MST: gestione complementare urologica ed infettivologica

Teresa Bini, MD
Università degli Studi di Milano
Clinica di Malattie Infettive e Tropicali
Ospedale San Paolo
Syphilis 2015
At 110 years from *T. pallidum* discovery
which news for an old disease?
Modern approaches to syphilis diagnosis and management

- «Reverse algorithm» screening
- PCR testing for diagnosis and PCR-identified bacteraemia
- Typing method for T pallidum and gene sequence analysis

Syphilis testing, typing, and treatment follow-up: a new era for an old disease

Syphilis and HIV

- How to Treat?
- Neurosyphilis
- How to prevent?

Syphilis treatment in the presence of HIV: the debate goes on
3° proposal: ECDC method
Both the screening and the confirmatory tests should be treponemal tests

Figure 2. Reverse sequence screening algorithm for syphilis infection. CIA indicates chemiluminescence immunoassay; EIA, enzyme immunoassay. Adapted from Centers for Disease Control and Prevention.3

Marrazzo, What’s New in Sexually Transmitted Infections in the HIV Care setting: Focus on Syphilis and Gonorrhea, Topics in Antiviral Medicine, 2014
**Why Should I?**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional algorithm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Will not diagnose patients with RPR-negative untreated disease (mostly early primary or late latent disease) &lt;sup&gt;[11]&lt;/sup&gt;.</td>
</tr>
<tr>
<td>RPR testing may be cheaper than some T. pallidum-specific serologic assays &lt;sup&gt;[9]&lt;/sup&gt;.</td>
<td></td>
</tr>
<tr>
<td>Reduces rate of false positive screening results, especially in low prevalence populations &lt;sup&gt;[10]&lt;/sup&gt;.</td>
<td></td>
</tr>
<tr>
<td>Reverse algorithm&lt;sup&gt;b&lt;/sup&gt;</td>
<td>May result in over-treatment and increased follow-up, especially in low prevalence areas &lt;sup&gt;[10]&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Suitable for high-throughput screening using automated platforms &lt;sup&gt;[12]&lt;/sup&gt;.</td>
<td></td>
</tr>
<tr>
<td>Identifies patients with all stages of disease.</td>
<td>Requires careful selection of sensitive and specific tests for screening and confirmation &lt;sup&gt;[13]&lt;/sup&gt;.</td>
</tr>
</tbody>
</table>

RPR, rapid plasma regain; TPPA, Treponema pallidum particle agglutination assay; VDRL, Venereal Disease Research Laboratory.

<sup>a</sup> Screening begins with a nontreponemal tests, such as RPR or VDRL and positive results are confirmed with TPPA or other T. pallidum-specific antibody testing.

<sup>b</sup> Screening begins with an anti-T. pallidum antibody test. Positive results are confirmed with RPR or VDRL. Negative confirmatory results (RPR or VDRL) are tested with a second anti T. pallidum antibody test.
Cross-Sectional study, 2749 patients
To evaluate the three algorithm methods, comparing each to the patient’s final clinical-microbiological diagnosis

- **Global sensitivity:** 75.8% TST vs 99.8% RSA vs 99.3% IUSTI method

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Serum Samples Tested</th>
<th>No. (%) of Serodiagnosis Positive by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Traditional Testing Algorithm</td>
</tr>
<tr>
<td>Primary</td>
<td>24</td>
<td>18 (75.00)</td>
</tr>
<tr>
<td>Secondary</td>
<td>365</td>
<td>362 (99.18)</td>
</tr>
<tr>
<td>Early latent</td>
<td>198</td>
<td>155 (78.28)</td>
</tr>
<tr>
<td>Late latent</td>
<td>1578</td>
<td>1153 (73.07)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>584</td>
<td>396 (67.81)</td>
</tr>
<tr>
<td>Total</td>
<td>2749</td>
<td>2084 (75.8)</td>
</tr>
</tbody>
</table>

Tong, Analysis of 3 Algorithms for Syphilis Serodiagnosis and Implications for Clinical Management, *Clin Infec Dis, 2014*
- **665 missed diagnosis** of which:
  - 52 early syphilis
  - 390 first syphilis infection
  - High prevalence of syphilis in the region of China studied

<table>
<thead>
<tr>
<th>Treatment History</th>
<th>Primary (Cases)</th>
<th>Secondary (Cases)</th>
<th>Early Latent (Cases)</th>
<th>Late Latent (Cases)</th>
<th>Tertiary (Cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>6</td>
<td>2</td>
<td>25</td>
<td>236</td>
<td>121</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recommended</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>103</td>
<td>31</td>
</tr>
<tr>
<td>Inappropriate treatment(^a)</td>
<td>0</td>
<td>0</td>
<td>12</td>
<td>86</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>3</td>
<td>43</td>
<td>425</td>
<td>188</td>
</tr>
</tbody>
</table>

\(^a\)Includes subjects with syphilis for whom it was unknown whether the recommended therapy was received.

Tp-PCR in Diagnosis vs DFM and Serology

- CDC considers Tp-PCR a valid diagnostic method along with dark-field microscopy, which is still considered the reference test (samples from syphilis pts that yield a negative DFM result and a positive Tp-PCR result are currently considered false-positive).
- PCR vs DFM: high-quality readings of DFM are difficult to obtain, especially when the test is not routinely performed; PCR relies less on human expertise, it is more reproducible and testing less costly if it’s performed on routine basis.
- Up to 20% of pts with syphilis may show a negative DFM.
- Its utility has been largely confined to ulcer and mucous membrane samples in I and II disease and his sensitivity in blood is inadequate for diagnosis in late-stage disease.
- Early syphilis diagnosis: Tp-PCR detection in lesions of early syphilis has the potential to identify serology-negative early infection.
- Qualitative detection of Tp DNA by PCR is influenced by disease stage and activity:
  - Tp detection sensitivity is highest in primary genital (78.4%) or anal chancre (95%).
  - In blood samples the highest sensitivities is found in congenital (83%) and secondary disease (52.2%).

