

# Diagnosis of syphilitic genital ulcer- the quest for a reliable laboratory method

## Abstract

**Objectives:** Dark field microscopy (DFM), once performed widely as a reliable method of diagnosing Syphilis, is rapidly losing ground to newer serological tests and molecular methods, including PCR. This study aimed at tracing the performance of Dark Field Microscopy and evaluating its usefulness in the present scenario. Also, doubts have been raised about the accuracy of the routinely used Syphilis non-specific screening tests such as VDRL and RPR, especially in populations with a high prevalence of HIV and malaria. The perils of untreated syphilis include dire consequences of latent or tertiary stages as well as transmission of infection from mother to foetus, with resultant congenital syphilis. An analysis of available diagnostic tests for syphilis was carried out to evaluate the performance of each method and assess its usefulness in providing an accurate diagnosis.

**Methods:** This study was part of an operational research project which involved analysis of data of one year, during which dark field microscopy was performed for genital ulcer disease, along with other serological tests like VDRL, TPHA and FTA-Abs, for the diagnosis of Syphilis.

**Results:** Of the 100 cases of genital ulcer diseases recruited in the study, over a period of one year, 7 were clinically diagnosed as Syphilis alone, while 4 were diagnosed as mixed infections of Syphilis along with Chancroid or Herpetic ulcer. The rest were clinically diagnosed cases of Herpes genitalis alone (78) or mixed infections of chancroid and herpes genitalis. From the study it was evident that DFM had poor sensitivity (33.3%), but high specificity (100%) with clinical diagnosis having 85% sensitivity. All other diagnostic methods, including VDRL, TPHA, FTA-Abs and ELISA-Tp, had 100% sensitivity and specificity.

**Conclusion:** With the advent of HIV infection, GUDs with two or more causative organisms are common, making aetiological diagnosis difficult without sophisticated laboratory tests. Serological tests for syphilis are simple to perform, but are only useful when the infection is not very recent. *Treponema pallidum* PCR, while more informative early in infection, is not available widely in developing countries, due to cost constraints. From the present study, it can be concluded that both non-specific and specific tests for diagnosis of Syphilis are reliable and can continue to be used for diagnosis, especially in resource poor settings where other diagnostic tests may be unavailable. Clinical diagnosis needs to be backed by laboratory methods to improve the accuracy, and institute appropriate treatment. Dark field microscopy, although not very sensitive, should continue to be used, as it is highly specific and offers an immediate diagnosis, which will be of immense value to the clinician and patient for early and appropriate treatment.

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Sumathi Muralidhar, Joginder Kumar, Deepa Anil Kumar, Nilofar Khan, Manju Bala, Ramesh V

Department of Apex Regional STD Centre & State Reference Laboratory for HIV, Vardhman Mahavir Medical College, India.

**Correspondence:** Sumathi Muralidhar Professor & Consultant Microbiologist, Department of Apex Regional STD Centre & State Reference Laboratory for HIV, Vardhman Mahavir Medical College Safdarjung Hospital, New Delhi, India, Email sumu3579@yahoo.com

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## Introduction

The diagnosis of Syphilitic genital ulcer poses a challenge to venereologists, because atypical presentations and multiple aetiologies are common occurrences. It is important to diagnose and manage these cases appropriately, as they are known to facilitate the spread and acquisition of HIV infection.<sup>1</sup> With the shift from bacterial to viral genital ulcer diseases (GUD), syphilitic genital ulcers are no longer as common as herpetic ulcers, and the HIV epidemic has been blamed for this.<sup>2-4</sup> For effective control of syphilis, it is important to have inexpensive and sensitive diagnostic tests, followed by an effective and affordable treatment. Lack of reliable diagnostic tests for syphilis, hampers the efforts at controlling the disease. The perils of untreated syphilis include dire consequences of latent or tertiary stages as well as transmission of infection from mother to foetus with resultant congenital syphilis.<sup>1</sup> Because *T.pallidum* is too fragile

an organism to be stained or cultured by simple methods, diagnostic tests rely on clinical evaluation, detecting the organism from lesions by dark field microscopy or fluorescent microscopy, and confirmation of the disease by serological diagnostic methods, since most infected individuals have no symptoms or have transient lesions.<sup>5</sup> Although serodiagnosis is the mainstay of diagnosis of all stages of syphilis, conventional serology is a two step approach with a non-specific test done first, followed by a specific test. Dark field microscopy (DFM) is a very useful diagnostic method that allows visualization of live treponemes obtained from cutaneous or mucous membrane lesions. But the sensitivity of this test is quite low, at less than 80%, and declines over time, or if the patient has applied topical antibiotics to the lesion. Oral specimens cannot be used for DFM, because of the possibility of false-positive results.<sup>6</sup> Molecular methods have emerged as very important tools for detection of *Treponema pallidum*. Polymerase chain reaction (PCR), and recently, the Realtime PCR,

have been proved by several studies to be very sensitive and specific, so much so that they could well become the gold standard tests of the future.<sup>4,7</sup> Also, doubts have been raised about the accuracy of the routinely used Syphilis non-specific screening tests such as VDRL and RPR, especially in populations with a high prevalence of HIV and malaria.<sup>8</sup> There is insufficient data on the utility and comparison of various diagnostic tests for syphilitic genital ulcers. This prompted the present study to analyse the available diagnostic tests for syphilis and evaluate the performance of each method to assess its usefulness in providing an accurate diagnosis.

