JCM
Journals.ASM.org

Detection of *Streptococcus pyogenes* by Use of Illumigene Group A *Streptococcus* Assay

Amanda M. Henson,[a,b] Donna Carter,[a] Kathleen Todd,[a] Stanford T. Shulman,[a,c] Xiaotian Zheng[a,c]

Ann and Robert H. Lurie Children's Hospital of Chicago, Chicago, Illinois, USA[a]; Rush University College of Health Sciences, Chicago, Illinois, USA[b]; Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA[c]

The performance of the Illumigene group A *Streptococcus* assay was evaluated by comparing it to culture using 437 consecutive throat swabs. The Illumigene assay was also directly compared to PCR with 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. A total of 8.8% of the 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 that prevents rheumatic fever and probably invasive infections (1). Current standard testing methods include rapid antigen detection, with about 70 to 80% sensitivity, and culture, which takes 24 to 48 h (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 and Robert H. Lurie Children's Hospital of Chicago.

Illumigene group A *Streptococcus* assay. One swab was plated onto 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, the sample in the supplied sample preparation buffer was heated to 95°C for 10 min. Heated samples were transferred to "test" and "control" chambers that contained lyophilized amplification reagents. Both amplification and detection took place on the Illumipro-10 device. The whole process time from preparation to result reading for 1 to 10 specimens was approximately 1 h.

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, KS (2). Erythromycin, clindamycin, and levofloxacin susceptibility testing was performed with MICroSTREP Plus panels, and the results were read using the MicroScan Walkaway instrument (Siemens Healthcare Diagnostics, Tarrytown, NY). Quality control was performed following the manufacturer's procedure using *Streptococcus pneumoniae* ATCC 49619. Interpretations were based on 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 (bioMérieux). The elution volume was 55 μl, with 200 μl of sample used for extraction. Real-time PCRs were performed on the LightCycler instrument with Roche 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 12 December 2012 to 30 January 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 showed 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 95.9% specificity. Twelve 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.

Received 16 July 2013 **Returned for modification** 8 August 2013
Accepted 12 September 2013
Published ahead of print 18 September 2013
Address correspondence to Xiaotian Zheng, x-zheng@northwestern.edu.

doi:10.1128/JCM.01892-13

TABLE 1 Comparison of Illumigene to culture results for 437 consecutive specimens

No. of specimens	Result by:		
	Culture	Illumigene	PCR
92	+	+	ND[a]
331	−	−	ND
12	−	+	+
2	−	+	−
0	+	−	ND

[a] ND, not done.

TABLE 2 Comparison of Illumigene to PCR results for 161 consecutive specimens

Result by Illumigene	No. of PCR results:	
	+	−
+	36	1
−	0	124

To compare the performance of the Illumigene group A *Streptococcus* assay to that of another molecular method, the first 161 consecutively collected specimens were also tested by real-time PCR (Table 2). Among them, 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.

Determination of antimicrobial susceptibility to erythromycin, clindamycin, and levofloxacin was performed with 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 with the 7 isolates that were initially found to be erythromycin resistant and clindamycin susceptible by the broth microdilution method (5). Five of these 7 isolates 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 the *Clostridium 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 an expensive instrument) and easy setup.

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 decrease 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 diagnosis of acute GAS pharyngitis in some laboratories (R. 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 resolution of 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 present study, we not only compared the performance of the Illumigene group A *Streptococcus* assay to bacterial culture on all 437 specimens but also performed full comparison to both culture and PCR with 161 consecutive specimens. We believe that this aids in assessment of 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 group 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 a continued need to maintain the capacity for culture to monitor resistance trends and to offer it selectively when there is a need. To assess current antimicrobial susceptibility trends, we have included GAS antimicrobial susceptibility testing of current isolates. Although GAS can be treated with β-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 United States, 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 isolates 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 United States are needed, our initial data showed a relatively moderate resistance rate of 8.8%. Reported clindamycin resistance was 1% in the United States 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 mutations in the quinolone resistance-determining regions (QRDRs), the first fluoroquinolone (levofloxacin)-resistant GAS was reported in 2000 (21), but it has rarely been found thereafter. All 102 GAS isolates in the present 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 1 h, with minimum hands-on time required. The rapid results and reliable performance may help clinicians improve patient management. The GAS macrolide and clindamycin resis-

tance rates are still moderate, and they need to be continually monitored.

ACKNOWLEDGMENTS

The Illumigene group A *Streptococcus* assay kits used for the study were supplied by Meridian Bioscience.

A.M.H. received an unrestricted travel grant from Meridian to attend an American Society for Microbiology meeting to present a poster.

