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CAT
Critically Appraised Topic

Title: Value of treponemal tests in follow-up of therapy in patients with syphilis.

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CLINICAL BOTTOM LINE

Follow-up of patients with syphilis is based on non-treponemal tests (rapid plasma reagin (RPR) test / Venereal Disease Research Laboratory (VDRL) test). Guidelines recommend evaluation of the non-treponemal titer at fixed time points after diagnosis. A four-fold increase in non-treponemal titer suggests reinfection or treatment failure. Instead of the non-treponemal tests, possibly an anti-treponemal IgM enzyme-linked immunosorbent assay can be used to follow treatment response in early syphilis, although applicability in clinical practice is unclear.
In UZ Leuven, the Architect Syphilis TP assay is used as screening test for syphilis. This treponemal test is an immunoassay for the qualitative detection of IgG and/or IgM to *Treponema pallidum*. The use of this assay has not yet been investigated before for follow-up, as the result may remain positive many years, sometimes lifelong.
A retrospective study was set up to describe the response of the Architect Syphilis TP assay in follow-up of patients with syphilis. Fifty-three patients with diagnosis of syphilis were included. A significant increase in treponemal signal was observed at the time of diagnosis of syphilis. Of great interest, at 6,12 and 24 months after diagnosis of syphilis, the signal of the Architect assay showed a significant decrease compared with the signal at diagnosis, indicating a correlation between the signal of the Architect Syphilis TP assay and treatment response. However, further studies will be necessary to find out if the Architect Syphilis TP assay will be useful in clinical practice to follow-up patients with syphilis.

CLINICAL/DIAGNOSTIC SCENARIO

Clinical introduction

Syphilis is a sexually transmitted disease caused by the spirochete *Treponema pallidum (T. pallidum)*. The disease continues to challenge clinicians with its nuances in diagnosis and management. Moreover syphilis is still a public health problem worldwide and recent outbreaks have been reported in several European countries[1]. The WHO estimates 12 million new syphilis cases every year, more than 90% of them in developing countries with a rapidly increasing number of cases in Eastern Europe. The incidence of syphilis has been rising in western countries since the year 2000, after having decreased to a historical minimum in the early 1990s[2]. Cases in men who have sex with men (MSM), human immunodeficiency virus (HIV)-infected persons and immigrants account for the most of the recent increase in syphilis prevalence in Europe and the United States. In 2009, 488 syphilis cases were reported in Belgium by sentinel networks, which meant a stabilisation after several years of increase in reported cases[3].

Syphilis is a chronic disease with if untreated different clinical stages: primary syphilis, secondary syphilis, latent syphilis, late syphilis and congenital early and late syphilis in infants (table 1.1.).

Following infection with *T. pallidum*, the initial clinical manifestations are termed primary syphilis. Since most chancres are painless, many persons with primary syphilis do not come to medical attention. Even without treatment, chancres usually heal within a few weeks.

Table 1.1. Different clinical stages of syphilis in adult patients[4,5].

stadium syphilis	time after exposure	clinical manifestations
Early (infectious) syphilis		
primary	9-90 days	chancer, regional lymphadenopathy
secondary	6 weeks - 6 months	systemic illness (mucocutaneous rash (involving palms and soles), malaise,
	(4-8 weeks after primary lesion)	low grade fever, headache, lymphadenopathy, condylomata lata, …)
(early) latent	< 1 year	no clinical symptoms, positive serology
Late (non-infectious) syphilis		
(late) latent	> 1 year	no clinical symptoms, positive serology
tertiary	1-30 years	neurosyphilis, cardiovascular syphilis, gummatous syphilis

From weeks to a few months later, approximately 25 percent of untreated individuals develop systemic illness with multisystem involvement. The secondary bacteremic stage is followed by a latent period. Patients infected have no symptoms but have infection demonstrable by serological testing.
The tertiary or late stage of syphilis is less seen today in the era of effective and prevalent antibiotic therapy. In about one-third of untreated cases a symptomatic late syphilis develops. Tertiary syphilis is a term often used synonymously with late symptomatic syphilis, but excludes meningovascular syphilis[4].

Recommendations for the treatment of syphilis in adult patients are shown in table 1.2.

Table 1.2. Treatment recommendations for adult patients with syphilis. (based on Centers for Disease Control guidelines)[5]

stadium syphilis	recommended regimen for adults*
early syphilis	benzathine penicilline G 2,4 million units IM in a single dose
(primary, secondary,	
early latent syphilis)	
late latent syphilis	benzathine penicilline G 7,2 million units total, administered
	as 3 doses of 2,4 million units IM each at 1-week interval
tertairy syphilis	benzathine penicilline G 7,2 million units total, administered
	as 3 doses of 2,4 million units IM each at 1-week interval
neurosyphilis	aqueous crystalline penicillin G 18-24 million units per day,
	administered as 3-4 million units IV every 4 hours or
	continuous infusion, for 10-14 days

* HIV-positive patients and pregnant women should also be treated according to these stage-specific recommendations

Diagnostic introduction

On the basis of the Wasserman test introduced 100 years ago[6], syphilis diagnosis continues to rely on serological assays because *T. pallidum* cannot be cultured in vitro. Furthermore, direct visualisation of the spirochete, which is the gold standard method, requires lesions and either fluorescent antibodies or a dark-field microscope, neither of which may be readily available. *T. pallidum* nucleic acid amplification tests are developed, but polymerase chain reaction (PCR) is not widely available for use by clinical laboratories. It may be used on oral or other lesions where contamination with commensal treponemes is likely. As none of the serological tests for syphilis differentiate between venereal syphilis (caused by *T. pallidum* subspecies *pallidum*)) and the other treponematoses (*T. pallidum* subspecies *perteneu* (yaws), *T. pallidum* subspecies *endemicum* (endemic syphilis) and *T. pallidum* subspecies *carateum* (pinta)), PCR can help to differentiate.

Serologic syphilis testing can be divided in treponemal and non-treponemal tests.

- Non-treponemal tests

Non-treponemal tests use antigens containing cardiolipin, lecithin and cholesterol, which flocculate on reaction with IgM and IgG antibodies: respectively the rapid plasma reagin (RPR) test, the Venereal Disease Research Laboratory (VDRL) test and the toluidine red unheated serum test. Their basis is still imperfectly understood because the antigens are normal components of host cells in humans. Apparently, *T. pallidum* infection results in binding of host lipids to the treponeme, converting inert lipids into immunogens in vivo[7]. Seroconversion typically occurs within 21 days of exposure but may occur up to 6 weeks after infection[8]. Advantages to these tests are that they are inexpensive and simple to perform. Furthermore, quantitative titers can establish a baseline to evaluate treatment response. However, they require treponemal-based confirmation because detectable antibodies can be produced by other inflammatory conditions. Sensitivities vary depending on the type of test and stage of infection with lower sensitivities in primary syphilis and late syphilis[9]. False-positive results are associated with viral infections, pregnancy, malignant neoplasms, autoimmune disease and advanced age[10].

- Treponemal tests

Treponemal tests have historically been more complex and expensive to perform, so they have traditionally been used as confirmatory tests for syphilis when the non-treponemal tests are reactive. Recent versions of these tests have been automated, enhancing simplicity and facilitating ease of use. The early specific treponemal tests include fluorescent treponemal antibody absorption assay (FTA-ABS), microhemagglutination assay for antibodies of *T. pallidum* (MHA-TP), *T. pallidum* particle agglutination assay (TP-PA) and *T. pallidum* hemagglutination assay (TPHA). But in the 1970s Veldkamp and Visser recognized the potential for an automated *T. pallidum* enzyme-linked immunosorbent assay (EIA). Commercially available tests followed. Most of these tests now use recombinant antigen and detect total anti-treponemal antibody (IgG and IgM). An evaluation of 10 EIA demonstrated sensitivities of 94,7%-99,1% and specificities of 100%[11]. The newer chemiluminescence immunoassays are likely to have the same role as screening tests as the current EIA tests. Advantages of these tests are their high sensitivities, also in early syphilis, their high specificity and their suitability for automation. However, treponemal tests cannot distinguish among recent, remote and previously treated infections.

- Follow-up

No simple test is available that allows to determine that syphilis has been cured within days or weeks after treatment. To assess treatment, the patient is asked to return for repeated serologic testing and clinical evaluation at varying intervals. For monitoring the serologic response to treatment, a quantitative RPR/VDRL test is recommended.

In UZ Leuven the primary screening test is a treponemal test, the Architect Syphilis TP assay (Abbott Germany co., Germany). Architect Syphilis TP is a two-step immunoassay for the qualitative detection of IgG and/or IgM to *T. pallidum* in human serum or plasma using chemiluminescent microparticle immunoassay (CLIA) technology. If the primary screening test is positive, a confirmatory test (RPR) is done[12].
In case of suspicion of neurosyphilis, UZ Leuven uses the same screening algorithm as for serum. When the serum treponemal test is positive, the cerebrospinal fluid (CSF) and serum is sent to ULB hospital Erasmus where the VDRL titer is measured in both samples[13].

To evaluate treatment response, the screening algorithm is used, but the clinicians only use the RPR titer to evaluate treatment response.

The aim of this critically appraised topic was to review serological strategies to follow treatment response in patients with syphilis. The second aim was to determine if the Architect CLIA is useful for the follow-up of syphilis.

QUESTION(S)

1. What is the best serological strategy to follow treatment response in patients with syphilis?

2. Is the Architect treponemal test useful for follow-up of treatment in patients with syphilis?

SEARCH TERMS

1) MeSH Database (PubMed): MeSH term: "syphilis, syphilis serodiagnosis, latent syphilis, syphilis late, syphilis early, syphilis and HIV, syphilis and treatment, neurosyphilis"
2) PubMed Clinical Queries (from 1966; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi): Systematic Reviews; Clinical Queries using Research Methodology Filters (diagnosis + specific, diagnosis + sensitive, prognosis + specific)
3) PubMed (Medline; from 1966), SUM Search (http://sumsearch.uthscsa.edu/), National Guideline Clearinghouse (http://www.ngc.org/), Cochrane (http://www.update-software.com/cochrane
4) UpToDate Online version 18.3 (2010)

1. What is the best serological strategy to follow treatment response in patients with syphilis?

1.1. Literature and guidelines

For screening and diagnosis of syphilis, different guidelines recommend different screening test combinations[14]. In 1982 the World Health Organization recommended both a non-treponemal and treponemal test for syphilis screening and diagnosis. Syphilis guidelines in the United Kingdom now recommend screening with either an EIA or a combination of VDRL and TPHA tests, using IgM-specific EIAs when early primary syphilis is suspected[15,16]. Other European guidelines for syphilis recommend either an EIA or TPPA as a screening test[17].

