**A Century of syphilis serology**

**Peter W Robertson**
SEALS Area Serology Laboratory
Prince of Wales Hospital
High Street, Randwick NSW 2031
Tel: (02) 9382 9153
Fax: (02) 9382 9151
Email: Peter.Robertson@sesiahs.health.nsw.gov.au

**Introduction**

The year 2006 represented the centenary of the first diagnostic serological test, the Wasserman complement fixation test for the diagnosis of syphilis. In this article I will relate some developments I have observed in the day-to-day running of a diagnostic serology laboratory over the thirty years preceding this centenary. I have assumed some background knowledge and elected to discuss only selected aspects of serological tests for syphilis (STS), rather than attempt a comprehensive review of the subject. A number of areas such as the serological diagnosis of congenital syphilis, the use of and interpretation of western blots in 'problem' sera and diagnosis of other treponemal diseases have not been included.

**The Role of Serology**

Recovery of the causative organism from an infected patient has traditionally been the means whereby the definitive diagnosis of most infectious diseases was made, and although *Treponema pallidum* was one of the first organisms to be identified as the cause of an infectious disease (syphilis) to this day culture of *T. pallidum* from an infected patient for the purpose of diagnosis has not been feasible. Thus, if the initial mucocutaneous lesion of primary syphilis is not recognised or properly investigated, the laboratory diagnosis of syphilis still remains the province of the serologist using STS. Recently molecular techniques have been shown to promise for diagnosis of syphilis, but the considerable volume and background of information on the characteristics of the serological tests in both the diagnosis and staging of the disease, as well as accumulation of data on interpretation of the serological response to treatment, mean that reliance on STS will continue for some years yet.

**Reagin testing**

In syphilis, the antibody that appears in serum and reacts with a mixture of different lipids is referred to as reagin. Since the original description of the Wasserman test, a variety of different synthetic antigens using different techniques have been used for detecting reagin. The most commonly used reagin assays are now the VDRL and RPR card tests. The RPR has the advantage of macroscopic reading, antigen stability and suitability for testing plasma. Present day reagin tests have been developed with the aim of increasing their sensitivity. Specificity is an inherent problem with all reagin tests because reagin antibody may be present in a number of other diseases and by the very nature of the tests biological false positives (BFP) cannot be eliminated completely.

As with all immune responses there is diversity in the pattern of appearance of antibodies in syphilis. Reagin levels usually begin to appear in serum in primary stage disease and peak in secondary syphilis. Therefore, a negative reagin test in a patient with suspected primary lesion does not exclude syphilis, but a negative reagin test in a patient with a rash should exclude syphilis (secondary) as the cause. Reagin assays alone are still used in some countries for blood donor screening based on the assumptions that they are usually present in high titres in the most infectious stages of the disease and reagin negative donors are therefore less infectious.

In addition to diagnostic screening, quantitative reagin tests are frequently required as part of investigations and management. Once a diagnosis of syphilis has been established, the effectiveness of treatment is assessed by demonstrating a significant fall in reagin antibody levels. Reinfection is often accompanied by a rise in reagin levels. These quantitative levels (titres) are determined by testing doubling dilutions of sera, most commonly using the RPR test. However, reading the end point of the titration is very subjective. This difficulty results in a lack of standardisation as was clearly demonstrated in a survey by the Royal College of Pathologists of Australasia QA Program in 2000 where RPR titres reported on a single serum ranged from 1 to 128 (Table 1). A similar spread of results was found with the VDRL. This lack of agreement precludes the comparisons of titres from serial samples unless these are tested simultaneously by the same operator using the same batch of reagent. In the modern day laboratory where accreditation requires attention to the measurement uncertainty in diagnostic assays, there are very few diseases where patient treatment is monitored by such subjective and unreliable criteria.

**Treponemal specific assays**

Because reagin antibody levels fall with advancing disease and eventually become negative with time, patients being investigated for a history of syphilis also require testing by a treponemal specific assay. Until recently the most commonly used *T. pallidum*
specific antibody assays have been the fluorescent treponemal antibody (FTA-abs) and the T. pallidum particle agglutination assay (TPPA). In the FTA-abs assay sera are absorbed with a ‘mash of spirochaetes’ to absorb antibodies against the non-pathogenic (Reiter) group of organisms. The TPPA uses inert gelatin particles sensitised with antigens of T. pallidum. The test was developed from the T. pallidum hemagglutination assay (TPHA) that used red cells as the carrier. In our experience the TPPA is easier to read, more stable and gives less non-specific reactions while maintaining the sensitivity of the TPHA. The TPPA is a convenient test that can be performed in smaller laboratories without any microscopy or other instruments.

Enzyme immunoassay and chemiluminescent techniques for T. pallidum-specific antibody have been developed over several years. Early versions of these immunoassays using single recombinant or other antigens were found to give false negative results at different stages of disease. As more recombinant antigens suitable for detecting antibodies in different stages of the disease were discovered, immunoassays using a mix of recombinant antigens were developed. Assays now include the 47kD antigen of T. pallidum, and this modification has enhanced the sensitivity of the assays in the diagnosis of primary syphilis.

The sensitivity, specificity and the convenience of easily adapting these assays to automated platforms have meant that these immunoassays are now the most popular STS for syphilis screening in developed countries.

