

Evaluation of the Lyra Direct Strep Assay To Detect Group A *Streptococcus* and Group C and G Beta-Hemolytic *Streptococcus* from Pharyngeal Specimens

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The Lyra Direct strep assay was compared to culture for its ability to detect *Streptococcus* group A and β -hemolytic groups C/G using rapid antigen-negative pharyngeal specimens ($n = 161$). The Lyra assay correctly detected all β -hemolytic streptococci (group A, $n = 19$; group C/G, $n = 5$). In batch mode, the Lyra assay reduced intralaboratory turnaround time by 60% (18.1 h versus 45.0 h) but increased hands-on time by 96% (3 min 16 s versus 1 min 40 s per specimen).

Various species of *Streptococcus* cause acute bacterial pharyngitis. Group A *Streptococcus* (GAS) (*Streptococcus pyogenes*) is the most common, but other β -hemolytic streptococci have also been implicated (1–3). These include *S. equi*, *S. dysgalactiae*, *S. equisimilis*, and *S. zooepidemicus*, and these in general are designated large-colony-forming or pyogenic Lancefield group C or G streptococci (4). The timely detection of these pathogens expedites appropriate antimicrobial therapy, thereby reducing illness duration and disease transmission, and in the case of GAS, non-suppurative complications (i.e., rheumatic fever and glomerulonephritis). To improve turnaround time, rapid antigen tests (RATs) have largely augmented bacterial culture (the gold standard). However, the performance of commercially available RATs varies greatly depending upon the manufacturer, methodology used (i.e., optical immunoassay, immunochromatographic, or enzyme immunoassay), and the patient population (i.e., pediatric versus adult) being tested (5). Due to these limitations, nucleic acid amplification tests (NAATs) are being implemented in clinical laboratories. One such test, the Lyra Direct strep assay (Quidel, San Diego, CA), was recently approved (22 April 2014) by the U.S. Food and Drug Administration (FDA) for the qualitative detection and differentiation of GAS and pyogenic group C/G β -hemolytic streptococci from throat swab specimens obtained from patients with signs and symptoms of pharyngitis. However, the manufacturer's package insert (M112en v2014APR21) also states that all negative Lyra test results should be confirmed by bacterial culture and should not be used as the sole basis for treatment. To date, no peer-reviewed publications pertaining to the Lyra assay are available. As such, the goal of this study was to evaluate the performance and workflow characteristics of the Lyra assay as a replacement for the conventional "backup" culture in the setting of an initial negative rapid antigen test result. This study was approved by the Human Investigative Committee/Internal Review Board of Beaumont Health, MI (no. 2015-171).

This evaluation included 161 unique and randomly selected outpatient subjects (pediatric [$n = 149$; mean age, 6.3 years; age range, 0.5 to 17 years] and adult [$n = 12$; mean age, 35.7 years; age range, 18 to 93 years]) with clinical findings supportive of acute bacterial pharyngitis. Specimens were collected with a flocced swab (ESwab) and placed into 1.0 ml of liquid Amies transport medium (Copan Diagnostics, Murrieta, CA). All specimens were

negative for group A *Streptococcus* by rapid antigen testing; the test method varied within each physician's office. Specimens were maintained at refrigeration temperature and tested within 24 h of collection at the Beaumont Laboratory. Reference culture was performed by direct inoculation of 50 μ l of liquid Amies to sheep blood agar and selective streptococcal agar (SBA and SXT, respectively) (Remel, Lenexa, KS). The inoculated plates were incubated (at 35°C to 37°C in 5% CO₂) and read at 24 and 48 h. Suspect colonies were identified with Gram stain, catalase, pyrrolidonyl arylamidase (PYR) (Remel), and PathoDx strep grouping (Remel). All non-group A *Streptococcus* isolates were fully identified using the MICroSTREP plus 1 panel (Beckman Coulter, Brea, CA) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (microflex LT and MALDI Biotyper real-time classification software [RTC version 4.0, build 11]; Bruker Daltonics, Billerica, MA). For MALDI-TOF MS, an in-house validated protocol similar to that previously described was utilized (6). In brief, colonies were spotted in duplicate on a stainless steel target plate using a toothpick, followed by air drying (15 min). One microliter of 70% formic acid with air drying (15 min) and then 1 μ l of matrix (saturated solution of α -cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid) with air drying (15 min) were temporally applied to each spotted specimen. The target plate, which included the appropriate controls, was loaded into the instrument and analyzed. Spectral matrix scores of ≥ 1.9 were deemed acceptable for species-level identification. The Lyra assay was performed and the results interpreted according to the manufacturer's instructions.