In contrast with screening, different guidelines give similar recommendations for follow-up of therapy[18,19](table 1.3.).
A quantitative RPR or VDRL test is recommended and a treponemal IgM EIA can be used for patients with negative RPR/VDRL. Non-treponemal titers usually correlate with disease activity, and results should be reported quantitatively. A fourfold change in titer, equivalent to a change of two dilutions, is considered necessary to demonstrate clinically significant difference between non-treponemal test results that were obtained using the same serologic test. Sequential serologic tests in individual patients should be performed by using the same testing method, preferably in the same laboratory. The RPR and VDRL are equally valid assays but quantitative results from the two tests cannot be compared directly because RPR titers are slightly higher than VDRL titers.

Table 1.3. Overview of guidelines: follow-up of therapy in immunocompetent patients with syphilis. (CDC: Centers for Disease Control; IUSTI: International Union against Sexually Transmitted Infections; BASHH: British Association for Sexual Health and HIV; RPR: rapid plasma reagin; VDRL: Venereal Disease Research Laboratory; EIA: enzyme immunosorbent assay).

Guidelines	Recommended tests	Follow-up
CDC 2010[16]	quantitative RPR or VDRL	indicative for treatment failure: *primary and secondary syphilis (6 and 12 months after treatment)* sustained 4-fold increased titer failure to decline 4-fold within 6 months signs and symptoms persist or recur *latent syphilis (6,12 and 24 months)* titers increase 4-fold initially high titer fails decline 4-fold within 12-24 months signs and symptoms develop *tertiary syphilis* limited information clinical response and follow-up
IUSTI 2008[17]	quantitative RPR or VDRL anti-treponemal IgM EIA in RPR/VDRL negative persons	indicative for treatment failure: 4-fold increase in titer confirmed on second specimen *early syphilis (1,2,3,6 and 12 months after treatment)* titer should decline 4-fold within 6 months *late syphilis* serological response often absent
BASHH 2008[18]	quantitative RPR or VDRL	indicative for treatment failure: sustained 4-fold or greater increase in titer recurrence of signs and symptoms *early syphilis (1,2,3,6 and 12 months after treatment, 6-monthly until serofast or negative)* *late syphilis (3-monthly until serofast)*

A quantitative VDRL/RPR should be performed on a specimen taken on the day the treatment is started as this provides an accurate baseline for monitoring response to treatment. An increase fourfold in a non-treponemal test, confirmed on a second specimen suggests reinfection or treatment failure. However, serological tests do not allow to distinguish treatment failure from a new *T. pallidum* infection. Moreover the rate of decrease of serologic titers is influenced by many factors as the history of previous syphilis, the stage of infection, the baseline serologic titers, the immune status and the administered treatment[20,21,22,23]. Therefore, there is actually no available gold standard test to determine if a patient is cured, serological titers are merely surrogate criteria of the actual evolution of disease.

Overall the interpretation and comparison of different miscellaneous studies, where serological response to treatment was followed, is complex. Even when a similar test (RPR or VDRL) is used, there is a high variability of treatment failure rate across studies which seems to have different explanations[24]. A summary of different studies and different guidelines gives us the following interpretation of serology in the different stages of syphilis.

- In primary and secondary syphilis titer should decline fourfold within six months after treatment. However, about 15% or more of primary and secondary syphilis HIV-negative patients do not have a fourfold decrease at 1 year after treatment, the significance of which is unknown[25]. In some patients non-treponemal antibodies can persist at a low titer for a long period of time, sometimes for the life of the patient (serofast reaction). Non-treponemal test titers might decline more slowly for persons who have previously had syphilis. Moreover decline in RPR dilutions also depends on the initial titer.
- In the follow-up of latent syphilis, quantitative non-treponemal tests should be repeated at 6,12 and 24 months. Small studies show less serological response in latent syphilis patients[26], however some guidelines recommend re-treatment in some cases. Patients with normal CSF examination should be re-treated if 1) titers increase four-fold, 2) an initial high titer (≥1/32) fails to decline at least fourfold within 12-24 months of therapy, or 3) signs or symptoms attributable to syphilis develop[16].
- Little is known about the serological non-treponemal response in late symptomatic syphilis. Only the BASHH guideline proposes to test three monthly until the patient is serofast.

The majority of patients who have reactive treponemal tests will have reactive tests for the remainder of their lives, regardless of treatment or disease activity.

1.2. Immunological anti-treponemal response

As guidelines recommend non-treponemal tests to follow-up syphilis, one can assume that the treponemal antibody responses are not useful to follow response of therapy in patients with syphilis. However, it is likely that IgM can play a role in the follow-up.

In the '80s several studies examined the anti-lipoidal and anti-treponemal antibody responses in patients with syphilis. The outer membrane of *T. pallidum* contains few proteins, minimizing surface-localized antigenic targets recognized by host antibodies or immune cells. The inflammatory effects are primarily mediated by certain membrane lipoproteins (TpN47, TpN17 and TpN15)[27]. TpN47 is highly immunogenic and activates endothelial cells, TpN17 and TpN15 induce antibody responses[28].
Serum IgM and IgG antibody responses to *T. pallidum* have been studied extensively in experimentally infected animals and humans[12]. Anti-treponemal IgM antibodies are produced approximately 2 weeks after exposure, followed by IgG antibodies 2 weeks after IgM production[29]. Both anti-treponemal IgM and IgG antibodies may be detectable within 3 days of lesion onset in primary syphilis[30]. Early responses are against TpN7 and some of the flagellar proteins, followed by TpN15 and TpN17[12]. IgG antibodies appear to be increased in patients with a longer duration of symptoms[15].

Patients with treated syphilis usually maintain anti-treponemal antibodies in their serum for years after they received adequate antibiotic therapy. However, 15-25% of patients treated during the primary stage revert to being serologically nonreactive after 2-3 years[31]. The persistent antibodies are presumably of the IgG class, specific IgM antibodies reportedly disappear after treatment. Merlin et al. showed that IgM antibodies subside and disappear within three months of treatment in patients with primary syphilis, and within two to 22 months in patients with secondary syphilis[32]. Other studies have shown that the time between starting treatment for primary syphilis and the disappearance of IgM antibodies was: one month[33], three to six months[34], and up to 12 month[35]. With respect to secondary syphilis, the interval ranged from four to eight months[33], or from three to 18 months[34,35].

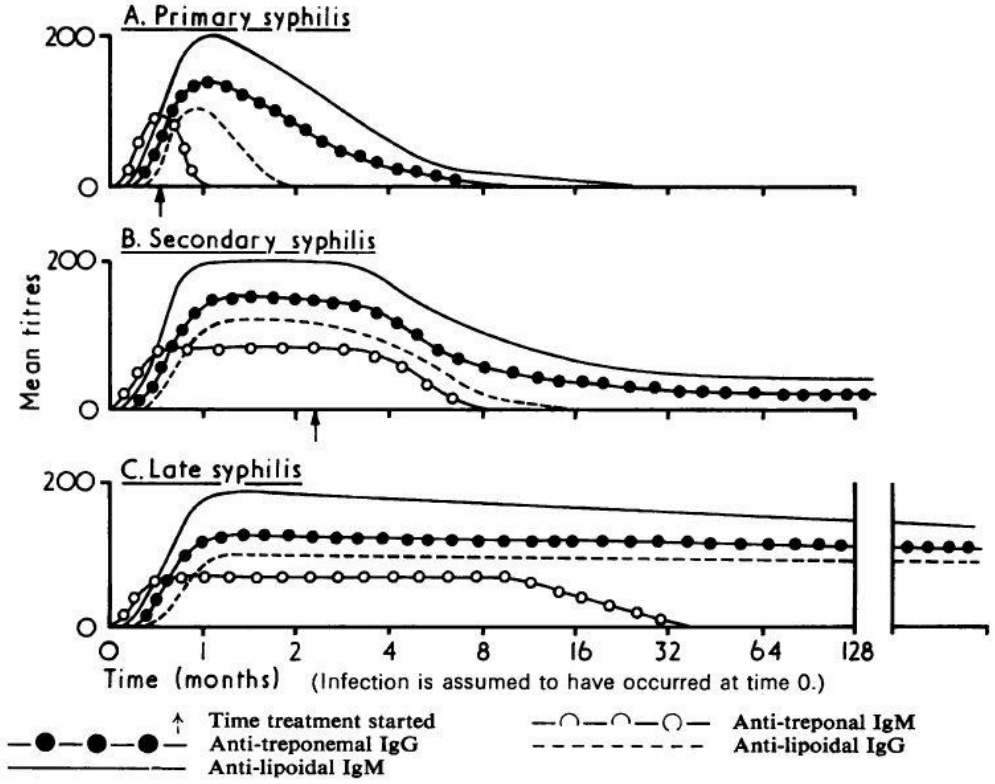


Figure 1.1. Time course of immunological response to syphilis and the influence of treatment[25].

Shannon et al. build up a picture of the time course of the typical immune response as reflected by the pattern of reactions and mean titers obtained from different serum tests[33]. Figure 1.1. illustrates an idealised version of serum antibodies of a case of treated primary syphilis. In this case, treatment began three weeks after infection. Had the treatment started earlier -that is before the production of lipoidal antibodies- it is likely that the response would have declined rapidly, thus leaving only anti-treponemal IgG showing a positive reaction. In patients with secondary syphilis all tests were positive before treatment and declined more slowly than in patients in the primary stage. Figure 1.1. graphically illustrates the idealised immunological history of a case of late syphilis. The presence of lipoidal IgG without treponemal IgM was characteristic of all cases of late syphilis.

