The Corneal Ulcer One-Touch Study: A Simplified Microbiological Specimen Collection Method

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PURPOSE: To determine if a new, single-sample device (ESwab; Copan Diagnostics, Inc) can simplify the traditional multi-sample approach to specimen collection in infectious keratitis.

DESIGN: Prospective, diagnostic test evaluation.

METHODS: In this institutional study, patients with suspected infectious keratitis meeting traditional criteria for diagnostic corneal specimen collection and culture were randomized to the order of first specimen collection method: ESwab or a sample directly plated for growth on chocolate agar. This was followed by standard samples for blood agar, Gram stain, Sabouraud agar, thioglycolate broth, and brain heart infusion broth in all cases. The specimens collected using the 2 approaches were analyzed separately by the laboratory in a masked fashion. The main outcome measure was positive growth on cultured media.

RESULTS: Eighty-one eyes from 80 consecutive patients were sampled. Culture positivity rate for the multi-sample method and ESwab was 70% and 69%, respectively, with a 75% agreement rate. ESwab sensitivity was 84% (95% confidence interval [CI]: 72%–93%), with a specificity of 67% (95% CI: 45%–84%). Positive and negative predictive values of the ESwab were 86% (95% CI: 74%–94%) and 64% (95% CI: 43%–82%), respectively. There was no difference in positive culture reports with respect to the order of specimen collection technique used.

CONCLUSIONS: The single-sample ESwab method is a more accessible and less cumbersome approach to corneal culturing for ophthalmologists, particularly those in the community setting who do not have access to the full set of traditional culture materials. Culture results using this single-sample approach were comparable to the multi-sample method.

MICROBIAL KERATITIS IS A SERIOUS CAUSE OF visual loss. This underscores the need for early and intensive treatment to minimize associated visual morbidity. The incidence of suspected microbial keratitis based on a large population-based sample in Northern California was 27.6 per 100 000 person-years (95% confidence interval [CI]: 24.6–30.9). The standard of care for suspected microbial keratitis in tertiary institutions includes culture and susceptibility-guided antimicrobial treatment for moderate to severe ulcers and any empirically treated ulcers that fail to improve.

Culture of corneal scrapings and antimicrobial susceptibility testing of recovered isolates allows for pathogen identification, targeted antimicrobial therapy, and determination of possible emerging antibiotic resistance. In moderate to severe infectious keratitis, the empiric use of antimicrobials increases the risk of inappropriate treatment choice and intensity, decreases pathogen recovery, and fails to identify antibiotic resistance.

Multiple corneal samples for culture on various growth media is the standard of care in the evaluation of microbial keratitis. This culturing method increases the probability of recovering a responsible pathogen from corneal tissue, which has a relatively low microbial load compared to other body sites. It is not, however, cost effective for most ophthalmologists, particularly among non–corneal specialists who may prefer to use empiric therapy.

Obtaining and maintaining fresh, unexpired media, stored and transported properly, is a deterrent for many ophthalmologists with the multiple corneal sampling approach. The notion of taking 5–7 corneal samples, some of which often require sharp instruments, is less than ideal for the patient. Finally, multiple samples also increase the likelihood of contamination from inappropriate technique, open plates, and specimen handling by non–laboratory-trained personnel. As such, there is an interest in simplifying microbiological sampling for suspected infectious keratitis.

The ESwab (Copan Diagnostics, Inc, Murrieta, California, USA) was selected as the collection device for the “one-touch” method. This nylon-tipped swab uses spray-on flocked fiber technology, improving sample collection with increased capillary action and hydraulic liquid uptake. The flocked fiber arrangement also improves specimen release, with less entrapment than Dacron, rayon, and cotton tips. The swab is placed in 1 mL of modified Amies...
medium (included in the collection device), which maintains bacterial sample viability for 48 hours (24 hours for Neisseria sp), and preserves the sample for polymerase chain reaction (PCR) analysis of bacterial, chlamydial, and viral pathogens for up to 6 months. The inoculated Amies medium can be aliquoted in the laboratory into ten 100-μL samples for further culturing and analysis. Shelf life at room temperature is 18 months. The ESwab has been validated for microbiological use, and is already in clinical use outside the area of ophthalmology as a superior sample collection method for a variety of specimen types that were collected using swabs.20–23

The purpose of this study was to compare the utility of the ESwab with the traditional multi-sample approach in the diagnostic evaluation of infectious keratitis.