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In brief, 50 μ l of liquid Amies from each patient specimen was transferred to a separate Lyra process buffer tube and heated at 95°C for 10 min. During this time, a sufficient quantity of lyophilized master mix (8 reactions per lyophilized master mix tube) was rehydrated. Using a 96-well plate, each respective well received 15 μ l of rehydrated Lyra master mix along with 5 μ l of post-heat-treated patient specimen. The plate that included the appropriate controls was loaded into an ABI 7500 fast real-time PCR instrument (Thermo Fisher, Waltham, MA). Following instrument programming, the TaqMan chemistry-based real-time PCR portion of the Lyra assay was completed in approximately 60 min. For the hands-on time component of the workflow analysis, two subsets ($n = 45$ and $n = 49$) of the 161 specimens were randomly selected to simulate the daily batch size of our laboratory. Assistant microbiology (E.M.D.) and assistant molecular (A.E.P.) supervisors independently observed and timed two certified microbiology technologists and two certified molecular technologists (each with ≥ 2 years of experience) as they completed all required steps in singleton for culture and Lyra testing, respectively. All steps for the Lyra assay and culture were outlined and reviewed with E.M.D. and A.E.P. prior to performing the time study to ensure the accuracy of data collection. All culture-based testing was performed in the microbiology laboratory. In brief, the microbiology technologists retrieved all plates from the incubator at 24 and 48 h, followed by rapid site reading and sorting of negative and suspected-positive plates. Negative plates were batch reported at 24 (preliminary report) and 48 (final report) h. Suspected-positive plates were evaluated as previously described. The time associated with a definitive identification of non-group A *Streptococcus* (MICroSTREP plus 1 panel and MALDI-TOF MS) was not included, as such modalities are not routinely utilized for culture-based identification of *Streptococcus* isolated from pharyngeal specimens. The Lyra testing was performed in the molecular laboratory using best practices (i.e., unidirectional workflow, pre- and post-PCR rooms, and aerosol-resistant pipette tips) to minimize specimen carryover and amplicon contamination. All steps for the Lyra assay setup took place in a designated dead air box (nonlaminar flow), except for the 10-min 95°C heat step. Following real-time PCR, the Lyra test results were printed and manually interpreted by each molecular technologist. Currently, the Lyra assay has neither computer-based test result interpretation nor a laboratory information system (LIS) interface. For the intralaboratory turnaround time (TAT) component of the workflow analysis, retrospective data were extracted from our LIS and analyzed to determine the average time interval from specimen receipt in the laboratory to final test result. These data (from approximately 1,500 pharyngeal specimens per month) consisted of the last two consecutive months (March and April 2015) of culture-based testing and the next two consecutive months (May and June 2015) of Lyra-based testing in our laboratory.

Table 1 summarizes the results of the 161 specimens included in this study. One hundred nineteen specimens were negative by culture and the Lyra assay. Nineteen specimens were positive for GAS by culture and the Lyra assay. Sixteen specimens were culture positive for *S. anginosus* (Lancefield group C; small-colony-forming non- β -hemolytic streptococci); all were negative by the Lyra assay. Four specimens were culture positive for *S. dysgalactiae* (Lancefield group C [$n = 2$] and group G [$n = 2$]; large-colony-forming β -hemolytic streptococci); all four were group C/G *Streptococcus* positive by the Lyra assay. Two were culture positive for *S.*

TABLE 1 Summary characteristics for assay results

n	Culture result	Lancefield typing			MALDI-TOF MS result	Lyra Direct strep result	
		A	C	G		A	C/G
119	Negative	NA ^a	NA	NA	NA	–	–
19	<i>S. pyogenes</i>	+	–	–	<i>S. pyogenes</i>	+	–
16	<i>S. anginosus</i>	–	+	–	<i>S. anginosus</i>	–	–
2	<i>S. dysgalactiae</i>	–	+	–	<i>S. dysgalactiae</i>	–	+
2	<i>S. dysgalactiae</i>	–	–	+	<i>S. dysgalactiae</i>	–	+
2	<i>S. salivarius</i>	–	+	–	<i>S. salivarius</i>	–	–
1	<i>S. equi</i>	–	+	–	<i>S. equi</i>	–	+

^a NA, not applicable.