Although examination of sera for anti-treponemal IgM antibodies may provide a useful indication of the active state of the disease in primary and secondary cases of syphilis, it appears to be unreliable in patients at later stages. Treatment of patients with latent syphilis usually produces a reduction in antibody concentrations to give the anti-lipoidal IgM and anti-treponemal IgG picture that is typical for successful treatment. When the patient enters the late symptomatic stage of the disease the anti-lipoidal IgM and IgG and anti-treponemal IgG responses remain typically raised and are unaffected by treatment. The persistence of the immune response in late syphilis may be due to a reservoir of treponemes in locations inaccessible to antibiotics or to their presence in non-dividing vegetative state and thus late syphilis may not respond serologically to treatment[36].

Different treponeme-specific IgM assays were developed. The 19S(IgM)FTA-ABS test, which is highly sensitive and specific for treponemal IgM antibodies in all stages of syphilis, is considered a reference method[37]. Lefevre et al. showed that in early stages of syphilis, it could be replaced by the Captia Syphilis-M assay, which is easier to perform and more economical. A decrease in IgM antibody as the stage of syphilis increased is reported by different authors[38]. In the case of reinfection the sensitivity of the Captia ELISA is 62% compared with the 19S(IgM)FTA-ABS. Nonreactive (IgM) FTA-ABS tests with sera from reinfected patients and patients with latent syphilis have also been reported[38,39]. Lefevre et al. stated that testing for anti-treponemal IgM is useful for detecting active disease early but also that the absence of IgM antibodies to treponemes does not imply that syphilis is absent.

Detection of IgM antibody is likely to be important for treatment follow-up or in surveillance[37,40]. Any appreciable decrease in IgM antibodies may be interpreted as evidence of therapeutic success. However, recommendations regarding the applicability of IgM testing for syphilis require further investigation.

1.3. Serological testing in HIV infected patients

In many geographical areas, up to half of patients with syphilis are co-infected with HIV. Unusual serologic responses have been observed among HIV-infected persons who have syphilis. The majority of reports have involved serologic titers that were higher than expected, but false negative serological test results and delayed appearance of seroreactivity also have been reported[41,42]. Unfortunately, there is a lack of large studies appraising the effect of HIV on the course of syphilis since the advent of highly active retroviral therapy (HAART). Indeed, the emergence of HAART in 1996 has deeply changed the clinical and biological impact of HIV infection[43]. For instance, in a large observational clinical cohort of HIV-infected patients, Ghanem et al recently demonstrated that the use of HAART was associated with a 60% reduction in the rate of serologic failure[44].
Guidelines recommend the same treatment for HIV positive as for HIV negative patients. As HIV positive patients who have early syphilis might be at increased risk for neurologic complications, careful follow-up for the development of symptomatic neurosyphilis after therapy is essential.

2. *Is the Architect treponemal test useful for follow-up of treatment in patients with syphilis?*

2.1. Materials and methods

Architect Syphilis TP assay is a two-step immunoassay for the qualitative detection of IgG and/or IgM to *T. pallidum* in human serum or plasma using CLIA technology. The details of the treponemal test are described in attachment 3. If the chemiluminescent signal in the specimen (S) is greater than or equal to the cut-off signal (CO), the specimen is considered reactive for anti-*T. pallidum*. Specimens with a S/CO value < 1,0 are considered nonreactive, specimens with S/CO ≥ 1,0 are considered as reactive[45].

2.2. Patient selection

All adult patients with syphilis presented at the department of Internal Medicine of UZ Leuven between December 2008 and December 2010 where retrospectively identified. Of the 95 patients who had more than one positive treponemal test, 41 patients without a treponemal test at time of diagnosis were excluded. Fifty-four patients who had a treponemal test at time of diagnosis, and one of more test results before and/or after diagnosis were included. Patient characteristics are described in table 2.1. In total 61 different syphilis cases are described in these 54 patients.

Table 2.1. Patient characteristics.

number of patients	54
age (year)	43 (28-59)
gender (male/female)	50/4
HIV status (positive/negative)	41/13
stadium first diagnosis in time period	
primary syphilis	9
relapse/reinfection	6
HIV positive	7
secondary syphilis	18
relapse/reinfection	6
HIV positive	14
latent syphilis	20
relapse/reinfection	12
HIV positive	18
tertiary syphilis	7
relapse/reinfection	0
HIV positive	2
total number of samples	291

Clinical diagnosis of syphilis is based on the combination of clinical signs of syphilis and an increased (positive) RPR result. In some cases, start of therapy was some months later after diagnosis. The treatment strategy in UZ Leuven was according to the CDC guidelines (table 1.2.).

2.3. Results

2.3.1. Treponemal test in different stadia of syphilis

Treponemal signals of all selected patients were analyzed in function of time and stage of infection.

2.3.1.1. Patients with primary syphilis

Nine patients had an initial diagnosis of primary syphilis in the selected time period (figure 2.1.). All of them showed a decline of signal in the treponemal test following therapy. The decline of signal was in all patients with follow-up samples minimum 5 S/CO between the sample at diagnosis and the last follow-up sample.

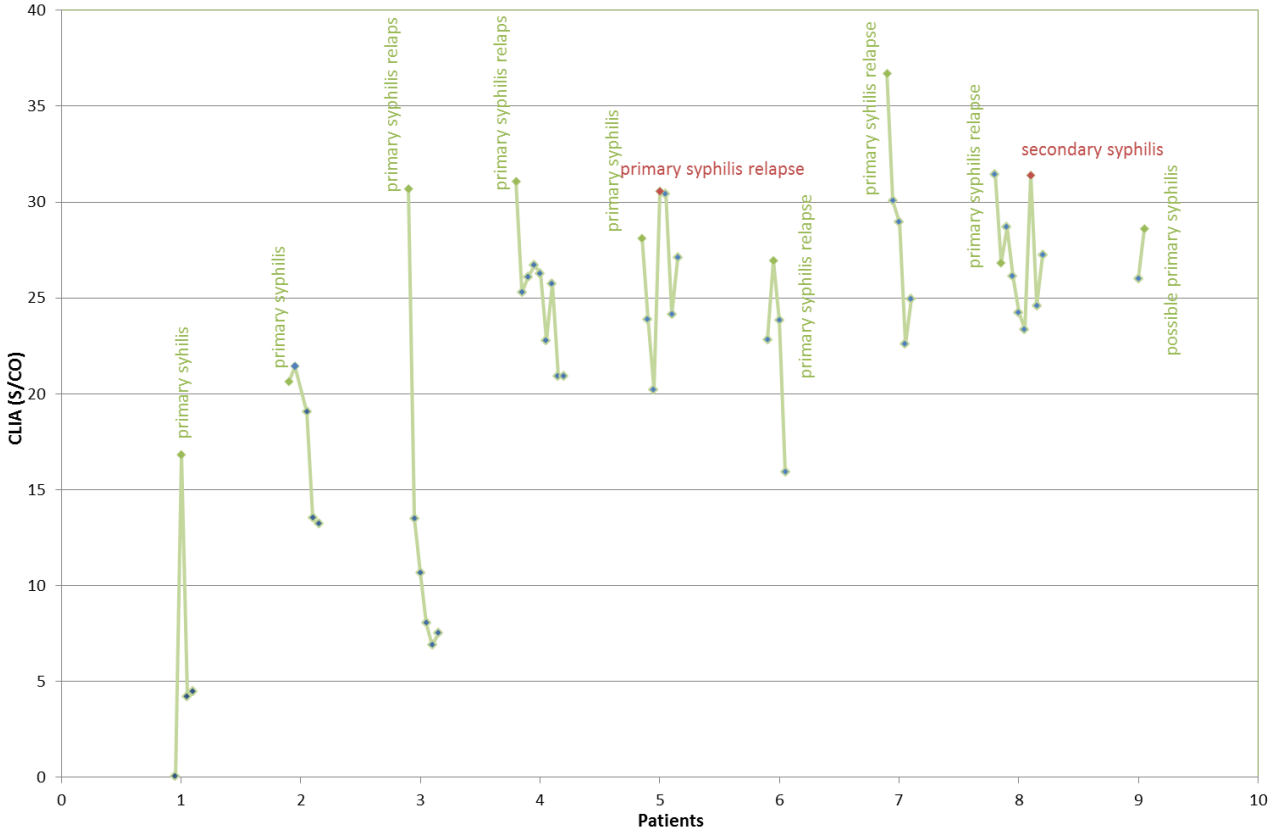


Figure 2.1. Treponemal test results in time course of follow-up of patients with primary syphilis (n=9). (green: test result at diagnosis primary syphilis; red: test result at diagnosis of relapse)

Two patients had an increase of the treponemal signal, which corresponded with a new diagnosis of respectively primary (patient 5) and secondary syphilis (patient 8). The RPR and treponemal test results are shown in more detail in table 2.2.

Table 2.2. Treponemal and non-treponemal test results from two patients with relapse during follow-up of primary syphilis.

patient	time before/after diagnosis (days)	CLIA (S/CO)	RPR(titre)	clinical information
patient 5	**0**	**28,11**	**1/64**	primary syphilis - start treatment
	103	23,88	1/4	evolution after treatment for primary syphilis
	194	20,23	1/1	evolution after treatment for primary syphilis
	292	**30,57**	**1/64**	primary syphilis, relapse, start treatment
	301	30,43	1/64	evolution (short) after treatment for primary syphilis relapse
	383	24,13	1/16	evolution after treatment for primary syphilis relapse
	456	27,1	1/8	evolution after treatment for primary syphilis relapse
patient 8	-66	31,45	1/2	evolution after treatment for secundary syphilis (second relapse)
	0	**26,83**	**1/4**	primary syphilis, 3th relapse - start treatment
	18	28,68	1/8	evolution after treatment for primairy syphilis
	95	26,13	1/1	evolution after treatment for primairy syphilis
	176	24,25	1/1	evolution after treatment for primairy syphilis
	207	23,37	1/2	evolution after treatment for primary syphilis, no clinical signs of relapse or reinfection.
	557	**31,40**	**1/512**	secondary syphilis, 4th relapes - start treatment
	641	24,60	1/64	evolution after treatment for secondary syphilis
	759	27,25	1/8	evolution after treatment for secondary syphilis

As a conclusion, the treponemal signal shows a good correlation with disease activity in patients with primary syphilis.