METHODS

ETHICS APPROVAL WAS OBTAINED FOR THIS PROSPECTIVE, randomized, single institution, open-label diagnostic test evaluation from the University of British Columbia’s Clinical Research Ethics Board in accordance with the Declaration of Helsinki.

All consecutive patients between March 2011 and August 2013 presenting with suspected microbial keratitis to our city’s only tertiary institution were considered for inclusion. These respective patients were directly referred from other ophthalmic practices or any of the city’s emergency departments. Inclusion criteria were any patient with suppurative infiltration of the corneal stroma with overlying epithelial breakdown that met 1 or more of the following: infiltrate greater than or equal to 1 × 1 mm in size, any infiltrate within the visual axis, failed initial treatment, or suspicion of an unusual organism. Patients under the age of 18, who were unable to provide consent, or in whom there was clinical suspicion of viral/protozoal/sterile keratitis were excluded.

Patients provided written informed consent to have specimens collected by both the ESwab and traditional sampling techniques. To detect any difference in sampling order, patients were randomized using a computerized number generator to assign them to the ESwab collection first, followed by a sample for growth on chocolate agar, or vice versa. The remainder of the collection order was the same between the 2 groups. This method avoided artificially high false-negative rates if the ESwab were to be randomized as the last specimen collected. The order for the traditional culture collection method was chocolate agar, ESwab, 5% sheep blood agar with Columbia agar base (SBA), Gram stain, Sabouraud agar, thioglycolate broth, and brain heart infusion broth. The order for the ESwab collection method was ESwab, chocolate agar, SBA, Gram stain, Sabouraud agar, thioglycolate broth, and brain heart infusion broth. Owing to its greater sensitivity to recover both fastidious and routine pathogens when compared to SBA, chocolate agar was used as the first medium to inoculate.

A complete medical history and full ocular examination were conducted, including best-corrected visual acuity (BCVA), slit-lamp biomicroscopy, and intraocular pressure measurement. BCVA was converted to the logarithm of the minimal angle of resolution (logMAR) for comparison and statistical analysis, including low vision values (counting fingers at 30 cm [CF]: logMAR 1.875; hand motions at 30 cm [HM]: logMAR 2.301; light perception [LP]: logMAR 2.700; no light perception [NLP]: logMAR 3.000).24 The dimensions, depth, and location of the ulcer; any associated thinning; and the presence of hypopyon were recorded. As most corneal ulcers are rarely perfectly circular, infiltrate area was calculated using the ellipse formula: \(a \times b \times \pi\), where \(a\) = height and \(b\) = width.

Sterile technique was used for all sampling, taking care to avoid any tissues except for the infiltrate. Specimen collection was undertaken by the cornea fellow and/or ophthalmology resident on the emergency service, after a training session on standardized sampling technique. After corneal anesthesia was obtained with sterile 0.5% tetracaine, samples were taken from the base and edges of each infiltrate and inoculated directly to agar plates, slides, broths, and the ESwab at the bedside. As is routine practice at our institution, number 15 scalpel blades were used for the agar plates, and sterile cotton swabs moistened with thioglycolate broth were used for the Gram stain and broths. Each sample was taken with a new sterile instrument. The sterile swab provided with the ESwab kit was used to sample the cornea in sterile fashion and was then broken off into the tube with the provided modified Amies medium.

All sample media were analyzed in the diagnostic microbiology laboratory at Vancouver General Hospital. The directly inoculated agar plates, broth, and Gram stain were processed, analyzed, and reported separately from the ESwab cultures in a masked fashion. All plated and liquid media for bacteria were incubated at 35 C in 3%–5% CO₂ for 5 days, read daily, and investigated using standard microbiology procedures. Sabouraud agar to recover fungal pathogens was incubated at room temperature and read at 5 days and 4 weeks. All media were from Oxoid, Hampshire, England. The ESwab sample was received by the microbiology laboratory and gently agitated by hand before inoculating the plates and broths as above. A total of 1 mL of the inoculated liquid media was used for inoculation and was divided among all media, including a slide for Gram stain.