## Material and methods

The study was carried out an Apex Regional STD centre of a tertiary care hospital in New Delhi, as part of an operational research project of one year duration. A total of 100 consecutive cases of genital ulcer disease, from both male and female patients attending the STI clinics, were included in the study. A detailed clinical and sexual history was taken and clinical examination performed for each case recruited in the study. When a case of genital ulcer disease was recruited, whatever the type of ulcer diagnosed clinically, all the cases were subjected to a battery of tests for Syphilis, which included –

- a. Dark field microscopy of specimens from genital ulcers- a specimen was collected from the ulcer by cleaning the area, compressing the base of the ulcer, and collecting the serous fluid with minimal blood, on to a glass slide. A cover glass was applied to this slide and examined under a microscope using a dark field condenser, within 20 minutes.
- b. Serological tests for syphilis (VDRL, TPHA, FTA-Abs and Tp-ELISA)- These tests were performed on sera separated from blood samples of patients with genital ulcers-
  - i. VDRL (Venereal Disease Research Laboratory) test is a non-specific, slide flocculation test where the antigen used is cardiolipin. Reaginic antibodies are detected in the patient's serum. The test is reported as non-reactive or reactive. If reactive, a quantitative test is performed by putting up dilutions and establishing the titre of antibodies.
  - ii. TPHA (*Treponema Pallidum* Haemagglutination) test- This is one of the confirmatory tests performed on VDRL reactive serum samples. It is a haemagglutination test performed on microtitre plates, as per kit manufacturer's instructions.
  - iii. FTA-Abs (IgG)(Fluorescent Treponemal Antibody Absorption) test-This is one of the confirmatory tests performed on VDRL reactive serum samples. It requires a fluorescent microscope and kits containing slides with formalinized *Treponema pallidum* (Nichol's strain) and fluorescent conjugates.
  - iv. ELISA-Tp (IgG)- this is an enzyme immunosorbent assay for the detection of IgG class antibody to *Treponema pallidum* in human serum or plasma. It is a specific test for syphilis.
- c. Realtime –PCR was performed on the swabs collected from the genital lesions as per prescribed methods. Special swabs provided with the specimen transport medium (STM) were used to collect the ulcer swabs. These swabs were then sealed in the provided STM tubes (Gentech Diagnostics), and stored at –20°C, till further processing. Realtime PCR using the kits from Biotron Healthcare

Pvt. Ltd. Realtime PCR was performed for *Treponema pallidum* in only 93 cases, due to exhaustion of extraction kits.

Appropriate controls were put up for all the tests and other methods of standardization were used to ensure quality of the test procedures. Clearance was obtained from the institutional ethics committee before the start of the study.

## Results

Of the 100 cases of genital ulcer diseases recruited in the study, over a period of one year, 7 were clinically diagnosed as Syphilis alone, while 4 were diagnosed as mixed infections of Syphilis along with Chancroid or Herpetic ulcer. The rest were clinically diagnosed cases of Herpes genitalis alone (78) or mixed infections of chancroid and herpes genitalis. Although Syphilis was clinically diagnosed in only 11 of the 100 cases of GUD, the diagnostic tests for Syphilis were performed on all the 100 GUD cases, except for the Realtime PCR test for *Treponema pallidum*, which was not performed on 7 cases due to exhaustion of the extraction kits. Table 1 demonstrates the results of the various diagnostic tests performed on the 11 cases of clinically diagnosed syphilis. From the table, it is evident that a clinical diagnosis of Syphilis is not necessarily confirmed as Syphilis by laboratory methods. Considering real time PCR as the gold standard, the sensitivity and specificity of other diagnostic methods for syphilis are shown in Table 2. From the above it is evident that dark field microscopy had poor sensitivity (33.3%), with clinical diagnosis having 85% sensitivity. But all other diagnostic methods, including VDRL, TPHA, FTA-Abs and ELISA-Tp, had 100% sensitivity and specificity. The sample size seems small for Syphilitic genital ulcers, but considering the declining incidence of bacterial GUDs and increasing viral GUDs over the last 2 decades, it is unlikely to have recruited more cases. Also, the main outcome of this study has been justified (to compare the various diagnostic modalities for syphilitic genital ulcers).

