A NEW IMMUNOCHROMATOGRAPHIC STRIP TEST FOR NEISSERIA GONORRHOEAE

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Revised Abstract

Objective: To develop a simple and rapid immunochromatographic strip (ICS) test for detection of Neisseria gonorrhoeae directly in clinical specimens.

Introduction: There is currently no rapid and simple diagnostic test or device for N. gonorrhoeae that is accurate, widely available, and can be used at the point of care in developing countries. Previous attempts using major outer membrane protein as the antigen target have been unsuccessful. Recently, we identified monoclonal and polyclonal antibodies to a new ribosomal protein, L7/L12, which has been demonstrated to have diagnostic utility in the optical immunoassay format. These antibodies have now been used to develop a simple and rapid ICS test.

Results: The detection limit for the gonococcal (GC) ICS test was found to be equivalent to 1.1-1.6x10^5 cells/ml. The test recognized all 92 N. gonorrhoeae clinical isolates tested. These isolates, obtained from 11 distinct geographical regions, collectively represent a spectrum of N. gonorrhoeae serovars. Other species of Neisseria, as well as commensal bacteria commonly present in the urethral and vaginal tracts, did not react when tested at 2 x 10^7 cells/ml. A retrospective clinical evaluation of the test, compared to the ligase chain reaction as the reference method, demonstrated a sensitivity and specificity in women of 63.6 % (14/22) and 97.6% (205/210), respectively, using endocervical specimens. In men, the test produced a sensitivity and specificity of 97.1% (34/35) and 98.9% (88/89), respectively, using urethral specimens.

Conclusions: The usefulness of the N. gonorrhoeae L7/L12 antigen as a target for a simple and rapid test appears to be promising.

Introduction

Gonorrhea is a sexually transmitted infection (STI) commonly manifested by urethritis, cervicitis, and salpingitis. A large number of cases progress asymptomatically, especially in women. If left undiagnosed and untreated, gonorrhea may progress to pelvic inflammatory disease and disseminated infection resulting in arthritis, endocarditis, and meningitis. It may also infect the eyes of newborns exposed to secretions from an infected mother.[1]

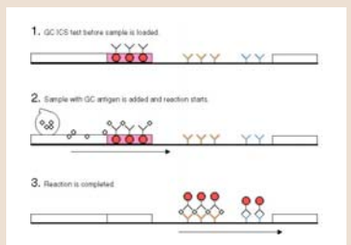
Neisseria gonorrhoeae, a gram-negative diplococcus, spreads mainly by unprotected sexual contact. Most modern diagnostic procedures are highly technical and logistically demanding. These include Gram stain, bacterial culture, biochemical, immunological, and molecular diagnostic methods. All of these methods are inappropriate for use in resource-limited settings in developing countries where the majority of infections still occur. A simple, cost-effective, and rapid test that can be used at the point of care is urgently needed and will be essential to meet the need for prompt recognition and treatment of gonococcal (GC) infection in developing countries. To date, despite advances in diagnostic technologies, there is only a single commercially available point-of-care test that provides for adequate performance,[2] but there may be others in development.

A point-of-care GC immunochromatographic strip (ICS) test that is easy to use and is capable of providing adequate sensitivity and specificity at an affordable price could become a valuable replacement for or supplement to syndromic diagnosis currently used in the developing world. The data presented here describe the development and evaluation of a simple and rapid point-of-care test for N. gonorrhoeae.

Principles of the Gonococcal Immunochromatographic Strip Test

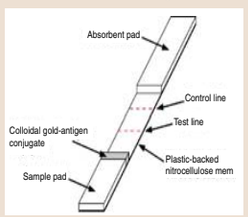
The GC ICS test is a lateral flow assay that uses monoclonal and polyclonal antibodies specific for the L7/L12 ribosomal protein. This previously described protein is part of the 50S ribosomal protein complex of N. gonorrhoeae and has a subunit molecular weight of 14kDa.[2] To conduct the GC ICS test, a swab specimen is extracted into a lysis buffer and the extract is then applied to the sample port of the test. This extract migrates by capillary action through a reagent zone on the test strip, which contains monoclonal antibodies conjugated to colloidal gold microspheres that serve as a signal reagent.
If the L7/L12 antigen is present in the sample, it will be captured by the conjugated antibodies.
The L7/L12-antibody-signal reagent complex is then wicked through a nitrocellulose membrane strip that contains a zone of immobilized polyclonal antibody specific for L7/L12. This zone captures the L7/L12 complex, concentrating the particles to form a visible line. If no L7/L12 antigen is present in the sample, no test line will form. The membrane also contains a procedural control line that will always form, regardless of the presence of L7/L12, ensuring the strip is working properly.
The GC ICS test takes approximately 20 minutes to perform.

