Serological diagnosis of syphilis

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Summary: *The availability of an increasing number of enzyme immunoassays (EIAs) for detecting syphilis antibodies makes it appropriate to review approaches to syphilis serology and to assess the role of syphilis EIAs in routine diagnostic microbiology laboratories. This paper summarises the principles and practice of syphilis serology and provides recommendations on the use of laboratory tests for syphilis in UK diagnostic microbiology laboratories.*

The main recommendations are summarised in a testing algorithm. Treponemal EIAs are an appropriate alternative to the use of combined Venereal Disease Research Laboratories/rapid plasma reagin and Treponema pallidum *haemagglutination assay (TPHA) tests for screening for syphilis. If a treponemal EIA is used for screening an alternative treponemal test, such as TPHA, should be used for confirmatory testing. The fluorescent treponemal antibody-absorbed test is probably best reserved for specimens giving discrepant results. Such specimens may be referred to the PHLS laboratories that provide confirmatory treponemal testing for reference testing and to facilitate collection of surveillance data on what remains an important public health problem.*

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Introduction

Syphilis is one of a group of diseases caused by spirochaete organisms of the genus *Treponema*. Sexually acquired syphilis occurs worldwide and is caused by *T. pallidum* subspecies *pallidum*. Related treponemes cause the non-venereal treponematoses bejel, or endemic syphilis (*T. pallidum endemicum*), yaws (*T. pallidum pertenue*), and pinta (*T. carateum*). Serology remains the mainstay of laboratory testing for syphilis, except during the very early stage of infection when direct detection of treponemes in material from lesions by darkground or fluorescent microscopy is necessary.

Certain characteristics of syphilis make it amenable to serological screening. It is an important health problem, there is a recognised latent phase, validated serological tests are widely available at relatively low cost, there are serious adverse effects if cases are missed (stillbirths, congenital syphilis, further adult sexual transmission, tertiary syphilis), and effective treatment is available.

There have been several developments in serological tests for syphilis in recent years, particularly the advent of enzyme immunoassays (EIAs) and, lately, the commercial availability of recombinant antigen-based tests[1]. This paper reviews these recent developments and provides guidance on the use of laboratory tests for syphilis for diagnostic microbiology laboratories in the United Kingdom (UK). Transfusion laboratories have different requirements and are not considered here.

Natural history of infection and immune response

The natural history of syphilis is very variable. The course of the infection spans many years and may lead to various clinical presentations, which are classified into early (infectious) and late (non-infectious) stages. Early syphilis may be further divided into primary, secondary, and early latent syphilis; late syphilis includes late latent and the various forms of tertiary syphilis (box 1). The immune response to syphilis involves production of antibodies to a broad range of antigens, including non-specific antibodies (cardiolipin or lipoidal antibody) and specific treponemal antibodies. The first demonstrable

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response to infection is the production of specific antitreponemal IgM, which may be detected towards the end of the second week of infection; antitreponemal IgG appears later, at about four weeks[2]. By the time that symptoms develop, most patients have detectable IgG and IgM[3]. The immune response can be affected by treatment and by HIV infection. The titres of non-specific antibody and specific IgM decline rapidly after adequate treatment of early syphilis but specific IgG antibody generally persists. HIV infection may reduce or delay the antibody response in primary syphilis but in most cases the response is normal or exaggerated[4].

Serological tests for syphilis and their application

Serological tests for syphilis may be classified into two groups.

Non-treponemal tests, which detect non-specific treponemal antibody. These include the Venereal Diseases Research Laboratory (VDRL) and rapid plasma reagin (RPR) tests.

Treponemal tests, which detect specific treponemal antibody. These include the *Treponema pallidum* haemagglutination assay (TPHA), the fluorescent treponemal antibody-absorbed test (FTA-abs), and most enzyme immunoassay (EIA) tests (boxes 2 and 3).

The details of the individual tests and their performance characteristics at different stages of syphilis have been reviewed elsewhere[1,2,5]. An important principle of syphilis serology is the detection of treponemal antibody by a screening test, followed by confirmation of a reactive screening test result by further testing. The confirmatory test, or tests, should ideally have equivalent sensitivity and greater specificity than the screening test and be independent methodologically, so as to reduce the chance of coincident false positive reactions. A second specimen should be tested to confirm the results obtained from the first specimen and to ensure that the patient details on the specimen were correct. A quantitative non-treponemal test and / or detection of specific treponemal IgM may be useful for assessment of the stage of infection and to monitor the effect of treatment. Serology cannot distinguish between the different treponematoses (syphilis, yaws, pinta, and bejel).

