Detection of *Streptococcus pyogenes* Using illumigene® Group A Streptococcus Assay

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Abstract

The performance of illumigene® Group A Streptococcus assay was evaluated by comparing to culture using 437 consecutive throat swabs. The illumigene® assay was also directly compared to PCR on 161 samples. This illumigene® assay is rapid and easy to perform. The assay also has high sensitivity (100%) compared to culture or PCR, and high specificity (99.2%) compared to PCR. 8.8% isolates were erythromycin resistant and 6.9% were clindamycin resistant.
Streptococcus pyogenes (Group A Streptococcus, GAS) causes various infections including acute pharyngitis in children. Rapid and accurate laboratory diagnosis is important for antibiotic therapy which prevents rheumatic fever and probably invasive infections (1). Current standard testing methods include rapid antigen detection with about 70-80% sensitivity and culture which takes 24 – 48 hours (2). A new molecular method, the illumigene® Group A Streptococcus assay, based on loop mediated isothermal amplification (LAMP) technology for testing GAS has been developed by Meridian Bioscience and has recently been cleared by the FDA. The target region of the assay is in the GAS pyrogenic exotoxin B (speB) gene (3, 4). In this prospective study, the illumigene® Group A Streptococcus assay was compared to standard bacterial culture and to PCR targeting the ptsI (phosphotransferase) gene to evaluate its clinical performance.

Specimens: Throat swabs routinely submitted for GAS testing by rapid antigen and culture methods were collected from symptomatic children at Ann and Robert H. Lurie Children’s Hospital of Chicago. Each specimen was collected with the double swab system with a liquid Stuart transport medium (Copan Diagnostics Inc., Murrieta, CA). One swab was used for rapid antigen assay. The second swab was used for culture and the illumigene® Group A Streptococcus assay. No patient was enrolled into the study more than once. The study was approved by the Institutional Review Board of Ann & Robert H. Lurie Children’s Hospital of Chicago.

illumigene® Group A Streptococcus assay: one swab was plated onto an agar plates for culture, and then was used to perform the illumigene® Group A Streptococcus assay as described in the manufacturer’s product insert. Briefly, sample in supplied Sample Preparation
buffer is heated to 95 °C for 10 minutes. Heated samples were transferred to TEST and
CONTROL chambers that contained lyophilized amplification reagents. Both amplification and
detection took place on the *illumi*pro-*10*™ device. The whole process time from preparation to
result reading for 1 to 10 specimens was approximately 1 hour.

Culture and susceptibility: Standard GAS culture was performed with 5% sheep blood
agar. GAS identification was based on colony morphology, gram stain, catalase test, and
serogrouping using reagents from PathoDX Strep Grouping distributed by Remel Lenexa, KA
(2). Erythromycin, clindamycin, and levofloxacin susceptibility testing was performed with the
MICRoSTREP *plus*® panels, and results were read using the MicroScan Walkaway instrument
(Siemens Healthcare Diagnostics, Tarrytown, NY). Quality control was performed following
manufacturer’s procedure using *Streptococcus pneumoniae* ATCC 49619. Interpretations were
based on the CLSI standards (5).

Real time PCR: Sample Preparation buffer, leftover from the *illumigene*® Group A
*Streptococcus* assay that contained bacterial remnants from the swabs and Tris Buffer solution
were used for extracting total nucleic acids on the easyMAG instrument (bioMerieux). The
elution volume was 55ul with 200 ul of sample used for extraction. Real time PCR reactions
were performed on the LightCycler instrument with Analyte Specific Reagents for GAS (Roche
Diagnostics, Indianapolis, IN) as previously reported (6). A 198-bp fragment of the *ptsI*
(phosphotransferase) gene of GAS was amplified and detected.

During the study period, to ensure that there would be sufficient number of positives to
assess for false positive and false negative results, a total of 440 consecutive specimens were
collected from Dec. 12, 2012 to Jan. 30, 2013. Three specimens were removed from the study
due to incomplete data collection/specimen testing. Patient ages ranged from 14 months to 37 years with 98% less than 18 years of age.

Among the 437 specimens tested, 92 (21.1%) were positive by culture. All 92 culture-positive specimens were also positive by the illumigene® Group A Streptococcus assay; this molecular assay was 100% sensitivity compared to culture. There were 331 specimens negative by both culture and the illumigene® Group A Streptococcus assay. Fourteen specimens were negative by culture but positive by the illumigene® assay giving a 95.9% specificity. 12 of these 14 specimens that were culture negative but illumigene® Group A Streptococcus assay positive were confirmed to be positive for GAS by real time PCR. No specimen was positive by culture but negative by the illumigene® Group A Streptococcus assay (Table 1). In contrast, in this study, the GAS rapid antigen assay gave 73.3% sensitivity and 89.1% specificity.

