td>Crusted papules</td>
<td>Oral mucosal lesions</td>
<td>Crusted papules and plaques</td>
</tr>
<tr>
<td><strong>Secondary</strong></td>
<td>Macular or papulosquamous</td>
<td>Papillomatous, and scarring</td>
<td>Mucous patches and condylomata lata</td>
<td>Scaly plaques</td>
</tr>
<tr>
<td><strong>Late</strong></td>
<td>Gummata and endarteritis</td>
<td>Gummata of bone and skin</td>
<td>Gummata of cartilage, bone, Dyschromia and skin</td>
<td></td>
</tr>
</tbody>
</table>

**Differences in transmission, invasiveness, crossage of placenta barrier!**
Syphilis – *T. pallidum subsp. pallidum*

- Bacteria (Spirochete) identified in testis of rabbit (Microscopy; 1905) – *in vitro* culture not described!
- First recorded epidemic after the conquest of Naples, Italy in 1495!
- From the New World ("the Americas") to Europe (Old World) by Columbus crew (1490s) OR existed (even in Europe) long before? – Emerged when, where, how?
Evolution of venereal and non-venereal treponematoses - hypotheses

**Unitarian hypothesis**
- Treponematoses coevolved with humans since Homo erectus
- Geographic and climate determined manifestations of the same disease and the same bacteria

**Columbian hypothesis**
- Modern venereal syphilis evolved from less virulent strains brought to Europe from the New World by Columbus in the 1490s
- Three different pathogens (tpr genes phylogeny)!

**New hypothesis**
- T. p. subsp. pallidum emerged 5,000-16,500 years
- Syphilis present in Europe before the Columbian era (misdiagnosed earlier?)
- Three different pathogens (tpr genes phylogeny)!

New and Old World distribution of pre-Columbian treponematoses cases (based on paleopathology)

Evolution of venereal and non-venereal treponematoses (paleopathology combined with phylogenetics)?

Unitarian hypothesis
- Treponematoses accompanied human evolution since Homo erectus, one disease and the same bacteria.

Columbian hypothesis
- Modern venereal syphilis evolved from less virulent strains brought to Europe from the New World by Columbus in the 1490s.

Most plausible theory!
- *T. p. subsp. pallidum* emerged 5,000-16,500 years ago
- Paleopathology, phylogenetics (+evol rate) and people migration ⇒ syphilis present in Europe in pre-Columbian era!
- Emerged in the New World (the Americas)?

**T. pallidum ssp. pallidum**

- **Gram (-) spiral-shaped spirochete**
  - 6-15 μm in length
  - 0.10-0.20 μm in diameter
  - 8-14 regular, tightly wound deep spirals

- Outer membrane (lack of LPS)

- Cytoplasmic membrane

- A thin layer of peptidoglycan between the membranes – provides structural stability

- 3 endoflagellae in the periplasmic space (between cytoplasmic membrane and the outer membrane)
Genome - Nichols
1 138 006 bp
1 041 genes
(function still unknown for many)

Virulence factors!?  
- Adhesins  
- Porins  
- Effector proteins e.g. ~ hemolysins  
- Chemotaxis  
- Motility (different types)  
- ................

Fraser CM et al. Science. 1998
Treponema pallidum ssp. pallidum:
- Additional genomes crucial to obtain!
- Causes multi-stage STI ("the great imitator") and is highly adapted to its obligate human parasitic lifestyle
The course of untreated syphilis

*Infection*

1. **Primary (Chancre)**
   - Incubation period: 9 – 90 days
   - Duration: 6 weeks to 6 months

2. **Secondary (Rash)**
   - Duration: Approx. 18 months

3. **Latent Syphilis (No signs of disease)**
   - Duration: Many years to a lifetime

4. **Tertiary**
   - Conditions: Benign gummatous, Cardio-vascular syphilis, Neurosyphilis
   - Duration: Many years to a lifetime

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**Early Syphilis**
- (Europe: 1 year; WHO and USA: 2 year)

