Laboratory Detection of Sexually Transmitted Diseases

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Abstract

Laboratory investigations required to diagnose STDs in a developing country like India are not always feasible or cost effective. Traditionally, diagnosis and treatment of STDs has been dependant on syndromic approach based on clinical symptoms but strengthening lab services for specific diagnosis is important to prevent overtreatment and development of antimicrobial resistance. Rapid and cost effective POC (point of contact) tests with reasonable sensitivity and specificity are being developed to address STDs at primary health care level and are especially useful in centres with low patient return rate. More specific, tedious, quality controlled procedures like culture and NAAT (nucleic acid amplification technique) assays can be carried out in reference laboratories with high sample load and high throughput.

Introduction

Sexually transmitted diseases are a major health concern in a developing country like India and pose number of challenges from diagnosis to treatment. Sexually Transmitted Diseases (STDs) can be caused by a variety of pathogens including bacteria, virus, fungus and protozoa. Excluding HIV, recent trends report a decline in bacterial STDs like gonorrhoea and chancroid with an upswing in viral STDs like herpes genitalis and condyloma acuminata [1]. Although both men and women are affected, the morbidity, complications and sequelae are much more significant in women like pelvic inflammatory disease (PID), ectopic pregnancy, infertility, cervical cancer etc. In the recent years,

syndromic approach was devised by World Health Organisation (WHO) for diagnosis and treatment of STDs in resource poor settings to circumvent expensive and laborious lab testing and has been successfully adopted by National AIDS Control Organisation (NACO) in India. Nevertheless, lab services need to be strengthened because apart from diagnosing STDs, lab tests also serve as useful tools for antimicrobial susceptibility testing, validation of syndromic management algorithms, screening of asymptomatic at risk individuals, disease surveillance and quality assurance [2].

In this review article, we discuss lab diagnosis of individual pathogens under three main headings according to presenting complaints and their differential diagnosis.

1.Patients presenting with cervicitis, vulvovaginitis, urethritis, proctitis and pharyngitis

1.1 Gonorrhoea

Gonorrhoea and chlamydia are the most common bacterial STDs prevalent worldwide [3]. The causative gram negative bacteria Neisseria gonorrhoea leads to lower urogenital infections, predominantly urethritis in males and cervicitis in females with purulent discharge. Proctitis and pharyngitis are seen more commonly in homosexual males. Conjunctivitis in adults and ophthalmia neonatorum in newborn (perinatal infection) is also seen.

Microscopy is highly sensitive and specific for urethral discharge samples in symptomatic males. Gram stained smears prepared from the discharge demonstrates intracellular gram negative diplococcii polymorphs. It is not recommended for rectal and pharyngeal samples due to lower sensitivity and large number of other obscuring organisms.

Culture of causative organism is inexpensive, highly sensitive and specific and also allows for antimicrobial susceptibility testing which is of utmost importance in gonorrhoea due to frequent emergence of antimicrobial resistance [4,5]. NAAT (nucleic acid amplification test) which detect specific DNA (deoxyribonucleic acid) or RNA (ribonucleic acid) sequences of the bacteria have been developed and are more sensitive than culture, especially for rectal and pharyngeal specimens but may lead to false positives (cross reactivity with commensal Neisseria species) which can be reduced by conducting a second NAAT using a different target sequence. Since no internationally licenced NAATs are yet available, they need to be validated in-house within the laboratories [2]. The ideal sample is first void urine from asymptomatic males and vaginal/cervical swab in females.

Serological tests and rapid POC (point of contact) tests are not yet approved and commercially available and are under research.

1.2 Chlamydia

Chlamydia trachomatis has 3 biovars based on tissue tropism, those that cause endemic blinding trachoma (serovar A-C), sexually transmitted urogenital infections (serovar D-K) and invasive lymphogranuloma venereum (serovar L1,L2,L3).