A positive culture was defined as any growth on plates or broths, unless contamination was suspected by the microbiology laboratory. Contamination was suspected if a single colony or scant growth appeared on the plate, but off the area of specimen inoculation or streaking. The definition of positive culture was chosen because of the difficulty in the management of clinical bacterial keratitis to differentiate...
TABLE 1. Baseline Characteristics of Participants Randomized to Receive Either the Traditional or ESwab Collection Device First for Corneal Sampling

<table>
<thead>
<tr>
<th></th>
<th>Culture Plates(^a)</th>
<th>ESwab(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y, mean [SD])</td>
<td>59 [21]</td>
<td>58 [21]</td>
</tr>
<tr>
<td>Sex (male)[%]</td>
<td>22 [54]</td>
<td>16 [40]</td>
</tr>
<tr>
<td>Eye involved (OD)[%]</td>
<td>22 [54]</td>
<td>23 [58]</td>
</tr>
<tr>
<td>BCVA (logMAR, mean [SD])</td>
<td>1.44 [0.91]</td>
<td>1.80 [0.93]</td>
</tr>
<tr>
<td>Ulcer location</td>
<td>19 [46]</td>
<td>21 [53]</td>
</tr>
<tr>
<td>Ulcer depth(^2), mean [SD]</td>
<td>18 [22]</td>
<td>16 [26]</td>
</tr>
<tr>
<td>Presence of hypopyon [%]</td>
<td>12 [29]</td>
<td>17 [43]</td>
</tr>
<tr>
<td>Presampling antibiotic use [%]</td>
<td>18 [44]</td>
<td>16 [40]</td>
</tr>
<tr>
<td>Contact lens wear [%]</td>
<td>11 [27]</td>
<td>12 [30]</td>
</tr>
<tr>
<td>Previous corneal transplant [%]</td>
<td>12 [29]</td>
<td>15 [38]</td>
</tr>
</tbody>
</table>

BCVA = best-corrected visual acuity.
\(^a\)Collection method performed first.
\(^b\)Reported as percent thinning.

Pathogenic from commensal organisms.\(^{25–30}\) Any positive growth on the directly inoculated multiple media in the traditional method was compared to positive growth on the indirectly inoculated media using the ESwab as the collection method.

Cohen kappa statistics were used to examine the level of agreement between the ESwab and the traditional culturing method with respect to the presence or absence of organisms; \(t\) tests were used to examine if the order of collection method was associated with lower specimen recovery. All analyses were performed using SAS software version 9.2 (SAS Institute, Inc, Cary, North Carolina, USA), using 2-sided tests of significance at the \(P < .05\) level.

**RESULTS**

A TOTAL OF 81 EYES FROM 80 PATIENTS WERE SAMPL ED, 41 with traditional culture plates first and 40 with ESwab first. Baseline characteristics between the 2 groups are shown in Table 1.

Age of participants ranged from 18 to 96 (mean: 59 years; SD: 21 years). BCVA ranged from 20/20 to NLP, with 47 eyes (58%) having vision of CF or worse (logMAR mean: 1.62; SD: 0.929). Ulcer area ranged from 0.8 to 78.5 mm\(^2\), with 75 of 81 ulcers (93%) measuring less than 20 mm\(^2\) (mean: 9.1; median: 5.7; SD: 13.1). Previous corneal transplant was present in 27 eyes (33%), 23 ulcers (28%) were associated with contact lens use, 10 patients (13%) had diabetes mellitus, 4 (5%) had human immunodeficiency virus (HIV), and 4 (5%) had previous refractive surgery (laser in situ keratomileusis or photorefractive keratectomy).

Empiric antibiotics had been used in 34 of 81 ulcers (42%). The antibiotics included: fluoroquinolones in 21 cases, an aminoglycoside in 5, a macrolide in 1, bacitracin/polymyxin B in 1, and a combination of different antibiotic categories in 6. The culture-positive rate for those with empiric antibiotic use was 77% (26 of 34 cases). The empiric antibiotics were changed or strengthened at the time of specimen collection in 23 of 34 cases (68%). The choice of empiric antibiotics was inappropriate, based on mechanism of action for the organism subsequently isolated in culture by the traditional method, in 5 cases (15%). These included aminoglycosides for gram-positive bacteria in 3 cases, a fluoroquinolone for a resistant strain of \(S\) pneumoniae, and an antibacterial agent for fungal keratitis.