**Late Syphilis**
Diagnosis of syphilis

- History (time), clinical features, and detailed examination of patient
- Laboratory diagnosis

pathogen → "Laboratory diagnosis" → human
Positive dark-field microscopy of ulcer specimen - characteristic shape and motility

DFA-TP positive smear of ulcer specimen

Table 1. Laboratory criteria for the diagnosis of syphilis

**Definitive (direct detection) diagnosis**
- Non-pathogenic commensal treponemes (Urogenital: *T. perfringens, T. phagedensis [reiteri]*, Oral: *T. denticola*) can be detected particularly in lesions from mouth and rectum (⇒PCR useful!)
- Not widely available (and QA), can miss cases, most present without symptoms/signs (ulcer sample needed!)
- Syphilis is mostly diagnosed with serology!

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plasma reagin test; MPR, microprecipitation reaction test; VDRL, Venereal Disease Research Laboratory test; TPHA, *T. pallidum* haemagglutination assay; TPPA, *T. pallidum* passive particle agglutination assay; ELISA, enzyme-linked immunosorbent assay; FTA-ABS, fluorescent treponemal antibody-absorption
Antibody response used in indirect diagnosis

Peeling et al.

Tuskegee study, Georgia, USA 1932-1972!!!
Serological tests for syphilis

Non-treponemal (reagin, lipoidal) tests (Cardiolipin, lecithin, cholesterol (IgM+IgG))

- Rapid plasma reagin (RPR) test
- VDRL (Venereal Disease Research Laboratory) test
- (TRUST and Microprecipitation reaction (MPR))
- Wasserman reaction test

Treponemal (specific) tests (whole cell lysates, recombinant proteins, synthetic peptides (TpN15, TpN17, (TpN44.5) and TpN47))

- FTA-ABS
- TPHA
- TPPA
- Western blots and pseudoblots
- Chemiluminescence assays
- Immunochromatographic formats
Rapid plasma reagin (RPR) test (Charcoal particles) (Qualitative/Quantitative)

Qualitative RPR

18mm Test Card

Use each test area once and discard.

R

Rm

N
Quantitative RPR test (follow-up)

When to perform quantitative RPR test:
- Positive screening, e.g. using qualitative RPR
- Positive sera using other test but negative using qualitative RPR
- Directed by the treating physician:
  i) Relapse/reinfection?
  ii) Effectiveness of therapy (decline by two dilutions (4-fold titre) within 6 months; 12 months for HIV+)?
Non-treponemal assays:
- Detect heterophile IgM and IgG associated with tissue damage and to lipids in the *T. pallidum* cell wall)
- Positive approximately 6 weeks after infection (one week after primary chancre)

Advantages:
Low cost, technically simple, rapid, and quantitation permits assessment of relapse/reinfection and effectiveness of therapy

Disadvantages:
- Not ideal sensitivity!
- Biological false-positives (mostly titer: ≤1:4; but 1:4 seen in late latent and tertiary disease), due to systemic disorders of parenchymatous organs (liver, kidneys, lungs), myocardial infection, atherosclerosis, acute viral infections (e.g. hepatitis, chickenpox, measles), leprosy, malaria, following immunization, and during pregnancy?
Reactivity of non-treponemal serological tests by stage of syphilis and influence of successful treatment

Sokolovsky, et al. JEADV. 2009
Serological tests for syphilis

Non-treponemal (reagin) tests (Cardiolipin-based)

- RPR test
- VDRL test
- (TRUST and MPR tests)
- Wasserman reaction test

Treponemal (specific) tests (whole cell lysates (Nichols strain), recombinant proteins, synthetic peptides (TpN15, TpN17, (TpN44.5) and TpN47) (usually IgM+IgG))

- (FTA-ABS (Fluorescent treponemal antibody – absorption))
- TPHA (Treponema pallidum haemagglutination)
- TPPA (Treponema pallidum passive particle agglutination)
- Western blot and pseudoblot
- ELISA or Chemiluminescence (e.g. Architect, Liaison)
- Immunochromatographic formats
T. pallidum passive particle agglutination (TPPA) assay

Performance characteristics
- High sensitivity, high specificity, simple to perform, relatively rapid, optimized and standardized reagents, does not require any special equipment

Compared to TPHA: no need of pre-absorption to remove anti-erythrocytic antibodies, easier to read and interpret, more stable reagents, and commonly less “background agglutination” (i.e. non-specific)

Interpretation of results
++     +     +     +/-      -       -
Advantages:

High sensitivity and high specificity!