salivarius (Lancefield group C; small-colony-forming non- β -hemolytic streptococci); both were negative by the Lyra assay. One specimen was culture positive for *S. equi* (Lancefield group C; large-colony-forming β -hemolytic *Streptococcus*) and was group C/G *Streptococcus* positive by the Lyra assay. In total, there was 100% agreement between culture and the Lyra assay for detecting only clinically significant β -hemolytic *Streptococcus*. The sensitivity, specificity, negative predictive value, and positive predictive value for the Lyra assay in this study were all 100%. Two specimens were initially inhibitory (1.24%) by the Lyra assay, but these resolved upon repeat testing. The cycle threshold (C_T) values (16.3 to 34.7 cycles) for positive specimens encompassed the breadth of the manufacturer's range (positive C_T cutoffs, ≥ 1 and < 40). The results (mean \pm standard deviation [SD]) for the workflow analysis demonstrated a 96% increase in hands-on time per specimen for the Lyra assay (3 min 16 s \pm 13 s) versus culture (1 min 40 s \pm 4 s), and a 60% decrease in intralaboratory TAT for the Lyra assay (18.1 \pm 10.1 h) versus culture (45.0 \pm 6.5 h). As anticipated, the hands-on time by culture was markedly less for negative (49.4 \pm 5.6 s) than for positive (5 min \pm 27 s) specimens. The hands-on time for the Lyra assay was independent of whether the specimens were positive or negative. The mean \pm SD analytical turnaround time (set up through reporting) for the Lyra assay was 3 h 57 min \pm 3 min using batch sizes of 45 and 49 samples.

Current guidelines from the Infectious Diseases Society of America (IDSA) recommend that RATs be used in lieu of culture to expedite the identification of GAS, and that negative RATs be followed by backup culture for pediatric patients (7). However, the main limitation of this algorithm is that the backup culture requires an additional 24 to 48 h to perform, significantly delaying appropriate patient management. An optimal solution is the use of highly sensitive and specific NAATs to replace GAS RATs and/or backup culture. In this regard, several commercially available NAATs have recently entered the diagnostic market. Some have the potential to replace GAS RATs, including Alere i strep A (Waltham, MA), AmpliVue GAS (Quidel, San Diego, CA), cobas Liat strep A (Roche, Basel, Switzerland), *illumigene* group A *Streptococcus* (Meridian Bioscience, Cincinnati, OH), and Simplexa group A strep direct (Focus Diagnostics, Cypress, CA). These NAATs, some of which are waived by Clinical Laboratory Improvement Amendments (CLIA), can be performed in < 1 h (15 to 60 min), require minimal hands-on time, and show equivalent-to-superior performance compared to that of culture-based methods (8–11). Other molecular methods are better suited to

batch mode testing and are ideal for replacing backup culture. These include the FDA-approved GasDirect test (GenProbe, San Diego, CA) and the LightCycler Strep-A assay (Roche Applied Science, Indianapolis, IN). The DNA probe-based GasDirect test has been available for approximately 2 decades but demonstrates suboptimal performance (12). In contrast, the Roche LightCycler Strep-A assay shows exceptional performance but requires extensive validation prior to implementation in the clinical laboratory, since it is not FDA approved (13).

The decision to replace RATs and/or backup culture with molecular-based technology is multifactorial and should be pursued using a multidisciplinary approach. The Lyra assay is not currently FDA approved as a replacement for backup culture. However, this study demonstrates the robustness of the Lyra assay to function in this capacity. In addition, we verified the specificity of the Lyra assay to detect only clinically significant β -hemolytic *Streptococcus*; non- β -hemolytic *S. anginosus* and *S. salivarius* did not yield false-positive results. Since implementing the Lyra assay, and despite performing the test only once per day (batch size, 45 to 50 specimens per day), our laboratory has observed a marked reduction in TAT and a modest increase in hands-on time. For perspective, the percentage of patients positive for GAS (19/161 [11.8%]) and β -hemolytic group C/G *Streptococcus* (5/161 [3.0%]) in this study was 14.8%. With a volume of 18,000 backup culture tests conducted per year in our laboratory, a significant number of patients with GAS ($n = 2,124$) and/or β -hemolytic group C/G ($n = 540$) *Streptococcus* infections might experience delays in therapeutic management using culture (45 h) instead of the Lyra assay (18.1 h). The clinical impact of treatment delay includes the prolongation of disease course and the promulgation of disease transmission. The amount of labor used per specimen for culture (1 min 40 s) and the Lyra assay (3 min 16 s) extrapolates to 0.24 and 0.47 full-time equivalents (FTEs), respectively, per year in our laboratory. Further improvements to intralaboratory TAT using the Lyra assay could be realized simply by performing batch mode testing more than once per day. In our opinion, the increased labor requirement is minimal and justified by providing a faster TAT using the Lyra assay.

The Lyra assay is well suited for use in laboratories with moderate to high test volumes (≥ 40 samples per day), such that up to 94 samples per run can be performed. An added benefit of the Lyra assay is a lower cost per test by minimizing instrument redundancy and the associated costs (i.e., annual maintenance fees and interinstrument comparability studies), and optimized personnel utilization via batch mode testing. For laboratories with smaller test volumes (< 40 per day) or those seeking a rapid NAAT option, there are alternative commercial assays available that can be performed in ≤ 30 min and are ideal for settings in which clinical decisions demand rapid TAT (i.e., emergency department). However, these rapid NAATs will likely be more expensive and might limit the ability of the laboratory to accommodate increased test volume without acquiring additional equipment.