In practice, serological tests for syphilis are used for:

- screening asymptomatic individuals with no history suggestive of syphilis, such as pregnant women;
- screening genitourinary medicine clinic attenders at recent risk of acquiring a sexually transmitted infection;
- screening blood and organ / tissue donors;
- detecting or excluding current or past syphilis in patients with HIV infection;
- testing patients whose history or clinical signs are consistent with syphilis – for example, genital ulceration or chronic neurological illness;
- confirmatory testing of specimens reactive in screening tests for syphilis;
- assessment of the stage of infection and monitoring the therapeutic response.

The testing strategy employed varies – either a non-treponemal test alone, a treponemal test alone, or both in combination may be used, depending on several factors, including whether the aim is to detect all stages of syphilis or only infectious syphilis. In the United States (US) and certain European countries, including France and Belgium, non-treponemal tests are used for screening[1]. One advantage of this approach is that it does not detect most adequately treated cases, thus simplifying patient assessment. There are, however, disadvantages with this approach. Screening undiluted specimens with a non-treponemal test alone can yield false negative reactions in the presence of high titres of antibody – the prozone phenomenon – for example, in early infection and with concomitant

BOX 1 Classification of syphilis

Early (infectious) syphilis	
Time after exposure	
9-90 days	Primary
6 weeks - 6 months (4-8 weeks after primary lesion)	Secondary
≤2 years	(Early) Latent
Late (non-infectious) syphilis	
>2 years	(Late) Latent
3-20 years	Tertiary Gummatous Cardiovascular Neurosyphilis
Congenital syphilis	
<2 years since birth (includes stillbirth)	Early congenital syphilis
≥2 years	Late congenital syphilis

Notes

Syphilis is most infectious to other adults through sexual contact during primary and secondary syphilis, but transmission has also been recorded during early latent syphilis.

Mother to child transmission can occur throughout early syphilis in the mother, which is also called congenitally transmissible syphilis. Transmission has been reported from mothers with late latent syphilis.

BOX 2 Basis of non-treponemal tests

Capture system	Test
Liposomes in suspension producing visible flocculation with lipoidal antibodies	VDRL
Liposomes in suspension + unattached charcoal particles producing dark coloured flocculation due to trapping of charcoal particles in lattice formed by antigen-antibody complex	RPR
VDRL antigen coated onto wells of microtitre plates and attached antibody detected by enzyme immunoasssay	EIA (Reagin)
VDRL antigen coated onto well of microtitre plates; attached antibody detected by anti-IgG plus anti-IgM-coated indicator red blood cells	SPEA (solid phase erythrocyte adherence)

HIV infection. Non-treponemal tests also lack sensitivity in late stage infection[1] and screening with a non-treponemal test alone may also yield false positive reactions in various acute and chronic conditions in the absence of syphilis (biological false positive reactions)[2]. Recently in the US, however, treponemal tests have been introduced for screening in blood banks[6] and their use has been also been advocated for screening psychiatric patients because of their greater ability to detect late stage infection[7].

In some European countries such as Germany and the Netherlands the TPHA is used for screening[1]. This provides a good screen for all stages of syphilis beyond the early primary stage but, because more primary infections are detected by a combination of VDRL and TPHA tests, the use of the TPHA alone has found limited favour in UK diagnostic laboratories where screening with both VDRL and TPHA has been common practice for many years[8]. The combination of VDRL and TPHA tests provides sensitive and specific screening for all stages of syphilis other than very early primary infection but it is more labour intensive than a single screening test, requires subjective interpretation, and cannot readily be automated[8]. With these practical disadvantages, and with the recent commercial availability of EIAs, the VDRL and TPHA combination for screening is being replaced increasingly in UK diagnostic microbiology laboratories by the use of EIA tests that detect treponemal IgG or IgG and IgM. The advantages of the EIA format include the production of objective results, the ability to link EIA plate readers directly to laboratory computer systems (reducing the potential for errors transcribing results), and the facility for automation. These factors make EIA attractive for laboratories with large workloads.

Commercial EIA tests have been developed since the World Health Organization recommended the use of a combination of a non-treponemal test and a treponemal test for screening and diagnostic purposes[9] and current practice in many centres in the UK has moved on. Published data show both that screening with a treponemal IgG EIA gives comparable results to the VDRL and TPHA combination[10,11] and that it may be a useful method for detecting treponemal antibody in patients who are infected with HIV[12]. Furthermore, a recent report suggested that a new recombinant antigen-based treponemal IgG and IgM EIA is the most sensitive treponemal test, and that it is also highly specific and thus suitable for screening[13].