Simple to perform, relatively rapid, optimized and standardized reagents, high-throughput (e.g. screening blood donors), automated!
Treponemal (specific) tests

Positive approximately 2-4 weeks after infection

**Advantages:**
High sensitivity and specificity

**Disadvantages:**
Can not be used to monitor disease activity, relapse/reinfection, or efficacy of therapy (may remain positive for life and do not reflect disease activity ⇒ quantitation of little value!)

**False-positives:**
- non-venereal treponematoses (possibly *Borrelia burgdorferi*)
- pregnancy?, HIV infection, autoimmune diseases
False-negatives in syphilis serodiagnosis

- Testing during the "window period" (early primary syphilis before seroconversion; 2-4 weeks after infection)
- "Prozone" effect in non-treponemal test: especially in secondary syphilis, early latent and early neurosyphilis, may give weak or negative reaction, due to excess of antibodies (dilute sera)!
- Non-treponemal test: in late stage syphilis; due to gradual reduction of cardiolipin antibodies over time?
- HIV-positive patients or patients with immunodeficiency caused by other etiology (relatively rare)
Reactivity of treponemal serological tests by stage of syphilis and influence of successful treatment

Sokolovsky, et al. JEADV. 2009
Table 4. **Interpretation of serological tests for syphilis**

<table>
<thead>
<tr>
<th>Result of serologic testing</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-treponemal test +ve, Treponemal test -ve</td>
<td>False positive non-treponemal screening test.</td>
</tr>
<tr>
<td>Non-trep test +ve, Trep test +ve</td>
<td>Untreated syphilis; Previously treated late syphilis.</td>
</tr>
<tr>
<td>Non-trep test -ve, Trep test +ve</td>
<td>Very early untreated syphilis; Previously treated early syphilis.</td>
</tr>
<tr>
<td>Non-trep test -ve, Trep test -ve</td>
<td>Not syphilis; Incubating syphilis; Very late syphilis; Syphilis with concomitant HIV infection and immunosupression.</td>
</tr>
</tbody>
</table>
Syphilis serologic screening algorithms

**Traditional algorithm**

- VDRL or RPR (non-treponemal)
  - Neg
  - Pos
    - Verify with TPPA, FTA-ABS or IgM

**Reverse algorithm**

- Screening: CIA, ELISA, or TPPA (treponemal)
  - Neg
  - Pos
    - Confirmation: TPPA, CIA, or ELISA + RPR or VDRL (quant.; >1:32)
      - Discrepancies: IgG immunoblot, IgM-FTA-Abs, IgM-ELISA
    - Repeat pos. on 2nd specimen!

French P, et al. IJSA. 2009
Syphilis serologic screening algorithms

**Traditional algorithm**
- Detects active infection
- Higher rate of biological false positives
- Can miss early primary and latent infection
- Prozone reaction can cause false negatives
- Can have a lower sensitivity ⇒ most sensitive non-treponemal test (**RPR**) crucial!

**Reverse algorithm**
- Detects early primary and latent infection more effective
- Detects all old, treated infections (problems in high-prevalent settings)
- Non-treponemal test to detect active infection
- Treponemal tests should have ideal specificity (CIA/many ELISA < TTPA) ⇒ low-prevalent populations problematic!
Evaluation of non-treponemal and treponemal assays and screening algorithms for serodiagnosis of syphilis

Malm K, Andersson S, Ballard R, Cox D, Fredlund H, Unemo M

- Non-treponemal:
  VDRL (Oxoid)
  RPR (Macro-Vue, BD)

- Treponemala screening:
  Syphilis TP (Abbott, Architect)
  Enzy-Well IgG (Diesse)
  Trep-SURE (Phoenix Biotech)
  Treponema pallidum particle agglutination (TPPA, Fujirebio)

- Treponemal confirmatory:
  Inno-LIA Syphilis Score (Innogenetics)

- Additional tests:
  Captia IgM (Trinity Biotech)
  Bioline Dual test, POC-test (Chembio)
Rapid point-of-care (POC) tests for syphilis – treponemal tests available many years!