In closing, the Lyra Direct strep assay provides a tangible solution for laboratories that desire to use a molecular approach for the detection of GAS from pharyngeal specimens. In addition, the

Lyra assay is the only FDA-approved option for the detection of β -hemolytic group C/G *Streptococcus*. Replacing backup culture with the Lyra assay requires additional validation studies, but this is not an insurmountable task. The incorporation of automation and computer-based test result interpretation would markedly improve the adoptability of the Lyra assay, especially for laboratories for which even nominal increases in labor requirements are critical.

REFERENCES

1. Cimolai N, Morrison BJ, MacCulloch L, Smith DF, Hlady J. 1991. Beta-haemolytic non-group A streptococci and pharyngitis: a case-control study. *Eur J Pediatr* 150:776–779. <http://dx.doi.org/10.1007/BF02026709>.
2. Gerber MA, Randolph MF, Martin NJ, Rizkallah MF, Cleary PP, Kaplan EL, Ayoub EM. 1991. Community-wide outbreak of group G streptococcal pharyngitis. *Pediatrics* 87:598–603.
3. Cherry JD. 2009. Pharyngitis (pharyngitis, tonsillitis, tonsillopharyngitis, and nasopharyngitis), p 160–169. In Feigin RD, Cherry J, Demmler-Harrison GJ, Kaplan SL (ed), Feigin & Cherry's textbook of pediatric infectious diseases, 6th ed. Saunders Elsevier, Philadelphia, PA.
4. Kaplan EL, Gerber MA. 2009. Group A, group C, and group G beta-hemolytic streptococcal infections, p 1225–1239. In Feigin RD, Cherry J, Demmler-Harrison GJ, Kaplan SL (ed), Feigin & Cherry's textbook of pediatric infectious diseases, 6th ed. Saunders Elsevier, Philadelphia, PA.
5. Stewart EH, Davis B, Clemans-Taylor BL, Littenberg B, Estrada CA, Centor RM. 2014. Rapid antigen group A streptococcus test to diagnose pharyngitis: a systematic review and meta-analysis. *PLoS One* 9:e111727. <http://dx.doi.org/10.1371/journal.pone.0111727>.
6. Alatoom AA, Cunningham SA, Ihde SM, Mandrekar J, Patel R. 2011. Comparison of direct colony method versus extraction method for identification of Gram-positive cocci by use of Bruker Biotyper matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol* 49:2868–2873. <http://dx.doi.org/10.1128/JCM.00506-11>.
7. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan EL, Lee G, Martin JM, Van Beneden C. 2012. Clinical practice guidelines 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. <http://dx.doi.org/10.1093/cid/cis847>.
8. Anderson NW, Buchan BW, Mayne D, Mortensen JE, Mackey TL, Ledebor NA. 2013. Multicenter clinical evaluation of the *illumigene* group A *Streptococcus* DNA amplification assay for detection of group A *Streptococcus* from pharyngeal swabs. *J Clin Microbiol* 51:1474–1477. <http://dx.doi.org/10.1128/JCM.00176-13>.
9. Cohen DM, Russo ME, Jaggi P, Kline J, Gluckman W, Parekh A. 2015. Multicenter clinical evaluation of the novel Alere i strep A isothermal nucleic acid amplification test. *J Clin Microbiol* 53:2258–2261. <http://dx.doi.org/10.1128/JCM.00490-15>.
10. Faron ML, Ledebor NA, Granato P, Daly JA, Pierce K, Pancholi P, Uphoff TS, Buchan BW. 2015. Detection of group A *Streptococcus* in pharyngeal swab specimens by use of the AmpliVue GAS isothermal helicase-dependent amplification assay. *J Clin Microbiol* 53:2365–2367. <http://dx.doi.org/10.1128/JCM.01085-15>.
11. Felsenstein S, Faddoul D, Sposto R, Batoon K, Polanco CM, Dien Bard J. 2014. Molecular and clinical diagnosis of group A streptococcal pharyngitis in children. *J Clin Microbiol* 52:3884–3889. <http://dx.doi.org/10.1128/JCM.01489-14>.
12. Pokorski SJ, Vetter EA, Wollan PC, Cockerill FR, III. 1994. Comparison of Gen-Probe group A streptococcus direct test with culture for diagnosing streptococcal pharyngitis. *J Clin Microbiol* 32:1440–1443.
13. Uhl JR, Adamson SC, Vetter EA, Schleck CD, Harmsen WS, Iverson LK, Santrach PJ, Henry NK, Cockerill FR. 2003. Comparison of Light-Cycler PCR, rapid antigen immunoassay, and culture for detection of group A streptococci from throat swabs. *J Clin Microbiol* 41:242–249. <http://dx.doi.org/10.1128/JCM.41.1.242-249.2003>.