2.3.1.2. Patients with secondary syphilis

Eighteen patients presented with an initial secondary syphilis in the selected time period (figure 2.2.). Three of the 18 patients developed after diagnosis of secondary syphilis another stadium of syphilis. Patient 12 had a secondary syphilis relapse, patient 15 an early latent relapse and patient 16 a not further defined relapse (probably gummata). At time of diagnosis of these relapses, the treponemal signal was in 2 of the 3 patients higher than in the last follow-up sample. In one patient (patient 15) the increase in the treponemal signal (25,73 – 29,95 S/CO) preceded one month the new diagnosis based on RPR and clinical findings.
In follow-up, most of the patients had a significant decline in treponemal signal. Moreover patient 12 had a negativation of the treponemal test at only one year after start therapy.
However, 3 patients had an increase of S/CO in follow-up, which could not be explained by increased RPR or the development of clinical symptoms. Further studies will have to clear out these findings.

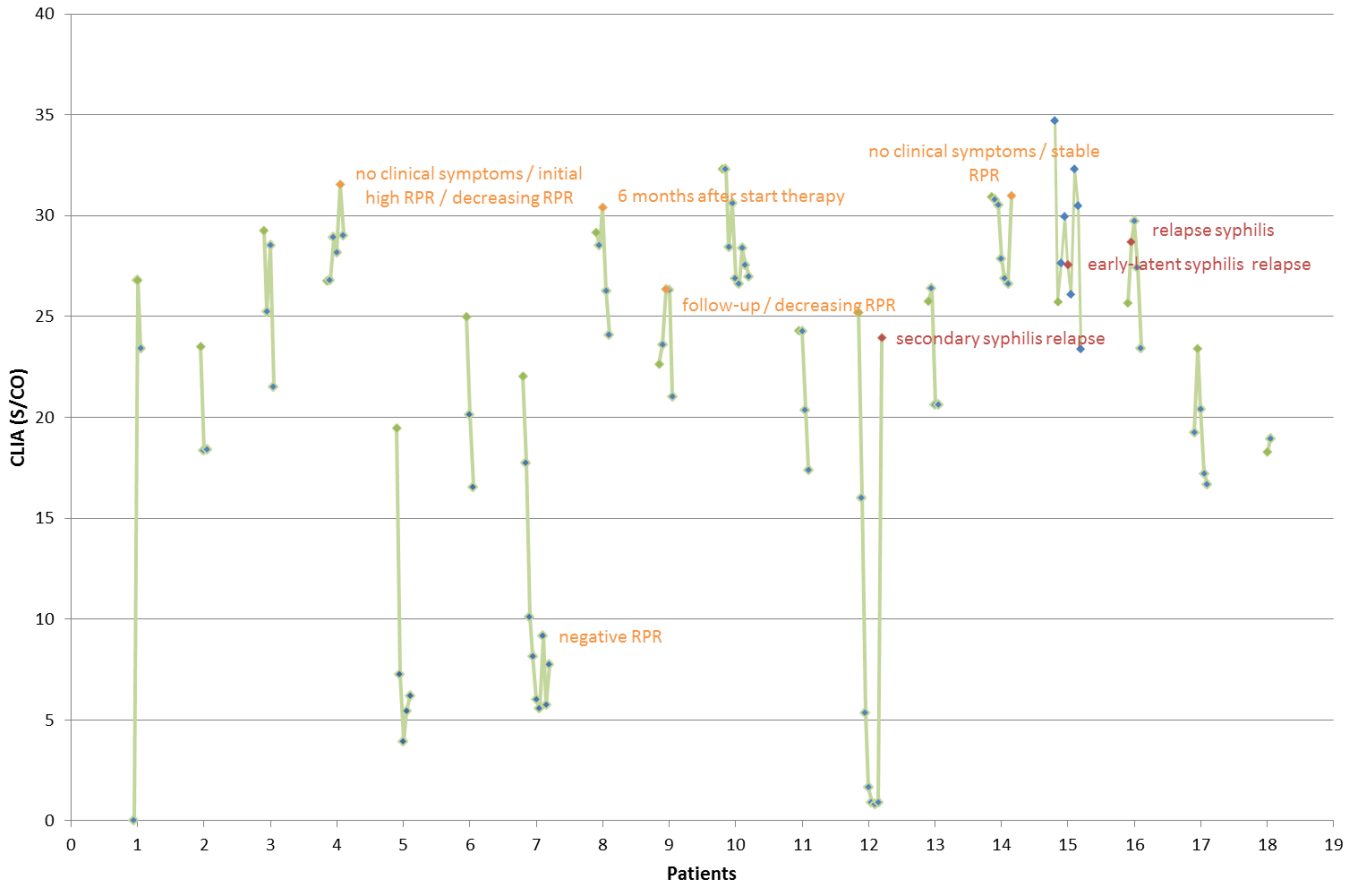


Figure 2.2. Treponemal test results in time course of follow-up of patients with secondary syphilis (n=18). (green: test result at diagnosis secondary syphilis; orange: increased test result without diagnosis; red: test result at diagnosis of relapse)

2.3.1.3. Patients with latent syphilis

Figure 2.3. shows the treponemal test results of 20 patients who were diagnosed in the selected time period with latent syphilis. Three patients had a new syphilis diagnosis in the period of follow-up. Patient 4 developed a primary syphilis, patients 16 and 17 developed an early-latent syphilis by reinfection. These patients had a treponemal signal that was higher than in the last follow-up sample, indicating a correlation of disease activity. At time of diagnosis of the new event, only patient 4 (primary syphilis) had a higher treponemal signal (29,08 S/CO) than at diagnosis of latent syphilis (26,33 S/CO).
Three patients (patient 1,8 and 12) had a complete negativation of the RPR at the end of the follow-up. In correlation, also the signal of the treponemal test decreased significantly in these patients. The other patients had a RPR that remained stable in the last follow-up samples. Also a stabilization of the treponemal signal was seen. However, a higher signal in follow-up samples than at time of diagnosis was seen in two patients (patient 13 and 19) although the RPR remained stable (positive) and no clinical signs of persistence or reinfection were present.
Two patients (patient 7 and patient 17) had already an increased signal in the sample preceding the sample at diagnosis. Patient 7 had a positive treponemal test (5,62 S/CO) in the first sample, although RPR was negative and

the patient had no clinical signs of disease activity. Six months later, the treponemal signal had increased (30,81 S/CO) and RPR was positive (1/64), making the diagnosis of early-latent syphilis. In this patient the treponemal test suggested a syphilis infection before the RPR had the time to become positive. Patient 17 had a higher signal prior diagnosis than at diagnosis, although in this patient interpretation is difficult. Treponemal signals of the different samples are close to each other.
In general a correlation is seen between disease activity and treponemal signals in patients with latent syphilis, although some patients have unexplainable test results.

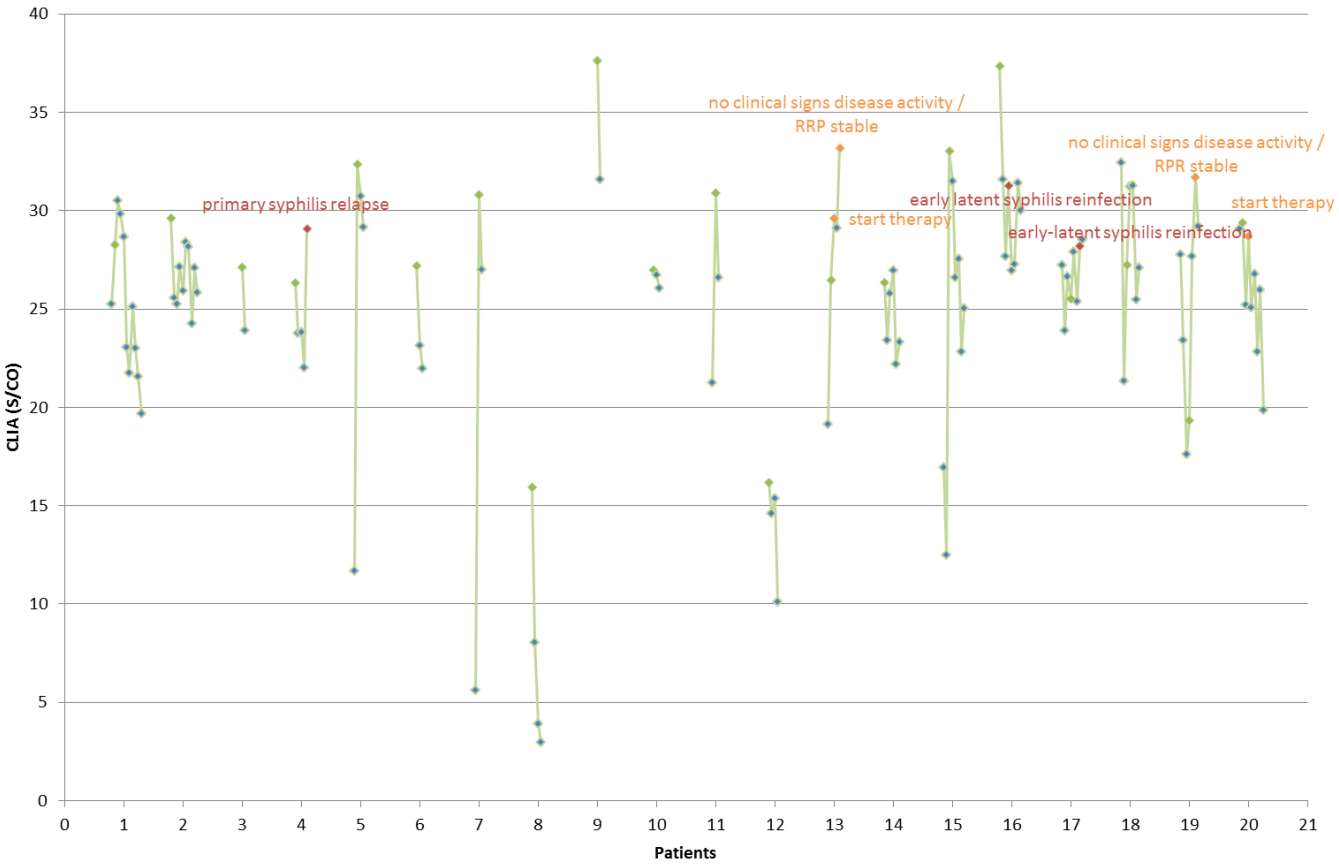


Figure 2.3. Treponemal test results in time course of follow-up of patients with latent syphilis (n=20). (green: test result at diagnosis latent syphilis; orange: increased treponemal test result without diagnosis of relapse or reinfection; red: test result at diagnosis of relapse or reinfection)

2.3.1.4. Patients with tertiary syphilis

Seven patients were diagnosed with tertiary syphilis among which 3 patients were diagnosed with neurosyphilis (figure 2.4.). All patients showed a decrease in treponemal signal in response to therapy. One patient with neurosyphilis (patient 7) had a small increase during follow-up (25,31–28,40 S/CO). In contrast, the RPR decreased with one titer at that moment, which means a disconcordance between the treponemal test and the RPR titer.
Although a relative small number of patients with tertiary syphilis were included, one can conclude that after therapy the treponemal signal decreases in function of the time and in correlation to the RPR.