<table>
<thead>
<tr>
<th>Name</th>
<th>Determine Syphilis TP</th>
<th>Syphilis Fast</th>
<th>Espline TP</th>
<th>Syphicheck-WB</th>
<th>SD Bioline Syphilis 3.0</th>
<th>VISITECT Syphilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Producer</td>
<td>Abbott Laboratories, USA</td>
<td>DIESSE Diagnostica Senese SpA, Italy</td>
<td>Fujirebio Inc.</td>
<td>Qualpro Diagnostics, India</td>
<td>Standard Diagnostics Inc., Korea</td>
<td>Omega Diagnostics Ltd. UK</td>
</tr>
<tr>
<td>Reaction</td>
<td>Immuno-Immunochromatography</td>
<td>Immuno-Immunochromatography</td>
<td>Immuno-Immunochromatography</td>
<td>Immuno-Immunochromatography</td>
<td>Immuno-Immunochromatography</td>
<td>Immuno-Immunochromatography</td>
</tr>
<tr>
<td>Material studied</td>
<td>Whole blood</td>
<td>Serum</td>
<td>Whole blood</td>
<td>Whole blood</td>
<td>Whole blood</td>
<td>Whole blood</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>97.7%</td>
<td>86.0%</td>
<td>97.7%</td>
<td>84.5%</td>
<td>95.0%</td>
<td>85.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>94.1%</td>
<td>92.8%</td>
<td>93.4%</td>
<td>97.7%</td>
<td>94.9%</td>
<td>98.0%</td>
</tr>
<tr>
<td>Time</td>
<td>5 min. do 24 h</td>
<td>8 min.</td>
<td>15 min.</td>
<td>15 min.</td>
<td>5-20 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>Cost/test</td>
<td>&lt;US$ 2,00</td>
<td>Not available</td>
<td>US$ 3,30</td>
<td>US$ 0,75</td>
<td>US$ 0,55</td>
<td>Not available</td>
</tr>
</tbody>
</table>

Point-of-care (POC) test:

**Basis:**
Essentially ELISAs performed on a solid nitrocellulosa matrix!

**Advantages:**
Relatively high sensitivity and high specificity, can use whole blood obtained by finger prick, inexpensive, no requirements of electricity, reliable results within 5-20 minutes (diagnosis, examination, treatment at a single clinic visit).

**Disadvantages:**
POC tests have been based on detection of treponemal antibodies ⇒ no indication of current infection


www.who.int/std_diagnostics
Chembio dual non-treponemal and treponemal test – dual lateral flow

- Non-Reactive Control Line Only
- Confirmed Reactive 3 Lines
- Reactive (2 Lines) Treponemal and Control (Old or treated case)
- Reactive (2 Lines) Non-treponemal and Control (False Positive)
Sensitivity of the Chembio POC test to detect anti-cardiolipin antibodies at various RPR titers

Unpublished data from CDC, USA (Courtesy: Ron Ballard)
Dual lateral flow test readers

Reader ⇒ simple, rapid and less subjective (particularly when poor lighting)
Thank you for your attention!
Routine syphilis testing (depends on setting)

- Pregnant women
- Blood and plasma donors
- At higher risk of having syphilis:
  1. newly diagnosed with other STI;
  2. patients with HIV, HBV, HCV;
  3. patients suspected of early neurosyphilis;
  4. patient with sexual behaviour putting them at higher risk, e.g. MSM, sex workers etc.
Nested PCR assay amplifying the *tpp47* gene of *T. pallidum* from ulcer swabs and blood

- 294 patients with suspected syphilis (87 primary, 103 secondary, 40 latent and 64 no syphilis) and 35 healthy subjects

- Overall results for **swabs**: sensitivity **82%**, specificity **95%**
  
  nPCR vs DFM  
  kappa = 0.53

- No agreement between nPCR in blood and diagnosis of syphilis
  - sensitivity 29%, 18%, 14.7% and 24% (vary by stage) for PBMC, plasma, serum and whole-blood
  - specificity 96%, 92%, 93% and 97%

- No influence of HIV status

- **PCR useful for ulcer swabs** (detection limit: ~10 GEQ)!