Internationally approved NAAT assays are the recommended test of choice for diagnosis and screening of both genital and extragenital chlamydial infections wherever feasible and are preferred over culture methods as they are more sensitive, do not require invasive sampling (urine or vaginal swabs can be used instead of urethral

or endocervical swabs) or stringent transport and storage (not dependant on organism viability), have lesser turn around time and can test for both chlamydia and gonorrhoea simultaneously [6]. In addition, antimicrobial susceptibility testing which requires culture is not routinely advised in chlamydia. Although NAATs may not be feasible in resource constrained settings, the problem can be addressed by creating regional reference labs especially in high prevalence areas where testing of large sample load and high through put automation will help reducing costs and turn around time while maintaining quality reporting.

Despite being less sensitive, POC tests which are ELISA (enzyme linked immunosorbent assay) based methods, offer the ability to test and treat the patient on site which is extremely valuable in resource poor, high prevalence settings and where patient follow up rate is low [7].

Serology measures antibody response to chlamydial antigens which may be delayed or not measurable in case of uncomplicated urogenital infections. It can be used for diagnosing complicated infections, LGV and neonatal pneumonia where antibody titres are high.

1.3 Trichomoniasis

It is the commonest non viral, non bacterial STD prevalent worldwide caused by flagellated protozoan Trichomonas vaginalis leading to cervicitis and frothy white discharge in females and urethritis in males. The ratio of infection in females to males is 4:1 [8,9]. Majority of infections are asymptomatic [10], yet lab diagnosis is important as trichomonas infection significantly increases the risk of HIV transmission and requires treatment to reduce genital compartment viral load.

Microscopy serves as a useful first line diagnostic test being highly specific but negative tests require further evaluation. Sensitivity is highest in symptomatic females. Vaginal swab in females and urine sediment in males is examined by wet mount microscopy for motile trichomonads within 10 minutes of collection.

POC tests are antigen detection assays and are more sensitive than microscopy but most of these can be used only for symptomatic females.

Culture is more sensitive than microscopy and POC tests but is more tedious and time consuming. Moreover, routine antimicrobial susceptibility testing is also not recommended. Hence, NAATs which are most sensitive tests and offer greater flexibility in sample collection are recommended. In addition, there are NAATs which test for gonorrhoea, chlamydia and trichomonas simultaneously.

1.4 Mycoplasma

Mycoplasma genitalium is causative organism of non gonococcal urethritis, cervicitis and upper genital tract infection in women while Mycoplasma hominis and Ureaplasmas are commensals.

NAAT is the only practical method for diagnosis but no assay has been FDA (Food and Drug Administration) approved yet and hence requires in-house validation. Sample preparation and assay sensitivity should be optimal since concentration of mycoplasma is 100 fold lower than chlamydia with multiplex assays having slightly lower sensitivity [2].

Due to high frequency of resistance to macrolides and fluoroquinolone, antimicrobial susceptibility testing is also recommended by using broth dilution minimal inhibitory concentration determination and detecting mutated sequences using molecular methods.

1.5 Bacterial Vaginosis

Bacterial vaginosis is not a sexually transmitted disease but sexual activity increases its risk of acquisition [11]. It's the most common cause of vaginal discharge in women of reproductive age group.

Amsel's criteria [12] used for diagnosing bacterial vaginosis include:

1. Homogenous greyish white adherent discharge
2. Vaginal pH of more than 4.5 measured by pH indicator strips (most sensitive but least specific as raised pH may occur due to contamination of vaginal fluid with cervical mucus, semen or menstrual blood and in T. vaginalis infection)
3. Release of fishy amine odour from vaginal fluid when mixed with 10% KOH (potassium hydroxide) solution
4. Clue cells on microscopic examination of vaginal fluid.

Gram stained vaginal smears under microscope are the preferred method for lab diagnosis and are interpreted using Ison-Hay criteria or Nugents score. Culture is of no diagnostic value.

2. Patients presenting with ulcers or lesions

2.1 Syphilis

Venereal transmission of the causative agent Treponema pallidum occurs through direct contact with treponeme rich ulcers of primary and secondary syphilis.

Direct diagnostic methods

1. Demonstration of motile T. pallidum by dark-field microscopy remains the simplest and reliable method, especially in treponeme rich ulcers of primary stage and immunodeficient individuals [13].
2. Direct fluorescent antibody (DFA) test employs fluorescein isothiocyanate-labelled antibody against treponemal antigen and does not require motile treponemes but it cannot differentiate venereal syphilis from endemic syphilis (yaws, pinta) [14].
3. PCR (polymerase chain reaction) based methods detect T. pallidum DNA with high sensitivity and specificity are test of choice for congenital syphilis, neurosyphilis and early primary syphilis [15, 16]. Multiplex PCR assays which detect T. pallidum, H. ducreyi and HSV simultaneously are also available now.