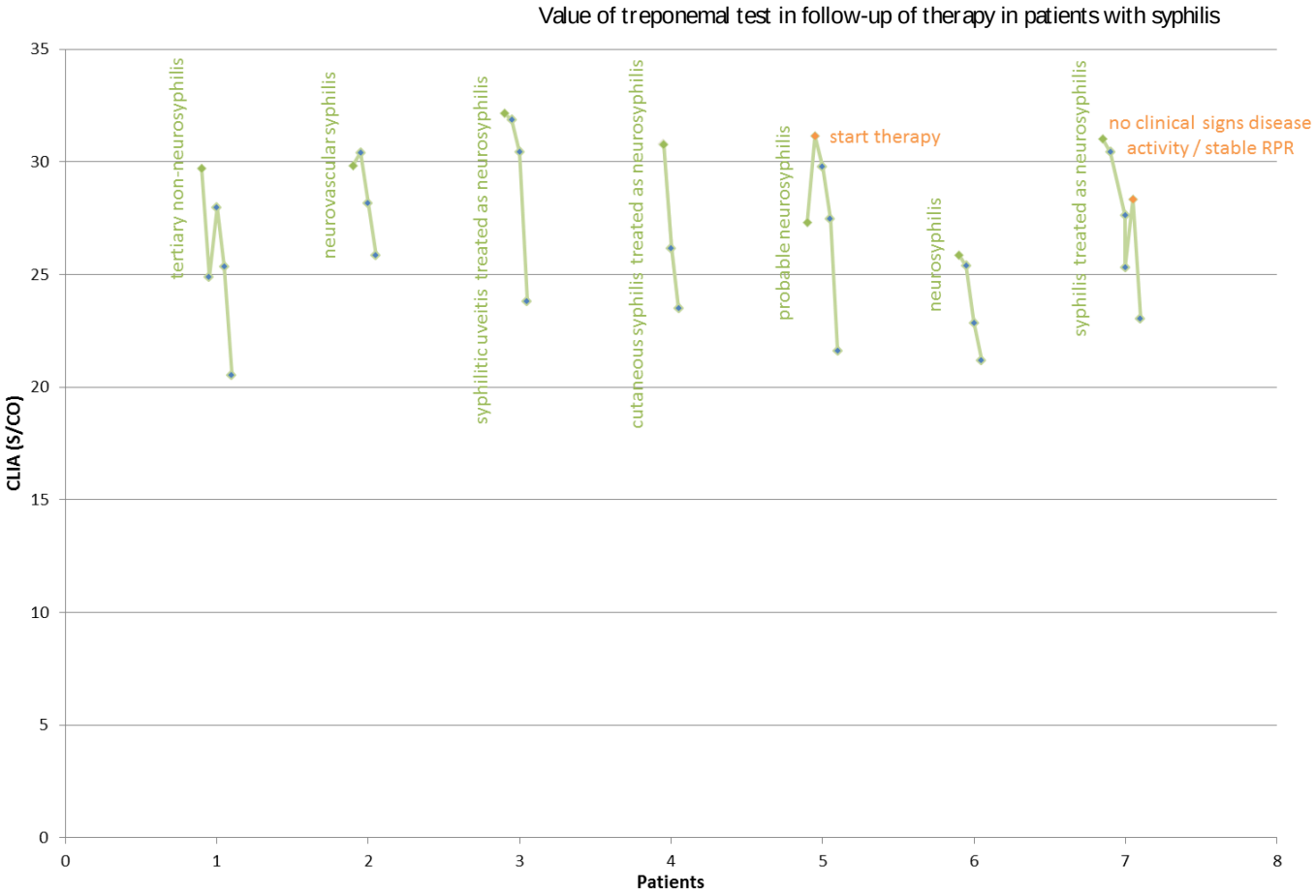


Figure 2.4. Treponemal test results in time course of follow-up of patients with tertiary syphilis (n=7). (green: test result at diagnosis; orange: increased test result without diagnosis of relapse or reinfection).

2.3.2. Treponemal test in follow-up

In this chapter, data from patients were reduced to data from single infections (diagnosis of syphilis), making 61 different syphilis cases in 54 patients. Characteristics of the different cases are summarized in table 2.3.

Table 2.3. Characteristics of infections used for analysis of Architect TP assay; prior diagnosis and in follow-up at 6,12 and 24 months after diagnosis.

infection cases	**total**	**prior**	relapse/ reinfection	HIV+	**6 months**	relapse/ reinfection	HIV+	**12 months**	relapse/ reinfection	HIV+	**24 months**	relapse/ reinfection	HIV+
primary syphilis	11	6	5	4	6	4	6	4	3	3	4	3	3
secondary syphilis	21	4	3	4	13	7	10	9	4	8	8	4	8
latent syphilis	23	13	11	13	16	11	16	13	8	12	7	4	6
tertiary syphilis	5	-	-	-	4	0	1	2	0	1	1	0	0
undefined	1	1	1	1	1	1	1	-	-	-	-	-	-
total	61	24	20	24	40	23	34	28	15	24	20	11	17

2.3.2.1. Treponemal test results prior to diagnosis in relationship to results at diagnosis

Twenty-three infection cases had a serum sample 5-330 days before diagnosis of syphilis. In cases with more samples before diagnosis, the sample closest to time of diagnosis was selected. The treponemal test results at diagnosis were significantly higher than before diagnosis (Wilcoxon, p<0,01) (figure 2.5.). However, 3 patients had a decrease in treponemal signal. These patients had already been diagnosed prior with syphilis (12, 14 and 15 months earlier). Of interest is that these three infection cases had the highest treponemal signal compared to the other cases. Possibly the high treponemal signal already indicates a new diagnosis, before the RPR has had the time to increase.

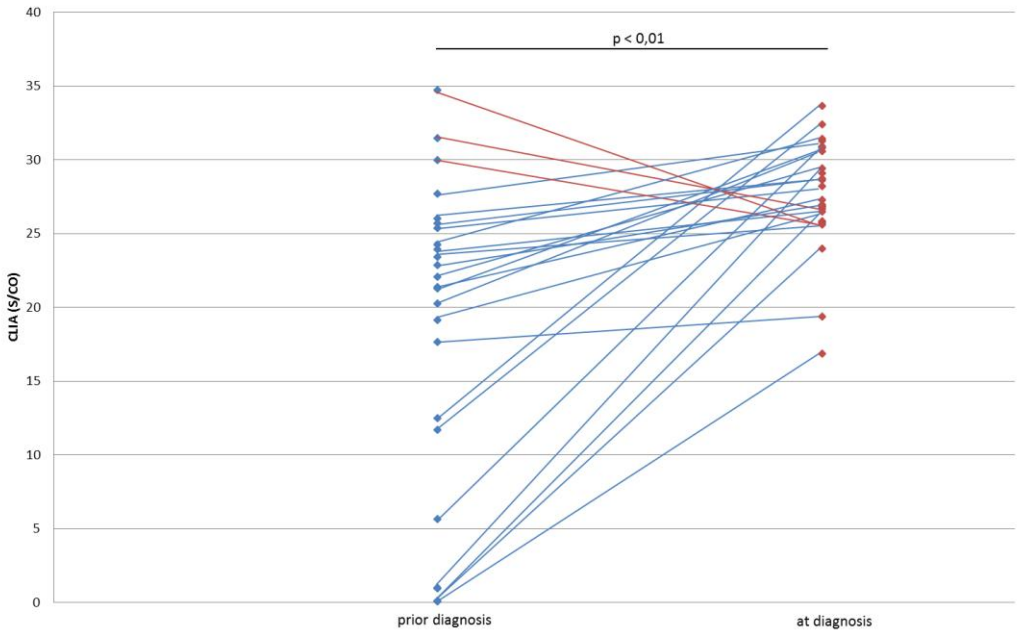


Figure 2.5. Evolution of treponemal test results prior diagnosis and at diagnosis (n=23). (red: decrease of treponemal signal; blue: increase of treponemal signal)

2.3.2.2. Treponemal test results after 6,12 and 24 months in relationship to results at diagnosis.

Infection cases that had one or more test results in the period of 150-300 days after diagnosis (6 months), 300-500 days after diagnosis (12 months) and 500-800 days after diagnosis (24 months) were selected. Data are shown respectively in figure 2.6, 2.7 and 2.8.

2.3.2.2.1. Treponemal test results at diagnosis compared with treponemal test results after 6 months.

After 6 months, results of the Architect Syphilis TP assay were significantly lower than at diagnosis (Wilcoxon, p<0,01) (figure 2.6.).

