

Testing time for syphilis in Kenya

Voluntary Service Overseas provides an opportunity to work in, and provide expertise to, remote populations and developing countries around the world. Here, Margaret Thumbi and Rita Drobner look at the value of screening for syphilis in East Africa.

In the UK, syphilis screening by the non-treponemal rapid plasma reagin (RPR) test is routinely followed up by more specific treponemal tests. However, false-positive screening test results occur in a variety of conditions unrelated to syphilis, such as in infectious mononucleosis, pregnancy, malaria, leprosy, systemic lupus erythematosus (SLE) and other autoimmune diseases. Therefore, most testing protocols state that positive RPR tests must be confirmed using a treponemal assay, and even the manufacturer advises that a positive test result be called 'RPR-reactive' rather than 'RPR-positive', and under no circumstances 'syphilis screen-positive'. Irrespective of the wording of the report, how helpful is the RPR result to clinical staff, particularly in developing countries such as Kenya?

SYPHILIS IN KENYA

Most healthcare providers in Kenya have sufficient resources to undertake the RPR test, but not to confirm it with the *Treponema pallidum* haemagglutination assay (TPHA). Reagent cost for the RPR test is eight Kenyan shillings (Ksh), which is about 12 pence, and this includes all the materials required. The confirmatory TPHA costs Ksh 50 (about 37 pence) per test in reagent costs, but also requires extra materials such as a microtitre plate and accurate pipettors, and will incur specimen referral costs. These extra costs must be covered by the patient, the primary healthcare provider or the referral laboratory. However, treatment for syphilis is free of charge, paid for by the Kenyan Ministry of Health as part of the country's national healthcare programme.

Unsurprisingly, therefore, tentative positive

results are more readily accepted, as the stigma of being diagnosed with syphilis appears to be lower than it would be in the UK. Among the population groups in the North Rift Valley, Western and Nyanza provinces of Kenya the main stigma is associated with human immunodeficiency virus (HIV) infection. Most patients in Kenya are enrolled in the HIV healthcare provisions of AMPATH (Academic Model for the Prevention and Treatment of HIV) programme. Therefore, an additional positive test result for syphilis – a disease that is treated easily and free of charge – is relatively inconsequential.

A further argument against confirmatory testing for syphilis is that this test may be of lower utility for detecting false screening positives in a population in which the incidence of syphilis is high. The RPR screening test detects the symptomatic primary, secondary and late stages of syphilis, but TPHA also detects antibodies that may be present many years after cure. Therefore, some false-positive RPR screens will be confirmed by TPHA, merely because the patient has a history of syphilis that had been treated successfully in the past.

In addition, samples that show non-

'False-positive screening results with the rapid plasma reagin test occur in a variety of conditions unrelated to syphilis'



Bordering on Uganda and Lake Victoria, the area and patient population is served by 19 AMPATH clinics. Reproduced by courtesy of IU-Kenya Partnership.

specific agglutination in the RPR test may also cross-react in the TPHA. Most cross-reactions will be apparent in the TPHA because each sample is tested against an internal control using unsensitised cells. Only if control cells do not agglutinate is the test result valid. False-positive TPHA results can still be obtained, due to the presence of other pathogenic treponemes not associated with syphilis (eg *T. pertenue* and *T. carateum*).

According to World Health Organization (WHO) statistics, sub-Saharan Africa has an estimated incidence of curable sexually transmitted diseases of about 250 per 1000 people of reproductive age (15–49 years). However, prevalence has been decreasing since the 1980s. Following a successful government health campaign, syphilis incidence was halved during the decade between 1990 and 2000 in antenatal clinics in Nairobi.

In light of the above, it would seem unreasonable to subject patients unnecessarily to three penicillin injections at the expense of the Kenyan Ministry of Health. Even if the patient had genital ulcers – making a diagnosis of syphilis more likely – penicillin is inappropriate and ineffective in treating *Haemophilus ducreyi*, herpes simplex virus 2, *Klebsiella granulomatis* and *Chlamydia trachomatis*, which are the other major causes of genital ulcers.

No accurate or independent data are available on the diagnosis or misdiagnosis of

syphilis among populations in the North Rift Valley, Western and Nyanza provinces. Therefore, the serology section of the AMPATH reference laboratories, which aim to promote evidence-based patient care and good laboratory practice, undertook a small study to discover the proportion RPR-reactive screens that could not be confirmed by TPHA.

CONFIRMATION OF SYPHILIS

The laboratory processed 1833 syphilis screens between May and July 2007. The RPR-reactive screens were tested by TPHA free of charge, and the additional results were made available to clinicians.

