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Editorial

Current issues in the development of laboratory investigations for syphilis

Since clinical manifestations of syphilis are protean, laboratory tests remain the cornerstone in the definitive diagnosis. Disease staging of syphilitic disease is usually performed through interpretation of clinical, epidemiological and a combination of syphilis tests. These most usually include non-treponemal and treponemal serological tests that are required for confirming or defining all stages of syphilis.

Traditional serological methods used for diagnosing syphilis detect either non-treponemal antibodies, viz: Venereal Disease Reference Laboratory (VDRL) test, rapid plasma reagin (RPR) test; or treponemal antibodies, viz: *Treponema pallidum* haemagglutination assay (TPHA), *Treponema pallidum* particle agglutination (TPPA) test, fluorescent treponemal antibody absorption (FTA-ABS) test, and *Treponema pallidum* immobilisation (TPI) assays.[1] In general, laboratory investigations of syphilis serve three main purposes: firstly, to diagnose a clinical case of syphilis, and stage it; secondly, to document treatment success; and lastly, to assist in case finding to stop spread of disease. All of these functions cannot be fulfilled without a properly quality controlled system that can generate reliable laboratory test results.

All nontreponemal antibody tests are often insensitive and poor in specificity, but they have been well studied and their titres correlated with disease activity. By contrast, treponema-specific antibody titres often correlate poorly with disease activity and cannot be used to assess disease stage. At present, FTA-ABS test is still used as a confirmatory test in samples showing reactivity in other assays, although titration is usually not performed in the diagnostic laboratory with this test. Moreover, its interpretation remains subjective and the test is not suitable for use in large-scale testing situations. TPI, a test done in the past to evaluate different stages of syphilis is difficult to perform, time-consuming and, therefore, very expensive. It is no longer used now. Monitoring of treatment progress for syphilis is currently done by determining decline in either anticardiolipin or, less often, antitreponemal IgM titre. Unfortunately, the combination of VDRL and TPPA does not lend itself readily to automation, and the FTA-ABS is both laborious and time-consuming.

At one time, Western blotting has been advocated and can detect antibodies to specific proteins separated by electrophoresis according to molecular weights.[2] The test is thus more specific than other treponemal antibody tests and still has problems with non-relevant proteins and false-positive reactivity patterns. These may induce indeterminate interpretations, and made it fall out of favour. Furthermore, the reported sensitivity of about 94% has resulted in their limited use. To this end, laboratory methods have been developed that include the enzyme immunoassay method (EIA) for detecting both IgG and IgM to *T. pallidum*, and has been shown to offer several advantages over the current tests: being simple and rapid, and gives objective results. Recent development of these new sensitive assays such as EIAs points to the questionable empirical threshold values that have been used in agglutination assays. Preliminary data on EIA use in antenatal screening in Hong Kong showed significant increase in sensitivity compared with traditional VDRL, although there is also increased number of single EIA positive cases that required follow-up. The present field experience, at large,

showed it to be both robust and sensitive. With increasing sensitivity, there can be an expected decline in positive predictive value especially in a low prevalence population. From a public health point of view, this strategy is fully justifiable. Cost-benefit analysis will further enable wider use in different at risk populations.

Recently, a new line immunoassay INNO-LIA Syphilis kit has been available and is valuable as a confirmatory assay for *T. pallidum* antibodies. This test uses recombinant antigens and synthetic peptides derived from *T. pallidum* (Nichols strain) membrane proteins. Antigens used consisted of three immunodominant proteins (TpN47, TpN17, and TpN15) expressed in *E. coli* and one synthetic peptide (TmpA) derived from transmembrane protein A.[1] Full-size *T. pallidum* genes expressing TpN47, TpN17, and TpN15 were amplified from *T. pallidum* (Nichols) by PCR with specific primers. The sensitivity and specificity results of the new assay compared favourably with consensus results based on the results of a variable number of classical serological methods and clinical information available.

In general, IgM tests are not intended for monitoring of treatment, and treponemal antibody tests should not be used for testing of CSF specimens. When a number of CSF samples was tested using INNO-LIA Syphilis kit, however, a higher degree of sensitivity than agglutination assays was reported.[3] Since IgM molecules do not cross the blood-brain barrier, detection of CSF treponemal antibodies may be somewhat less sensitive by agglutination techniques (TPHA, VDRL) than by other assay formats. Because goat anti-human IgG labelled with alkaline phosphatase is used as conjugate, this test can only detect IgG but not IgM. Despite the high costs, this line immunoassay gives the prospect of possibly replacing a number of testing strategies for confirming the diagnosis of syphilis infections, which currently requires the performance of several assays. At present, it appears that the major advantage of INNO-LIA is in the classification of indeterminate cases based on other serological tests, and not in its sensitivity.

Development of polymerase chain reaction (PCR) amplification and restriction endonuclease digestion of PCR products has enabled a molecular subtyping scheme for *T. pallidum* for laboratory strains as well as clinical specimens.[4] Two genes exhibiting intrastrain variability were identified as potential targets for strain differentiation: the acidic repeat protein (*arp*) gene, and a member of the treponemal pallidum repeat (*tpr*) gene family. Twelve different subtypes were distinguishable among 63 strains studied, with most (54.2%) possessing *arp* genes with 14 repeats. To the sexual health epidemiologist, these developments using molecular typing are well worth watching.

At present, the Public Health Laboratory Centre, Department of Health, provides a Quality Assurance (QA) Scheme for laboratories that perform serology tests for syphilis routinely. This QA service is provided free of charge to participating laboratories intending to continually improve on their work performance in the field of syphilis testing. With rise of sexually transmitted infections in parts of southern China, future laboratory investigations for syphilis would have to rely on highly sensitive and specific, rapid and automated tests. It is high time that due prudence be given to the diagnoses, treatment and case finding of this old disease.

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