## Discussion

In the present study, although 100 cases of genital ulcer disease were recruited in the study group, only 11 were clinically diagnosed as Syphilis, either alone or with other genital ulcer diseases. This indicates that other causes of GUD outnumber Syphilis, especially herpetic ulcers. Clinical diagnosis of Syphilis had a sensitivity of 85%, which indicates that 15% of the times Syphilis is falsely diagnosed. The sensitivity of dark field microscopy in the present study was only 33.3%, compared to 78.8% and 73% in studies by Romanowski et al and Daniel et al, respectively.<sup>9,10</sup> The sensitivity of dark field microscopy has a bearing on the timing of the procedure after appearance of the ulcer. It is also affected by application of antibiotic or other creams to the ulcerative wounds, or the quality of the specimen collected and sent to the laboratory.<sup>11</sup> The poor sensitivity may even be explained by the fact that the dark field microscopy was performed by several faculty members with varying degrees of experience and expertise. A 100% specificity for dark field microscopy makes it a valuable option for diagnosing syphilitic genital ulcers, especially as a primary screening tool. This will be very useful in instituting early treatment of cases during the first visit itself. All the specific tests for diagnosis of Syphilis viz TPHA, FTA-Abs and ELISA-Tp IgG, showed a 100% sensitivity and specificity, which means they can reliably be used for the diagnosis of Syphilis. Other studies corroborate these findings.<sup>1,5,12</sup>

**Table 1** Results of various diagnostic tests performed on clinically diagnosed Syphilitic genital ulcer

Sl. No.	Clinical Diagnosis	Dark field microscopy	VDRL	TPHA	FTA-Abs	ELISA- Tp IgG	Real time PCR for Tp
1	Syphilis	Positive	1:32	++++	+++	+	+
2	Syphilis	Negative	Non reactive	-	-	-	Negative
3	Syphilis	Negative	Non reactive	-	-	-	Negative
4	Syphilis	Negative	1 : 64	++++	+	+	+
5	Syphilis	Negative	1:32	++++	+	+	+
6	Syphilis and Herpetic ulcer	Negative	1:02	++++	+	+	+
7	Syphilis + Herpetic ulcer	Negative	Non reactive	-	-	-	Negative
8	Syphilis	Positive	1:16	+	+	+	+
9	Syphilis+ Herpetic ulcer	Negative	1:16	+	+	+	+
10	Syphilis + Herpetic ulcer + chancroid	Negative	Non reactive	-	-	-	Negative
11	Syphilis	Negative	Non reactive	-	-	-	Negative

**Note:** There was one case diagnosed clinically as Herpetic ulcer, but was reactive by VDRL (1:4) and positive by TPHA, FTA-Abs, ELISA- Tp IgG and Real time PCR for Syphilis.

**Table 2** Considering real time PCR as the gold standard, the sensitivity and specificity of other diagnostic methods for syphilis

Diagnostic method	Sensitivity (in %)	Specificity (in %)
Clinical diagnosis	85	-
Dark field microscopy	33.3	100
VDRL	100	100
TPHA	100	100
FTA-Abs	100	100
ELISA- Tp	100	100

**Limitations of the study:** There were a few limitations of this study which may have had a bearing on the results. These include-

- Small sample size- this can be explained by the fact that the incidence of syphilitic (bacterial) genital ulcers has declined over the last two decades. Also, the sample size could have been increased if the study period had not been restricted to one year.
- Real time PCR kits were limited in number and hence, not all samples could be subjected to this test. This restricted the numbers for comparison of all the diagnostic tests for syphilitic genital ulcers.

## Conclusion

Syphilis control is facilitated by the availability of inexpensive and reliable diagnostic tests, followed by affordable treatment. Early detection and treatment is also critical in preventing severe long term complications in the patient and onward transmission to sexual partners.<sup>2</sup> Although molecular methods of diagnosing genital ulcer diseases offer very high sensitivity and specificity, the time consumed and cost incurred in generating the results, as compared to traditional methods, prohibit their use in screening specimens, especially in the developing nations. From the present study, it can

be concluded that both non-specific and specific tests for diagnosis of Syphilis are reliable and can continue to be used for diagnosis, especially in resource poor settings where other diagnostic tests may be unavailable. Clinical diagnosis should be backed by laboratory methods to improve the accuracy, and institute appropriate treatment. Dark field microscopy, although not very sensitive, should continue to be used where the facility is available, as it is highly specific and offers an immediate diagnosis, which will be of immense value to the clinician and patient for early and appropriate treatment. It may provide a critical complementary role in the diagnosis of syphilitic ulcers, in resource poor settings. An added advantage of dark field microscopy is that, unlike other serological tests for syphilis, it does not yield a delayed or reduced response when there is concomitant HIV infection or full blown AIDS, when antibody responses may be erratic.<sup>12</sup> Thus, there is still a role for dark field microscopy in the diagnosis of Syphilis. Measures need to be taken to maintain the quality of the procedure and to expand testing sites which see a high prevalence of primary and secondary syphilis.

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## Conflict of interest

None.

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