REFERENCES

1. **Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C.** 2012. Clinical practice guideline for the diagnosis and management of group A streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. Clin. Infect. Dis. **55:**1279–1282.
2. **Garcia LS, Isenberg HD.** 2007. Group A Streptococcus: culture and non-culture tests, p 3.11.8.1–3.11.8.7. *In* Garcia LS, Isenberg HD (ed), Clinical microbiology procedures handbook, 3rd ed. ASM Press, Washington, DC.
3. **Billal DS, Hotomi M, Shimada J, Fujihara K, Ubukata K, Sugita R, Yamanaka N.** 2008. Prevalence of *Streptococcus* invasive locus (*sil*) and its relationship with macrolide resistance among group A *Streptococcus* strains. J. Clin. Microbiol. **46:**1563–1564.
4. **Jing HB, Ning BA, Hao HJ, Zheng YL, Chang D, Jiang W, Jiang YQ.** 2006. Epidemiological analysis of group A streptococci recovered from patients in China. J. Med. Microbiol. **55:**1101–1107.
5. **CLSI.** 2011. Performance standards for antimicrobial susceptibility testing: 21st informational supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
6. **Uhl JR, Adamson SC, Vetter EA, Schleck CD, Harmsen WS, Iverson LK, Santrach PJ, Henry NK, Cockerill FR.** 2003. Comparison of LightCycler PCR, rapid antigen immunoassay, and culture for detection of group A streptococci from throat swabs. J. Clin. Microbiol. **41:**242–249.
7. **Lalande V, Barrault L, Wadel S, Eckert C, Petit J, Barbut F.** 2011. Evaluation of a loop-mediated isothermal amplification assay for diagnosis of *Clostridium difficile* infections. J. Clin. Microbiol. **49:**2714–2716.
8. **Doing KM, Hintz MS.** 2012. Prospective evaluation of the Meridian Illumigene loop-mediated amplification assay and the Gen Probe ProGastro Cd polymerase chain reaction assay for the direct detection of toxigenic *Clostridium difficile*. Diagn. Microbiol. Infect. Dis. **72:**8–13.
9. **Boyanton BL, Sural P, Loomis C, Pesta C, Gonzalez-Krellwitz L, Robinson-Dunn B, Riska P.** 2012. Loop-mediated isothermal amplification compared to real-time PCR and enzyme immunoassay for toxigenic *Clostridium difficile* detection. J. Clin. Microbiol. **50:**640–645.
10. **Slinger R, Goldfarb D, Rajakumar D, Moldovan I, Barrowman N, Tam R, Chan F.** 2011. Rapid PCR detection of group A streptococcus from flocked throat swabs: a retrospective clinical study. Ann. Clin. Microbiol. Antimicrob. **10:**33. doi:10.1186/1476-0711-10-33.
11. **Anderson NW, Buchan BW, Mayne D, Mortensen JE, Mackey TA, Ledeboer NA.** 2013. Multicenter clinical evaluation of the *illumi*gene group A *Streptococcus* DNA amplification assay for detection of group A Streptococcus from pharyngeal swabs. J. Clin. Microbiol. **51:**1474–1477.
12. **Green TA, Black CM, Johnson RE.** 1998. Evaluation of bias in diagnostic-test sensitivity and specificity estimates computed by discrepant analysis. J. Clin. Microbiol. **36:**375–381.
13. **McAdam AJ.** 2000. Discrepant analysis: how can we test a test? J. Clin. Microbiol. **38:**2027–2029.
14. **Hadgu A.** 2000. Discrepant analysis is an inappropriate and unscientific method. J. Clin. Microbiol. **38:**4301–4302.
15. **Sternberg M.** 2001. Discrepant analysis is still at large. J. Clin. Microbiol. **39:**826–827.
16. **Tanz RR, Shulman ST, Shortridge VD, Kabat W, Kabat K, Cederlund E, Rippe J, Beyer J, Doktor S, Beal BW, North American Streptococcal Pharyngitis Surveillance Group.** 2004. Community-based surveillance in the United States of macrolide-resistant pediatric pharyngeal group A streptococci during 3 respiratory disease seasons. Clin. Infect. Dis. **39:**1794–1801.
17. **Bley C, van der Linden M, Reinert RR.** 2011. *mef*(A) is the predominant macrolide resistance determinant in *Streptococcus pneumoniae* and *Streptococcus pyogenes* in Germany. Int. J. Antimicrob. Agents **37:**425–431.
18. **Rubio-Lopez V, Valdezate S, Alvarez D, Villalon P, Medina MJ, Salcedo C, Saez-Neito J.** 2012. Molecular epidemiology, antimicrobial susceptibilities and resistance mechanisms of *Streptococcus pyogenes* isolates resistant to erythromycin and tetracycline in Spain (1994–2006). BMC Microbiol. **12:**215. doi:10.1186/1471-2180-12-215.
19. **Liang Y, Liu X, Chang H, Ji L, Huang G, Fu Z, Zheng Y, Wang L, Li C, Shen Y, Yu S, Yao K, Ma L, Shen X, Yang Y.** 2012. Epidemiological and molecular characteristics of clinical isolates of *Streptococcus pyogenes* collected between 2005 and 2008 from Chinese children. J. Med. Microbiol. **61:**975–983.
20. **Yang P, Peng X, Zhang D, Wu S, Liu Y, Cui S, Lu G, Duan W, Shi W, Liu S, Li J, Wang Q.** 2013. Group A Streptococcus strains circulating during scarlet fever epidemic, Beijing, China, 2011. Emerg. Infect. Dis. **19:**909–915.
21. **Yan SS, Fox ML, Holland SM, Stock F, Gill VI, Fedorko DP.** 2000. Resistance to multiple fluoroquinolones in a clinical isolate of *Streptococcus pyogenes*: identification of gyrA and parC and specification of point mutations associated with resistance. Antimicrob. Agents Chemother. **44:**3196–3198.