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Standard serologic testing for syphilis in individual patients: the European view  
(paper nr 1)

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Introduction

The aim of this paper is to describe the current view regarding the diagnosis of syphilis by serologic tests in Europe. The method used is asking key-questions and giving answers to those questions, having reviewed the data available. The concept-paper including proposed answers to key questions was discussed in the IUSTI/WHO Europe Workshop Syphilis Management at the IUSTI Europe Conference on STI, October 7-9, 2004, 19 experts participating (IUSTI Europe: 13; WHO Europe: 6; USA: 1), in addition to 4 observers. Here the final version is given, after discussion in the workshop. The subject of this paper is not (anonymous) epidemiological screening of large populations, but standard serologic testing for syphilis of individual patients at risk for STI.

Key question 1. How valid is the VDRL or RPR test as primary screening test?

Both are non-treponemal tests, derived from the Wassermann reaction for cardiolipin. Antibodies to cardiolipin become detectable early in the infection around 7-10 days after the appearance of the primary chancre or 3-5 weeks after acquiring the infection. Overall sensitivity for the VDRL/RPR test is approximately 70%-85% in the primary stage with the potential to reach 100% in the secondary stage.[1,2]

The most serious, and often under-estimated, disadvantage is the occurrence of the prozone phenomenon, i.e. a false negative reaction resulting from inhibition of agglutination due to excess antibody in the serum. The prozone phenomenon is generally considered to occur in 1-2% of patients with secondary syphilis although this may well be an underestimate. False negative VDRL/RPR tests also occur in late-stage syphilis, but these are not due to the prozone phenomenon.[3] False negative VDRL/RPR tests are especially relevant in pregnant women.[4]

The occurrence of false positive reactions should not be a major problem, assuming additional (treponemal) tests are performed if the VDRL/RPR test is used as primary screening test.

The VDRL/RPR test is cheap and simple to perform, but labour intensive and not suited for automatisation, while interpretation is subjective.

Because the VDRL/RPR test can be performed within 30 minutes, it is ideal for rapid testing in individuals, who present in emergency departments and in patients, where there is a strong clinical suspicion of syphilis (in combination with the standard primary screening test). In these circumstances diluted as well as undiluted serum should be tested as part of the standard procedure to prevent a false-negative test due to the prozone phenomenon. An alternative option for rapid serologic testing may be a treponemal EIA in an automated situation.

Answer: The VDRL/RPR test is not recommended as primary screening test for early and late syphilis. The RPR test may be used as rapid test for detection of early symptomatic syphilis in at-risk patients (supplemented with a standard primary screening test). In these circumstances diluted as well as

1 Answers to key questions are based on proposed answers, which were discussed in the workshop.
undiluted serum should be tested as part of the standard procedure to prevent a false-negative test due to the prozone phenomenon. It may also be a useful test in patients suspected of very early syphilis.

**Key question 2. How valid is the TPHA or TPPA as primary screening test?**
The *Treponema pallidum* Haemagglutination Assay (TPHA), which uses red blood cells, and the *Treponema pallidum* Particle Assay (TPPA), which uses gelatin particles, are both treponemal tests. TPHA reactivity may be detectable around the 4th week of infection. The overall sensitivity in the untreated primary stage is in the 70%-80% range reaching 100% beyond this stage. When false negative reactions occur, they are usually associated with early primary stage infection.

The TPPA is superior to the TPHA in detecting cases of primary syphilis. A recent evaluation found that the TPPA was more sensitive and more specific than four commercial TPHA kits and concluded, that the TPPA was the most suitable of the assays examined for screening for all disease stages.[5] The risk of a false positive test, generally a rare occurrence, may be slightly increased using the TPPA compared to the TPHA.[6] After treatment of early syphilis the TPPA titre is more likely to show a decrease than the TPHA titre.[7]. Interpretation of agglutination is subjective.

The TPPA/TPHA is not ideally suited to automation. The TPPA/TPHA will also detect cases that have been adequately treated.

**Answer:** TPHA or TPPA are recommended as primary screening test in early and late syphilis. The TPPA may be preferred.

**Key question 3. How valid is the treponemal EIA as primary screening test?**
The EIA, a treponemal test, has an overall sensitivity of 80-85% in the primary stage of infection, reaching 100% beyond this stage.[8] Specificity is similar to that of the TPHA. The test is quantified. Tests based on recombinant antigens are available, which should aid standardisation of reagents. Major advantages of EIA include objective reading and the potential for automation and electronic report generation. The EIA will also detect cases that have been adequately treated.

**Answer:** The treponemal EIA is recommended as primary screening test.

**Key question 4. Which test should be used as primary screening test?**
What to use as primary screening test will be governed to some extent by the resources available (money and personnel) and the volume of testing to be performed.

