

Gonococcal antigen detection by Immunochromatographic assay: A reliable and point-of-care diagnostic test for gonorrhoea

Md. Shah Alam¹, Md. Abdus Salam²

ABSTRACT

Objective: The study was carried out to assess the reliability of Immunochromatographic test for gonococcal antigen in urethral swabs from suspected male patients to validate it as rapid and point-of-care test for gonorrhoea.

Methodology: A total of 80 clinically suspected cases of gonorrhoea in males of different age groups attending at the Skin and VD out patient department of Rajshahi Medical College Hospital (RMCH), Bangladesh during January to December, 2007 were enrolled. Urethral and/or prostatic secretions were collected aseptically for bacterial culture in Chocolate agar media, Gram-stained smear microscopy for gram-negative diplococci and rapid test by Immunochromatographic assay.

Results: Out of 80 samples, culture yielded growth of *Neisseria gonorrhoeae* in 47 (58.75%) cases, and microscopy revealed gram-negative diplococci in 45 (56.25%) cases. Immunochromatographic test was performed by following manufacturer's instructions in randomly selected 50 cases including 38 urethral swabs and 12 prostatic secretions with 35 cases found positive against 36 of those culture-positive cases. Considering culture as gold standard of diagnosis, sensitivity, specificity, positive and negative predictive values of Immunochromatographic test were calculated as 97.22% (95% CI 83-99%), 100% (95% CI 73-100%), 100%, and 93.33% respectively.

Conclusion: This limited study reinforces that detection of gonococcal antigen by Immunochromatographic assay is a rapid, easy to perform and reliable diagnostic tool for early detection of acute male gonorrhoea with high sensitivity and specificity. It may be a useful test for screening clinically suspected case of gonococcal infections in male, particularly suitable as point-of-care test.

KEY WORDS: Gonorrhoea, Immunochromatographic test, Sensitivity, Specificity.

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INTRODUCTION

Gonorrhoea is a sexually transmitted disease caused by *Neisseria gonorrhoeae*, an obligate human pathogen which is highly adapted to its ecological niche.¹ The disease has been recognized since antiquity and still remains as one of the most common venereal diseases ensuring as a global health problem.² The World Health Organization estimates that more than 62 million cases of gonorrhoea occur each year, with high rates consistently are seen in the developing countries, in particular South and South East Asia.³ Despite

global health efforts, high rates of gonorrhoea currently are either stable or increasing.⁴ Data on the prevalence of sexually transmitted infections (STIs) are scanty in Bangladesh, however, a few studies conducted in Bangladesh have documented a high prevalence of STIs (N. gonorrhoeae 35.8%, C. trachomatis 43.5% and syphilis 8.5%) among female sex workers (FSWs).^{5,6}

Acute gonococcal infection in men is characterized primarily by urethritis but repeated gonococcal infections can cause dreaded complications like urethral stricture. Women are mostly asymptomatic but ascending gonococcal infection can cause pelvic inflammatory diseases (PID), ectopic pregnancy or infertility.⁷ Gonorrhoea has been identified as a co-factor in HIV transmission, an important reason for proper and timely treatment of gonorrhoea.⁸

The gold standard test for the detection of N. gonorrhoeae is culture, which has high sensitivity and specificity. However, it requires well trained staff and its performance is affected by specimen transport conditions. Microscopy is important in the rapid detection and treatment of gonorrhoea,⁹ but infections with certain serovars are less likely to be detected by microscopy, where culture from different ano-genital sites is essential to maximize detection of gonorrhoea.¹⁰

Nucleic acid amplification tests (NAATs) have higher sensitivity and can be used on non-invasive samples like urine but cross-reaction with other Neisseria species, expense, highly trained staff and sophisticated equipment are the limiting factors. In settings where patients are asked to return for laboratory results, some infected patients never receive treatment as they fail to return for their test results. This reduction in treatment and the possible onward transmission of N. gonorrhoeae during any delay in treatment are practical problems.⁹ To address the problem, WHO has developed the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment free and Deliverable) criteria for rapid STI tests to provide guidance when evaluating new rapid diagnostic tests.^{11,12} Diagnosis and treatment in a single visit is an important step in infection control in areas where limited health care facilities make arranging return visits for test results difficult and, therefore receiving treatment improbable.¹³

There is no specific and sensitive serological test available to detect recent gonococcal infections through the demonstration of N. gonorrhoeae-specific antibodies in patients' sera.¹⁴ However, rapid and early antigen detection method with Immunochromatographic assay for Neisseria

gonorrhoeae by using urethral swab from patients with suspected gonococcal infection in males is available currently. The sensitivity and specificity of the rapid Immunochromatographic test (ICT) compared with the results of standard culture were claimed to be 94.1% and 95.8% respectively.¹⁵ Considering the rapidity and ease of this method which does not require any specialized laboratory or highly skilled personnel and can be done at point-of-care, gonorrhoea rapid test has a possibility to be accepted as a popular screening test. Our attempt was first of its kind to evaluate the diagnostic sensitivity and specificity of this Immunochromatographic test for detection of gonococcal antigen in male urethral swabs in Bangladesh.