Pregnancy

- Tp bacterial load predicts syphilis stage, disease activity and risk of adverse neonatal outcomes.
Monitoring treatment can be achieved by measuring a two-diluision (four-fold) fall in the titre of VDRL or RPR. The requisite fall in titre may not be observed for several months up to 1 year, could be problematic due to the non-specific nature of serum RPR and only a small proportion (13%) of «serofast» patients respond serologically to re-treatment.

Measuring Tp levels in blood and ulcer exudate through PCR may be a useful measure of treatment success in early syphilis.

First study that detected Tp nucleic acids to measure treatment response.

4 pts with early Syphilis (3/4 HIV+)

A Tp DNA in ulcer
B Tp RNA in ulcer

Tipple, Rapid T pallidum clearance from blood and ulcer samples following single dose benzathine penicillin treatment of early syphilis, PLoS Negl Trop Dis, 2015
Clearance of *Tp* nucleic acids from blood and ulcer exudate within 56 hours of treatment with Benzathine Penicillin im

Half-life for blood clearance of *Tp* of 5.7 hours for DNA and of 3.9 hours for RNA

For an ulcer, bacterial DNA and RNA clearance half-lives were 3.2 and 4.1 hours respectively

All patients were cured by the treatment showing subsequently both clinical and serologic response

A-C. *Tp* DNA in blood

D-F. *Tp* RNA in blood

Tipple, Rapid *T. pallidum* clearance from blood and ulcer samples following single dose benzathine penicillin treatment of early syphilis, PLoS Negl Trop Dis, 2015
Tp-PCR in treatment management

Macrolide Resistance

- A2058G and A2059G rRNA mutations confer macrolide resistance and can be detected by PCR and restriction digestion. These resistances are showing a rising prevalence.
- A real-time triplex PCR which amplifies both copies of Tp 23S rRNA gene and uses molecular probes to detect wild-type A2058G and A2059G sequences has been described and could become a quick, sensitive and specific method for macrolide resistance screening, useful for the treatment of penicillin-allergic pts with early syphilis.
To Study in deep...


- Tipple C, Syphilis testing, typing and treatment follow-up: a new era for an old disease, Curr Opin Infect Dis, 2015

- Casal C, Risk factors and pregnancy outcomes in women with syphilis diagnosed using a molecular approach, Sex Transm Infect, 2013

- Shields M, A longitudinal evaluation of Treponema pallidum PCR testing in early syphilis, BMC Infect Dis, 2012


- Gayet-Ageron, Use of Treponema pallidum PCR in testing of ulcers for diagnosis of primary Syphilis, Emerg Infect Dis, 2015
Syphilis in HIV: How to Treat?

- The optimal antimicrobial regimen to treat syphilis in HIV is still unknown and no treatment regimens have been demonstrated to be more effective in preventing neurosyphilis than the regimens recommended for HIV-negative patients.

- Despite international guidelines, a survey conducted amongst American infectious disease physicians found that the majority favoured managing early syphilis in HIV-positive pts with 3 doses of BPG.

<table>
<thead>
<tr>
<th>Syphilis Stage</th>
<th>USA CDC 2010</th>
<th>UK guidelines 2008</th>
<th>European IUSTI 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Syphilis (&lt; 2 years)</td>
<td></td>
<td>BPG 2.4 MU IM stat</td>
<td></td>
</tr>
<tr>
<td>Late Syphilis (&gt;2 years)</td>
<td></td>
<td>BPG 2.4 MU IM stat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 doses at weekly intervals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurosyphilis</td>
<td>Benzyl Penicillin 18-24 MU/day, 3-4 MU iv every 4 h for 10-14 days</td>
<td>Procaine Penicillin 2.4 MU IM/day + Probenecid 500 mg qds for 17 days</td>
<td>Benzylo Penicillin iv every 4 hours for 10-28 days</td>
</tr>
<tr>
<td>Difference in HIV</td>
<td>Antibiotic choice same as HIV-negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Neuro Syphilis 
and 
Asymptomatic Neuro Syphilis

- Neurosyphilis, defined as positive VDRL and/or FTA in CSF and an increased number of mononuclear cells (usually > 20 cells/μL), can occur at any stage of syphilis.

- CNS invasion occurs early during infection in approximately 30-40% of cases and is asymptomatic in the majority of pts.

- Pts should be carefully evaluated for neurologic, ophtalmic and otologic symptoms and a lumbar puncture should be performed for those who are symptomatic.

- Marra et al, comparing neurocognitive assessment in 82 HIV+ pts who had previous syphilis infection with 84 who had never been infected with syphilis, found that prior syphilis infection was associated with a greater level of neurocognitive impairment, in the absence of a diagnosis of neurosyphilis.

- Previous syphilis infection, in the absence of neurosyphilis diagnosis, has been associated to impaired learning skills, poor memory and higher Global Deficit Score.
(Asymptomatic) Neuro Syphilis Management

- CNS invasion is more likely to occur among HIV-infected pts with **CD4+ cell counts ≤ 350 cells/μL and/or RPR/VDRL titres ≥ 1:32**; these two combined criteria are the most reliable in identifying **asymptomatic neurosyphilis** (sens 100%, spec 87%)
- **CSF CXCL13 levels** have been recently proposed as **useful diagnostic marker**, independently of CSF VDRL and pleocytosis
- It may be wise to have a low threshold for performing a LP, but currently **there are no data supporting LP execution and a better outcomes of neurosyphilis treatment among pts without neurologic symptoms**

Marra CM et al, CXCL13 as a cerebrospinal fluid marker for neurosyphilis in HIV-infected patients with syphilis, Sex Transm Dis, 2010
To Study in deep...

