Real-Time PCR vs Standard Culture Detection of Group A β-Hemolytic Streptococci at Various Anatomic Sites in Tonsillectomy Patients

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Objective: To compare rates of group A β-hemolytic streptococci (GABHS) detection by real-time polymerase chain reaction (rtPCR) and standard culture (SCx) at different anatomic sites to determine whether a more patient-friendly site (eg, retromolar trigone or gingivo-buccal sulcus) would yield results similar to the tonsillar surface. Real-time polymerase chain reaction can detect GABHS at rates equal to SCx, and results require only a few hours.

Design: Prospective study.

Setting: Tertiary care setting.

Patients: The study population comprised 130 patients undergoing tonsillectomy or adenotonsillectomy.

Intervention: At tonsillectomy, swabs were taken of pharyngeal tonsil surface, pharyngeal tonsillar core, inferior gingivobuccal sulcus, and retromolar trigone. Tissue samples were taken from tonsil core and adenoid. All comparisons between methods and sites were made using the McNemar test for comparing correlated proportions. All calculated P values were 2-sided.

Main Outcome Measure: Detection of GABHS by rtPCR and SCx.

Results: In 41 cases (32%), GABHS was detected at 1 or more sampled sites, and 29 of those positive were detected on the tonsil surface—SCx and rtPCR results were both positive in 28 (97%). Of the 29 cases, results from the gingivobuccal site were positive by both rtPCR and SCx in 4 (14%), rtPCR only in 3 (10%), and SCx only in 3 (10%). Of the 7 tonsil surface–positive cases with retromolar trigone swabs, results were positive by rtPCR only in 1 (14%) and SCx only in 2 (29%).

Conclusion: Whether rtPCR or SCx is used, swabs of gingivobuccal sulcus and retromolar trigone do not accurately reflect GABHS populations on the tonsil surface.


Group A β-hemolytic streptococci (GABHS) are the most common bacterial pathogens associated with pharyngotonsillitis.1 Although most patients who present with symptoms of acute pharyngotonsillitis experience only a limited disease course, a substantial portion of them receive pharmacologic treatment and eventually tonsillectomy, a procedure performed on approximately 800 000 patients annually.2 Most previous studies relied on the use of standard culture (SCx) to identify the presence of GABHS in the oropharynx. This technique is suboptimal because it is slow (an average of 2 days is required for culture results) and is often uncomfortable for patients (samples are taken from the tonsil surface and posterior pharyngeal wall with swabs). Furthermore, studies have demonstrated that surface SCx techniques do not reliably reflect populations of GABHS that may be present in the tonsil core.3–6 In response to the need for rapid detection of GABHS, streptococcal antigen immunoassays have been developed to provide same-day, point-of-care test results for patients with acute pharyngotonsillitis. However, these antigen immunoassays have been found to be less sensitive than SCx (the gold standard) for the detection of GABHS and are often used in protocols that require negative results be confirmed with SCx.7 When immunoassay false-negative cases are identified on SCx, treatment is delayed, additional cost is incurred, and no advantage over SCx alone is realized.

Recently, Uhl and associates7 described a real-time polymerase chain reaction (rtPCR) technique for detecting GABHS from pharyngeal throat swabs of patients with acute pharyngotonsillitis in an ambulatory outpatient clinic setting. Compared with both SCx and a rapid strep antigen immunoassay, the rtPCR technique yielded...
more true-positive cases than SCx and was found to be more sensitive than the immunoassay. When compared with the standard approach for detection of GABHS (immunoassay with negative results confirmed by culture), the rtPCR method required less than half the personnel time and was generally completed in approximately 1 hour. This method is currently used at our institution in all settings in which pharyngeal throat swabs are used to detect GABHS. To our knowledge, no study has been conducted using rtPCR to detect GABHS in patients undergoing tonsillectomy.

Even if rtPCR offers advantages over existing techniques in the detection of GABHS, the method still requires a swab of the tonsil surface and posterior pharyngeal wall—a procedure that is often difficult, especially for nonotolaryngologists, and traumatic for patients. If a more accessible site for sample collection in the oral cavity or oropharynx could be identified and could accurately reflect GABHS populations in the pharyngotonsillar region, it would be widely accepted by clinicians. With the natural drainage of pharyngotonsillar secretions to the anterior tonsil pillars and to the retromolar trigone and inferior gingivobuccal sulcus, these areas may be such an alternative site. Because rtPCR requires only the presence of GABHS DNA for detection, it may provide a better detection rate than SCx in these more clinically accessible sites.

