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Point-Counterpoint: A Nucleic Acid Amplification Test for *Streptococcus pyogenes* Should Replace Antigen Detection and Culture for Detection of Bacterial Pharyngitis

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Nucleic acid amplification tests (NAATs) have frequently been the standard diagnostic approach when specific infectious agents are sought in a clinic specimen. They can be applied for specific agents such as *S. pyogenes*, or commercial multiplex NAATs for detection of a variety of pathogens in gastrointestinal, bloodstream, and respiratory infections may be used. NAATs are both rapid and sensitive. For many years, *S. pyogenes* testing algorithms used a rapid and specific group A streptococcal antigen test to screen throat specimens, followed, in some clinical settings, by a throat culture for *S. pyogenes* to increase the sensitivity of its detection. Now *S. pyogenes* NAATs are being used with increasing frequency. Given their accuracy, rapidity, and ease of use, should they replace antigen detection and culture for the detection of bacterial pharyngitis? Bobbi Pritt and Robin Patel of the Mayo Clinic, where *S. pyogenes* NAATs have been used for well over a decade with great success, will explain the advantages of this approach, while Richard (Tom) Thomson and Tom Kirn of the NorthShore University HealthSystem will discuss their concerns about this approach to diagnosing bacterial pharyngitis.

POINT

Acute pharyngitis is one of the most common diagnoses made in outpatient settings (1). Although viruses are responsible for the majority of cases, *Streptococcus pyogenes* (beta-hemolytic group A streptococci [GAS]) and, less commonly, other bacteria are estimated to cause 25% of the cases in adults and nearly 40% of the cases in children (2–4). Most cases of GAS pharyngitis are mild and self-limited, although potential complications include peritonsillar abscesses, otitis media, mastoiditis, cervical lymphadenitis, pneumonia, rheumatic fever, and poststreptococcal glomerulonephritis. Antimicrobial therapy may prevent these complications and may also shorten the duration of illness and potentially minimize the spread of infection to others; for these reasons, antibiotics are frequently administered, particularly to children and to adults with severe GAS pharyngitis (5, 6).

We acknowledge that implicit in any diagnostic strategy is an assumption that results will be actionable, which, in the case of GAS pharyngitis, means that treatment would be administered. We realize that GAS pharyngitis, especially when it is nonsevere, is not universally treated and that there are geographic practice differences. There are a number of reasons for this, including that antibiotics have a relatively small effect in reducing symptoms and symptom duration, that rheumatic fever and poststreptococcal glomerulonephritis are rare in certain populations, that antibiotics risk disturbing the microbiome (and consequently increasing the risk of conditions such as thrush and *Clostridium difficile*-associated diarrhea), that antimicrobial use may result in allergies and other adverse drug effects, and also because of the associated cost and logistics of testing and treatment. Despite these controversies, which we will subsequently propose justify an outcome-based, cost-effectiveness study using modern diagnostics, we assume herein that making a diagnosis of GAS is generally desired and that therefore the ideal way to do so should be used.

A seemingly straightforward way to guide antibiotic use is for clinicians to apply various clinical prediction rules, such as the Centor criteria, which attempt to differentiate viral from GAS pharyngitis (7). Unfortunately, none of these prediction rules have demonstrated acceptable sensitivity for justifying the elimination of laboratory testing (5, 8, 9). Methods for laboratory detection of *S. pyogenes* include rapid antigen detection tests (RADTs), bacterial culture, and nucleic acid amplification tests (NAATs). RADTs are commercially available and widely used for detection of *S. pyogenes* in point-of-care settings because of their ease of use, low cost, and ability to produce results rapidly. They generally exhibit high specificity for detection of *S. pyogenes*, and thus, positive results do not need to be routinely confirmed by another method. However, RADTs have relatively low sensitivity, with most reported levels ranging from 70 to 90% (10, 11). Further, test sensitivity is dependent on the severity of disease, with poorer sensitivity (47 to 65%) in patients with lower modified Centor scores (12). For these reasons, it is common practice to confirm negative RADT results with bacterial culture. National and European guidelines provide guidance for performing confirmatory testing but differ in their recommendations (13). The Infectious Diseases Society of America and the American Heart As-

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sociation recommend performing bacterial cultures for children and adolescents with negative RADT results but do not recommend reflex cultures for adults with negative RADT results, given the lower incidence of *S. pyogenes* pharyngitis and rheumatic fever in this population (5, 14). However, new evidence suggests that reflexive culture may be indicated for adults, as well as children and adolescents, since RADTs fail to detect GAS pharyngitis in a significant number of adults (13). Some groups, such as the American College of Physicians and the American Society of Internal Medicine, use clinical (Centor) criteria rather than patient age to guide testing (15, 16). Given the conflicting information, many clinical microbiology laboratories opt to perform culture on all patients with negative RADTs. Culture confirmation of negative results is also required by the United States Food and Drug Administration for some RADTs.

