

Diagnostics

Guidelines for serological testing for syphilis

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Although we may wish it were not so, syphilis, like the poor, will always be with us—at least for the foreseeable future. The levels of both are determined to a large extent, by political instability and socioeconomic deprivation. Overall, the incidence of syphilis is low in Western Europe (approximately 0.3 cases/100 000 in England in 1998)¹ although it has reached epidemic proportions in the Russian Federation where the levels in 1996 exceeded 900 cases/100 000 in men and women in the 20–29 year old age group.² The need to maintain effective strategies for syphilis control, which must include diagnosis and management, in areas of low prevalence such as the United Kingdom, is reinforced by the recent local outbreak of heterosexually acquired syphilis in South West England³ as well as the marked increase in homosexually acquired infection in the Manchester area.⁴ A significant proportion of the infected men in Manchester were HIV positive so the overall community health gain from rapid and effective diagnosis extends well beyond syphilis: ulcerative sexually transmitted infections promote HIV transmission by augmenting HIV infectiousness and HIV susceptibility via a variety of biological mechanisms.⁵ The importance of the serological diagnosis of syphilis has now been recognised with the publication of the excellent guidelines for serological testing for syphilis in diagnostic microbiology laboratories by the PHLS Syphilis Serology Working Group.⁶ These complement the recent national guidelines on the management of syphilis^{7,8} and together they should improve the overall diagnosis and management of syphilis within the United Kingdom and beyond.

Guidelines for serological diagnosis for syphilis are long overdue. The last guidelines which were produced by the World Health Organization in 1982⁹ recommended the use of a cardiolipin antigen test such as the Venereal Diseases Reference Laboratory (VDRL) or rapid plasma reagin (RPR) test and the *Treponema pallidum* haemagglutination assay (TPHA) for screening for syphilis. The new recommendations⁶ extend the WHO guidelines by suggesting that treponemal antigen based enzyme immunoassays (EIAs) are an appropriate alternative to the combined VDRL/RPR and TPHA screen. EIA as a single screening test was shown to give similar results to the VDRL/RPR and TPHA combination some years ago¹⁰ and is already used by many laboratories, particularly those with large workloads. Results of the UK National External Quality Assessment Scheme for Microbiology syphilis serology distribution in April 2000 showed that 56% (130/232) of responding laboratories within the United Kingdom used

an EIA (quoted with permission of the UK NEQAS organiser). Advantages of EIA include automated (or semiautomated) processing, objective reading of results, and interfacing with the laboratory computer system to allow electronic report generation. The widespread and growing use of automation and computerisation in laboratories has led to the reorganisation and rationalisation of microbial serology to meet the continuous demands for increased cost effectiveness in service delivery. Several requests to the author for advice suggest that, owing to rationalisation of services and developments in automation and computerisation, there is a trend for syphilis testing to move from bacteriology laboratories to dedicated microbial serology laboratories that traditionally may have dealt mainly with viral serology. These changes make the guidelines particularly timely as many laboratories may be taking on syphilis testing for the first time. There is also a trend for fewer laboratories to perform confirmatory testing, preferring to forward specimens reactive on screening to a specialised laboratory or centre.

The guidelines outline the current serological tests for syphilis and highlight the differences in screening practice between the United Kingdom and the United States. They contain an excellent algorithm for “Treponemal antibody screening and confirmatory testing” which is based on the key recommendations of the group. The guidelines recognise that there are a number of commercial tests of any given format and that these can vary in performance characteristics. Decisions on which test a laboratory uses will be based on many factors including cost, ease of use, suitability for automation, compatibility with the format of other tests already in use in the laboratory, as well as performance characteristics. Sadly, the changes described above inevitably decrease the weighting of performance characteristics in test selection. There is an enormous choice of test reagents, manufactured and/or supplied by different companies. For example, of the UK laboratories participating in the NEQAS scheme there were 19 different cardiolipin tests, 13 TPHA/TPPA tests, nine EIAs, and seven FTA-abs in use (quoted with permission of the UK NEQAS organiser). It is important that laboratories do not change reagents frequently in order that they and their users such as genitourinary medicine physicians become fully conversant with the performance characteristics of the particular tests used. Published performance criteria following stringent evaluation in independent centres are available for very few of the numerous tests (and their modifications) produced by different manufacturers. For example, there is a paucity