Indirect diagnostic methods

1. Non treponemal serologic tests are rapid, simple, inexpensive micro-flocculation tests and include VDRL (venereal disease research laboratory) slide test and rapid plasma reagin (RPR) card test. These tests help to establish a baseline titre for evaluation of recent infection, response to treatment and detecting reinfection/relapse in persons with persistently reactive titre. Sensitivity is reduced in primary syphilis and late latent syphilis. Prozone reactions and cross reactivity lead to false negative and positive results respectively. [17].
2. Treponemal serological tests include Treponema pallidum particle agglutination (TP-PA), EIA (enzyme immune-assay) and western blot tests. These tests are more specific but may remain reactive for years and do not differentiate venereal syphilis from endemic syphilis. Hence, these tests are used mainly to verify reactivity in non treponemal tests.

2.2 HSV

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are large double-stranded DNA viruses with the former acquired in childhood causing oral lesions while the latter causes sexually transmitted vesicular genital lesions.

Direct cytological examination is not used for diagnosis owing to low sensitivity and specificity.

Detection of viral antigen in lesional material is done using direct immunofluorescence (IF), immunoperoxidase (IP) staining or capture ELISA. Direct IF and IP staining have similar sensitivity but the former is more time-consuming and expensive (requires fluorescence microscope). Capture ELISA has comparable or higher sensitivity than culture for typical presentations but lower when cervical or urethral swabs are used.

Culture and molecular methods are the mainstay for diagnosis [18]. Cell cultures demonstrating cytopathic effects are further confirmed and typed using direct IF, IP staining or NAAT assay. Sensitivity of culture decreases as lesions begin to crust. NAAT assays are most sensitive and even detect asymptomatic HSV shedding. Serological assays are only used for screening of HSV exposure in high risk group like HIV infected individuals and asymptomatic partners of HSV infected individuals.

2.3 Chancroid

Chancroid is caused by Haemophilus ducreyi and produces painful penile, perianal and vulval ulcers alongwith suppurative inguinal lymphadenitis. Unlike syphilitic ulcer, chancroid ulcer is painful, irregular with undermined edges and usually not indurated.

Direct microscopy has very low sensitivity and specificity due to secondary contamination of ulcer by polymicrobial flora. Bacteriological culture and biochemical reactions for H. ducreyi remains the gold standard for diagnosis of chancroid [19]. Successful culture is critically dependent on using freshly made media (ideally fewer than 7 days old) and correct incubation conditions. H. ducreyi colonies are positive for oxidase, nitrate reduction and porphyrin test and negative for catalase, indole and urease tests [20].

Serological assays and molecular methods are not yet commercially available and are under research.

2.4 Donovanosis

Donovanosis or granuloma inguinale is caused by Klebsiella granulomatis, a Gram-negative bacterium leading to beefy red, hypertrophic, granulomatous genital ulcers. Pseudo-buboes are formed after secondary infection.

Laboratory diagnosis depends on the visualization of 'Donovan bodies' within cytoplasmic vacuoles of histiocytes, plasma cells and polymorphs in smears prepared from lesions or histological sections of tissue biopsies stained using Warthin–Starry silver impregnation reagent [21]. The 'Donovan bodies' appear as blue to purple coloured organisms surrounded by a prominent clear to acidophilic pink capsule and resemble closed safety pins.

Culture is tedious and done only in specialist centres. NAAT or serological assays are currently not available to assist in diagnosis [22].

2.5 HPV

Human papillomavirus is divided into high risk (HR HPV – 16,18,31,33 etc) and low risk (LR HPV-6,11) types with the former causing precancerous and cancerous anogenital lesions while the latter

causing anogenital warts (condyloma acuminata) and non-precancerous lesions. It is a fairly common sexually transmitted, transient infection with slow clearance following cell mediated immune response [23]. Persistent infection is a pre-requisite for development of cancer. The humoral response mounted after infection is neither able to clear the virus, nor useful diagnostically but may be used for surveillance purpose. Since the virus is not readily cultivable, diagnosis mainly relies on molecular methods for demonstration of HPV DNA and have excellent negative predictive value. When combined with cytology (pap smear), HPV DNA testing becomes more cost effective for screening of cervical carcinoma [24, 25].