- Marra CM et al, Neurocognitive impairment in HIV-infected individuals with previous syphilis, Int J STD AIDS, 2013
- Marra CM et al, CXCL13 as a cerebrospinal fluid marker for neurosyphilis in HIV-infected patients with syphilis, Sex Transm Dis, 2010
- Ghanem KG et al, Lumbar puncture in HIV-infected patients with syphilis and no neurologic symptoms, Clin Infect Dis, 2009
- Libois A et al, HIV and syphilis: when to perform a lumbar puncture, Sex Transm Dis, 2007
Warwick et al (Brighton and Sussex University Hospitals Trust) treated all coinfected pts (130) with a neuropenetrative regimen regardless of syphilis stage (17 days of Procain Penicillin 2.4 MU IM + Probenecid 500 mg PO 4 times a day). He found a serological treatment success rate of 98%, higher than has previously been recorded elsewhere.

Ghanem et al reports a 60% reduction in rate of serological failure to syphilis treatment in HIV-patients on ART.

Patients with HIV, particularly those not on ART, have slower serological response and it’s possible that serological failure is merely a result of a slower serological response to treatment and a consensus needs to be made about appropriate follow-up time in HIV.

Warwick, Should syphilis be treated differently in HIV-positive and HIV-negative individuals? Treatment outcomes at a university hospital, Brighton, UK, Int J STD AIDS, 2009

Ghanem, Antiretroviral therapy is associated with reduced serologic failure rates for syphilis among HIV-infected patients, Clin Infect Dis, 2008

Knaute, Serological response to treatment of syphilis according to disease stage and HIV status, Clin Infect Dis, 2012
High-dose Oral Amoxicillin plus Probenecid is highly effective for Syphilis in patients with HIV infection
Tanizaky R et al
Clinical Infectious Diseases, March 31, 2015

- Retrospective observational cohort study
- 286 HIV+ syphilis male pts (54.5% on ART)
- 69.6% with early syphilis and 30.4% with late syphilis (including syphilis with unknown duration)
- Treated with 3 g/day of oral Amoxicillin + 0.75-1.5 g/day of Probenecid for 2-4 weeks
- 100% showed a clinical treatment response and 95.5% decreased RPR titre by 4-fold of which 96.3% within 12 months

### Disease Stage

<table>
<thead>
<tr>
<th>Disease Stage</th>
<th>Primary</th>
<th>Secondary</th>
<th>Early latent</th>
<th>Late latent</th>
<th>Unknown duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment efficacy</td>
<td>93.8%</td>
<td>97.3%</td>
<td>100%</td>
<td>85.7%</td>
<td>92.4%</td>
</tr>
</tbody>
</table>

**Late syphilis** (OR 3.9, p.019) and **high HIV-1 load before treatment** (OR 0.9, p.033) were associated with treatment failure.
- **1° study** that reported the **treatment efficacy of high-dose oral amoxicillin plus probenecid for syphilis**, regardless of HIV-1 infection status. Previous studies were either only pharmacokinetics or anecdotal, published in 1970s and 1980s.

- **Amoxicillin**, similar to aqueous penicillin, **crosses BBB**, whereas BPG does not. This might be particularly important for HIV-infected pts, because these pts likely present with ANS and progression to NS is faster.

- Highly tolerable regimen (only 28 pts experienced ADR) which requires only a single hospital visit.

<table>
<thead>
<tr>
<th>Disease Stage</th>
<th>Treatment Proposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Syphilis</td>
<td>2 <strong>weeks</strong> of 3 g oral Amoxicillin plus 750 mg Probenecid a day</td>
</tr>
<tr>
<td>Late Syphilis</td>
<td>4 <strong>weeks</strong> of 3 g oral Amoxicillin plus 750 mg Probenecid a day</td>
</tr>
</tbody>
</table>

Prophylaxis for Syphilis in HIV: is it possible?

Doxycycline Prophylaxis to Reduce Incident Syphilis among HIV-Infected Men who have Sex with Men who Continue to Engage in High Risk Sex: A Randomized, Controlled Pilot Study

Robert K. Bolan, MD* Sex Transm Dis. 2015 February ; 42(2): 98–103

- Pilot study, randomized double arm trial to determine if daily prophylactic 100 mg doxycycline is efficacious in reducing STDs among high risk population
- 30 HIV+ MSM with at least 2 documented and adequately treated episodes of syphilis since HIV diagnosis
- 48 week follow up: visit at baseline and week 12, 24, 36 and 48
- Behavioral sexual risk self-reported questionnaires during each visit
- 2 arms: doxycycline arm (15 pts) vs money incentivization for remaining STD-free (15 pts)

<table>
<thead>
<tr>
<th>STI contraction</th>
<th>Doxy Arm</th>
<th>CM Arm</th>
<th>p value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonorrhea/Chlamydia only</td>
<td>4</td>
<td>8</td>
<td>0.18</td>
<td>0.36 (0.08-1.56)</td>
</tr>
<tr>
<td>Syphilis Only</td>
<td>2</td>
<td>7</td>
<td>0.10</td>
<td>0.24 (0.04-1.33)</td>
</tr>
<tr>
<td>Any STD</td>
<td>6</td>
<td>15</td>
<td>0.02</td>
<td>0.27 (0.09-0.83)</td>
</tr>
</tbody>
</table>

No differences in self reported data about sexual behaviour
Prophylaxis for HIV in Syphilis: is it feasible in PrEP Era?

- In USA, 20-50% of MSM with syphilis have concurrent HIV infection
- HIV-uninfected men who had syphilis have an elevated HIV risk (iPrEX study)

The high risk of an HIV diagnosis following a diagnosis of syphilis: A population-level analysis of New York City men

Preeti Pathela

Clinical Infectious Diseases Advance Access published April 13, 2015

- They measured HIV incidence following primary or secondary syphilis diagnosis from 2000 June to 2010 June in male cases reported to the New York City HIV/AIDS ans STD surveillance registries
- Of 2805 men with syphilis contributing to 11714 person-years of follow-up, 423 (15.1%) acquired HIV with a median time to HIV diagnosis of 1.6 years
- Global annual HIV incidence was 3.61%, and it was higher among MSM (5.56% vs 1.2% of MSW), male with secondary syphilis (4.10% vs 2.64% of primary syphilis) and males diagnosed with another bacterial STD after syphilis (7.89%)
A new syphilis diagnosis in an HIV-negative man, especially MSM and secondary syphilis, offers a key opportunity for PrEP initiation.