The aims of our study were (1) to determine if rtPCR could be used to detect GABHS in anterior oral cavity sites of patients with GABHS present on the tonsill surface, thereby providing a clinical testing site that would avoid the inherent difficulties of swabbing oropharyngeal sites (eg, children who refuse to open their mouths or gag) and (2) to use rtPCR to further scrutinize the previous observation of GABHS detected, with SCx, in the tonsil core when GABHS was not present on the tonsil surface.

### METHODS

#### STUDY DESIGN

This was a prospective study of consecutive patients undergoing tonsillectomy with or without adenoidectomy at Mayo Clinic, Rochester, Minnesota. Approval for this study was obtained from the Mayo Clinic institutional review board, and informed consent was obtained from all patients.

#### STUDY SAMPLE

All patients who presented to the Department of Otorhinolaryngology/Head & Neck Surgery over a 6-month period for tonsillectomy with or without adenoidectomy were considered candidates for enrollment. Exclusion criteria included history of radiation to the head or neck region, tonsillectomy for oncologic purposes, cleft lip or palate, immunodeficiency, adenoidectomy alone, a contraindication to tonsillectomy or general anesthesia, or refusal to participate in the study.

### SPECIMEN COLLECTION AND ANALYSIS

All specimens were collected at the time of surgery. After the induction of anesthesia, but before surgical incision, independent swabs were used to collect specimens from the inferior gingivobuccal sulcus, the retromolar trigone, the tonsil surface, and the adenoid surface by the operating surgeon using a double-swab collection and transport system (CultureSwab; Becton Dickinson Microbiology Systems, Cockeysville, Maryland) (Figure 1). One swab was used for SCx and the other for rtPCR analysis. For patients undergoing concomitant adenoidectomy, a biopsy specimen from the central adenoid was taken with sterile curved biopsy forceps, and the tissue was stored for transfer in a sterile container. The operating surgeon then performed the planned tonsillectomy (with or without adenoidectomy).

The tonsil specimens were bathed in an iodine bath for 2 minutes, rinsed in alcohol, and placed on a sterile field. A sterile scalpel was used to bisect the tonsil, starting from the lateral (cauterized) surface. The interior cross-section of the tonsil was swabbed with another double swab, a biopsy specimen from the interior was harvested with a sterile curved scalpel, and all the samples were collected for sterile transport to the microbiology laboratory. In total, 7 samples each were processed for analysis by both rtPCR and SCx (1 gingivobuccal sulcus swab, 1 retromolar trigone swab, 1 tonsil surface swab of both tonsils, 1 tonsil core swab of both tonsils, 1 tonsil core tissue biopsy of both tonsils, 1 adenoid surface swab, and 1 adenoid tissue biopsy if adenoidectomy was performed) (Figure 2).

All swab samples were analyzed according to protocols previously detailed elsewhere. Briefly, bacteria on the swabs were lysed by shaking with glass beads, and 5 mL of the sample was
analyzed by rtPCR using the LightCycler 1.5 (Roche Applied Science, Indianapolis, Indiana) with fluorescence resonance energy transfer probes. The primers, probes, and internal control were provided as analyte-specific reagents from Roche Applied Science. The internal control was used to demonstrate the lack of PCR inhibition of the rtPCR assay for negative specimens. For rtPCR detection of GABHS in tissue samples, a piece of tissue was placed in a sterile 1.5-mL Sarstedt tube (Sarstedt Inc, Newton, North Carolina) and mixed with 400 µL of 1× Tris-EDTA, 100 µL of proteinase K, and 50 µL of 10% sodium dodecyl sulfate. This was then vortexed and placed in a thermomixer overnight at 55°C at a mixing speed of 500 rpm. Next, 200 µL of supernatant was transferred to the MagNA Pure device (Roche Applied Science) for extraction, and the samples were run through the LightCycler Assay as previously described.8 For SCx detection of GABHS in tissue samples, a small piece of the collected tissue was homogenized using a stomacher for 90 seconds in 10 mL of beef broth before proceeding with SCx techniques.7

**DATA ANALYSIS**

The primary outcome measures were detection of GABHS by rtPCR and by SCx for each of the 7 samples. All comparisons between methods and sites were made with the McNemar test for comparing correlated proportions. All calculated P values were 2-sided, and P < .05 was considered statistically significant.

**RESULTS**

The 130 patients enrolled in the study included 102 children (78%) and 28 adults (age > 18 years) (22%). Ninety-one patients (70%) underwent adenoidectomy at the time of tonsillectomy. For all sampled sites, SCx or rtPCR showed that 41 patients (32%) were positive for GABHS. By rtPCR, the tonsil core was positive for GABHS in 35 patients (27%) and the tonsil surface was positive in 29 (22%).