The VDRL and RPR test constitute the large majority of syphilis tests in the Public Health Laboratories in the USA anno 2004, as they are used as primary screening tests, with mostly the qualitative RPR test being performed.[9] The VDRL/RPR test is cheap, easy to perform, and is suited to testing relatively small numbers of specimens. However, because of the prozone phenomenon, the VDRL/RPR test has serious limitations as a single screening test for early syphilis. The tendency of the prozone phenomenon to occur in sera with high VDRL/RPR test titers is particularly important in patients with concomitant HIV infection,[10] as sera from HIV infected patients have been shown to have significantly higher initial RPR titers than non-HIV infected controls with syphilis.[11] Also, patients re-infected with syphilis, even in cases of primary syphilis, appear to produce higher antibody titres than during the initial infection, [12] while HIV infected patients are significantly more likely to have a history of previous syphilis.[11] Thus there appears to be an increased risk of the prozone phenomenon, i.e. a false negative VDRL/RPR test, occurring in sera from HIV-positive patients and patients re-infected with syphilis. This is particularly relevant because of the ongoing syphilis outbreak in Europe among homosexuals with persistent risk behaviour, who are at risk of being HIV-positive and at risk for reinfection with syphilis, whether they are HIV-infected or not. In late latent syphilis a false-negative VDRL/RPR test may occur. And, as stated in paper nr 6 (D. Mabey: “Prevention of congenital syphilis in Europe”), the VDRL/RPR test is not recommended as single primary screening test for pregnant women.[4]

The TPHA on its own is a good primary screening test for syphilis at all stages beyond the early primary stage. Because of the importance attached to detecting early primary stage infection the TPHA has not been advocated as single screening test for diagnostic laboratories in the UK. If the TPHA is used as screening test, sera should also be tested by the VDRL/RPR (UK recommendation).[1] as the combination of these two tests will detect more cases of early primary syphilis than either test alone. As the TPPA has higher sensitivity than the TPHA in primary stage syphilis, it would be a suitable single primary screening test, particularly when smaller batches of samples require to be tested.

EIA as single screening test has a performance similar to the TPHA and VDRL/RPR test combination.[8,13-15] It is particularly suited to laboratories with large workloads. Several blood borne
diseases can be screened for at the same time using the same specimen.

Whichever screening test is used, there is likely to be a short window phase of 1-2 weeks when conventional non-treponemal and treponemal screening tests are negative. As an alternative to repeated testing 1-2 weeks later, an EIA for specific anti-treponemal IgM may be considered in those, who may have a very early primary infection. Of course dark field (dark ground) microscopy as rapid test in a patient suspected of primary syphilis (in the genital area) is also an option.

When the primary treponemal screening test is positive, i.e. TPPA or EIA, a quantitative non-treponemal test, e.g. the VDRL or RPR test, should be performed (in combination with a confirmatory treponemal test) to guide management (staging the disease, assessing the need for treatment and monitoring the serological response after treatment).

**Answer:** A treponemal test, TPPA or EIA, is recommended as primary screening test, the choice being decided by local circumstances (volume of tests, costs, regional organisation, etc). The use of a non-treponemal test, e.g. the VDRL or RPR test, as a single primary screening test is not advocated. When the primary treponemal screening test is positive, a quantitative non-treponemal test, e.g. the VDRL or RPR test, should be performed (in combination with a confirmatory treponemal test) to guide management (staging the disease, assessing the need for treatment and monitoring the serological response after treatment).

**Key question 5. What to use as a confirmatory test?**
Again, this will be governed to some extent by the resources available (money and personnel), the volume of testing to be performed and the primary screening test used. Ideally a reactive screening result should be confirmed with a treponemal antigen test of a different type from that used for screening. Therefore the TPPA/TPHA can be used to confirm a reactive EIA or an EIA to confirm a reactive TPPA/TPHA: the practicalities of laboratory testing mean, that the former scenario (confirmation of reactive EIA by TPPA/TPHA) is more likely. For the *T. pallidum* IgG-immunoblot test as confirmatory test: see key question 7.

Quantification of the titer of the TPPA/TPHA and especially adding at least one quantitative non-treponemal test (e.g. the VDRL/RPR test) to the confirmatory test is essential for realising an optimal test profile, as it may help to stage the disease. The addition of at least one quantitative non-treponemal test also serves to monitor the serological response (decrease of titer) to treatment.

**Answer:** If the EIA is used as primary screening test, the TPPA (TPHA) can serve as a confirmatory test; if the TPPA (TPHA) is used as primary screening test, the EIA can serve as confirmatory test. A quantitative non-treponemal test (e.g. the VDRL or RPR test) should be performed to guide management (staging the disease, assessing the need for treatment and monitoring the serological response after treatment). For an optimal test profile a quantitative TPPA (TPHA) may be considered.