To identify MSM population that would benefit the most from PrEP, Buchbinder et al showed a population attributable fraction (PAF) for syphilis of about 10%; of 11 other HIV-associated risks that were examined, only condomless receptive anal intercourse and reporting more than 5 recent sex partners had higher PAFs.

Approximately 30 persons with prior syphilis infection would need to be on PrEP for a year to prevent 1 HIV infection.

Currently CDC recommends PrEP for MSM with recent (past 6 months) histories of bacterial STD, but probably this timing cut off must be revised.

**To Study in deep...**

- Buchbinder SP et al, HIV pre-exposure prophylaxis in men who have sex with men and transgender women: a secondary analysis of a phase 3 randomised controlled efficacy trial, Lancet Infect Dis, 2014

- Solomon MM et al, Syphilis predicts HIV incidence among men and transgender women who have sex with men in a preexposure prophylaxis trial, Clin Infect Dis, 2014
Non-Gonococcal Urethritis 2015
Slithering down the simplest things

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>C trachomatis</td>
<td>11-50%</td>
</tr>
<tr>
<td>M genitalium</td>
<td>6-50%</td>
</tr>
<tr>
<td>U urealyticum</td>
<td>11-26%</td>
</tr>
<tr>
<td>T vaginalis</td>
<td>1-20%</td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>2-4%</td>
</tr>
<tr>
<td>HSV</td>
<td>2-3%</td>
</tr>
</tbody>
</table>

Schwebke, Re-Evaluating the Treatment of Nongonococcal Urethritis: Emphasizing Emerging Pathogens–A Randomized Clinical Trial, Clin Infect Dis, 2011
Diagnosis:

- The diagnosis of urethritis should be confirmed by demonstrating PMNLs (≥ 5/HPF) from the anterior urethra using a Gram stained or methylene-blue stained urethral smear.

- For symptomatic pts with a negative urethral smear use a leucocyte esterase dipstick on the remains of the first-void urine specimen (≥1+). If both are negative, advise the patient to re-attend for an early morning smear if his symptoms do not settle.

- Empirical treatment without verifying the presence of urethritis is not recommended.

- In settings without microscopy, a diagnosis can be made by the presence of mucopurulent or purulent urethral discharge on examination, ≥1+ on a leucocyte esterase dipstick on a FVU specimen or the presence of threads in a FVU specimen.
Treatment:

- For severe symptoms should be initiated as soon as the diagnosis is made, without waiting for the results of tests.
- With mild symptoms it’s possible to review the patient after 3-7 days, when the results of the NAATs and gonorrhoea culture are available, as sometimes urethritis can resolve spontaneously.
- All sexual partners at risk should be assessed and offered epidemiological treatment. The duration of «look back» is arbitrary and 4 weeks is suggested for symptomatic men.
- A test of cure 4-5 weeks after treatment in those who tested positive for M genitalium should be performed.

<table>
<thead>
<tr>
<th>Recommended Regimen</th>
<th>Alternative Regimen</th>
<th>Recommended Regime if patient is, or suspected to be, M genitalium positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline 100 mg twice a day orally for 7 days</td>
<td>Azythromycin 1 g stat</td>
<td>Azithromycin 500 mg 1 day, then 250 mg/die for 4 days</td>
</tr>
<tr>
<td></td>
<td>Lymecycline 300 mg bd for 10 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetracycline hydrochloride 500 mg bd for 10 days</td>
<td></td>
</tr>
</tbody>
</table>
1° randomized controlled trial using the 5-day extended Azithromycin regimen performed

- Eradication rate for
  - C trachomatis 98.8%
  - M genitalium 81%

- 5-day extended Azithromycin regimen also mitigate the emergence of macrolide resistance in M genitalium

- Dual antimicrobial therapy might need to be considered
46 males positive for *M genitalium* (microscopy and rt-PCR)

All pre-treatment *M genitalium* specimens had wild-type 23S rRNA

Treated with *Josamycin* 500 mg 3 times a day for 10 days

The elimination of *M genitalium* DNA was substantially faster in pts with lower pre-treatment bacterial load.

Rate of eradication was 93.5%

Of 6 pts with high bacterial load, 3 (50%) remained positive post-treatment and these positive specimens contained **macrolide resistance mutations** (A2059G and A2062G)

The pre-treatment *M genitalium* load might be an effective predictor of eradication efficacy with macrolide and selection of macrolide resistance
Persistent and Recurrent NGU:

- **Persistent NGU** happens when symptoms do not resolve following treatment (15-25% cases).
- **Recurrent NGU** is empirically defined as the recurrence of symptomatic urethritis within 30-90 days following treatment of acute NGU (10-20%).
- **Diagnosis** of P/RNGU can be made only undertaking a Gram or methylene blue stained urethral smear in men who are symptomatic.
- Consider testing for *T vaginalis* if it’s prevalent in the local population, for *M genitalium* and for *U urealyticum*.
- Ensure that the patient has completed the initial course of therapy and the reinfection is not a possible cause.
- Only treat if pt has definite symptoms or physical signs AND microscopic evidence of urethritis.
- Any treatment of PNGU should cover *M genitalium* (20-40% of PNGU) and *T vaginalis* (10% PNGU).