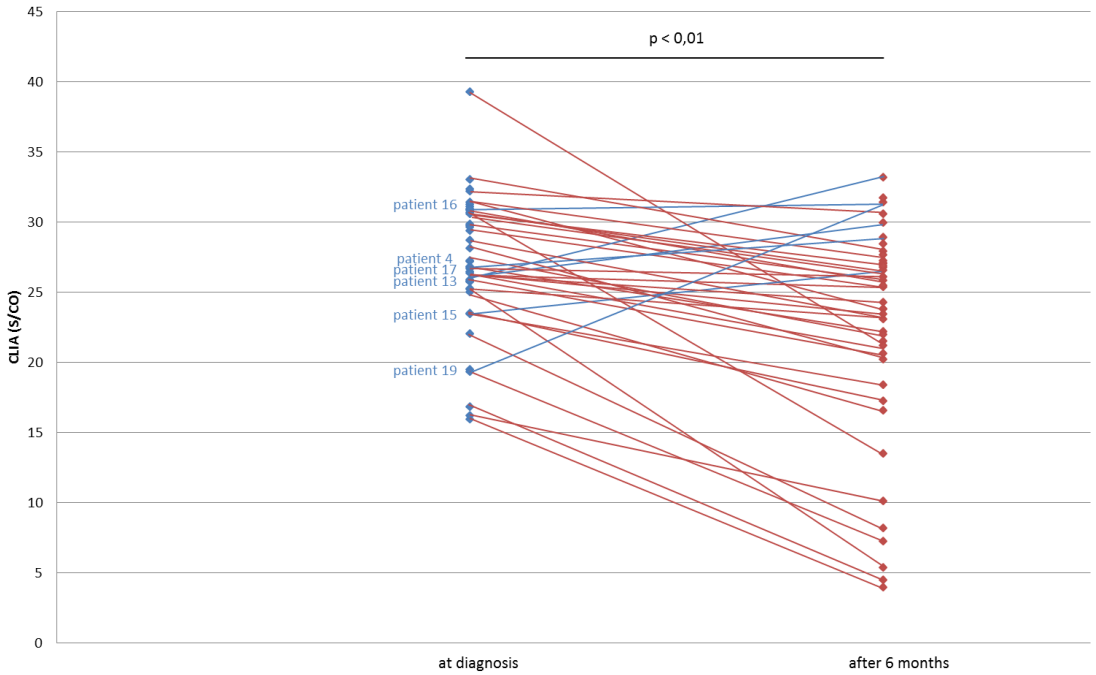


Figure 2.6. Evolution of treponemal test results 6 months after diagnosis (n=40). (red: decrease of treponemal signal; blue: increase of treponemal signal)

However, 6 out of 40 cases showed an increase in treponemal signal. Four of them were diagnosed with latent syphilis (patient 13,16,17 and 19 in figure 2.3.) and two cases were diagnosed as secondary syphilis by the clinician (patient 4 and 15 in figure 2.2.). All 6 patients had however at least a two-fold decrease in RPR titer, indicating a slower response of the treponemal test in these patients.
Possibly the increased signal from the case of patient 15 could be explained by the fact that 3,5 months later early-latent syphilis was diagnosed.

2.3.2.2.2. Treponemal test results at diagnosis compared with treponemal test results after 12 months.

Likewise, after 12 months, the treponemal signal was significantly lower than at diagnosis (Wilcoxon, p<0,01) (figure 2.7.). However, 5 out of 28 cases showed an increase. One patient was initially diagnosed with primary syphilis (patient 7 in figure 2.1.), two with secondary syphilis (patient 4 and 14 in figure 2.2.), one patient with latent syphilis (patient 19 figure 2.3.) and one case was diagnosed as neurosyphilis (patient 5 in figure 2.4.). However, in 2 out of these 5 patients (patient 7 and patient 5), the treponemal signal did decrease after 24 months, indicating slower treponemal response, compared with the RPR.

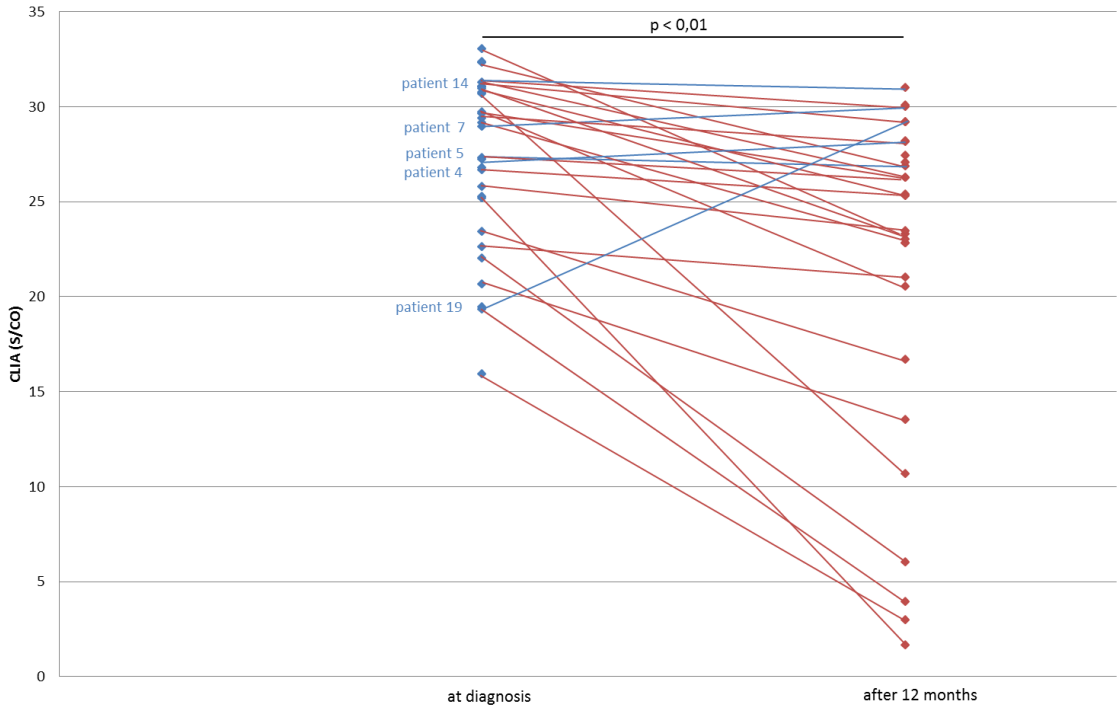


Figure 2.7. Evolution of treponemal test results 12 months after diagnosis (n=28). (red: decrease of treponemal signal; blue: increase of treponemal signal)

2.3.2.2.3. Treponemal test results at diagnosis compared with treponemal test results after 24 months.

After 24 months only one patient (patient 4, secondary syphilis) had a small increase in treponemal signal (26,77-29,2 S/CO) (figure 2.8.). Although, in this patient, RPR had decreased four-fold in one year, and no clinical symptoms indicated disease activity. In general, the treponemal test results were significantly higher at diagnosis than after 2 years (Wilcoxon, p<0,01).

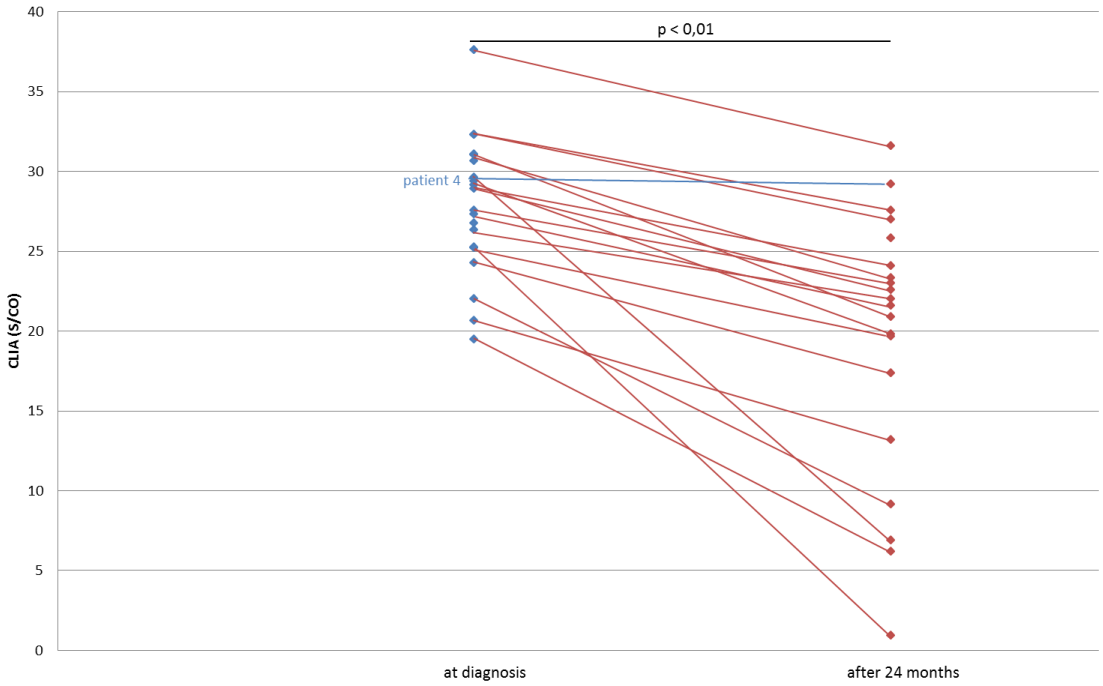


Figure 2.8. Evolution of treponemal test results 24 months after diagnosis (n=20). (red: decrease of treponemal signal; blue: increase of treponemal signal)

2.4. Economic impact of treponemal and non-treponemal tests in UZ Leuven

The total cost for the BD RPR Macro-Vue test (€9,84/test) is approximately two-fold the cost for the Architect Syphilis TP Assay (€4,82/test). Details are shown in attachment 4. The higher cost of the RPR test is due to the high labor cost, as this is a manual test, and the small number of tests, making a higher distribution cost.