Of 81 corneal ulcers, an organism was identified by microbiological culture using either method in 65 cases (80%) (Table 2). Of the 65 positive cultures, 35 cases (54%) yielded a single bacterial organism, 28 (43%) yielded 2 or more bacterial organisms, 1 case (1.5%) grew a single fungal organism (\(Pneumocystis\) jirovecii), and 1 case (1.5%) grew mixed fungal/bacterial organisms (\(Candida\) sp/\(S\) epidermidis/other coagulase-negative \(Staphylococcus\)). Four isolates from the ESwab and 6 isolates from the traditional-method cultures were interpreted as “likely contaminants” by the laboratory based on growth pattern, and were not interpreted as a positive culture. Eleven of the 36 single organism cases (31%) yielded gram-negative bacteria, while 24 (67%) yielded gram-positive organisms, and 1 (2.8%) grew a fungus. Of the 29 multiorganism cases, all included at least one gram-positive organism, 11 included a gram-negative organism, and 1 included a fungus (Table 2). Of these 29 cases, at least 1 pathogenic organism not considered normal flora contiguous with the ocular surface (eg, \(Pneumocystis, S\) viridans) was isolated in 19 of 29 cases (66%), while 10 of 29 cases (34%) yielded possible flora contiguous with the ocular surface only (coagulase-negative \(Staphylococcus, Corynebacterium\) sp, \(P\) acnes). As referenced above, however, it is difficult to distinguish the pathogenicity of these organisms when recovered from clinically infected tissue, especially in light of clinical reports in the literature.\(^{3,12,17,25,28–31}\)

The Gram stain was positive in 13% (7/56) of positive cultures recovered by the ESwab and in 27% (14/52) of positive cultures recovered by the traditional method where the Gram stain was performed, in each case reflecting the ultimate organism grown in culture. Gram staining was performed in 100% of the ESwab samples and 89% (72/81) of traditional samples, not being performed in 9 cases owing to inadequate sampling or poor quality upon receipt at the laboratory. Five of the 9 traditional samples without Gram stain had positive cultures.

The traditional method yielded a culture-positive rate of 70% (57 of 81 cases). The ESwab culture-positive rate was 69% (56 of 81 cases). When commensal organisms were
ignored, the 2 methods agreed on the identity of the causative organism in 61 cases (75%). When a positive test was defined as growth of any organism, the 2 methods agreed in 64 cases (79%). These included agreement in cases with no growth by either method. Discordant culture results between the 2 approaches to specimen collection occurred in 17 cases (21%). When specifically examining treatment-naïve cases, the recovery rates for the ESwab and traditional method were 74% (35/47 cases) and 70% (33/47 cases), respectively, with concordant results in 79% (37/47 cases). The Cohen kappa coefficient for total results demonstrated good agreement between the 2 methods at 0.65 (95% CI: 0.56–0.74) (Table 2).

The traditional method had a higher proportion of culture results with 2 or more organisms from a single patient than did the ESwab (42% vs 23%, respectively). All such multi-organism cases included an organism known to be contiguous with the ocular surface, specifically *S. epidermidis*, other coagulase-negative staphylococci, *Corynebacterium* sp, and *P. acnes*. When these organisms were removed from the analysis, recovery rates between the ESwab and traditional method were 74% (35/47 cases) and 70% (33/47 cases), respectively, with concordant results in 79% (37/47 cases). The Cohen kappa coefficient for total results demonstrated good agreement between the 2 methods at 0.65 (95% CI: 0.56–0.74) (Table 2).

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To examine the sensitivity of the ESwab compared to the traditional method, we used a 2 × 2 table and associated assay validation statistics (Table 3). From this table, the sensitivity of the ESwab to determine a bacterial or fungal cause for infectious keratitis was 84% (95% CI: 72%–93%), while the specificity was 67% (95% CI: 45%–84%). The positive predictive value (PPV) was 86% (95% CI: 74%–94%) and the negative predictive value (NPV) was 64% (95% CI: 43%–82%), with a calculated disease prevalence of 70%.