METHODOLOGY

Patients: Eighty (80) clinically suspected cases of gonorrhoea in males of different age groups attending at the Skin and VD out patient department of Rajshahi Medical College Hospital (RMCH), Bangladesh from January to December, 2007 were included. Selection criteria of cases were based on history of exposure, painful or burning urination, purulent urethral discharge, irritation inside the penis, redness at the opening of the urethra, swollen and/or painful testicles. Informed written consent was obtained from each patient before collection of sample. The research protocol was approved by the Institutional Review Board (IRB) of Rajshahi Medical College for issues of ethical clearance.

Sample collection: Urethral discharges (45) and prostatic secretions (35) were collected by using sterile cotton swab introduced into the urethra for about 2–4 cm to permit absorption of the exudates. For prostatic secretion, per rectal prostatic massage was given till prostatic secretion was obtained. Samples were collected before introduction of antibiotics or within 24 hours of antibiotics therapy. Three swabs of urethral discharge or prostatic secretion (for culture, smear preparation and antigen detection) were taken from each patient.

Laboratory methods: Culture: First swab containing urethral discharge or prostatic secretion was inoculated soon after collection on to Chocolate agar medium aseptically following multiple strokes technique of inoculation. The inoculated plate was kept into CO₂ extinction jar and was incubated at 37°C for maximum up to 48 hours. The plate was routinely checked after 24 hours of incubation for growth and considered as no growth in absence of colonies after 48 hours. Presumptive identification was done by colony morphology, oxidase test,

Table-I: Detection rate of *N. gonorrhoeae* by microscopy and culture (n=80).

Specimens	Number positive	
	Microscopy	Culture
Urethral discharge 45 (56.25)	38 (84.44)	39 (86.66)
Prostatic secretion 35 (43.75)	07 (20.00)	08 (22.85)
Total 80 (100)	45 (56.25)	47 (58.75)

Figures in the parenthesis indicate percentage

superoxol test and gram-staining. Confirmatory identification was done by rapid carbohydrate utilization test.

Microscopic examination of gram-stained smear: The second urethral or prostatic swab was smeared on a clean glass slide and fixed with a drop of methanol. Fixed smear was stained by Gram's method following Jensen's modification. Then the slide was examined under microscope (Olympus CH-20, Japan) using oil immersion lens for the presence of intra and extra cellular gram-negative diplococci.

Immunochromatographic test: The gonorrhoea rapid test strip (Blue Cross Biotech Corporation, Philippine) is a qualitative, lateral flow immunoassay for the detection of gonorrhoea antigen from male urethral swab specimens. In this test, antibody specific to the gonorrhoea antigen is coated on the test line region of the strip.

Five full drops of Reagent A (0.15M NaOH) were added to an extraction tube and third urethral or prostatic swab was inserted immediately and compressed at the bottom of the tube. The swab was rotated 15 times and stood for 2 minutes. Then 4 full drops of Reagent B (0.2N HCl) were added to the

Table-II: Rate of detection of gonorrhoea by Immunochromatographic test against culture-positive cases (n=50).

Specimens	No. (%)	ICT-positive n (%)	Culture-positive n (%)
Urethral discharges	38 (76.00)	30 (78.95)	30 (78.95)
Prostatic secretions	12 (24.00)	05 (41.67)	06 (50.00)
Total	50 (100.00)	35 (70.00)	36 (72.00)

extraction tube. The swab was again compressed at the bottom of the tube and rotated 15 times and stood for 1 minute. Finally the swab was pressed against the side of the tube before withdrawn. The test strip with arrows pointing toward the specimen was immersed vertically into the extracted specimen solution and waited for the colored line (s) to appear. The result was read at 10 minutes. Two colored lines, one in the control line region (C) and another in the test line region (T) were considered positive, while only colour line in the control region and absence in the test region was marked as negative (Fig.1).

Case definition for Gonorrhoea: Culture positive for *N. gonorrhoeae* was considered as gonorrhoea patient.

Statistical calculation: Sensitivity, specificity and their 95% CI were calculated with Clinical Calculator One.¹⁶

RESULTS

Detection rate of *N. gonorrhoeae* by microscopy and culture among suspected cases are shown in Table-I. Out of 80 samples including 45 urethral discharges and 35 prostatic secretions, culture yielded growth in 47 (58.75%) and microscopy was positive in 45 (56.25%) cases.

For detection of gonococcal antigen by rapid Immunochromatographic test (ICT) in the urethral and/or prostatic secretions, fifty samples out of total 80 cases were done due of constraint of availability of test strips. These 50 samples included randomly selected 38 urethral discharges and 12 prostatic secretions from total collected cases. ICT was found positive in 35 (70%) cases while culture yielded 36 (72%) as positive among these randomly selected 50 samples. Table-II shows rate of detection of gonorrhoea by Immunochromatographic test against culture-positive cases.