Beyond culture being considered the gold standard for the diagnosis of GAS pharyngitis, it can be used to detect other causes of bacterial pharyngitis, such as group C and G streptococci, *Arcanobacterium haemolyticum*, and *Fusobacterium necrophorum*, depending on the procedures applied. However, culture is relatively labor-intensive, must be performed in a clinical laboratory, requires the use of proper laboratory techniques, and takes 24 to 48 h to generate a result (5). NAATs provide an equally sensitive and faster alternative to conventional bacterial culture and are now available in rapid and easy-to-use commercial formats. NAATs have replaced culture for the detection of many organisms. Given the limitations of other testing options, we propose that NAATs are poised to replace antigen detection and culture for the detection of GAS pharyngitis.

Introduction of a NAAT for *S. pyogenes* in our laboratory. We were early adopters of a NAAT for the diagnosis of GAS pharyngitis; we replaced RADT-reflex culture algorithms with a rapid, real-time *S. pyogenes* PCR assay in our routine clinical practice in 2002 (17). The PCR assay we use adopted analyte-specific reagent primers and fluorescence resonance energy transfer probes (Roche Diagnostics, Indianapolis, IN) as previously described (17). When considering NAAT implementation, we compared the performance of this PCR assay to that of the Directigen 1-2-3 Group A Strep Test kit (BD Diagnostic Systems, Sparks, MD), which was in use at the Mayo Clinic at the time the PCR assay was adopted. We also performed bacterial culture of all specimens. Compared to culture, the Directigen and PCR assays showed sensitivities of 55 and 93%, respectively (17). These data supported our decision to replace the RADT-reflex culture method with PCR and allowed us to gain the support of our clinical practice required to make this change. Two additional components facilitated our successful transition from RADT-culture to PCR; our abilities to get timely results to our patients and to link the filling of a prescription with positive results (Fig. 1). Our patients are typically tested in urgent-care center, outpatient clinic, or emergency department settings. Systems are in place to rapidly deliver specimens to the laboratory by using pneumatic tubes or electronic transport vehicles or, for off-site locations, frequent courier deliveries. Also, at specimen collection, clinicians determine how a patient would be treated were their result to be positive and where the patient would prefer to fill the prescription if one were needed. The patient is also given a phone number and a time to call to get the result. The prescription travels with the specimen to the laboratory. Our average turnaround time for this assay is 3 h from receipt in the laboratory. As soon as the result is entered into the laboratory information system, it is available to the patient via an automated telephone line. As mentioned, patients call in for their results and listen to an automated message. If the result is negative, they are told that this is the case. If it is positive, they learn this and are told to pick up the prescription at the designated pharmacy. The laboratory technologist entering the positive result in the laboratory information system faxes the prescription to the patient's pharmacy of choice.

FIG 1 A theranostic approach to *S. pyogenes* PCR testing, linking the laboratory result to delivery of a prescription for antibiotics. A sore throat prompts a patient or caregiver to call a phone triage line (A), where a health care provider uses a standardized phone questionnaire to assess the patient's condition. If indicated, the patient is instructed to report to the outpatient clinic (B), where a pharyngeal swab is obtained for *S. pyogenes* PCR (C). Eligible patients may elect to collect their own swab in lieu of waiting to be seen. The swab is then delivered to the clinical microbiology laboratory for testing (D), along with a prescription for antibiotics from the patient's provider. If the PCR result is positive, the prescription is faxed to the patient's pharmacy. The patient receives the test result by an automated phone system (E), along with information to pick up the prescription if the test is positive (F).

Recently, we have shown that patients can collect their own throat swabs (and parents can collect their child's throat swab), yielding PCR results equivalent to those from health care worker-collected swabs (18). As a result, we have incorporated the option of patient self-swabbing (or parental swabbing of children) into our process. As with any disease, diagnostic testing must be incorporated in a comprehensive system for patient evaluation prior to treatment, so the self-swabbing process incorporates a health care worker screening tool that identifies which patients qualify for self-swabbing. The reason for establishing and maintaining the above-described system over the last 14 years is that it has, until now, been the fastest way to provide the most sensitive means of diagnosing GAS pharyngitis. Although a panel-based molecular approach to pharyngitis (targeting bacteria and viruses) could be considered, in our opinion, the clinical impact, both positive (e.g., more rapid symptom resolution, prevention of complications) and negative (e.g., false-positive results for rare agents such as *Corynebacterium diphtheriae*, detection of herpes simplex virus in latently infected patients), as well as economic issues, should be addressed before the widespread adoption of such an approach.