Of the 29 patients in whom GABHS was detected on the tonsil surface, 10 (34%) were positive for GABHS in the inferior gingivobuccal sulcus: 3 by rtPCR alone, 3 by SCx alone, and 4 by both methods. Only 7 of these 29 patients had a retromolar trigone swab, 3 (43%) of which were positive for GABHS (1 by rtPCR and 2 by SCx). Thus, GABHS populations detected in the inferior gingivobuccal sulcus and retromolar trigone did not reliably predict the presence of GABHS on the tonsil surface.

If all 7 anatomical sites of the study are considered together, rtPCR was not significantly better than SCx in the detection of GABHS (positivity rates, 16.5% vs 15.7%; P = .42). As given in Table 1, of 778 total samples, rtPCR and SCx were concordant in 722 (93%), and rtPCR detected GABHS in 31 samples (4%) that SCx did not. However, in samples of core tonsil tissue, rtPCR was significantly better than SCx in the detection of GABHS (positivity rates, 21.7% vs 10.9%; P = .002). This was also true in samples of adenoid tissue (positivity rates, 25.6% vs 17.8%; P = .004). There was no significant difference between the rtPCR and SCx detection rates for any other tested site (data not shown).

Table 2 summarizes the GABHS detection results comparing the tonsil core with the tonsil surface separately for the 2 methods of detection. Of the 35 patients with GABHS detected with rtPCR in the tonsil core, GABHS was not detected on the tonsil surface in 7 (20%). Standard culture detected GABHS in the tonsil core of 27 patients, of which GABHS was not detected on the tonsil surface in 2 (7%). Considering all 130 patients, rtPCR demonstrated a significant difference in detection of GABHS populations present in the tonsil core compared with on the tonsil surface (positivity rates, 26.9%

<table>
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<tr>
<th>Site</th>
<th>rtPCR+ SCx+</th>
<th>rtPCR+ SCx−</th>
<th>rtPCR− SCx+</th>
<th>rtPCR− SCx−</th>
<th>rtPCR</th>
<th>SCx</th>
<th>P Value</th>
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<tr>
<td>Tonsil core tissue</td>
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<td>0</td>
<td>72</td>
<td>21.7</td>
<td>10.9</td>
<td>.002</td>
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<tr>
<td>Adenoid tissue</td>
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<td>11</td>
<td>1</td>
<td>95</td>
<td>25.6</td>
<td>17.8</td>
<td>.004</td>
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<td>625</td>
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<td>15.7</td>
<td>.42</td>
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</table>

Abbreviations: GABHS, group A β-hemolytic streptococci; rtPCR, real-time polymerase chain reaction; SCx, standard culture; +, positive result; −, negative result.

<table>
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<tr>
<th>Method of Detection</th>
<th>Surface+ Core+</th>
<th>Surface+ Core−</th>
<th>Surface− Core+</th>
<th>Surface− Core−</th>
<th>Surface</th>
<th>Core</th>
<th>P Value</th>
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<tbody>
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<td>7</td>
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<td>26.9</td>
<td>.03</td>
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<tr>
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<td>3</td>
<td>2</td>
<td>100</td>
<td>21.5</td>
<td>20.8</td>
<td>.65</td>
</tr>
</tbody>
</table>

Abbreviations: GABHS, group A β-hemolytic streptococci; rtPCR, real-time polymerase chain reaction; SCx, standard culture; +, positive result; −, negative result.

Table 1. Comparison of rtPCR and SCx in Detecting GABHS at Various Sites

Table 2. Comparison of rtPCR and SCx in Detecting GABHS in the Tonsil Core and on the Tonsil Surface

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In our study, the rate of GABHS was detected on the tonsil surface. Previous studies that examined the use of SCx in detecting GABHS in the retromolar trigone of tonsillectomy patients have demonstrated that SCx can be used to detect GABHS in the inferior gingivobuccal sulcus and the retromolar trigone of tonsillectomy patients because PCR detection requires only the presence of DNA and not necessarily viable organisms.

In a recent study of 384 throat swabs for GABHS from an outpatient urgent care center, rtPCR yielded 7 more true-positive results than SCx. For this reason and those previously stated herein, we predicted that rtPCR would detect GABHS in the inferior gingivobuccal sulcus and the retromolar trigone of tonsillectomy patients in whom GABHS was detected on the tonsil surface. Previous studies of patients with GABHS detected on the tonsil surface have demonstrated that SCx can be used to detect GABHS in the anterior oral cavity in only approximately 10% to 33% of cases. In our study, the rate of rtPCR detection of GABHS in the anterior oral cavity increased to 35% in the gingivobuccal sulcus and to 43% in the retromolar trigone.