3. HIV

HIV is transmitted through body fluids via sexual route or contaminated blood products. The two major types of virus, HIV-1 (sub types A-K, N and O) and HIV-2 cross react heavily and require specific antigen testing for differentiation. Diagnostic tests for HIV are mainly serological and include:

3.1 Enzyme immunoassays (EIA)

The latest third and fourth generation assays can detect Ig M antibodies and viral antigen respectively in addition to viral antibodies thereby enhancing chances of detection in the window period.

3.2 Rapid tests

These are based on same principle as EIA but provide faster results due to utilisation of high antigen concentration and more sensitive colour detection reagents. Being highly sensitive, these tests are the first line of investigation with negative results reported as negative and positive results repeated with kits using different antigens and other confirmatory assays [26, 27].

3.3 Confirmatory assays

IFA (Immunofluorescence assay) have been largely replaced by WB (Western Blot) and LIA (Line Immunoassays). But unlike EIA, none of these can detect Ig M antibodies or viral antigens and are more expensive, hence serial or parallel EIA testing algorithms can suffice at a much reduced cost in resource poor settings.

3.4 Molecular methods like RT (reverse transcriptase)

PCR and NAAT are used for detecting viral RNA, DNA, enzymes and proteins. These methods are useful for early diagnosis in infants born to seropositive mothers [28], diagnosing acutely infected individuals in window period and assessing viral load for follow-up of infected patients.

For monitoring HIV infection, CD4 counting is done by flow cytometry.

Conclusion

Simple, cost effective and sensitive lab tests should be selected for screening followed by repeat testing or testing with more specific methods like culture or PCR for confirmation of diagnosis. Collecting the sample from ideal site, ensuring optimal transport conditions and maintaining strict quality control in labs are essential for maintaining quality reporting which has direct effect on patient care.

References

1. Narayanan B. A retrospective study of the pattern of sexually transmitted diseases during a ten year period. Indian J Dermatol 2002;47:10-8.
2. WHO – Lab diagnosis of sexually transmitted infections, including human immunodeficiency virus 2013. http://who.int/reproductivehealth/publications/rtis/9789241505840/en/ [Last accessed on 07-04-2017].