To compare the performance of the illumigene® Group A Streptococcus assay to another molecular method, the first 161 consecutively collected specimens were also tested by real time PCR. Among them, thirty-six (36) specimens were positive by both the illumigene® Group A Streptococcus assay and PCR assays, and 124 were negative by both methods. One specimen was positive by the illumigene® Group A Streptococcus assay but was negative by PCR. Therefore, compared to PCR results, the illumigene® Group A Streptococcus assay is 100% sensitive and 99.2% specific.

Antimicrobial susceptibility to erythromycin, clindamycin, and levofloxacin was performed on all 92 GAS isolates from the study and an additional 10 isolates collected immediately following the study conclusion. Nine of these 102 isolates (8.8%) were resistant to erythromycin, and 2 (2%) were constitutively resistant to clindamycin. The 2 clindamycin resistant isolates were also erythromycin resistant. Using the disk diffusion method, inducible
Clindamycin resistance was assessed on the 7 isolates that were initially found to be erythromycin resistant and clindamycin susceptible by the broth microdilution method (5). Five of these 7 showed inducible resistance to clindamycin. Therefore, overall clindamycin resistance was 6.9% (5 inducible and 2 constitutive). All isolates tested were susceptible to levofloxacin.

In recent years, LAMP technology has been studied for the detection of various infectious agents. Compared to more widely used molecular amplification technologies such as PCR, LAMP based assays have been shown to be similarly sensitive and specific. The first commercialized assay was for the detection of *C. difficile* toxin gene and has been successfully used by many clinical laboratories (7-9). The advantages of such assays include the ease of adoption (no need for expensive instrument) and easy set up.

In this prospective study, we evaluated the clinical performance of the illumigene® Group A Streptococcus assay. Standardized antigen detection and bacterial culture have been widely used by most laboratories to assist in the diagnosis of acute pharyngitis caused by GAS. To improve detection sensitivity and to decreases turnaround times, real time PCR has been shown to be sensitive and rapid (6,10) and therefore has been successfully used to replace both antigen detection and culture for diagnosing acute GAS pharyngitis in some laboratories (Robin Patel, personal communication). The illumigene® Group A Streptococcus assay is a new FDA cleared molecular amplification test. At this writing, there is one published report regarding performance of this assay (11). In this multicenter clinical trial comparing the illumigene® Group A Streptococcus assay to culture, specimens with discrepant results were tested using a PCR assay. After resolving any discrepancies, the illumigene® Group A Streptococcus assay was found to be 99.0% sensitive and 99.6% specific (11).
In the present prospective study, we expanded comparisons to include both culture and PCR. Because of its convenience, discrepant analysis has been used in many studies. However, this type of analysis may be biased (12-15). To address this potential limitation, in our current study, we not only compared the performance of the illumigene® Group A Streptococcus assay to bacterial culture on all 437 specimens, but have also performed full comparison to both culture and PCR on 161 consecutive specimens. We believe that this aids in assessing the true performance of the assay with confidence.

One of the limitations of the illumigene® Group A Streptococcus assay is the lack of detection of groups C and G streptococci. In addition, if this assay is used to completely replace throat culture, there would be no viable organisms available for antimicrobial susceptibility testing when needed. Therefore, even when implementing a sensitive molecular assay, there may be continued need for maintaining capacity for culture for monitoring resistance trend and for offering it selectively when there is a need. To assess current antimicrobial susceptibility trends, we have included GAS antimicrobial susceptibility testing on current isolates. Although GAS can be treated with beta-lactam drugs such as penicillin and amoxicillin because of its universal susceptibility to the class, macrolides are alternatives for patients allergic to penicillins (1). GAS resistance to macrolides varies significantly by geographic location, and it has been increasing in some areas (16-20). In the U.S., based on a multicenter study conducted about a decade ago, erythromycin resistance of GAS ranged from 3.8% to 4.3% in different years (16). Such resistance is higher in Europe and in Canada. It is reported to be 8.2% in Germany (17) and 32.8% in Spain (18). It is highest in China where more than 96% of GAS are resistant (19,20). It has also been found that this is often clonally related with certain emm genotypes predominant. While more recent and complete GAS susceptibility data in the U.S. are needed,
our initial data showed a relatively moderate resistance rate of 8.8%. Reported clindamycin resistance was 1% in the U.S. about 10 years ago (16). We observed a somewhat higher rate in our study with 2% constitutive and 5% inducible resistance to clindamycin. Due to a mutations in the quinolone resistance determining regions (QRDR), the first fluoroquinolone (levofloxacin) resistant GAS was reported in 2000 (21) but has rarely been found thereafter. All 102 GAS isolates in the current study were susceptible to levofloxacin.

In summary, the present study shows that compared to standard and reference methods, the illumigene® Group A Streptococcus assay is highly sensitive and specific. It can be completed in less than one hour with minimum hands on time required. The rapid results and reliable performance may help clinicians to improve patient management. The GAS macrolide and clindamycin resistance rates are still moderate, and they need to be continually monitored.

Acknowledgements

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REFERENCES


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ND: not performed
Table 2. Comparison of *illumigene®* results to PCR on 161 consecutive specimens

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