As noted above, GAS is not the only bacterial cause of pharyngitis. Whether or not diagnosis of other bacterial causes of phar-

yngitis should be routinely pursued is an open question. For example, a study of the effect of antibiotic treatment on the outcome of pharyngitis associated with detection of *F. necrophorum* in an associated throat swab could be considered (19, 20).

What is new for *S. pyogenes* NAATs? The advent of Clinical Laboratory Improvement Amendments (CLIA)-waived rapid NAATs for *S. pyogenes* detection, such as the Roche Cobas Liat and the i Strep A (Alere, Waltham, MA) (21, 22), provides new opportunities for the rapid diagnosis of GAS pharyngitis. These NAATs are as easily and quickly performed as RADTs. We recently compared the performance of the Cobas Liat Strep A assay with our PCR assay by using residual material from 200 throat swabs that were submitted for *S. pyogenes* testing and showed the two assays to have equivalent performance characteristics (21). Of the 200 specimens tested, 114 were negative and 84 were positive by both assays. The remaining two specimens were positive only with the Liat assay but had originally tested positive by our PCR assay. These assays take ≤15 min, meaning that they can be performed and results can be obtained at the point of care. At this time, they are not necessarily interfaced to the electronic medical record, which is necessary for ideal patient care, and certain quality control questions, such as whether or not monitoring for contamination (as these tests are performed outside routine laboratory settings) is needed, remain to be addressed.

The cost of *S. pyogenes* NAATs. The main perceived drawback of *S. pyogenes* testing overall is cost, and NAATs are certainly not inexpensive. However, it is important to compare not only the cost of NAAT and RADT reagents but also the cost of bacterial culture for patients with negative RADT results. Depending on the season and population tested, ≥70% of RADTs may require reflex culture testing, adding a significant burden to the laboratory and health care system. Another consideration is the additional time required to obtain a culture result, during which the untreated patient may experience ongoing symptoms. Alternatively, the prolonged turnaround time of confirmatory culture may cause clinicians to forgo recommended testing guidelines and prescribe antibiotics based only on clinical features or to both test and prescribe antibiotics regardless of the test result. The latter approach would lead to unnecessary antibiotic use and possibly increase the risk of antimicrobial resistance. There are also issues of patient and health care provider satisfaction and costs avoided by not needing to follow up on delayed culture results that may now be realized with point-of-care NAATs for GAS pharyngitis.

Conclusions. NAATs offer significant advantages over RADTs with reflexive culture for the detection of GAS pharyngitis. They are as sensitive as either culture alone or RADTs with reflexive culture and can rapidly provide definitive and actionable results. With the availability of CLIA-waived rapid NAATs, we are now entering the next frontier in molecular diagnostics. These tests are easy to perform and can be used in many settings, including, but not limited to, outpatient clinics, urgent-care centers, and hospital laboratories. These state-of-the-art diagnostics for GAS pharyngitis will provide new opportunities to streamline the testing and treatment of patients with pharyngitis in a myriad of settings, including traditional health care settings and nontraditional locales. They also provide a tool for performing a definitive, outcome-based, cost-effectiveness study to define which pharyngitis patients should be tested (and how) and which should be treated in modern clinical practice. Finally, future tests for GAS pharyngitis may need to assess macrolide susceptibility, given that not all GAS strains are macrolide susceptible and that macrolides may be prescribed to penicillin-allergic patients with GAS pharyngitis.

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COUNTERPOINT

Acute pharyngitis is a disease entity encountered frequently by physicians in an outpatient setting. In 2007, >12 million U.S. ambulatory care visits (1% of all visits) were associated with a diagnosis of acute pharyngitis and another 4 million were classified as "streptococcal sore throat" (1). Although many etiologic agents may result in the clinical presentation of acute pharyngitis, *Streptococcus pyogenes* (group A streptococcus, GAS) is the major organism targeted for identification through the application of clinical prediction rules and diagnostic testing (2). Focus on this organism is driven by the well-described risks of suppurative (invasive infection) and nonsuppurative (rheumatic fever) sequelae following GAS pharyngitis and the knowledge that antimicrobial therapy mitigates these risks (3, 4). What is overlooked by many in the clinical and diagnostic professions are complications, including pharyngeal abscess, bacteremia, pneumonia, metastatic infection involving other organs, and Lemierre's syndrome, arising from bacterial pharyngitis caused by etiologies other than GAS that require culture or multiplex molecular testing for detection. In addition to added morbidity and occasional death, loss of work or school days, spread of contagious pathogens, and public health lapses all result from a lack of full laboratory evaluation (5). Whether to use PCR for the detection of GAS or culture-multiplex PCR for the detection of multiple pathogens depends on clinical exam findings, patient demographics, resources available to the clinician, and test performance characteristics. Focusing solely on the detection of GAS in all settings is not optimal patient care.