Materials required included the Immuntrep RPR test kit (Omega Diagnostics), Immuntrep TPHA test kit (Omega Diagnostics), microtitre plates and plate covers, pipettors (25 μ L, 75 μ L and 100 μ L) and a timer.

Blood samples were received from clinics in Vacutainers without anticoagulant and stored at ambient temperature (20–24 °C) until tested (up to five days). The kit manufacturer's instructions were followed for testing.

Rapid plasma reagin procedure

Briefly, one drop (50 μ L) of patient serum or control was added to a test card and spread to cover the entire circle. One drop (16 μ L) of antigen was added from the dispensing bottle (with needle assembled) to the test specimen. The test card was rotated at 100 rpm for eight minutes on an automatic rotator. The results were then inspected with the aid of a good light source. Reactive samples showed macroscopically visible aggregates, while non-reactive samples showed a smooth grey appearance with no aggregates.

Treponema pallidum haemagglutination assay procedure

Briefly, 25 μ L of diluent was dispensed in rows 1, 3, and 4, and 100 μ L of diluent was dispensed in row 2 of a microtitre plate. Then, 25 μ L of sample or control was added to row 1, mixed and 25 μ L was transferred to row 2. The contents of row 2 were mixed and 25- μ L amounts were transferred to row 3, mixed and 25 μ L was discarded. Then, 25- μ L amounts were transferred from row 2 to row 4, mixed and 25 μ L was discarded. Final dilutions in rows 3 and 4 were 1 in 80. Finally, 75- μ L amounts of mixed control (unsensitised) cells were added to row 3, and the same amount of



Laboratory training session at the AMPATH reference laboratory in Eldoret.

mixed test (sensitised) cells were added to row 4. The plate was tapped gently to mix and results were read after standing for 45–60 minutes at ambient temperature.

Agglutination in row 4 but not in row 3 indicated a positive result. Agglutinated cells formed an even layer or a diffuse ring over the bottom of the well. Non-agglutinated test cells formed a compact button in the bottom of the well and indicate a negative result. Agglutination in the control well indicated a non-valid result.

TO SCREEN, OR NOT TO SCREEN

Out of the 1833 patients screened, 56 (3.1%) samples were reactive by the RPR test, of which 39 were positive by TPHA (Table 1). Females represented a higher proportion (61%) of false positives by RPR; however, while this may not be significant due to the low numbers included in the study, SLE and pregnancy are two conditions that result in false-positive results in women.

Non-specific agglutination in the patient population studied was very high, although cross-reaction in the RPR test does not necessarily lead to cross-reaction in the TPHA. Of the 56 positive results obtained, not a single test was invalidated as a result of the agglutination of control cells.

Unfortunately, it was not possible to address the problem of the low sensitivity of the RPR test (70% in primary syphilis, 100% in secondary syphilis, and 70% in the late stages of the disease), but a treponemal confirmatory test clearly improved test result specificity.

If RPR tests are all that laboratories can

afford in the North Rift Valley in Kenya then they should not be used as a screening tool (ie when enrolling newly diagnosed HIV patients on healthcare programmes). The limitations of the test must be explained well to clinical staff, and the laboratory reports must state clearly that the RPR test alone cannot confirm or exclude syphilis infection.

ACADEMIC MODEL FOR PREVENTION AND TREATMENT OF HIV

The Academic Model for Prevention and Treatment of HIV (AMPATH; <http://medicine.iupui.edu/kenya/hiv.aid.html>) was started in 2000. It has grown to become one of the largest HIV care providers in East Africa. AMPATH cares for more than 52,000 HIV-infected adults and children, with nearly half of all patients on antiretroviral drugs.

Enrolment on the programme has risen to 2000 patients per month in 2007. Nineteen clinical centres across three Kenyan districts and an outreach department provide local care. The aims of AMPATH as a whole are to promote comprehensive care through medical access, in concert with initiatives for family preservation and secure livelihoods, food security, public mobilisation and education, and care for vulnerable children and orphans. ■

FURTHER READING

- American Proficiency Institute. *Educational commentary – laboratory testing for syphilis*. 2005. www.api-pt.com
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- World Health Organization. *Global prevalence and incidence of selected curable sexually transmitted diseases*. www.who/hiv_aids/2001.02

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Table 1. Breakdown of positive rapid plasma reagin screening tests.

	Patients		Split according to gender			
	Number	Percentage	Number		Percentage	
TPHA-positive	39	70%	Female	22	Female	56%
			Male	17	Male	44%
TPHA-negative	17	30%	Female	12	Female	71%
			Male	5	Male	29%
Total	56	100%	Female	34	Female	61%
			Male	22	Male	39%