BOX 3 Basis of treponemal tests

Antigen	Capture system	Test
Intact treponemes	Treponemes fixed onto microscope slides	FTA-ABS
Purified and sonicated treponemes	Attached to red blood cells	TPHA
	Attached to gelatin particles	TPPA
	Attached to microtitre Plates	EIA
	Proteins separated by PAGE and transferred to filters by Western blotting	Immunoblots
Recombinant antigens	Attached to microtitre plates	Recombinant EIA
	Attached to latex particles	Latex agglutination

Confirmatory tests

The FTA-abs is still generally regarded as the 'gold standard', but it has a number of limitations. It is a subjective test and difficult to standardise. It is sensitive, but the TPHA is more sensitive, except in the third and fourth weeks of infection; the TPHA is also more specific[2]. In addition, false negative FTA-abs results have been described in HIV infection[14] and false positive FTA-abs results have been described in association with false reactivity on screening with a particular commercial (antiglobulin) treponemal IgG EIA that has been used widely in the UK[15]. The TPHA is therefore the most appropriate test for confirming reactive EIA results at present; equally, if TPHA is used for screening, an EIA can be used as the confirmatory test[8]. Although immunoblotting has been suggested as a possible confirmatory test[16] further evaluation is needed in order to define its precise role. Potentially, an EIA of a different format[17] to the screening EIA could be employed for confirmatory testing but this also requires further evaluation.

Assessment of the stage of infection and monitoring the effect of treatment

In treponemal infection, a quantitative non-treponemal test and/or a test for specific anti-treponemal IgM helps to assess of the stage of infection, and provides a baseline for monitoring the effect of treatment. In general, IgM becomes undetectable within three to nine months after adequate treatment of early syphilis, but it may persist for 12 to 18 months after treatment of late disease[2]. Detection of specific anti-treponemal IgM in patients with no history of recent treatment suggests active disease and the need for treatment. Quantitative non-treponemal tests such as the VDRL/RPR remain the method of choice for follow up testing, the object being to demonstrate a decline in titre, depending on a range of factors including the initial titre, stage of infection when treated, treatment regimen, and HIV status[8].

Detection of specific antitreponemal IgM may also be useful in the diagnosis of congenital infection but, because a negative result around the time of delivery does not exclude congenital infection, serological follow up is necessary. This should include repeat IgM testing, together with repeat quantitative non-treponemal and treponemal testing to demonstrate loss of passive maternal antibody.[8]

Conclusions

The following recommendations extend previous guidance[9] by suggesting that treponemal EIAs are an appropriate alternative to the use of combined VDRL/RPR and TPHA tests for screening for syphilis. If a treponemal EIA is used as the screening test an alternative treponemal test, such as TPHA, should be used for confirmatory testing. The FTA-abs is probably best reserved for serum specimens that yield discrepant results. The role of immunoblotting, or EIA for confirmatory testing of sera screened by EIA, requires further evaluation before it can be recommended for routine use.