<table>
<thead>
<tr>
<th>Doxycycline 100 mg bid 7 days As first line</th>
<th>Azithromycin 500 mg stat then 250 mg/die 4 days As first line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin 500 mg stat then 250 mg for the next 4 days (IIIb) + Metronidazole 400 mg twice daily for 5 days depending on local T vaginalis prevalence (IVC)</td>
<td>Moxifloxacin 400 mg orally once daily for 7-14 days (IIIB) + Metronidazole 400 mg twice daily for 5 days (IVC)</td>
</tr>
</tbody>
</table>
Continuing Symptoms:

- Patients who either remain symptomatic following a second course of treatment or who have frequent recurrences after treatment

- **Moxifloxacin 400 mg orally once daily for 7-14 days** (IIIB)

- **Erythromycin 500 mg 4 times daily for 3 weeks** has been shown to be effective, but before the new macrolides were available (clarithromycin is now recommended in pts with *U urealyticum*)

- Urological investigation is usually normal unless the patient has urinary flow problems and is not recommended (IVC)

- Chronic abacterial prostatitis, chronic pelvic pain syndrome and psychosexual causes should be considered

To Study in deep...

European guideline for the management of *Chlamydia trachomatis* infections: July 2010

Lanjouw, Ossewaarde, Stary and Boag

Diagnostic Assays:
- **Nucleic acid amplification techniques (NAATs):** cryptic plasmid, MOMP or rRNA; attention to plasmid free and plasmid mutant strains
- Isolation in cell culture
- Enzyme immunoassays (EIA)
- Direct fluorescence assays (DFA)

Choice of Specimen:
- 1° choice: **first-void urine** in both sex or **vaginal swab** in women (better 4 weeks after the last menstrual bleeding; III)
- Less adequate: **pharyngeal, conjunctival and rectal specimens**
- Not recommended semen!

Diagnostic Challenges:
- Emergence of **LGV** among MSM (genotyping necessary)
- Emergence of **Swedish C trachomatis variant**

From 2003 outbreaks in European countries (UK: 2000 cases 2003-2012; Barcelona: 146 cases 2007-2011; Netherlands, France)
Serology:
- Only **invasive disease** will lead to Ab levels useful for diagnostic purposes (no value in uncomplicated urethritis, cervicitis; limited value in ascending infections and infertility workup; high titres of IgG/IgA and IgM can be diagnostic in LGV and Neonatal pneumonia respectively)
- Only synthetic peptide-based EIAs show no cross-reactions
- **Duration of Ab-positivity is not known**

Treatment of uncomplicated infections:
- Treatment is recommended and has to include the partners
- Resistance may occur at very low frequencies

<table>
<thead>
<tr>
<th>First Choice Treatment IA</th>
<th>Alternative Treatment IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 g Azithromycin one shot or 100 mg Doxycycline twice a day for 7 days</td>
<td>500-1000 mg Josamycin twice a day for 7 days or Another macrolide</td>
</tr>
</tbody>
</table>

- In **pregnancy**: 1 g Azythromycin or 500 mg Amoxicillin 4 times a day for 7 days (IA); in high prevalence populations (>5%) pregnant women should be screened (IIB)
Rectal infection treatment:

- Higher failure rate of the standard single dose of Azythromycin has been described in rectal infections, but usually a distinction between non-LGV and LGV chlamydial infections is not made.

<table>
<thead>
<tr>
<th>Non-LGV Rectal infection IIIB</th>
<th>LGV Rectal Infection IIIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg Doxycycline twice a day for 7 days</td>
<td>100 mg Doxycycline twice a day for 21 days</td>
</tr>
</tbody>
</table>

Therapy failure:

- Repeat a longer course (10-14 days) with doxycycline or a macrolide. Suggested rifampicin plus a macrolide.
- It might be also caused by persistence of chlamydial strains or reinfection from an untreated partner.
- Consider concurrent STIs: screen for at least HBV, syphilis, gonorrhoea, HIV (IA) and M genitalium.
- NAATs cannot discriminate between live and dead microorganisms; up until 4-6 weeks after therapy a test result may still be positive, thus a test of cure is not recommended.
Gonococcal Urethritis 2015
New resistant «SuperBug» Gonorrhea

2008

2011

2013

75.6%

69.6%

66.1%
Diagnosis of uncomplicated gonorrhoea is established by **identification of N gonorrhoeae in genital, rectal, pharyngeal or ocular secretions.** Ng can be detected by:

- **Microscopy** has a good sensitivity ($\geq 95\%$) and specificity in symptomatic men, while has poor sensitivity ($<55\%$) in asymptomatic men and in identifying endocervical ($<55\%$) or rectal infection ($\leq 40\%$) and cannot be recommended as a test of exclusion. Microscopy is not recommended for pharyngeal infection.

- **Culture** is fundamental in monitoring evolving antimicrobial resistance and testing pts with persisting infection or symptoms following treatment. It is appropriate for endocervical, urethral, rectal, pharyngeal and conjunctival specimens, but **not for urine**

- **NAATs** are more sensitive than culture ($>96\%$) in both symptomatic and asymptomatic infection and offer testing on a wider range of specimens (even if urine samples show lower sensitivity than swabs from the genital tract). NAATs are the test of choice for screening for rectal and pharyngeal gonococcal infection. Positive NAAT results may be observed within 1-2 days of infection.

---

2012 European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults
C Bignell and M Unemo
### Who has to be tested?

<table>
<thead>
<tr>
<th>Indications for testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms or signs of urethral discharge in men</td>
</tr>
<tr>
<td>Vaginal discharge with risk factor for STI</td>
</tr>
<tr>
<td>Mucopurulent cervicitis</td>
</tr>
<tr>
<td>Diagnosis with any other STI</td>
</tr>
<tr>
<td>Sexual partner of persons with an STI or PID</td>
</tr>
<tr>
<td>Acute epididymo-orchitis in a male aged &lt;40 years</td>
</tr>
<tr>
<td>Screening young adults (&lt;25 years) for sexually transmitted infection</td>
</tr>
<tr>
<td>Screening individuals with new or multiple recent sexual partners</td>
</tr>
<tr>
<td>Purulent conjunctivitis in a neonate or adult</td>
</tr>
<tr>
<td>Mother of a newborn with ophtalmia neonatorum</td>
</tr>
<tr>
<td>Acute PID</td>
</tr>
</tbody>
</table>

### Who has to be treated?

<table>
<thead>
<tr>
<th>Indications for treating</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification of intracellular diplococci at genital site by microscopy</td>
</tr>
<tr>
<td>Positive culture or confirmed NAAT from any site</td>
</tr>
<tr>
<td>If a recent partner has confirmed gonococcal infection</td>
</tr>
<tr>
<td>Mother of neonate with confirmed gonococcal infection</td>
</tr>
<tr>
<td>Considering after sexual assault</td>
</tr>
<tr>
<td>Purulent urethral discharge or mucopurulent cervicitis when rapid diagnostic test are not available and after specimen collection for laboratory testing</td>
</tr>
</tbody>
</table>

Bignell & Unemo, European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults, 2012
Gonococci resistant to third-generation cephalosporins have been reported in Japan, France and Spain.