2.5. Conclusion

The findings of this study show a significant response of the Architect Syphilis TP signal to treatment. In some cases, an earlier response of the treponemal test, indicating relapse or reinfection, was seen compared to the RPR. On the other hand, in a number of patients, the treponemal signal had a slower response to treatment compared with the RPR test. However, larger studies are needed to determine if this test, originally developed as screening test, is useful for the follow-up of syphilis in clinical practice. There were several limitations in our study. Primary, patients were retrospectively identified. Secondary, only a small number of patients was tested, whether a majority of the patients had already had a syphilis infection and were HIV positive. Thirdly, a relative small time period was selected to follow patients. It will be important in new studies to include patients with and without HIV and newly diagnosed and reinfection cases. A comparison with the RPR and clinical signs and symptoms will be necessary to calculate sensitivities and specificities, making it possible to calculate the performance of the Architect Syphilis TP assay in follow-up.

COMMENT

This retrospective study was approved by the Ethics Committee/IRB UZ K.U.Leuven.

TO DO/ACTIONS

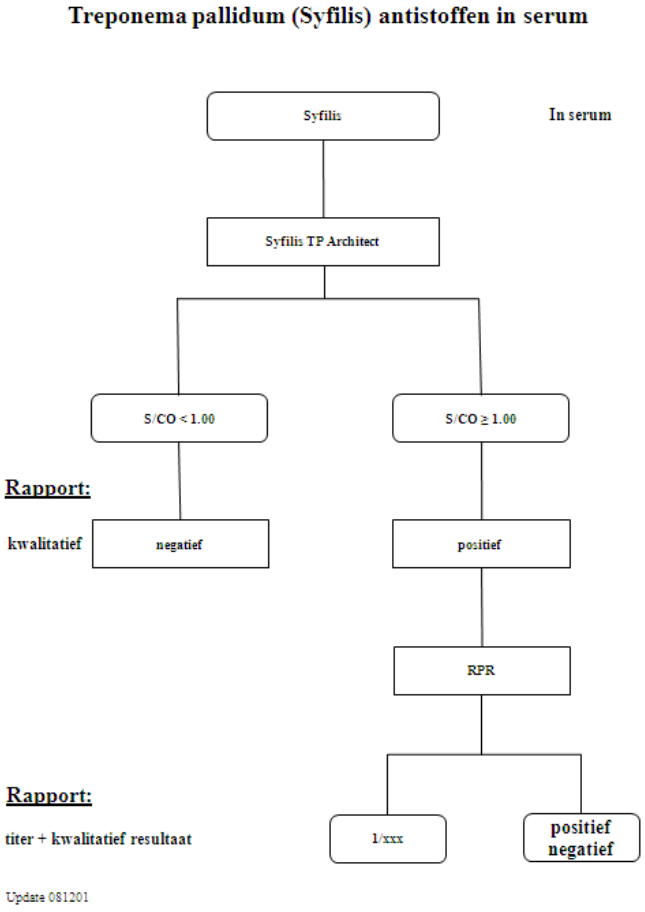
1) Writing down these findings in the form of a letter which can be published in an international journal.

2) A larger study will have to clear out if the Architect Syphilis TP assay may be useful in clinical practice. The use of a golden standard, coupled with the calculations of sensitivities and specificities will give more information about the performance of this test in follow-up.

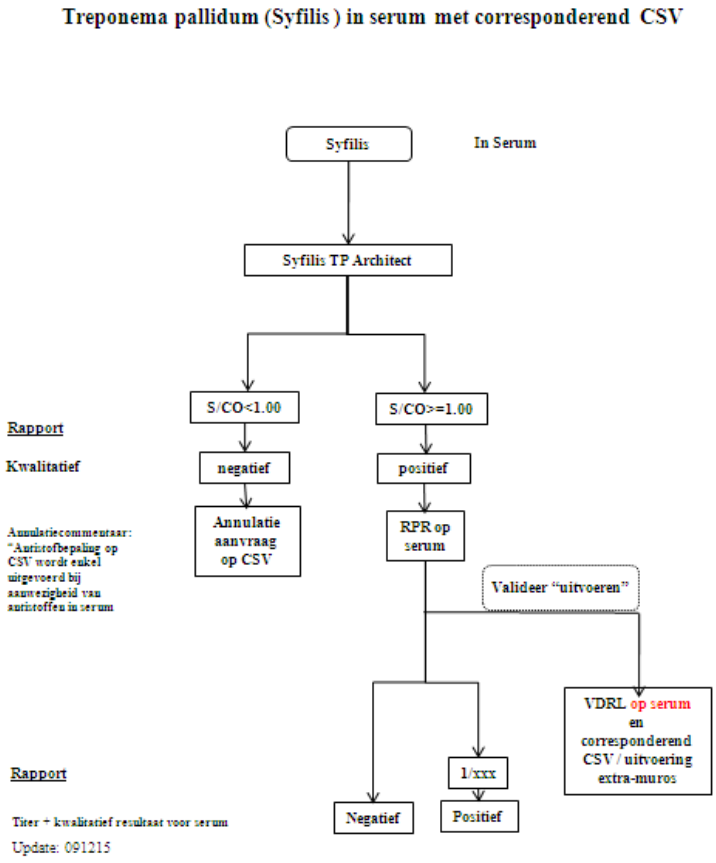
3) For the time being there will be no change in reporting the treponemal test result. Only the qualitative result (positive/negative) will be reported, pending more clinical evidence.

ATTACHMENTS

Attachment 1: Flowchart used in UZ Leuven for screening serum for syphilis[12]



Attachment 2 : Flowchart used in UZ Leuven for screening serum and correspondent cerebrospinal fluid for syphilis[13]



Attachment 3 : Procedure Architect TP Syphilis assay

In the first step, sample microparticles coated with recombinant *T. pallidum* antigens (TpN15,TpN17 and TpN47) that detect serum IgG and IgM antibodies against three antigenic domains of *T. pallidum* and diluent are combined. Anti-*T. pallidum* antibodies present in the sample bind to the coated microparticles. After washing, the acridinium-labelled anti-human IgG and IgM conjugate is added in the second step (minimum concentration in conjugate (anti-IgG) 26,6 ng/mL / (anti-IgM) 1,34 ng/mL.). Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-*T. pallidum* antibodies in the sample and the RLUs detected by the Architect immunoassay optical system. The presence or absence of anti-*T. pallidum* antibodies in the specimen is determined by comparing the chemiluminescent signal (S) in the reaction to the cut-off signal (CO) determined from a previous Architect Syphilis TP calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cut-off signal, the specimen is considered reactive for anti-*T. pallidum*. Specimens with a S/CO values < 1,0 are considered nonreactive, specimens with S/CO ≥ 1,0 are considered as reactive.

Attachment 4 : Total costs of Architect Syphilis TP assay and BD Macro-Vue RPR Card Test in UZ Leuven (2011). (MLT, medical laboratory technologist; MSU, medical supervision)

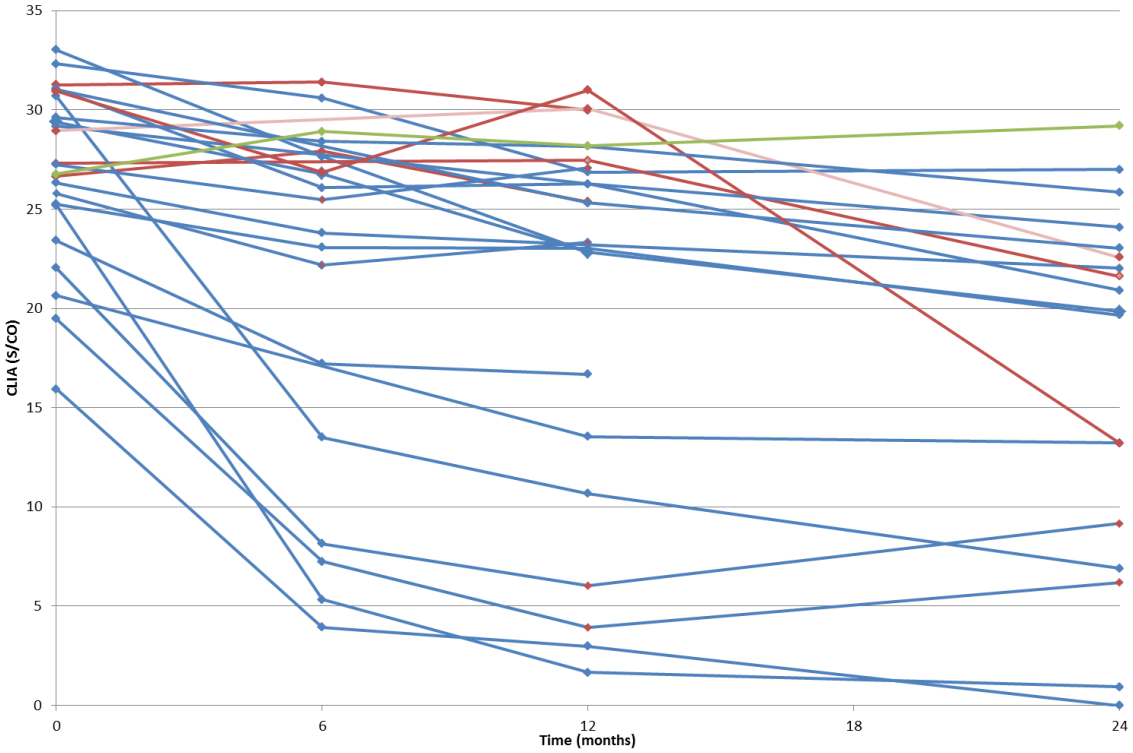
syphilis test	number of tests	costs (euro)			
	/ quarter	distribution	labor cost MLT + logistics	support + MSU	total
Architect Syphilis TP assay	1464	0,81	1,19	2,82	4,82
BD Macro-Vue RPR Card Test	143	3,66	2,85	3,33	9,84

Attachment 5 : RIZIV (Rijksinstituut voor ziekte- en invaliditeitsverzekering) reimbursement (Art 24. 1/2/2011)[46].