Orle et al. JCM. 1996; Chen et al. JCM. 2006; Gayet-Ageron et al. STD. 2009; Liu et al. JCM. 2001; Marfin et al. DMID. 2001
Detection of IgM anti-treponemal antibodies

- IgM –FTA-ABS (high sens but poor spec.), 19S – IgM-FTA-ABS (columns for separation of IG fractions no longer available in many countries)
- IgM solid phase haemadsorption assay (SPHA), TP-IgM-HA
- IgM-capturing ELISA
- *T. pallidum* IgM-immunoblot (Western blot)

**Applications?**:

- the earliest stage of syphilis (but IgM might also be present in latent period and in late disease ⇒ limits the value in diagnosis of syphilis in adults!)
- assessment of the disease activity
- diagnosis of congenital syphilis
- (diagnosis of neurosyphilis)
Serodiagnosis of syphilis in HIV positive patients

- Usually serological response to *T. pallidum* is unchanged independent of HIV positivity
- The syphilis lab. diagnostics usually do not differ dependent on HIV status
- However, atypical results might be observed:
  - False positive VDRL: up to 11%
  - False negative VDRL: particularly in Stage I and II
  - More frequent prozone phenomenon
Lumbar puncture in a patient with positive syphilis serology:

| CDC, MMWR 2010; | 1. Symptoms of neurological or ocular involvement  
2. Symptoms of S III  
3. Treatment failure  
4. Late syphilis in HIV (+) patient and  
   a) symptoms suggestive of neurosyphilis or ocular syphilis (or)  
   b) RPR>1:32,  
   c) CD4 count: <350/µL |
|-------------------|--------------------------------------------------------------------------------------------------|
2. Symptoms of ocular syphilis  
3. Auricular (otological) symptoms possibly caused by syphilis otitis  
3. Concomitant HIV infection, especially if CD4 count is <350/µL and/or the serum RPR test titre is >1:32 |
Diagnosis of neurosyphilis

- Neurological examination, incl. imaging studies (MRI)
- Positive syphilis serology
- Examination of CSF (lumbar puncture)

No single laboratory test or clinical feature can diagnose neurosyphilis and diagnosis is usually made on a combination!
Examination of CSF

- General examination:
  - number of mononuclear cells
  - proteins
  - albumins
  - glucose

- Serological tests: non-treponemal (VDRL) and treponemal (TPHA, TPPA, FTA-Abs)

- (indexes assessing the blood-brain barrier)
Laboratory diagnostic criteria for neurosyphilis – Traditional (not any index) (IUSTI/WHO, 2008)

Mononuclear cells >5-10/mm³ in CSF

plus

Positive TPHA/TPPA/MHA-TP and/or FTA-ABS in CSF

OR

Positive VDRL/RPR in CSF
Serological response in congenital syphilis

- Passive maternal IgG treponemal antibodies
- IgG Abs produced by a child
- IgM Abs produced by a child
- Passive maternal anticardiolipin Abs
**Confirmed congenital syphilis diagnostic criteria**