MDR N gonorrhoeae includes resistance to:
- At least 1 of the antibiotics belonging to category I (like ceftriaxone, cefixime, spectinomycin)
- 2 or more antibiotics belonging to category II (like penicillin, ciprofloxacin, azithromycin)

WHO «Global Action Plan to Control the Spread and Impact of Antimicrobial Resistance in Neisseria gonorrhoeae»

ECDC «Response Plan to Control and Manage the Threat of Multidrug-Resistant Gonorrhoea in Europe»

Combination Antimicrobial Therapy with extended-spectrum cephalosporins and azithromycin!
Uncomplicated infection of urethra, cervix and rectum of adults and adolescents when antimicrobial sensitivity is unknown

Recommended treatments

Ceftriaxone 500 mg im as single dose + Azithromycin 2 g as single oral dose

Alternative:

 ✓ Cefixime 400 mg as single oral dose + Azithromycin 2 g as single oral dose (if ceftriaxone not available, or injectable antimicrobials is not possible or refused)
 ✓ Ceftriaxone 500 mg im as single dose (if azithromycin is not available)
 ✓ Spectinomycin 2 g im as a single dose + Azithromycin 2 g as single oral dose (if resistance to cephalosporins is identified or suspected, history of penicillin anaphylaxis or cephalosporin allergy)

Bignell & Unemo, European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults, 2012
Recommended treatments

Uncomplicated infection of **pharynx**

- **Ceftriaxone** 500 mg im as single dose
  + **Azithromycin** 2 g as single oral dose

**Alternative:**
- Ceftriaxone 500 mg im as single dose (if azithromycin is not available)
- Ciprofloxacin 500 mg im as a single dose or Ofloxacin 400 mg as a single oral dose or Azithromycin 2 g as a single oral dose (if resistance to cephalosporins is identified or suspected, history of penicillin anaphylaxis or cephalosporin allergy and fluoroquinolone or azithromycin resistance are excluded by susceptibility testing)

CDC: 250 mg Ceftriaxone + 1 g Azithromycin

Bignell & Unemo, European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults, 2012
Recommended treatments

**Genital, anorectal and pharyngeal infection** when **extended-spectrum cephalosporin resistance** identified

- **Ceftriaxone** 1 g im as single dose
  + **Azithromycin** 2 g as single oral dose

- **Gentamicin** 240 mg im as a single dose
  + **Azithromycin** 2 g as single oral dose

Bignell & Unemo, European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults, 2012
**Recommended treatments**

- **Pregnancy and breastfeeding:**
  - Ceftriaxone 500 mg im as a single dose or Spectinomycin 2 g im as a single dose

- **Penicillin allergy:**
  - Spectinomycin 2 g im as a single dose + Azithromycin 2 g as a single oral dose

- **Epididymo-orchitis:**
  - Ceftriaxone 500 mg im as a single dose + Doxycycline 100 mg oral twice a day for 10-14 days

- **PID:**
  - Ceftriaxone 500 mg im as a single dose + Doxycycline 100 mg oral twice a day + Metronidazole 400 mg oral twice a day for 14 days

- **Disseminated gonococcal infection:**
  - Initial therapy Ceftriaxone 1 g im or iv/24 h Spectinomycin 2 g im/12 h for 7 days but check for switching at 24-48 h to: Cefixime 400 mg oral twice daily or, if fluoroquinolone sensitivity is confirmed, ciprofloxacin 500 mg oral or ofloxacin 400 mg oral twice daily

Bignell & Unemo, European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults, 2012
After treatment…

- Sex partners should be contacted and offered testing together with treatment and counseling; all sex partners within the preceding 60 days of onset of symptoms or diagnosis should be evaluated and treated.

- Assessment after treatment is recommended to confirm compliance, resolution of symptoms and signs and to exclude re-infection.

- **A test of cure is recommended in all cases** to identify persisting infection and emerging resistance (CDC recommend it only in pts treated without ceftriaxone).

- When symptoms persist after treatment, **culture is recommended** and may be performed 3-7 days after completion of therapy, possibly supplemented a week later with a NAAT to increase sensitivity.

- **Test of cure in asymptomatic pts can be performed with a NAAT 2 weeks after completion of therapy** and all positives should be cultured to test antibiotic susceptibility before further treatment is given.

---

Bignell and Unemo, European Guideline on the Diagnosis and Treatment of Gonorrhoea in Adults, 2012
1430 isolates, 68% from italians
Sites of isolations: urogenital 81.5%, rectum 11%, throat 1.2%, double sites 0.94%
Results:

- All isolates were fully responsive to ceftriaxone and spectinomycin.

- 81/1430 (5.7%) were MDR: 84% isolated from men, of which 44% MSM.

- Among MDR, 11 different resistance patterns were identified. The most frequent was ciprofloxacin, penicillin and tetracycline (36/81) and ciprofloxacin was found in the majority of MDR patterns (10/11), while cefixime in 7/11.

- 1 isolate from a MSM from the north of Italy in 2009 showed resistance to 5 antimicrobials (azithromycin, cefixime, ciprofloxacin, penicillin and tetracycline).