**Key question 6. Is there still a role for the FTA-absorption test as confirmatory test?**
The FTA-absorption test is a treponemal test. In the Public Health Laboratories in the USA a Captia Syphilis IgG EIA constitutes the large majority of (confirmatory) treponemal tests, with next in frequency the FTA-absorption test and TPPA being performed in about equal numbers.[9] The FTA-absorption test performs well, when used as a confirmatory test for sera found to be positive on screening with the VDRL/RPR test. It performs less well in confirming the treponemal nature of VDRL/RPR negative sera, that are detected by screening with EIA (or TPHA/TPPA).[16] It is technically more complex to undertake than the TPPA/TPHA or EIA. Although sensitivity is high, the specificity of the FTA-absorption test is poorer than that of the other treponemal tests. Possibly false negative results have been reported in HIV infection.[17] The reputation for high specificity stems from the principle of dual testing; i.e. the FTA-absorption test is used to test sera, that have been pre-selected, thus increasing considerably the probability that they contain anti-treponemal antibodies.

Problems with the FTA-absorption test include considerable variation in test performance between commercial kits and the high subjectivity of reading. Some experienced laboratories may decide however to continue using the FTA-absorption test as standard confirmatory test.

**Answer:** the FTA-absorption test is not recommended as a confirmatory test.

**Key question 7. Can we use the *T. pallidum* IgG-immunoblot test as confirmatory test?**
Immunoblotting has considerable advantages over the FTA-absorption test as a supplementary test for use, when the first-line confirmatory test fails to confirm the positive primary screening test. Initially there were problems in defining a positive immunoblot result for tests based on native *T. pallidum* antigen, but such tests have been largely superseded by tests based on recombinant antigens. E.g., the
INNO-LIA Syphilis Kit determines an antibody response to three recombinant antigens (TpN15, TpN17 and TpN47), as well as a synthetic peptide based on TpN44.5a (TnpA) deposited as distinct lines on a strip.[18] This makes interpretation easier. Depending on resources and the number of confirmatory tests to be performed, it may be advantageous to use the IgG-immunoblot test as a confirmatory test. Technically the IgG-immunoblot test has superseded the Treponema pallidum Immobilization (TPI) test.

**Answer:** The IgG-immunoblot test can be used as a confirmatory test. It is the recommended test, when a positive screening EIA is not confirmed by the TPHA/TPPA test or a positive screening TPHA/TPPA is not confirmed by the EIA test.

**Key question 8. Any further interesting diagnostic laboratory developments?**

In the (distant) future new developments may occur as the genome of *T. pallidum* has been mapped completely.[19] For the present IgM tests (EIA or immunoblot) may be used for screening neonates for congenital syphilis (IgM class antibodies do not cross the placenta) in combination with the PCR test for *T. pallidum*, which then replaces the rabbit infectivity test.[20,21] In neurosyphilis the value of PCR testing is limited.[22] Furthermore nucleic acid amplification tests, e.g. the multiplex PCR, are useful for detecting *T. pallidum* DNA in mucocutaneous ulcers (Genital Ulcerative Disease) and provide a more sensitive gold standard for diagnosing early syphilis.[22-24] At the moment PCR is not a substitute for rapid testing with dark field (dark ground) microscopy because of lack of availability within laboratories and the time taken to obtain a result. For rapid treponemal tests for screening, see paper nr 6; D. Mably, “Prevention of congenital syphilis in Europe”.

The serology test protocol recommended in 1999 in the Russian Federation,[25] has been updated.[26] In 2001 the Ministry of Health of the Russian Federation (MHRF) issued an order according to which Wassermann’s reaction, TPI-test and FTA-abs-test were excluded and RPR-test, TPHA, ELISA (with common Ig and separate IgM/IgG determination) and FTA-abs-test were included in the diagnostic serology protocol. RPR-test and TPHA/ELISA are intended for screening and TPHA, ELISA and FTA-abs-test and sometimes immunoblotting for confirmatory tests. According to this protocol the diagnostic procedure recommended is the use of the RPR-test and TPHA or ELISA simultaneously. It is regarded as very important, that the serologic treatment response is ascertained by determining the titer of the RPR-test, instead of valuing it in Wassermann’s ‘pluses’. Implementation of this order will take several years (not implemented yet in 2004, but is expected to be so in 2006).

**Answer:** IgM EIA and IgM immunoblot and PCR for *T. pallidum* DNA are valuable diagnostic tools for diagnosing congenital syphilis and PCR for *T. pallidum* appears to be the new gold standard for diagnosing early syphilis, but is no substitute yet for dark field (dark ground) microscopy. In the Russian Federation the syphilis serology test protocol has been updated in 2001, recommending a combination of a non-treponemal and treponemal test for screening, a treponemal test for confirmation and the titer of the RPR-test for monitoring the serologic response.

**References**