Infectious etiologies of acute pharyngitis. As a group, viruses, including influenza virus, Epstein Barr virus, cytomegalovirus, herpes simplex virus, and human immunodeficiency virus, represent the most common cause of infectious acute pharyngitis (approximately 50%) (3). GAS is estimated to cause 5 to 15% of acute pharyngitis cases in adults and 15 to 30% of all cases of pharyngitis in children aged 5 to 15 years (6-10). Other bacterial etiologies include non-group A beta-hemolytic streptococci (specifically, groups C and G), *Arcanobacterium haemolyticum*, *Corynebacterium diphtheriae*, *Neisseria gonorrhoeae*, *Chlamydia pneumoniae*, *Francisella tularensis*, *Mycoplasma pneumoniae*, and *Fusobacterium necrophorum*. Of concern, *A. haemolyticum* was found in 2.5% of symptomatic adolescents with pharyngitis and 0% of controls in a Canadian study, while *F. necrophorum* was cultured from 10% of throat specimens collected from patients with a diagnosis of pharyngitis or persistent sore throat, a percentage equal to that of patients with GAS detected (11, 12). GAS detection was more common in patients <20 years old, with *F. necrophorum* more common in patients >20 years old (12).

Consequences of a failure to make an etiologic diagnosis. The consequences of a failure to identify and treat GAS pharyngitis with antibiotics are well documented and regarded as significant by clinicians. However, some of the other bacterial etiologies of acute pharyngitis may also carry the risk of complications when they are not appropriately treated. Both group C and group G streptococci cause sporadic and epidemic pharyngitis that is clinically indistinguishable from GAS in school age children and in adults (13-16). Early treatment may reduce the duration of symptoms, limit spread to susceptible contacts, and prevent invasive infections (17, 18). *A. haemolyticum* causes pharyngitis primarily in adolescents and young adults presenting with clinical features that overlap those of GAS pharyngitis (11, 19, 20). Serious invasive infections caused by *A. haemolyticum* have been reported and include peritonsillar and pharyngeal abscesses, bacteremia, and pneumonia (21, 22). The pathogenesis of *F. necrophorum* invasive disease and its link to antecedent pharyngitis are clear. *F. necrophorum* causes most cases of Lemierre's syndrome, which is characterized by necrotizing tonsillopharyngitis, followed by bacteremia, septic thrombophlebitis of the internal jugular vein, and septic pulmonary emboli. Additionally, there is evidence that *F. necrophorum* causes endemic pharyngitis in adolescents and young adults in the absence of Lemierre's syndrome at a rate similar to that of GAS, and on the basis of published epidemiologic data, *F. necrophorum* is estimated to cause Lemierre's syndrome at a higher incidence than that at which GAS causes acute rheumatic fever (23, 24). It should be noted that evidence demonstrating if and/or how often pharyngitis caused by *F. necrophorum* directly leads to Lemierre's syndrome and if treatment with antibiotic therapy would prevent it does not exist (25).

Choosing a diagnostic test for acute pharyngitis. Despite the wide etiologic differential for acute pharyngitis, diagnostic testing for most patients is limited to methods that target GAS. The most commonly used diagnostic tests include bacterial culture, GAS antigen detection, and GAS nucleic acid amplification assays.

TABLE 1 Three approaches to the laboratory diagnosis of pharyngitis with etiologies detected

Etiology	Pharyngitis GAS PCR	Pharyngitis culture based	Pharyngitis molecular syndromic
GAS	X	X	X
Group C and G streptococci		X	X
A. haemolyticum		X	X
F. necrophorum		X	X
N. gonorrhoeae		X	X
C. trachomatis			X
HIV			X
Enteroviruses			X
Herpes simplex virus			X
M. pneumoniae			X
C. diphtheriae			X
Respiratory viruses			X