- Resistance to previously recommended antimicrobials, such as ciprofloxacin, persists, even though the agent has not been recommended for gonorrhoea treatment for years.
HPV 2015
A Matter of Prevention

KEEP CALM
AND DO
YOUR ANTI-HPV VACCINE
Screening per HPV in Italia

- Lo screening tramite Pap-test viene raccomandato una volta ogni 3 anni a tutte le donne tra i 25 e i 64 anni.

- Attivi numerosi programmi di screening pubblici, che però non coprono l'intero territorio nazionale.

- Circa il 60-70% delle donne nella fascia d'età indicata si sottoponga a Pap-test almeno ogni 3 anni: il 20-25% tramite programmi pubblici, il 40-45% a pagamento.

- Non si sono mai sottoposte a screening il 20% delle donne.

Osservatorio nazionale screening 4° rapporto, Studio Passi 2005
Sulla base delle evidenze attualmente disponibili, il Consiglio Superiore di Sanità ritiene che la strategia vaccinale di più sicuro impatto per la prevenzione delle infezioni da HPV è quella che interviene nella fase pre-adolescenziale (9 - 12 anni)
Strategie di prevenzione: vaccinazione

Il 12° anno di vita si configura come l’età ideale per diversi motivi:

- **Scuola secondaria.** In questo contesto ragazze e genitori possono essere informati adeguatamente sull’infezione e sulla vaccinazione.

- **Possibilità di recuperare nella terza classe della scuola secondaria** le dosi mancanti del ciclo vaccinale e di riproporre l’immunizzazione in caso di mancata adesione.

- Influenza dei genitori nelle scelte.

- **Possibilità di inserire la vaccinazione nella stessa fase di vita in cui sono previste tutte le altre vaccinazioni previste dal calendario nazionale** per configurare la vaccinazione anti-HPV come intervento di normale prevenzione immunitaria.

- Recentemente positiva esperienza del programma di vaccinazione contro l’epatite B nella pre-adolescenza, con possibilità di utilizzare la rete di rapporti e le procedure organizzative già usate tra il 1991 e il 2003 dai servizi vaccinali.
ISTITUTO SUPERIORE DI SANITÀ, Progetto VALORE (VAlutazione LOcale e Regionale delle campagne di vaccinazione contro l’HPV): favorire l’adesione consapevole alla vaccinazione

- L’ultima ricognizione disponibile (30 giugno 2013) ha rilevato una copertura nazionale media per tre dosi di vaccino HPV pari al 69%, in riferimento alle coorti di nascita 1997-1999.

- È stata, inoltre, rilevata un’ampia variabilità tra le Regioni: dal 25-26% nella PA di Bolzano, all’85% in Sardegna per la coorte 1997 e 81% in Toscana per le coorti 1998 e 1999.

- Differenze sono state riportate anche tra ASL della stessa regione.

Strategie di prevenzione: costi

- **Gratuita**, attraverso strutture SSN alle ragazze tra gli 11 e i 12 anni

- **188,15 euro/dose** in farmacia. **67 euro/dose** in ambito ospedaliero regione Lombardia estensione fino a 45 anni da gennaio 2011.
GARDASIL: composizione del vaccino

Il Vaccino Quadrivalente è indicato per la prevenzione:
- delle lesioni genitali precancerose del collo dell’utero
- del cancro del collo dell’utero
- delle lesioni genitali (pre)cancerose della vulva
- delle lesioni genitali (pre)cancerose della vagina
- delle lesioni genitali esterne (condilomi acuminati)

© 2023 AIFA
Vaccino quadrivalente (6 – 11 - 16 – 18)

**Future II study group, Lancet; 2007**
*Studio fase III randomizzato, multicentrico*

<table>
<thead>
<tr>
<th>Popolazione:</th>
<th>16–23 aa, ≤ 4 partners, Follow-up 3 aa</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Popolazione naive:</td>
<td>HPV negative e PAP normale</td>
</tr>
<tr>
<td>- Popolazione mista:</td>
<td>con o senza HPV e/o PAP anomalo</td>
</tr>
</tbody>
</table>

**Efficacia:**

<table>
<thead>
<tr>
<th>Popolazione naive</th>
<th>↓ 100% CIN 2/3 da HPV 16/18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Popolazione mista</td>
<td>↓ 44% CIN 2/3 da HPV 16/18</td>
</tr>
<tr>
<td>Popolazione mista</td>
<td>↓ 17% CIN 2/3 da qualsiasi HPV</td>
</tr>
</tbody>
</table>
Percentuale di sieroconversione all’HPV 6, 11, 16, 18 al settimo mese dall’arruolamento

<table>
<thead>
<tr>
<th></th>
<th>HPV 6</th>
<th>HPV 11</th>
<th>HPV 16</th>
<th>HPV 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donne, 16-23 anni</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>99,1%</td>
</tr>
<tr>
<td>Ragazze, 10-15 anni</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Ragazzi, 10-15 anni</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>99,7%</td>
</tr>
</tbody>
</table>

*Block et al, Pediatrics 2007*
Gardasil: Medie Geometriche anticorpali dopo terza dose, per tipo di HPV e anno di età
Efficacia:

Popolazione mista: HPV negative con o senza PAP anomalo

Efficacia:

Popolazione naive: HPV negative e PAP normale

Popolazione naive: ↓ 100% CIN 2/3 da HPV 16/18

Popolazione naive: ↓ 68% CIN 2/3 da qualsiasi HPV

Popolazione mista: ↓ 90% CIN 2/3 da HPV 16/18

Cervarix

Vaccino **bivalente** diretto contro i genotipi **16, 18**

Nuovo adiuvante di GSK: Alluminio+MPL

Somministrazione in 2 dosi i.m.: 0 – 1 mese

Autorizzato per l'uso (EMEA), registrato in Italia

**Studio 008** (Paavonen, Lancet 2007)

**Studio 001-007** (Harper, Lancet 2006)

Studi fase III randomizzati, multicentrici
Cervarix: Persistenza dei titoli anticorpali fino a 4.5 anni dalla terza dose

I vaccini anti-HPV **NON HANNO EFFETTO TERAPEUTICO** sulle lesioni HPV-relate e/o sulle infezioni da HPV già presenti al momento della vaccinazione: target ideale → popolazione pre-adolescente

Stanley, 2012

Benchè l’infezione naturale generi Ab specifici anti-HPV capaci di clearare completamente il virus, **i titoli non sono alti comparati con quelli indotti dalla vaccinazione anti-HPV** → “protection against reinfection is not absolute; this role could potentially be filled through HPV vaccination”

Villa, 2006; Schwarz and Leo, 2008
Il futuro

Vaccino nonavalente diretto contro HPV associati al 90% dei cervicoarcinomi
HPV 6/11/16/18/31/33/45/52/58

HPV Vaccines in Development

Here are some vaccines now in company pipelines.