3. Global incidence and prevalence of selected curable sexually transmitted infections —2008. Geneva, World Health Organization, 2012. http://www.who.int/reproductivehealth/publications/rtis/stisestimates/en/ [Last accessed on 07-04-2017].
4. Tapsall JW, Ndowa F, Lewis DA et al. Meeting the public health challenge of multidrug- and extensively drug-resistant Neisseria gonorrhoeae. Expert Review of Anti-Infective Therapy,2009, 7(7):821–834.
5. Ohnishi M, Golparian D, Shimuta K et al. Is Neisseria gonorrhoeae initiating a future era ofuntreatable gonorrhea?: detailed characterization of the first strain with high-level resistance to ceftriaxone. Antimicrobial Agents and Chemotherapy, 2011, 55(7):3538–3545.
6. Association of Public Health Laboratories (APHL). Laboratory diagnostic testing for Chlamydia trachomatis and Neisseria gonorrhoeae. Expert consultation meeting summary report, 13–15 January 2009 Atlanta, GA. Silver Spring, MD, APHL, 2009. https://www.aphl.org/programs/infectious_disease/std/Documents/ID_2009Jan_Laboratory-Guidelines-Treponema-pallidum-Meeting-Report.pdf [Last accessed on 07-04-2017].
7. Gift TL, Pate MS, Hook EW et al. The rapid test paradox: when fewer cases detected lead to more cases treated: a decision analysis of tests for Chlamydia trachomatis. Sexually Transmitted Diseases, 1999, 26(4):232–240.
8. Van Der Pol B, Kraft CS, Williams JA. Use of an adaptation of a commercially available PCR assay aimed at diagnosis of chlamydia and gonorrhea to detect Trichomonas vaginalis in urogenital specimens. Journal of Clinical Microbiology, 2006, 44(2):366–373.
9. Miller WC, Swygard H, Hobbs MM et al. The prevalence of trichomoniasis in young adults in the United States. Sexually Transmitted Diseases, 2005, 32(10):593–598
10. Seña AC, Miller WC, Hobbs MM et al. Trichomonas vaginalis infection in male sexual partners: implications for diagnosis, treatment, and prevention. Clinical Infectious Diseases, 2007, 44(1):13–22.
11. Bradshaw CS, Morton AN, Hocking J et al. High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. Journal of Infectious Diseases, 2006, 193(11):1478–1486.
12. Amsel R, Totten PA, Spiegel CA et al. Non specific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. American Journal of Medicine, 1983, 74(1):14–22.
13. Wheeler HL, Agarwal S, Goh BT. Dark ground microscopy and treponemal serological tests in the diagnosis of early syphilis. Sexually Transmitted Infections, 2004, 80(5):411–414.
14. Hook EW III, Roddy RE, Lukehart SA et al. Detection of Treponema pallidum in lesion exudate with a pathogen-specific monoclonal antibody. Journal of Clinical Microbiology, 1985, 22(2):241–244.
15. Gayet–Ageron A, Ninet B, Toutous-Trellu L et al. Assessment of a real–time PCR test to diagnose syphilis from diverse biological samples. Sexually Transmitted Infections, 2009, 85(4):264–269.
16. Liu H, Rodes B, Chen CY et al. New tests for syphilis: rational design of a PCR method for detection of Treponema pallidum in clinical specimens using unique regions of the DNA polymerase I gene. Journal of Clinical Microbiology, 2001, 39(5):1941–1946.
17. Ratnam S. The laboratory diagnosis of syphilis. Canadian Journal of Infectious Diseases and Medical Microbiology, 2005, 16(1):45–51.
18. Centers for Diseases Control, Sexually transmitted diseases treatment guidelines 2010. Morbidity and Mortality Weekly Report, 2010, 59(RR–12). https://www.cdc.gov/mmwr/pdf/rr/rr5912.pdf [Last accessed on 07-04-2017].
19. Lewis DA. Diagnostic tests for chancroid. Sexually Transmitted Infections, 2000, 76(2):137–141.
20. Morse SA, Trees DL, Htun Y et al. Comparison of clinical diagnosis and standard laboratory and molecular methods for the diagnosis of genital ulcer disease in Lesotho: association with human immunodeficiency virus infection. Journal of Infectious Diseases, 1997, 175(3):583–589.
21. Freinkel AL. Histological aspects of sexually transmitted genital lesions. Histopathology, 1987, 11(8):819–831.
22. Carter JS, Bowden FJ, Sriprakash KS et al. Diagnostic polymerase chain reaction for donovanosis. Clinical Infectious Diseases, 1999, 28(5):1168–1169.
23. Stanley M. Immunobiology of HPV and HPV vaccines. Gynecologic Oncology, 2008, 109(2 Suppl):S15–21.
24. Ronco G, Giorgi-Rossi P, Carozzi F et al. Efficacy of human papillomavirus testing for the detection of invasive cervical cancers and cervical intraepithelial neoplasia: a randomised controlled trial. Lancet Oncology, 2010, 11(3):249–257.
25. Cuzick J, Arbyn M, Sankarnarayanan R et al. Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. Vaccine, 2008,26(Suppl 10):K29–K41.
26. WHO, UNAIDS, CDC. HIV rapid testing: training package. Atlanta, GA, USA, Centers for Disease Control and Prevention, 2006.
27. WHO, UNAIDS. HIV assays: operational characteristics (phase 1). Report 14, simple/rapid tests. Geneva, World Health Organization, 2004. http://apps.who.int/iris/bitstream/10665/43059/1/9241592370.pdf [Last accessed on 07-04-2017].
28. Ou CY, Fiscus S, Ellenberger D et al. Early diagnosis of HIV infection in the breastfed infant. Advances in Experimental Medicine and Biology, 2012, 743:51–65.