GC ICS Test Principle



Principles (cont.)

GC ICS Test Design



Example of a Positive Test Result

Example of a Negative Test Result

- Test results were read after 20 minutes
- Positive reaction was generated using N. gonorrhoeae strain Mel
- Negative reaction was generated using extraction buffer control

Test Procedure

Females

- Place the patient's swab into a flexible tube
- Add 12 drops extraction buffer
- Agitate and allow to stand for 5-7 minutes
- Mix all tubes by vortexing
- Thoroughly express the buffer from each swab
- Remove and discard the swab

Males

- Place the patient's swab into a flexible tube
- Add 6 drops of extraction buffer
- Agitate and allow to stand for 5-7 minutes
- Mix all tubes by vortexing
- Remove and discard the swab

- Cover the tube, invert it, and transfer 3-4 drops to the test cassette
- Time the reaction for 20 minutes
- Read the results in a well-lighted area

Methods and Materials

Minimum Analytical Detection Level

To determine the minimum analytical detection level of the GC ICS test, viable N. gonorrhoeae cells were plated on modified Thayer-Martin medium (VWR, Brisbane, CA) and incubated for 16 hours in a carbon dioxide-rich atmosphere at 35°C. After 16 hours, cells were replated at the same conditions and harvested after an additional 16 hours. N. gonorrhoeae cells were removed from the plate with a bacteriological loop and suspended in Amies GC transport medium (Becton Dickinson, Sparks, MD). The concentration of harvested cells was determined by measuring the optical density of the cell suspension at 550 nm (visible light) using a Shimadzu Biospec 1601 spectrophotometer. Bacterial cell concentration was determined from the optical density, using the McFarland standard curve.[3] A series of two-fold dilutions was then prepared from the initial cell suspension in Amies GC transport medium. Sterile Dacron® swabs (Puritan Hardwood Products LLC, Guilford, MI) were saturated with each dilution. Each swab was tested with the assay protocol described above until the visible test line (positive signal) completely disappeared. The last dilution, producing a visible test line on the GC ICS test membrane determined the minimal detection level.

Assay Cross-Reactivity

To determine the cross-reactivity of the GC ICS test with other bacterial and fungal flora commonly present in vaginal and urinary tracts, viable commensal strains of Neisseria and other organisms (Bactrol™ Plus, Becton Dickinson, Sparks, MD) were inoculated onto appropriate media and cultured in appropriate conditions for 24 hours. Initial cell concentrations were determined spectrophotometrically as previously described. The organisms were diluted to 2x10^7 cells/ml in PBS, pH 7.2. Sterile Dacron® swabs were saturated in each cell suspension. Each swab was tested in accordance with the previously described assay protocol.

Methods and Materials (cont.)

Detection of Cultured N. gonorrhoeae

To determine the reactivity of different strains of N. gonorrhoeae, clinical isolates were obtained from Thermo Electron Corporation. Each isolate was pre-diluted in PBS, pH 7.2, and sterile Dacron® swabs were saturated in each dilution. Swabs were tested in accordance with an assay protocol, as previously described.

Evaluation of Clinical Specimens

Ethical clearances were obtained from both the PATH Human Subjects Protection Committee and the University of Alabama's Institutional Review Board. Swabs from patients attending the sexually transmitted disease (STD) clinic at the University of Alabama were collected and stored frozen (-70°C) until the time of testing. Each swab was then thawed and tested in accordance with an assay protocol, as previously described. Reference Ligase Chain Reaction (LCR, Abbott Diagnostics, Chicago, IL) testing was routinely performed by the University of Alabama. LCR results were not revealed until they were matched with the results of the GC ICS Test.