<table>
<thead>
<tr>
<th>Company</th>
<th>Immunogen</th>
<th>Vaccine type</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merck</td>
<td>Nonavalent L1 in yeast</td>
<td>Preventive</td>
<td>Phase III</td>
</tr>
<tr>
<td>ISA Pharmaceuticals</td>
<td>Synthetic long peptides of E6 and E7</td>
<td>Therapeutic</td>
<td>Phase II</td>
</tr>
<tr>
<td>Hoffman–La Roche</td>
<td>E6, E7, and interleukin 2 in recombinant vaccinia (cowpox virus)</td>
<td>Therapeutic</td>
<td>Phase II</td>
</tr>
<tr>
<td>Advaxis</td>
<td>E7 in attenuated, live <em>Listeria</em> vaccine</td>
<td>Therapeutic</td>
<td>Phase II</td>
</tr>
<tr>
<td>BioSidus</td>
<td>E7 fused to L1</td>
<td>Preventive and therapeutic</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Sanofi</td>
<td>Polymeric L2</td>
<td>Preventive</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Indian Immunologicals</td>
<td>L1 in recombinant typhoid vaccine</td>
<td>Preventive</td>
<td>Preclinical</td>
</tr>
<tr>
<td>Cadila</td>
<td>L1 in recombinant measles vaccine</td>
<td>Preventive</td>
<td>Preclinical</td>
</tr>
</tbody>
</table>

V503 9-Valent HPV Vaccine (Merck)

ClinicalTrials.gov Identifier: NCT01304498
- inizio arruolamento Gennaio 2011
- fine Dicembre 2011
- 600 ragazze di età 9-15 anni

ClinicalTrials.gov Identifier: NCT01254643
- inizio arruolamento Gennaio 2011
- fine Settembre 2013
- 100 ragazze Giapponesi di età 9-15 anni

Nessuna pubblicazione disponibile
### Raccomandazioni internazionali: vaccinazione di routine nel maschio

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinazione di routine per i bambini di 11 o 12 anni + catch-up dai 13 ai 21 anni</td>
<td>Vaccinazione nelle scuole per i ragazzi di 12-13 anni + 2 anni di catch-up per i ragazzi di 14-15 anni</td>
<td>Vaccinazione per i maschi dai 9 ai 26 anni (vaccinazione di routine tra i 9 e i 13 anni)</td>
</tr>
</tbody>
</table>

- Nel 2011, l’European Medicines Agency (EMA) ha dato parere positivo all’estensione delle indicazioni del vaccino quadrivalente nei maschi fino a 26 anni
- In Europa raccomandano la vaccinazione anche degli adolescenti maschi: Austria; Sassonia (da gen.2013)
33% of the women had high-risk HPV in the cervix and 55% had high-risk HPV in the anal canal.

23% women had high-risk HPV in both the cervix and anal canal, 31% had anal but not cervical high-risk HPV, 10% had cervical but not anal high-risk HPV, and 35% were negative for high-risk HPV at both sites.

In a multivariate analysis, factors associated with high-risk anal HPV included a history of cervical lesions, infection with HPV types other than HPV-16, a current CD4 count below 350 cells/mm3, and being in the 40-49 year age range.

Factors associated with high-risk cervical HPV included a history of cervical lesions, anal infection with HPV-16 or other types, nadir (but not current) CD4 count below 350 cells/mm3, and being from sub-Saharan Africa.
Human immunodeficiency virus and human papilloma virus - why HPV-induced lesions do not spontaneously resolve and why therapeutic vaccination can be successful
Sjoerd H van der Burg*1,2 and Joel M Palefsky1,2

needed in this area, HPV seems to alter transcriptional activity of the IFNβ and NFκB-pathways resulting in a decreased ability of keratinocytes to produce the necessary cytokines and chemokines to attract the adaptive immune system [48-50]. The identification of HPV-induced low-
tic progression [51]. In addition, the development of such lesions is associated with a locally altered cytokine environment with an increase in IL-10 and a decrease in proinflammatory cytokines [52-54]. The progression rate of

A comparison of immune presentation of opportunistic pathogens and HPV indicates that there is less inflammation and there are lower amounts of antigens available to the immune system with HPV infection. One could com-

Immunogenicità del vaccino HPV nelle donne HIV+, 16-23 anni

- Open-label, 48-wk phase II trial in HIV+ women, age 16-23 yrs (n = 99)
  - Group A: ART naive or no ART for ≥ 6 mos
  - Group B: on ART for ≥ 6 mos, with 2 VL < 400 c/mL
  - Historical controls: HIV- women aged 16-23 yrs (n = 267)
- All pts received quadrivalent HPV vaccine at Day 1, Wk 8, and Wk 24, then followed for 24 wks
- No AEs > grade 3 evaluated as related to vaccine

- High levels of vaccine seroconversion in both groups at 48 wks

Vaccinazione anti HPV nei maschi HIV+

AIDS Malignancy Consortium protocol 052: Singolo braccio, open label, multicentrico. Vaccino quadrivalente (sierotipi 6,11,16,18)

End point: 1. sieroconversione a 28 settimane, 2. Safety

Sieroconversione per tutti i 4 tipi: tipo 6 (98%), tipo 11 (99%), tipo 16 (100%), tipo 18 (95%)

Wilkin, 2010