### TABLE 2. Organism Growth From Suspected Cases of Microbial Keratitis Recovered by Both the ESwab and Traditional Culture Methods, and by Each Method Alone, Irrespective of Sampling Order

<table>
<thead>
<tr>
<th>Organism</th>
<th>Recovery by Both Methods</th>
<th>ESwab Only</th>
<th>Traditional Method Only</th>
<th>Negative by Both Methods</th>
<th>Cohen Kappa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>11</td>
<td>5</td>
<td>12</td>
<td>53</td>
<td>0.43 (0.21–0.65)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>73</td>
<td>0.64 (0.31–0.97)</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>68</td>
<td>0.31 (–0.01–0.63)</td>
</tr>
<tr>
<td><em>Streptococcus viridans grp</em></td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>75</td>
<td>0.79 (0.50–1.00)</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>79</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td><em>Streptococcus group G</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>80</td>
<td>0.00 (0.00–0.00)</td>
</tr>
<tr>
<td><em>Corynebacterium sp</em></td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>70</td>
<td>0.67 (0.40–0.94)</td>
</tr>
<tr>
<td><em>Propionibacterium acnes</em></td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>75</td>
<td>0.65 (0.28–1.00)</td>
</tr>
<tr>
<td><em>Bacillus sp</em></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>78</td>
<td>–0.02 (–0.04–0.01)</td>
</tr>
<tr>
<td><em>Rothia mucilaginosa</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td><em>Micrococcus sp</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>80</td>
<td>0.00 (0.00–0.00)</td>
</tr>
<tr>
<td><em>Dolosigranulum pigrum</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td><em>Eubacterium aerofaciens</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>80</td>
<td>0.00 (0.00–0.00)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10</td>
<td>1</td>
<td>0</td>
<td>70</td>
<td>0.95 (0.84–1.00)</td>
</tr>
<tr>
<td><em>Moraxella sp</em></td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>76</td>
<td>0.88 (0.66–1.00)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>77</td>
<td>0.85 (0.56–1.00)</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>80</td>
<td>0.00 (0.00–0.00)</td>
</tr>
<tr>
<td><em>Pantoea agglomerans</em></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>80</td>
<td>0.00 (0.00–0.00)</td>
</tr>
<tr>
<td><em>Paecilomyces</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>80</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td><em>Candida</em> sp*</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>80</td>
<td>0.00 (0.00–0.00)</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>20</td>
<td>33</td>
<td>1514</td>
<td>0.65 (0.56–0.74)</td>
</tr>
</tbody>
</table>

*aGram-negative isolates.

### TABLE 3. Comparison Between Traditional Culture Method and ESwab for Pathogen Isolation in Cases of Clinical Microbial Keratitis

<table>
<thead>
<tr>
<th></th>
<th>Traditional Culture Method (Multiple Samples)</th>
<th>ESwab</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>48</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>9</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

*aResults were counted in the Positive category where a microbial pathogen was isolated by the sampling technique in question. Results were considered Negative when there was no growth.*
We examined if collection method order (ESwab first or second) was associated with lower specimen recovery. When the ESwab was used first, recovery rates were 68% (27/40) for the ESwab cultures and 65% (26/40) for traditional cultures. When the chocolate agar sample was taken first, the traditional culture recovery was 76% (31/41), while the ESwab culture recovery was 71% (29/41). There was no evidence of an association (P = .3) between swabbing order and the number of different species the traditional plating technique recovered. We cannot exclude, however, the possibility that a larger sample size would yield a statistically significant association with the traditional plating technique. In addition, as reported by the laboratory using semi-quantitative methods, quantification of organisms present was not different for the 2 methods (data not shown).

False negatives with sampling order were analyzed to examine if yield was artificially lower for the second swab. Of the 41 cases where the ESwab was performed second after the chocolate sample, 12 cases were ESwab negative. Of these 12, 4 were false negatives (growth occurred by the traditional method) (30%). Of the 40 cases where the ESwab was performed first, 14 cases were negative by the traditional method. Of these 14, 5 were “false negatives” (growth occurred by the ESwab) (36%).