Calculation of diagnostic indices of Immunochromatographic test in comparison to culture is shown in Table-III. Sensitivity, Specificity, Positive predictive value and Negative predictive

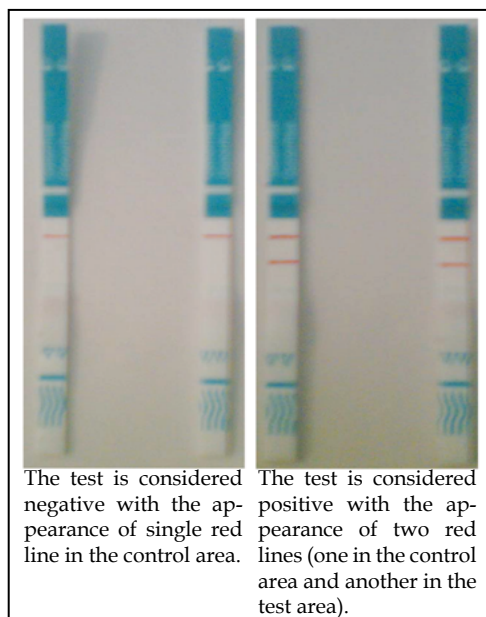


Fig.1: Immunochromatographic Test.

Table-III: Calculation of diagnostic indices of Immunochromatographic test in respect to culture.

	Culture		Total
	Positive	Negative	
ICT			
Positive	35 (a)	0 (b)	35
Negative	01 (c)	14 (d)	15
Total	36	14	50

$\text{Sensitivity} = \frac{a \times 100}{a+c} = 97.22\% \text{ (95\% CI 83-99\%)}$
 $\text{Specificity} = \frac{d \times 100}{b+d} = 100\% \text{ (95\% CI 73-100\%)}$
 $\text{Positive predictive value} = \frac{a \times 100}{a+b} = 100\%$
 $\text{Negative predictive value} = \frac{d \times 100}{c+d} = 93.33\%$

value of Immunochromatographic test were 97.22%, 100%, 100% and 93.33% respectively.

DISCUSSION

Innovation of rapid and point-of-care diagnostic test for infectious diseases is gaining importance in the recent years. For effective treatment and reduction of transmission for a sexually transmitted disease like gonorrhoea, Immunochromatographic test that detects a unique target antigen found in all strains of *N. gonorrhoeae* is of paramount importance.

Suzuki et al reported 58.6% culture positivity among suspected male gonococcal urethritis patients in their evaluation study of NOW gonorrhoea test. The sensitivity, specificity, positive and negative predictive values of the rapid test using urine samples were noted 91.4%, 95.8%, 96.9% and 92% respectively.¹⁵ Rate of culture positivity and diagnostic indices observed in this study are in good concordance with Suzuki et al and our previous report.¹⁷ Use of urethral discharges and/or prostatic secretions instead of urine and samples collected before or within 24 hours of antibiotic therapy in symptomatic males could be correlated with higher detection rates and increased sensitivity and specificity in this series. The rate of culture-positive cases among total 80 clinically suspected patients of gonorrhoea was 58.75% while it increased to 72% among randomly selected 50 cases out of 80 for which both Immunochromatographic test and culture were done. This discrepancy was due to random selection of 50 cases for obvious purpose of rapid Immunochromatographic test that could have been possible to carry out for only 50 cases. Although, we have recorded a very high sensitivity and specificity of Immunochromatographic test for male gonorrhoea, but other rapid tests in detecting gonococcal infections in female showed sensitivity

and specificity ranged 60 to 70% and 90 to 97% respectively.¹⁸

Despite much advances made in the areas of rapid diagnosis of common sexually transmitted diseases, there are limitations of rapid tests including gonorrhoea. First, the rapid tests perform better in symptomatic patients than patients with low likelihood. Second, the accuracy of collection of sample also greatly influences the test results. Third, the gender and nature of specimen are important considerations too.¹⁸ Although culture and microscopy are two standard methods for diagnosis of gonococcal infection, but requirement of costly machine, reagents, incubator, electricity and trained personnel are all limiting factors for their wide use. Moreover, waiting time for culture report is also a factor for overall effective management of patients suffering from gonorrhoea, especially in the developing countries. In comparison, Immunochromatographic test to detect gonococcal antigen is a simple, cheap and rapid test with very high diagnostic accuracy has been found in our study. Its application is free from all those limiting factors and the ICT can be performed even by the paramedics with easy interpretation of the results.

The proposed advantage of diagnosis and treatment of STIs in a single visit is not limited to developing countries but a common problem in many US STI clinics where patients presenting for testing do not return for results.¹⁹ Currently the rapid tests are best suited to environments with a population at high risk for the disease and immediate treatment is preferable because of unlikely follow-up or because of significant risk of transmission to others. Considering the usefulness of Immunochromatographic assay as point-of-care test, we emphasize on further evaluation of gonorrhoea rapid test taking both male and female cases and using different samples in order to maximize its diagnostic accuracy before recommending it as a versatile point-of-care test.

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