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of performance data for the various TPHA kits, supplied by 12 different companies, used in the United Kingdom. There are also few evaluations of the *Treponema pallidum* particle agglutination assay (TPPA), which uses gelatin particles as a carrier rather than erythrocytes, and has replaced the TPHA in many laboratories. An early developmental report¹¹ found that the microcapsule agglutination method was superior to the TPHA in detecting cases of primary syphilis. More recently, Pope and colleagues in the United States¹² reported that the TPPA was an appropriate substitute for the TPHA. Another recent report found that the TPPA was significantly more sensitive than the FTA-abs and marginally more sensitive than the TPHA,¹³ which makes the TPPA very suitable as a confirmatory test.

The starting point for the algorithm is the result of screening (either VDRL/RPR and TPHA or EIA alone). Screening with a non-treponemal test alone is not recommended because of the potential for false negative results as a result of the prozone phenomenon.¹⁴ A negative screening result is reported as "Treponemal antibody NOT detected but advise repeat if at risk of recent infection." The importance of repeat testing is well founded because approximately 15% of patients with primary syphilis will be seronegative at initial presentation.¹⁵ A reactive screening result should be confirmed with a treponemal antigen test different from that used in screening (for example, TPHA if EIA is used for screening) and a quantitative non-treponemal test (VDRL/RPR). The various scenarios which are then dealt with in detail include: (a) confirmatory and non-treponemal tests reactive; (b) confirmatory tests reactive but non-treponemal test negative; and (c) confirmatory test negative with a negative or reactive non-treponemal test. Group (a) is the group most likely to include untreated syphilis (or other treponemal disease) at any stage and it is suggested that an EIA for specific antitreponemal IgM should be considered on the basis of non-treponemal test titre and clinical details. Positive IgM reactions are considered to be consistent with recent/active treponemal infection: the commercially available Captia Syphilis-M EIA has a sensitivity of 93% in primary infection, 85% in secondary infection, and 64% in early latent infection.¹⁶ However, it is noted that in the absence of a history of adequate treatment, a negative result does not exclude the need for treatment. Irrespective of the IgM result VDRL titres greater than 16 are rarely found in adequately treated infections.¹⁷ Sera in group (b) are most likely to be from treated patients or those with untreated late latent infection. Occasionally, however, sera from patients with primary infection may give this pattern: I am aware of two recent cases of primary syphilis where the VDRL test was negative yet the screening EIA was positive. Although the algorithm does not include IgM testing of this group, IgM testing should be undertaken in all cases of suspected primary infection irrespective of the initial screening results. In the absence of a history of

adequate treatment, a negative VDRL result, like a negative IgM, does not exclude the need for treatment: in one study¹⁶ all 33 patients with untreated late latent syphilis gave a negative Captia Syphilis-M EIA result. Group (c) is the only group where *additional* confirmatory testing is recommended. Where the first line confirmatory treponemal antigen test is negative and the additional confirmatory treponemal antigen test(s) and VDRL are negative then the specimen can be reported as a false positive screening test. If at least one additional confirmatory test is reactive then this signifies a low level of treponemal antibodies, which could be the result of a treated or longstanding infection or to an early primary infection. IgM EIA testing will differentiate between these possibilities. If the only reactive screening test were the VDRL/RPR, and primary syphilis was not suspected, the negative result in the treponemal confirmatory test normally used would seem sufficient to denote a biological false positive reaction (if quantitative VDRL/RPR positive) or a false positive VDRL/RPR screening test (if quantitative VDRL/RPR negative). Again, an IgM EIA test should be performed when primary syphilis is suspected.