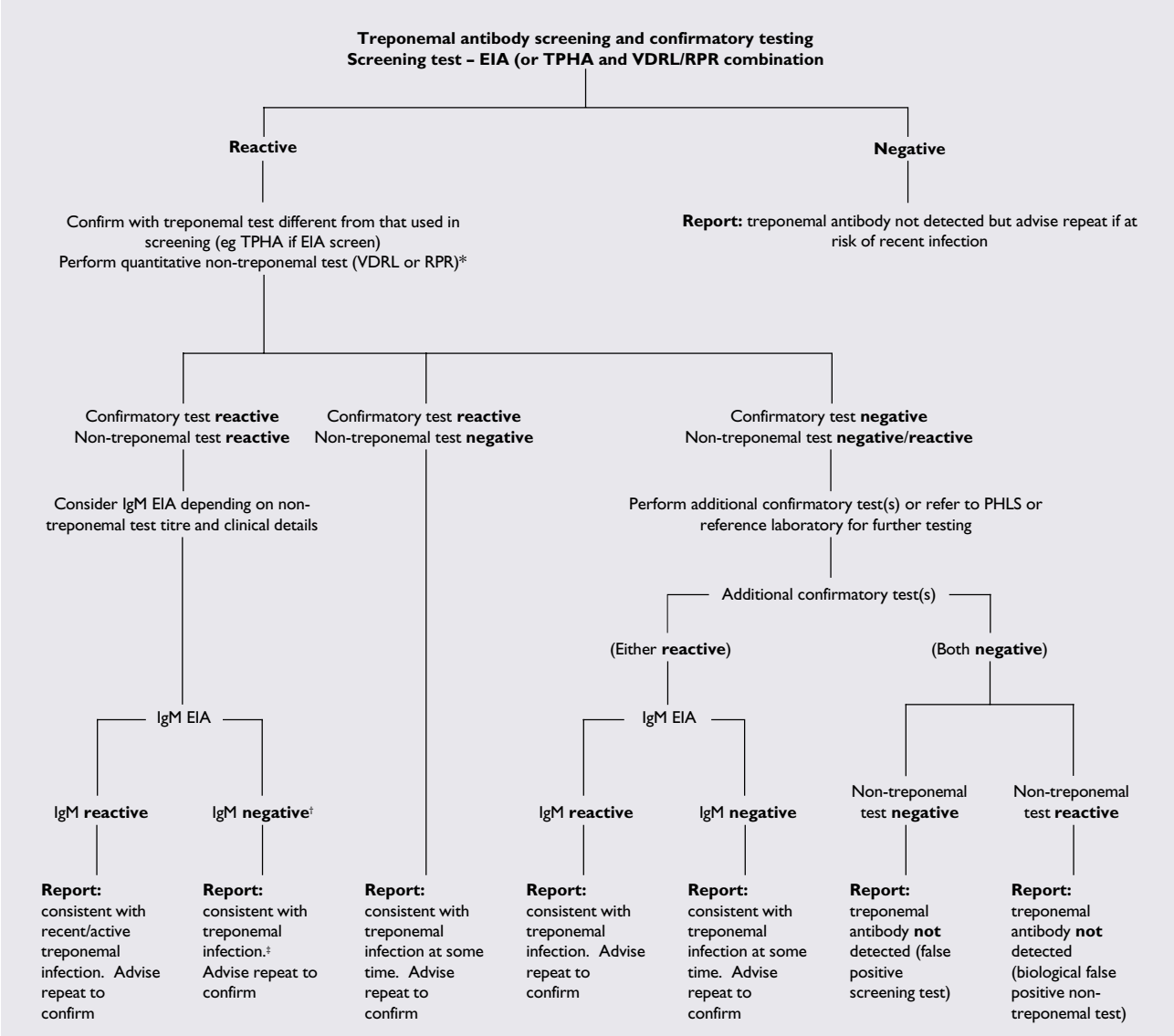
Many public health laboratories (PHLs) in England and Wales, and other large diagnostic laboratories in the UK, now use EIAs for screening rather than the conventional combination of a VDRL/TPHA screen. Either approach is suitable for screening for all stages of syphilis, but EIA has practical advantages as a screening test and it is recommended that laboratories with large workloads (above 20 000 tests a year) use this approach – depending on local circumstances it may also be appropriate for lower workloads**.** Details of several EIAs with acceptable performance characteristics have been published, but it is important to note that there are variations in treponemal EIA performance, as with other EIA tests (SI Egglestone, unpublished observations). Decisions on which EIA to use will also need to take account of other factors, including cost, ease of use, availability of suitable automated processing equipment, and compatibility with other EIA format tests already used in the laboratory.

Recommendations (see algorithm)

1. Follow up of seronegative patients at recent risk of acquiring a sexually transmitted infection is essential because of the seronegative window in early primary syphilis.

2. Screening with a non-treponemal test alone is not

Algorithm for treponemal antibody screening and confirmatory testing



* Testing up to a dilution of, for example, 1 in 6 will detect a prozone; reactive sera should not be titrated to the endpoint.

† In the absence of a history of adequate treatment, a negative result does not exclude the need for treatment.

‡ Add 'at some time' if VDRL titre <1 in 16.

recommended because of the potential for false negative results.

3. A treponemal EIA alone (IgG or IgG/IgM), or a combination of a non-treponemal test and a treponemal test (VDRL/RPR and TPHA), is appropriate for screening in the UK.

4. Specimens that are reactive on screening require confirmatory testing with a different treponemal test of equal sensitivity to that used for screening and, ideally, greater specificity.

5. Specimens giving discrepant treponemal test results on confirmatory testing need further testing; the PHLs that perform confirmatory treponemal testing – Birmingham PHL, Bristol PHL, Manchester PHL, Newcastle PHL and Sheffield PHL – can provide this service.

6. All confirmed reactive specimens should be referred to one of the above PHLs for reference testing and to allow collection of surveillance data.

7. Following confirmation of a reactive specimen, a second specimen should be tested to confirm the results and the correct identification of the patient. Consideration should then be given to referring the patient to a consultant in genitourinary medicine for expert advice on clinical management.

8. In treponemal infection a quantitative non-treponemal test and/or a test for specific treponemal IgM should be performed as part of the assessment of the stage of infection and to monitor the efficacy of treatment.

Detailed guidance for the UK on specific areas of syphilis serology – congenital infection, neurosyphilis, and in patients with HIV infection – is to be prepared.

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