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INVESTIGATIVE REPORT

Evaluation of Three Serological Tests Manufactured in Belarus for the Diagnosis of Syphilis

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The performance of three serological tests manufactured in Belarus for the diagnosis of syphilis, i.e. a microprecipitation reaction (MPR) and two enzyme-linked immunosorbent assays (ELISAs) were compared with internationally recognized assays, namely the rapid plasma reagin test and the *Treponema pallidum* passive particle agglutination assay (TPPA). Sera from 392 consecutive patients attending Brest (Belarus) regional dermatovenereological dispensaries were tested. The sensitivity of the MPR test was low (77.3%) compared with the rapid plasma reagin test, while the specificity was high (100%). In contrast, both Belarusian ELISAs performed well when compared with the TPPA (sensitivities of 99.2% and 100%, specificities of 98.7% and 99.0%, respectively). There is a clear need to improve the sensitivity of the existing Belarusian MPR test or to use a more sensitive screening test in order to improve diagnosis of the disease in Belarus. *Key words: syphilis; serology; laboratory diagnosis; Treponema pallidum passive particle agglutination; rapid plasma reagin; Belarus.*

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Belarus is an Eastern European country with a population of just under 10 million, of whom two-thirds are urban and one-third are rural dwellers (1). Syphilis remains a significant public health problem in the country, with an incidence rate of 27.1/100,000 inhabitants in 2007 (2). Approximately 451,000 serum samples are tested for syphilis each month in 140 serological laboratories. Of these laboratories, 93% use a nationally-manufactured non-treponemal test (the microprecipitation reaction (MPR)) as the initial serological screening test for the disease (3). The MPR, which is extensively used in the Newly Independent States (NIS) (4–6) is analogous to the internationally well-recognized Venereal Disease Research Laboratory (VDRL) test, but without the use of a microscope. This method can be applied to serum or to plasma specimens and detects non-specific antibodies to cardiolipin. The reagents for the MPR test are produced by several manufacturers in the NIS. In addition, nationally-manufactured enzyme-linked immunosorbent assays (ELISAs), which detect specific treponemal antibodies, are widely used in the region as confirmatory tests for the disease. In a recent survey of diagnostic laboratories in Belarus, 57% used these nationally-manufactured treponemal ELISAs (3).

While many laboratories adopt a conventional approach to serological testing for syphilis, namely screening with the non-treponemal test, followed by confirmation of reactive sera using a treponemal assay, many others have adopted an approach whereby both non-treponemal and treponemal tests are performed simultaneously and treatment is provided if either test proves positive. This approach has emerged largely as a result of a loss of faith in the performance of the MPR test, arising from the situation where many patients have anti-treponemal antibody responses, but show no reactivity in the non-treponemal MPR test.

Despite their widespread use, there are no data available concerning the performance characteristics of these nationally-manufactured tests and no direct evaluations have been made comparing these local tests with internationally-acknowledged "gold-standard" tests.

Unfortunately, Belarus is not alone in this regard. Monitoring of the quality of laboratory tests in other Eastern European countries does not occur systematically. Among the NIS, Russia is the largest producer of diagnostic tests and these are widely distributed throughout the region. However, many countries also have small manufacturers that provide national laboratories with relatively inexpensive reagents compared with those available in the West.

The present study aimed to evaluate three tests for the serological diagnosis of syphilis manufactured in Belarus against internationally, well-recognized and validated non-treponemal and treponemal tests, namely the rapid plasma reagin (RPR) and *Treponema pallidum* passive particle agglutination assay (TPPA)

and to suggest appropriate approaches to provide a rational basis for the serological diagnosis of syphilis in that country.

PATIENTS AND METHODS

Patients and specimens

The patients included in the study comprised 392 consecutive attendees at the Brest regional skin and venereal disease (SVD) clinics, Brest, Belarus, between April and June, 2008. These patients presented after referral from peripheral clinics for specialist management of sexually transmitted infection (STI)-related conditions, including syphilis.