Compared to antigen detection, both culture-based methods and NAATs for GAS demonstrate better and essentially equivalent sensitivity (26). Compared to standard culture techniques, NAATs require less personnel time to perform and can be completed in a much shorter time frame (1 to 3 h versus 16 to 18 h). While GAS NAATs may be superior to culture-based methods with regard to turnaround time, this approach suffers a significant drawback in the inability to detect potential pathogens other than GAS. From both diagnostic and clinical standpoints, causes of acute pharyngitis other than GAS have been largely ignored when addressing patients presenting with acute pharyngitis. Disease frequency and severity and serious sequelae suggest the following approach. Pediatric patients need GAS testing. Adolescents and young adults need GAS, *Streptococcus* group C and G, and *A. haemolyticum* culture, with testing for *F. necrophorum* to follow as soon as an acceptable test is developed. Early treatment of symptomatic patients with a positive test result may shorten the duration of symptoms, will prevent transmission to susceptible contacts, and, most importantly, can prevent severe, life-threatening sequelae (27). Pharyngitis in older adults is less common, with culture diagnosis infrequently used. If needed, the broad group of etiologies should be sought by culture. It is important to note that the detection of other beta-hemolytic streptococci and *A. haemolyticum* requires experience by the laboratory technologist but not additional cost, since blood agar is the preferred medium for all pathogens.

New technologies and the paradigm shift in laboratory diagnosis. There are benefits to considering a rapid, syndrome-based testing approach to acute pharyngitis, analogous to those that have been implemented for patients with respiratory symptoms, diarrheal illnesses, and meningitis/encephalitis (28-31). Such a panel might include a broad range of common or especially virulent bacterial and viral causes of acute pharyngitis and may be tailored to specific age groups (Table 1). As diagnostic technology continues to evolve, this approach becomes more feasible from a financial perspective and will, in all likelihood, be performed at the point of care, as has occurred for influenza virus and respiratory syncytial virus NAATs (32-34). The advantages of a syndromic diagnostic approach for pharyngitis are many. (i) Etiologic diagnosis is always known. (ii) Therapy is directed, not empiric, favoring antimicrobial stewardship. (iii) Complications, e.g., those following *A. haemolyticum* and *F. necrophorum* infections, are prevented. (iv) Epidemiology is robust, enhancing community and health care prevention efforts. (v) Clinical acumen is improved, as etiologic answers are known, not assumed. (vi) Sexually transmitted diseases are identified and contained by education and contact tracing. Although the cost to the laboratory with comprehensive testing will increase, the overall cost to the health care system may decrease, with better patient management and public health. The rapid clinical acceptance of multiplex respiratory, gastrointestinal, and central nervous system panels underscores the practical understanding of the syndromic approach in general (35). The shift toward having all, rather than just some, of the diagnostic information is compelling and argues for a syndromic approach to the diagnosis of pharyngitis.

Summary. The rapid and sensitive detection of GAS by PCR in patients with pharyngitis is an important improvement. The expanding knowledge of the etiologies, epidemiology, and clinical consequences of pharyngitis argues for additional diagnostic testing in appropriate patient settings. Molecular multiplex testing that provides a syndromic approach by detecting many pathogens is around the corner. The nature of microbiology laboratory testing is changing, and we in the laboratory need to lead this change.

Thomas J. Kirn and Richard B. Thomson, Jr.

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SUMMARY

Points of agreement

1. Clinical prediction rules do not differentiate GAS from viral pharyngitis. The decision to use antimicrobial therapy to treat this infection is driven by results of laboratory tests that detect GAS. NAATs have been shown to be a rapid and highly accurate means to detect GAS on throat swabs.
2. Rapid detection and reporting of GAS pharyngitis greatly facilitates antimicrobial stewardship.
3. Bacterial throat cultures on 5% sheep blood agar plates can be used to detect multiple agents of pharyngitis, including GAS and group C and G streptococci, as well as *A. haemolyticum*. However, results will likely be available 24 to 48 h later than NAAT results.

Issues to be resolved

1. In an era of extremely low rates of poststreptococcal sequelae, does the benefit of antimicrobial treatment of GAS pharyngitis outweigh the risks, including increased rates of colonization with multidrug-resistant *Streptococcus pneumoniae*, allergic reactions to penicillins, and alteration of the microbiome of the patient?

2. There is an increasing body of evidence that suggest that *F. necrophorum* is an important agent of acute pharyngitis. Unfortunately, a simplified laboratory test method that can rapidly and accurately diagnose this infection does not currently exist.
3. Rigorous outcome studies are needed to demonstrate the benefit of antimicrobial treatment of group C and G streptococcal, *F. necrophorum*, and *A. haemolyticum* infections.
4. Syndromic multiplex NAATs may ultimately be used to more efficiently determine the etiology of acute pharyngitis. Given the expense of this testing, the clinical and economic benefits must be proven.

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