In all categories of treponemal infection a repeat specimen is advised to confirm the findings; this will normally be a week or so after the initial specimen. It is also recommended that all reactive specimens should be referred to one of the PHLS syphilis specialist laboratories listed at the end of the guidelines for reference testing and to allow collection of surveillance data.

While the detection of early infectious syphilis is the priority of any syphilis testing programme it must also be recognised that in the United Kingdom most newly diagnosed cases are late stage infection.¹ The screening schedules proposed in the guidelines take account of this and achieve high sensitivity in all stages of infection. However, depending on the particular tests used and the quality of the clinical/laboratory liaison there may be a failure to detect a small proportion of untreated primary infections at one end of the spectrum and markers of long standing treated treponemal infection at the other. The extent of these failures will vary slightly and depend on the particular tests used. By the time that signs and symptoms of primary syphilis are present most patients have detectable IgG and IgM¹⁸ but before this there is a short window around 2–4 weeks post-infection when only IgM is detectable. Provided that the specificity of EIAs that detect both IgM and IgG is as good as those that detect only IgG, and the sensitivity in late stage infection is also as good, it would be an advantage to use the former type of EIA even although primary syphilis is rare. Specificity is important in terms of the cost and workload involved in confirmatory testing and referral: a 0.5% decrease in specificity of a test used in a laboratory screening 20 000 specimens per year means an extra 100 referrals for confirmation at a cost of around £1500 to £3000.

Even when highly specific screening tests are used confirmatory testing is essential because

of the low prevalence of syphilis. For example, treponemal EIAs generally give a specificity on screening of around 99.5%. However, if the prevalence of syphilis in the test population is 0.5% (it will be lower in populations such as antenatal patients) then the predictive value of a positive screening test result is only 50%. Applying a confirmatory test with the same specificity to the sera reactive on screening (prevalence of syphilis now 50% in this population) will give a positive predictive value of 99%. The guideline recommendation that specimens that are reactive on screening require confirmatory testing with a different treponemal test, of equal sensitivity, from that used for screening and, ideally, greater specificity highlights the shortcomings of the FTA-abs, previously considered the "gold standard" confirmatory test. Indeed the FTA-abs is not recommended as the first line confirmatory test. The specificity of the FTA-abs is poorer than that of the other treponemal antigen screening tests¹⁷⁻¹⁹ while certain newer EIAs are significantly more sensitive than the FTA-abs¹³⁻²⁰ in detecting markers of past infection which means that the FTA-abs will fail to confirm a small number of genuinely reactive EIAs. The TPHA/TPPA is comparable with the newer EIAs which means that the most accurate confirmation of treponemal antibodies will result from using the TPHA/TPPA to confirm a reactive EIA or an EIA to confirm a reactive TPHA/TPPA: the practicalities of laboratory testing mean that the former scenario is more likely. Although the FTA-abs may have slightly greater sensitivity in early primary infection a positive FTA-abs result alone has poor specificity and in one study more than 90% of such reactions were the result of conditions other than syphilis.²¹ The use of an anti-treponemal IgM to supplement standard screening and confirmatory procedures is a better approach to maximising the detection of early primary infection than relying on the FTA-abs. Other limitations of the FTA-abs in initial confirmation include the finding that false reactivity in the FTA-abs was significantly associated with false reactive EIA results²²; and the subjective interpretation of the test that may lead to bias when other test results are known.²³

These guidelines will not only be of immediate benefit to laboratories involved in syphilis serology they will also act as an impetus to review the provision of syphilis serology services throughout the United Kingdom. Review could lead to improvements in reliability and cost effectiveness of laboratory testing via increased standardisation of screening tests with clearly identified policies for referral of confirmatory tests to appropriately resourced regional or supraregional specialist/reference

centres. Such changes would also have enormous benefit for the prompt surveillance of infectious syphilis. Further guidelines dealing with specific areas of syphilis serology such as congenital infection, neurosyphilis and co-existing HIV are promised.⁶ Guidance on direct detection of *Treponema pallidum* in genital ulcers would also be welcome.

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