Serum obtained from each patient (one per patient) was heat inactivated and tested using the three Belarusian and the two international comparator tests.

Laboratory methods

A single cardiolipin-based non-treponemal MPR test, manufactured by the Institute of Bioorganic Chemistry of the Academy of Sciences of Belarus (BAS), Minsk, Belarus, was evaluated by comparing its performance against a quantitative RPR test (Macro Vue RPR Card Test, Becton Dickinson, USA). The MPR test is a flocculation-based test, similar to the VDRL test. It is performed in microtitre plates without a charcoal indicator, but is not read using a microscope. Flocculation reactions are read using a 4+ scoring system. Samples showing reactivity using undiluted serum were titrated using two-fold dilutions up to a 1:64 dilution.

In addition, two locally produced treponemal assays were evaluated, namely the "ELISA-syph-summaric assay" (Farmland, Minsk, Belarus), which detects IgA, IgM and IgG antibodies to Tp17 and Tp41 antigens and the "ELISA-trep" test (BAS), which detects IgG antibodies to Tp15, Tp17, Tp41 and Tp47 antigens of *T. pallidum* were compared with the TPPA (Serodia–TPPA, Fujirebio, Japan).

All tests were performed and interpreted according to manufacturers' instructions.

RESULTS

The results of testing 392 sera using the MPR, RPR, TPPA, ELISA-syph-summaric and ELISA-trep are presented in Table I.

Table I. *Different combinations of the sera testing results using microprecipitation reaction (MPR), rapid plasma reagin (RPR),* Treponema pallidum *passive particle agglutination test (TPPA), enzyme-linked immunosorbent assay (ELISA)-syph-summaric and ELISA-trep*

No. of sera reacting in different tests	MRP	RPR	TPPA	ELISA-syph-summaric	ELISA-trep
61	+	+	+	+	+
3	+	+	–	–	–
1	+	+	–	–	+
19	–	+	+	+	+
32	–	–	+	+	+
5	–	–	–	+	+
3	–	–	–	–	+[a]
1	–	–	–	+[a]	–
267	–	–	–	–	–

[a]Test result regarded as false positive.

Of the 392 sera tested, 65 were found to be reactive in both the MPR and RPR tests. However, 19 additional sera were reactive in the RPR test, but non-reactive in the MPR test. While the majority ($n = 11$) of those 19 sera were reactive at low titres ($\leq$ 1:2) in the RPR test, eight sera had higher titres ($\geq$ 1:4), and two of these sera had an RPR titre of 1:16. All 19 sera that were RPR-positive and MPR-negative, were found to be reactive in all the treponemal tests used in the study, indicating that they were true positives. In addition, three sera were found to be reactive in both non-treponemal tests, but non-reactive when tested by all the three treponemal tests. These were considered as biological false-positive reactions. Overall, the MPR test proved to have a low sensitivity (77.3%), but was highly specific (100%) when compared directly with the RPR test. However, both non-treponemal tests produced biological false-positive reactions. At a high seroprevalence of 21.4%, the MPR test had a positive predictive value of 100% and a negative predictive value of 94.7% compared with the RPR test.

In contrast, of the 392 sera tested with the three treponemal tests, 121 were found to be reactive by at least one treponemal test. Five of these were non-reactive in the TPPA, but positive when tested by both the ELISA tests. Three were reactive in the ELISA-trep test, but were non-reactive in all other non-treponemal and treponemal tests. One serum specimen was reactive only in the ELISA-trep test and positive by both the MPR and RPR tests, indicating that the ELISA-format is marginally more sensitive than the TPPA test.

The overall performance characteristics of the Belarusian tests and the TPPA test in this high-prevalence setting are presented in Table II.