Our results, in combination with those of previous studies that examined the use of SCx in detecting GABHS in the anterior oral cavity, suggest that GABHS has a propensity for the tonsil and cannot be detected reliably enough in the anterior oral cavity sites for clinical testing. The likely explanation is that although rtPCR detects the DNA of viable and nonviable organisms, a certain DNA load is required to register a positive result. If only a small amount of GABHS DNA is present or if that small amount is not represented in the small portion (5 µL) of the sample analyzed, rtPCR may still provide a negative result. It appears that neither the retromolar trigone nor the gingivobuccal sulcus contains an adequate DNA load; thus, clinicians need to continue sampling the tonsil surface to obtain appropriate specimens. However, in an acute care setting, the yield may have been higher.

In a study of tonsillectomy patients in 1929, Polvogt and Crowe11 cultured GABHS in 91 of 100 specimens. Since then, multiple studies have demonstrated the presence of GABHS in the tonsils of patients undergoing tonsillectomy for either recurrent acute pharyngotonsillitis or adenotonsillar hypertrophy. Typically, patients who undergo tonsillectomy for infection have been treated with multiple courses of antibiotics that failed to eradicate the infection completely or have had multiple recurrences of a presumed “new” infection. In searching for the cause of these treatment failures and frequent recurrences, some authors have posited that the pathogens reside deep in the tonsil parenchyma, tonsil crypts, or adenoid tissue. It has been presumed that antimicrobial penetration is poor in these areas, and neighboring pathogens with β-lactamase capacity provide some degree of local protection against antimicrobials. To explore these possibilities, previous studies used SCx to compare surface and core tonsil pathogenic flora after tonsillectomy and found discrepancies in the GABHS populations, indicating that core populations may not be reflected on the tonsil surface.

In previous studies of tonsillectomy patients, SCx isolates of GABHS were present in up to 43% of specimens from the tonsil core when they were not present on the tonsil surface. A possible reason for this discrepancy is that SCx is not sensitive enough to detect smaller populations of GABHS on the tonsil surface. As previously mentioned, we suspected that rtPCR of the tonsil surface would provide a better reflection of the populations in the tonsil core because this technique can detect GABHS DNA and does not require the presence of viable organisms, as does SCx.

In a comparison of the tonsil surface and tonsil core sites (Table 2), the rtPCR findings are consistent with those of previous studies that used SCx, namely, in 7 cases (20%), we detected GABHS in the tonsil core when none was detected on the tonsil surface. Standard culture, however, demonstrated a good concordance of the GABHS populations in the tonsil core and on the surface. Of all of the anatomical sites studied, rtPCR was significantly better than SCx in detecting GABHS only in tonsil core tissue and adenoid tissue specimens (Table 1).

The rtPCR results from the tonsil surface and core support the suggestion of previous studies that GABHS may be sequestered either in the crypts of the tonsil or intra-cellularly in the tonsil core, even when these populations are undetectable on the surface. Together with the superiority of rtPCR over SCx in detecting GABHS in the tonsil core and adenoid tissue specimens, the results suggest that the standard digestion protocols used in our study to prepare the tissues for SCx are inferior to the extraction methods used to isolate DNA for PCR. With the use of SCx, our study showed no significant differences in GABHS populations in the tonsil core and on the surface, contrary to previous studies that reported discrepancies. Because an increasing number of patients are currently undergoing tonsillectomy because of obstructive (not infectious) reasons, our patient population possibly included more patients with adenotonsillar hypertrophy than did earlier studies. Generally, these patients would be less likely to receive antibiotic therapy before tonsillectomy and, thus, could potentially have more robust populations of GABHS on the tonsil surface. Although this would explain why we did not find a significant difference in the detection of GABHS on the tonsil surface and in the tonsil core with SCx, this would not explain—and in fact be contrary to—the rtPCR findings that demonstrated discrepancies between the tonsil surface and core.

CONCLUSIONS

With the use of either rtPCR or SCx, swab samples taken from the inferior gingivobuccal sulcus and the retromolar trigone do not accurately reflect the GABHS popula-
tions on the tonsil surface. Accurate detection of GABHS by any technique requires traditional swabbing of the tonsil surface.

We had predicted that because of increased sensitivity in the detection of GABHS, rtPCR would demonstrate equal populations of GABHS on the tonsil surface and core. Consistent with the results of previous studies, however, rtPCR detected GABHS in the tonsil core at a higher rate than on the tonsil surface. This finding supports the hypothesis that a survival advantage is conferred in the tonsil core and that tonsillectomy patients may have populations of GABHS in the tonsil core that are not detected with swabbing of the tonsil surface.

In tonsillectomy patients, the rate of detection of GABHS is the same for rtPCR and SCx, but rtPCR has the added advantage of same-day results. For the detection of GABHS in tissue from the tonsil core and the adenoid, rtPCR is superior to SCx. Real-time PCR is an accurate same-day alternative to SCx for the clinical detection of GABHS.

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REFERENCES