<table>
<thead>
<tr>
<th>IUSTI/WHO, 2008</th>
<th>CDC, 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Detection of <em>T. pallidum</em> (in placenta, cutaneous or mucosal lesions, on autopsy)</td>
<td>- Clinical picture consistent with congenital syphilis</td>
</tr>
<tr>
<td>- DFA-Tp, DFM, NAAT</td>
<td>- titer of anticardiolipin antibodies in a child $\geq 4$-fold higher than in mother</td>
</tr>
<tr>
<td></td>
<td>or</td>
</tr>
<tr>
<td></td>
<td>- Detection of <em>T. pallidum</em> (in placenta, cutaneous or mucosal lesions, on autopsy)</td>
</tr>
<tr>
<td></td>
<td>- DFA-Tp, DFM, NAAT</td>
</tr>
<tr>
<td>Stage of syphilis</td>
<td>Therapy</td>
</tr>
<tr>
<td>-------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Early syphilis (primary, secondary and early latent)</td>
<td>Benzathine penicillin 2.4 million units i.m.</td>
</tr>
<tr>
<td>Early syphilis (primary, secondary and early latent)</td>
<td>Procaine penicillin 600,000 units i.m.</td>
</tr>
<tr>
<td>Early syphilis (primary, secondary and early latent)</td>
<td>Doxycycline 200 mg daily orally (either 100 mg twice daily or a single 200 mg dose)</td>
</tr>
<tr>
<td>Early syphilis (primary, secondary and early latent)</td>
<td>Tetracycline 500 mg orally 4× daily for 14 days</td>
</tr>
<tr>
<td>Early syphilis (primary, secondary and early latent)</td>
<td>Erythromycin 500 mg 4× daily orally for 14 days</td>
</tr>
<tr>
<td>Early syphilis (primary, secondary and early latent)</td>
<td>Azithromycin 2 g orally as a single dose</td>
</tr>
<tr>
<td>Late latent syphilis, cardiovascular and gummatous syphilis</td>
<td>Benzathine penicillin 2.4 million units i.m. weekly for 2 weeks (Day 1, 8, 15)</td>
</tr>
<tr>
<td>Late latent syphilis, cardiovascular and gummatous syphilis</td>
<td>Procaine penicillin 600.00 U i.m. for 17–21 days</td>
</tr>
<tr>
<td>Late latent syphilis, cardiovascular and gummatous syphilis</td>
<td>Doxycycline 200 mg daily (either 100 mg twice daily or as a single 200 mg dose) for 21–28 days</td>
</tr>
<tr>
<td>Late latent syphilis, cardiovascular and gummatous syphilis</td>
<td>Tetracycline 500 mg 4× orally for 28 days</td>
</tr>
<tr>
<td>Late latent syphilis, cardiovascular and gummatous syphilis</td>
<td>Erythromycin 500 mg 4× daily for 28 days</td>
</tr>
<tr>
<td>Neurosyphilis and ocular syphilis</td>
<td>Benzyl penicillin 18–24 million units i.v. daily, as 3–4 million units every 4 hours for 10–21 days</td>
</tr>
<tr>
<td>Neurosyphilis and ocular syphilis</td>
<td>Benzyl penicillin 0.15 million units/kg/day i.v. over six doses (every 4 hours) for 10–14 days</td>
</tr>
<tr>
<td>Neurosyphilis and ocular syphilis</td>
<td>Procaine penicillin 1.2–2.4 million units i.m. daily PLUS Probénecid 500 mg 4× daily, for 10–14 days</td>
</tr>
<tr>
<td>Neurosyphilis and ocular syphilis</td>
<td>Doxycycline 200 mg twice daily orally for 28 days</td>
</tr>
<tr>
<td>Early syphilis in pregnancy (primary, secondary and early latent syphilis)</td>
<td>Benzathine penicillin 2.4 million units i.m. as a single dose</td>
</tr>
<tr>
<td>Early syphilis in pregnancy (primary, secondary and early latent syphilis)</td>
<td>Benzathine penicillin 2.4 million units 2 doses (Day 1 and Day 8)</td>
</tr>
<tr>
<td>Early syphilis in pregnancy (primary, secondary and early latent syphilis presenting in the last trimester of pregnancy)</td>
<td>Procaine penicillin 600,000 units – 1.2 million units i.m. daily for 10–14 days</td>
</tr>
<tr>
<td>Congenital syphilis</td>
<td>Benzyl penicillin 150,000 units/kg i.v. daily (administered in six doses every 4 hours) for 10–14 days</td>
</tr>
<tr>
<td>Congenital syphilis</td>
<td>Procaine penicillin 50,000 U/kg i.m. daily for 10–14 days</td>
</tr>
<tr>
<td>Congenital syphilis (if CSF examination is normal)</td>
<td>Benzathine penicillin 50,000 U/kg i.m. as a single dose</td>
</tr>
</tbody>
</table>