RIZIV Nomenclature		
552731 552742	Diagnose van een infectie door Treponema in bloed of cerebrospinaal vocht met een techniek waarbij een specifiek antigeen wordt gebruikt (Maximum 1) (Cumulregel 326)	B 250
552716 552720	Diagnose van infectie door Treponema in bloed of cerebrospinaal vocht met een techniek waarbij een niet specifiek antigeen (type RPR of VDRL) wordt gebruikt (Maximum 1) (Cumulregel 326)	B 80

Attachment 6 : Evolution of treponemal test result after diagnosis of syphilis.

Twenty-four cases had two follow-up samples at 6,12 and/or 24 months. All these cases showed a gradual decrease in RPR titer. The results of the Architect Syphilis TP assay for these patients are shown in the figure. In the period of 2 years after diagnosis, 16 of the 24 patients also had a gradual decrease in treponemal signal. Five patients had one sample with a higher signal than at diagnosis. Although after 2 years the treponemal signal was lower than at diagnosis. Furthermore one patient had a signal that slightly increased. An explanation of these single higher results is difficult because RPR and clinical symptoms did not indicate any disease activity.



Treponemal test results from different patients with different stadia of syphilis at 6,12 and 24 months after diagnosis. (red: cases with one treponemal signal higher than the signal at diagnosis; green: case with higher signal in last sample than at diagnosis; blue: cases with decreasing signal in time; red points: samples with increased signal compared with previous sample).

RELEVANT EVIDENCE/REFERENCES

1) Guidelines and Recommendations

[16] Workowski K, Berman S. Sexually transmitted diseases treatment guidelines 2010. MMWR 2010;59:1-110.

[17]French P, Gomberg M, Janier M et al. IUSTI: 2008 European guidelines on the management of syphilis. International Journal of STD & AIDS 2009; 20:300-309.

[18]Kingston M, French P, Goh B et al. UK international guidelines on the management of syphilis 2008. International Journal of STS & AIDS 2008; 19:729-740.

[15]Egglestone S, Turner A. Serological diagnosis of syphilis. Commun Dis Public Health 2000; 3:158-62.

[19]Young H. Guidelines for serological testing for syphilis. Sex Transm Infect 2000; 76:403-405.

2) Review

[14]A Sena, B White and P Sparling. Novel Treponema pallidum serologic tests: a paradigm shift in syphilis screening for the 21st century. CID 2010; 51(6):700-708.

3) All references (guidelines, recommendations, reviews, original articles, handbooks)

[1] Fenton A Breban B, Vardavas R. Infectious syphilis in high-income settings in the 21st century. Lancet Infect Dis 2008:8:244-253.

[2] Annual epidemiological report on communicable diseases 2010. Syphilis. [Online 19/4/11] http://www.ecdc.europa.eu/en/publications/Publications/1011_SUR_Annual_Epidemiological_Report_on_Communicable_DisDisea_in_Europe.pdf

[3] WIV: Syphilis. Surveillance van Infectieuze Aandoeningen door een Netwerk van Laboratoria voor Microbiologie 2009. [Online 19/4/11] http://www.iph.fgov.be/epidemio/epinl/plabnl/plabannl/09_048n_v.pdf

[4] Mandell G, bennett J, Dolin R. Principles and practice of infectious diseases. 6th edition. Elsevier, 2005:2768-2783

[5] Workowski K, BermanS. Sexually transmitted diseases treatment guidelines 2006. MMWR 2006; 55:1-94

[6] Wasserman A, Neisser A, Bruck C. Eine serodiagnostische reaction

[7] LaFond RE, Lukehart SA. Biological basis for syphilis. Clin Microbiol Rev 2006; 19:29-49.

[8] BallardRC. Gentital ulcer adenopathy syndrome. In: Holmes KK, Sparling PF, Stamm WE, et al. Sexually treansmitted diseases. 4th ed. New York: McGraw hill, 2008: 1201-1208

[9] Van der Sluis JJ. Laboratory techniques in the diagnosis of syphilis: a review. Genitourin Med 1992;68:413-419.

[10] Larsen SA, Steiner BM, Rudolph AH. Laboratory diagnosis and interpretation of tests for syphilis. Clin Microbiol Rev 1995;8:1-21.

[11] Cole MJ, Perry RK, Parry V. Comparative evaluation of 15 serological assays for the detection of syphilis infection. Eur J Clin Microbiol Infect Dis 2007;51:118-124.

[12] *Treponema pallidum* (Syphilis) antistoffen in serum. Kwaliteitssysteem LAG-UZ-KULeuven. BMP-SER bijlage 2, p48. Versie 8-9-2010.

[13] *Treponemal pallidum* (Syphilis) in serum met corresponderend CSV. Kwaliteitssysteem LAG-UZ-KULeuven. BMP-SER bijlage 2, p49. Versie 8-9-2010.

[14] Sena A, White B, Sparling P. Novel *Treponema pallidum* serologic tests: a paradigm shift in syphilis screening for the 21st century. CID 2010; 51(6):700-708.

[15] Egglestone S, Turner A. Serological diagnosis of syphilis. Commun Dis Public Health 2000; 3:158-62.

[16] Workowski K, BermanS. Sexually transmitted diseases treatment guidelines 2010. MMWR Recomm Rep 2010; 59:1-110.

[17] French P, Gomberg M, Janier M et al. IUSTI: 2008 European guidelines on the management of syphilis. International Journal of STD & AIDS 2009; 20:300-309.

[18] Kingston M, French P, Goh B et al. UK international guidelines on the management of syphilis 2008. International Journal of STS & AIDS 2008; 19:729-740.

[19] Young H. Guidelines for serological testing for syphilis. Sex Transm Infect 2000; 76:403-405.

[20] Rolfs RT, Joesoef MR, Hendershot EF et al. A randomized trial of enhanced therapy for early syphilis in patients with and without human immunodeficiency virus infection. The syphilis and HIV Studu group. N Eng J Med 1997;337:307-314.

[21] Lukehart S. Serologic testing after therapy for syphilis: is there a test for cure? Ann Inter Med 1991;114:1057-1058.

[22] Fiumara N. Treatment of seropositive primary syphilis: an evaluation of 196 patients. Sex Trans Dis 1977;4:92-95.

[23] Fiumara N. Reinfection primary, secondary and latent syphilis: the serologic response after treatment. Sex Trans Dis 1980;7:111-115.

[24] Farhi D, Dupin N. Origins of syphilis and management in the immunocompetent patient: facts and controversies. Clinics in Derlatology 2010;28:533-538.

[25] Rolfs RT. Treatment of syphilis,1993. Clin Infect Dis 1995;20:S23-28.

[26] Romanowski B, Sutherland R, Gordon H et al. Serologic response to treatment of infectiuous syphilis. Annals Int Med 1991;114:1005-1009.

[27] Lukehart S. Biology of treponemes. In: Holmes KK, Sparling PF, Stamm WE, et al. Sezxually transmitted diseases. 5-4th ed. New York: McGraw Hill, 2008:647-659.

[28] Baughn RE, McNeely MC, Jorrizo JL et al. Characterization of the antigenis determinants and host components in immune complexes from patients with secondary syphilis. J Immunol 198,136:1406-1414.

[29] Luger A. Serological diagnosis of syphilis; current methods. In: Young H, McMillan A. Immunological diagnosis of sexually transmitted diseases. New York: Marcel Dekker,1998:249-274.

[30] Baker-Zande SA, Roddy RE, Handsfield HH et al. IgG and IGM antibody reactivity to antigens of *Treponema pallidum* after treatment of syphilis. Sex Transm Dis 1986;13:214-220.

[31] Romanowski B, Sutherland D. Serologic response to treatment of infectious syphilis. Ann Intern Med 1991;114:1005-1009.

[32] Merlin S, Andre J, Alacoque B et al. Importance of specific IgM antibodies in 116 patients with various stages of syphilis. Genitourin Med 1985;61:82-87.

[33] Shannon R, Booth SD. The pattern of immunological responses at various stages of syphilis. Brit J Ven Dis 1977,53:281-286.

[34] O'Neil P, Nicol CS. IgM class antitreponemal antibody in treated and untreated syphilis. Br J Vener Dis 1972;48:460-463.

[35] Shannon R,Copley C, Morrison G. Immunological responses in late syphilis. Br J Vener Dis 1980;56:372-376.

[36] Collart P, Borel LJ, Durel P. Significance of spiral organisms found after treatment in late human and experimental syphilis. Br J Vener Dis 1964;40:81-89.

[37] Luger A.1987. Serological diagnosis of syphilis: current methods, p 249-274. In H Yoeng and A McMillan, Immunological diagnosis of sexually transmitted diseasess. Marcel Dekker, Inc., New York.

[38] Lefevre J, Bertrand M, Bauriaud R. Evaluation of the captia enzyme immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. J Clin Microb 1990;28:1704-1707.

[39] Sato T, Kubo E, Yokota M et al. *Treponema pallidum* specific IgM hemagluttination test for serodiegnosis of syphilis. Br J Vener Dis 1982;60:364-370.

[40] Lefevre J, Prere M, Abbal M et al. Apport du FTA abs IgM quantitatif aux différents stades de la syphilis avant et après traitement. Ann Dermatol Venereol 1983;110:425-430.

[41] Yinnon A, Coury P, Pliolito R. Serologic response to treatment of syphilis in patients with HIV infection. Arch Intern Med 1996;156:321-325.

[42] Rolfs R, Joesoef M, Hendershot E et al. A randomised trial of enhanced therapy for early syphilis in patientswith and without human immunodeficiency virus infection. The syphilis and HIV Study group. N Eng J Med 1997;337:307-314.

[43] Farhi D, Benhaddou N, Grange P et al. Clinical and serologic baseline and follow-up features of syphilis according to HIV status in the post-HAART era. Medicine 2009;88:331-340.

[44] Ghanem K, Moore R, Rompalo A et al. Antiretroviral therapy is associated with reduced serologic failure rates for syphilis among HIV-infected patients. CID 2008;47:258-265.

[45] Package insert Architect Syphilis TP assay. Abbott Diagnostics, Wiesbaden, Germany. September 2007.

[46] Riziv nomenclatuur art. 24. Versie in werking sinds 1/2/2011 [Online 15/5/11] http://www.riziv.be/care/nl/nomenclature/pdf/art24.pdf