Results

Minimum Analytical Detection Level

Minimum analytical detection levels were determined for 4 strains representing different serovars from 1A group and 6 strains representing different serovars from 1B group. Results are presented in the table below.

Detection Limits for Gonococcal Strains

Strain	Serovar	Minimal Detection level (cells/ml)
Pittsburgh 4804	IB-4	1.1x10^5
Cambodia 1211	IB-8	1.2x10^5
Georgia 2	IB-2	1.2x10^5
ANC 2-4	IA-6	1.3x10^5
Pittsburgh 8396	IA-21	1.3x10^5
Dominican Republic 056	IB-3	1.3x10^5
Senegal P292	IB-32	1.4x10^5
Philippines 104	IB-1	1.5x10^5
Peru (Alg) 033	IA 4	1.5x10^5
ANC 1-1	IA1,2	1.6x10^5

Assay Cross-Reactivity

The commensal bacteria listed below were evaluated with the GC ICS test. All bacterial strains did not react when tested at a concentration of 2 x 10^7 cells/ml. The 5 Neisseria species tested reacted in the GC ICS test only at concentrations of more than 2 x 10^7 cells/ml.

Bacteria Tested for Reactivity in the GC ICS Test

Organism	Strain	Organism	Strain
Neisseria lactamica	ATCC 23970	Proeus vulgaris	ATCC 13315
Neisseria meningitidis	ATCC 13090	Pseudomonas aeruginosa	ATCC 27853
Neisseria sicca	ATCC 9913	Salmonella choleraesuis	ATCC 14028
Neisseria cineria	196	Serratia marcescens	ATCC 8100
Neisseria flavescens	30008	Staphylococcus aureus	ATCC 25923
Candida albicans	090195	Staphylococcus epidermidis	ATCC 12228
Proteus mirabilis	16728	Streptococcus pyogenes	ATCC 19615
Enterobacter cloacae	ATCC 23355	Haemophilus influenzae	ATCC 35056
Escherichia coli	ATCC 25922	Streptococcus pneumoniae	ATCC 6303
Klebsiella pheumoniae	ATCC 13883	Streptococcus agalactiae	ATCC 13813

Detection of Cultured N. gonorrhoeae

Results from a retrospective evaluation of the GC ICS test with 92 N. gonorrhoeae clinical isolates indicated they were 100% reactive on the GC ICS test. The strains were obtained from 11 distinct geographical regions, representing a spectrum of N. gonorrhoeae serovars. Summarized results are presented immediately below.

GC ICS Test Results for Cultured Clinical Isolates

Region	Number of strains	Positive results
Alaska, US	6	6/6
Georgia, US	10	10/10
Pennsylvania, US	10	10/10
Washington, US	27	27/27
Dominican Republic	5	5/5
Peru	6	6/6
Cambodia	5	5/5
Philippines	5	5/5
Kenya	8	8/8
Senegal	5	5/5
Denmark	5	5/5
Negative control	1	0/1

Results (cont.)

Evaluation of Clinical Specimens

Clinical performance data for female and male samples from the University of Alabama are presented below. Data were obtained from a total of 356 clinical samples (232 female and 124 male). The prevalence of N. gonorrhoeae infection as determined by LCR was 17.7% in males and 15.1% in females. Sensitivity, specificity, and predictive values are presented below.

Female Endocervical Swabs

GC ICS test \ LCR results	+	-
+	14	5
-	8	205

Sensitivity = 63.6%
Specificity = 97.6%
Positive Predictive Value = 60.8%
Negative Predictive Value = 95.7%

Male Urethral Swabs

GC ICS test \ LCR results	+	-
+	34	1
-	1	88

Sensitivity = 97.1%
Specificity = 98.9%
Positive Predictive Value = 97.1%
Negative Predictive Value = 98.9%

Conclusions

- We have developed a rapid and simple ICS test, using the L7/L12 N. gonorrhoeae target antigen.
- Test results indicate a high sensitivity for urethral specimens and lower sensitivity for endocervical samples, compared with reference diagnostic methods such as the LCR.
- Specificity for both urethral and cervical samples is high.
- Test results, including sample collection, may be obtained in less than 30 minutes.
- Further clinical studies to evaluate the GC ICS test performance are underway.

References

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