DISCUSSION

This study represents the first ever attempt to evaluate the performance of diagnostic test systems produced in Belarus for the serological diagnosis of syphilis, using internationally recognized standards, by evaluating a Belarusian non-treponemal MPR test against RPR and

Table II. *Performance characteristics of the microprecipitation reaction assay (MPR), enzyme-linked immunosorbent assay (ELISA)-syph-summaric and ELISA-trep tests manufactured in Belarus, following testing of sera (one per patient) from 392 consecutive patients with suspicion of syphilis*

Performance characteristics	MPR	ELISA-syph-summaric test	ELISA-trep test	Serodia-TPPA
Sensitivity (%)	77.3	99.2	100	94.9
Specificity (%)	100	98.7	99	100
PPV (%)	100	96.7	97.5	100
NPV (%)	94.7	99.7	100	98.1

TPPA: *Treponema pallidum* passive particle agglutination test; PPV: positive predictive value; NPV: negative predictive value.

two Belarusian treponemal ELISAs against TPPA. The MPR test is widely used in practice in NIS countries (4–6) and is designed to serve the needs of high-volume laboratories. It is run both as a qualitative, as well as a quantitative, test and is therefore used routinely to monitor the effectiveness of therapy.

The main drawback of the MPR test is its dependence on interpretation by highly experienced laboratory personnel, since there is no charcoal indicator or microscope to facilitate reading. In Belarus the MPR test evaluated here is used for initial screening, with positive results being confirmed by a complex series of serological tests that include a further non-treponemal test and two confirmatory treponemal tests (3). This study indicated that when the test is used as the initial screening test, approximately one-quarter of all reactive cases would remain undetected. In addition, it should be noted that, while the specificity of the MPR test was 100% (compared with the RPR test), three biological false-positive reactions were detected in this relatively small series, using both non-treponemal tests. Under these circumstances, the Belarusian MPR test cannot be recommended as the initial serological screening test for syphilis in its present form. It is clear that further studies are required to determine whether the lack of sensitivity of the MPR test recorded here is unique to the Belarusian brand, or whether there is an intrinsic lack of sensitivity in the MPR test *per se*. This is likely, since a sensitivity of 41.7% was recorded for a Russian MPR test when it was used to diagnose syphilis in a low-prevalence (1.2%) population in Moscow (7).

In contrast, the performance characteristics of both Belarusian ELISAs were excellent, with an indication that they were marginally (but not statistically) more sensitive than the TPPA – a fact that has also been observed by others (8). It is clear that either Belarusian ELISA test can be used with confidence as a confirmatory test.

However, the results of this study highlight a dilemma for public health authorities in Belarus, where considerable funds are spent on serological testing for syphilis. Ideally, the local manufacturers should improve the performance characteristics of the Belarusian MPR test or give consideration to local production of an RPR test or a TRUST test (in which toluidine red is used as an indicator in place of charcoal) as a replacement.

The major obstacle with respect to the high-quality Western analogues for the countries of Eastern Europe is their expense, which makes them unaffordable in many countries. Eastern European tests are five to ten times cheaper than their Western analogues. However, in order to continue to use locally produced tests, the question of test validation against internationally acknowledged standards must be addressed.

If local production of a sensitive non-treponemal test is not possible, the Belarusian authorities should consider an alternative algorithm for syphilis serological testing. In the USA and Western Europe, many laboratories use treponemal ELISAs (which can be automated easily) as primary screening assays. All sera that are found to be reactive should then be tested with a non-treponemal test (9) and treatment provided only when both tests are found to be reactive. Under these circumstances, the country could import a relatively small number of internationally validated RPR tests as "confirmatory" assays. However, it is important to bear in mind that treponemal serological tests remain positive even after successful treatment; they therefore cannot be used alone to establish a diagnosis of syphilis.

In conclusion, bearing in mind financial constraints and the need to provide a more rational basis for serological testing for syphilis, this may be an opportune time to revise policies regarding syphilis screening in Belarus. At present, testing of kindergarten staff, handlers and other food industry employees, education and healthcare providers is required by law. These groups are at low risk of disease and do not present a risk of spread of infection as a result of their occupation. A targeted approach to screening, which reduces spread of disease among high-risk groups, such as STI patients and their contacts, commercial sex workers and men who have sex with men, and which prevents consequences of the disease (by improving antenatal screening) would prove more cost-effective than existing practices (10–13).

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