The cut-off value for immunoassays used in screening assays is set to maximise sensitivity. Consequently false-positive results can occur. However, not all positive results in STS immunoassays that are negative in other assays should be dismissed as false-positive. We have reported that isolated positive results in the Abbott-Murex ICE immunoassay were more common in STD patients than in other groups, suggesting that isolated false-positive results may reflect past exposure and should prompt further investigation. These isolated positives usually have low readings and clinicians in STD clinics serviced by this laboratory have developed a ‘feel’ for this assay to the extent that we now report the OD/cut-off ratio with all positive results.

For many years it was generally accepted that treponemal specific antibodies could be detected for life after syphilitic infection. The concept of seroreversion was first described when both FTA and TPPA were shown to become negative in patients with HIV infection. We have also demonstrated a case where seroreversion occurred following treatment for syphilis in an HIV negative patient (Table 2). In this case the EIA assay was the only test that remained positive.

### Reagin negative patients

One frequently encountered finding is patients who are positive on T. pallidum specific assays, including FTA and TPPA, but negative on reagin testing. This pattern can be found in treated patients or in untreated patients where the disease may have progressed over time. Serological testing cannot distinguish between these cases. We encounter this situation not infrequently when undertaking screening in clinical settings such as some psychiatric admissions where tertiary syphilis is part of the differential diagnosis. An awkward situation occasionally arises when an elderly person has offered blood/product donation to a relative and where STS testing reveals this pattern. This result prompts a response that includes referral to a physician and testing of partners and children. The management of patients with this pattern and an uncertain history of treatment seem to vary between clinicians, but lumbar puncture is frequently performed as part of the assessment to exclude neurosyphilis.

### Examination of CSF

Laboratory investigations on the CSF to diagnose neurosyphilis include non-specific investigations of cell count and total protein as well as syphilis serology. The serological diagnosis of neurosyphilis is made by demonstrating that an antibody response has occurred in the central nervous system. Reagin tests alone are not suitable for testing the CSF for evidence of neurosyphilis. The RPR is difficult to read if used for testing CSF and is not recommended for use and the sensitivity of the VDRL has been estimated to be as low as 27%.

The FTA in CSF has been shown to be 100% sensitive in the diagnosis of neurosyphilis. As almost all patients with neurosyphilis will have FTA antibodies in serum and the test has a low limit of detection (high sensitivity) for detecting antibody, it is essential to confirm that the presence of FTA antibodies in the CSF are present as the result of CNS production of antibody and not from contamination of the CSF with serum. This contamination can result from a traumatic lumbar puncture or

### Table 1. RCPA Quality Assurance Program, Item 2000:2 Spec B

<table>
<thead>
<tr>
<th>RPR titre reported</th>
<th>No. of labs. Reporting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>&gt;8</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>32</td>
<td>3</td>
</tr>
<tr>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>128</td>
<td>1</td>
</tr>
</tbody>
</table>

### Table 2. STS Seroreversion in a 24-year-old male.

<table>
<thead>
<tr>
<th>Year/Result</th>
<th>1996</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTA ABS</td>
<td>Reactive</td>
<td>Non Reactive</td>
</tr>
<tr>
<td>TPPA</td>
<td>Reactive (=320)</td>
<td>Non Reactive (&lt;80)</td>
</tr>
<tr>
<td>RPR</td>
<td>Non Reactive</td>
<td>Non Reactive</td>
</tr>
<tr>
<td>Abbott Murex ICE</td>
<td>Reactive</td>
<td>Reactive</td>
</tr>
</tbody>
</table>
damage to the blood brain barrier that allows a leak of serum protein into the CSF. Laboratories reporting the results of positive CSF serology without regard to serum contamination can therefore mislead clinicians.

Various techniques have been used to identify serum contamination of CSF. The ratio of serum to CSF albumin has been used to estimate the proportion of antibody in that been produced intrathecally. For many years we have used the presence of adenovirus antibody as a marker of ‘serum contamination’ of the CSF 6.

Syphilis and HIV infection

Concurrent HIV and syphilis infection frequently occurs and the incidence of syphilis in homosexually active men is increasing in Australia 7. Because HIV-induced immune impairment may increase the risk of neurosyphilis, it is essential to identify syphilis infection in HIV positive patients 8. Testing for syphilis in these patients requires special consideration. Higher rates of asymptomatic primary disease and delayed seroconversion in STS have been described in HIV infection 9 and the incidence of BFP with the reagin screening tests is more common in HIV infection. The finding that seroreversion of STS is more common in these patients dictates that the most sensitive assays available should be used to screen these patients. Since the pattern of seroreversion of the different tests is not consistent, we test both FTA and TPPA to confirm positive immunoassay results.

Conclusion

Over the last century the accuracy of the STS have improved considerably, and the availability of recombinant antigens of Treponema pallidum has only recently seen a progression towards use of automated methods and a movement away from manual or semi-automated techniques. In contrast, assays for HIV and hepatitis using recombinant antigens on automated platforms have been available for several years. The reluctance to adopt modern techniques in this area of laboratory medicine may be due to the aura, mystery and legend that surrounds syphilis or simply the conservatism of those organisations and persons who lead the way in this area. In this article I have attempted to identify some areas where rethinking of the conventional wisdom behind current practices is required.

Acknowledgements

I am grateful to Prof Sydney Bell for advice and assistance with preparation of this article and Dr Ian Gardner for permission to use the QAP results.

References