DISCUSSION

CURRENT STANDARD OF CARE IN THE EVALUATION OF suspected infectious keratitis includes 5–6 specimens collected from each patient using a combination of different possible collection materials. The high proportion of patients with corneal ulcers who receive empiric treatment may be in part attributable to the low cost-effectiveness and inconvenience of this approach, particularly for non–tertiary care ophthalmologists, who rarely stock multiple fresh culture media in the office. Agar plates require refrigeration and have a shelf life of only 60 days, while broths can be stored for 1 year at room temperature. Multiple attempts at corneal sampling may also increase the possibility of accidental specimen contamination, both at the time of collection and at inoculation on open media.

Empirc therapy is appropriate in certain mild cases of suspected infectious keratitis. However, the institution of empiric therapy in moderate to severe keratitis increases the risk of compromising subsequent pathogen recovery on corneal scrapings, delay in pathogen identification, failure to identify possible antibiotic resistance, and, occasionally, mis-treatment of the etiologic pathogen altogether, such as in fungal keratitis. Simplication of corneal sampling may improve the uptake of specimen collection for culture by non–tertiary care clinicians, thereby addressing the above concerns.

As described above, the ESwab was chosen given its improved microbial uptake and release technology, its 18-month shelf life at room temperature, its successful use for specimen collection in other areas of medicine, and the successful use of indirect inoculation with liquid Amies medium in corneal ulcer sampling. This is the first study of this new collection system for the evaluation of infectious keratitis. Despite being a single sample, the ESwab demonstrated good sensitivity (84%) and PPV (86%), with moderate specificity (67%) and NPV (64%). Therefore, a clinician without access to traditional corneal sampling methods will be relatively confident that his or her single sample will grow the causative organism if that organism would have grown by the traditional method, while needing to maintain a high suspicion if the result is negative. It is also important to consider the possibility of a lower calculated specificity value for the ESwab being an artifact of its comparison with a method that may be less sensitive.

To determine a cause for microbial keratitis, previous studies have shown that specimen collection using a swab placed in liquid media with just 1 sampling has comparable sensitivity for organism recovery when compared to the traditional method of direct inoculation of culture media requiring multiple sampling. The traditional method had a higher number of culture results in this study with 2 or more organisms from a single patient than did the ESwab (42% vs 23%, respectively), but this was largely owing to the lower recovery using the ESwab of S epidermidis and other coagulase-negative staphylococci, which were recovered in every case where multiple organisms were identified. Given that these organisms are common commensal flora on the skin near the eyes and on the hands, this difference is possibly a reflection of increased contamination using the traditional method, which requires multiple manual steps, rather than decreased sensitivity of the ESwab, since the ESwab did demonstrate recovery of these organisms. When organisms known to be contiguous with the ocular surface are removed from analysis (S epidermidis, coagulase-negative staphylococci, Corynebacterium sp, and P acne), bacterial recovery rates are comparable (ESwab: 79%; traditional method: 83%). Comparable recovery rates between the 2 methods were also shown for gram-negative organisms (ESwab: 90%; traditional method: 86%).

Where the ESwab has the greatest potential for clinical relevance is in the community setting, where access to microbiological sampling materials is limited and investigations are often forgone. The ESwab is not a replacement for the traditional multi-sample method of corneal culturing, which is the standard of care in the tertiary setting. In this setting, the ESwab demonstrated similar culture positivity rates, an acceptable agreement rate, and a similar profile of missed organisms when compared to the standard method.

This study has some limitations. With respect to study design, no prior use of the ESwab in ophthalmic
applications existed to determine an appropriate sample size, and as such our sample size was kept consistent with previous similar prospective diagnostic test evaluations in infectious keratitis that used different sampling methods.\textsuperscript{3,14,17,38} Second, this study was conducted in a large tertiary center, differing from the community setting where the reliance upon empiric treatment is high and where the ESwab may be most beneficial. However, the inclusion criteria incorporated treatment-naïve keratitis (47 ulcers, 58%), which presumably emulates those cases treated by community ophthalmologists. In this setting, the ESwab recovery rate was comparable to the traditional method (74% and 70%, respectively). In addition, at the outset of the study there were no data to understand if sampling order was associated with lower specimen recovery for either the traditional plating technique or the ESwab. Despite the first corneal sample presumably having the highest yield owing to the greatest pathogen load, a single-sample approach loses the opportunity of multiple attempts at detecting the pathogen in an often pathogen-poor tissue. The possibility existed of increased false negatives of the second swab, if perhaps the first sampling removed the majority of the pathogen. Although limited by low sample size, our results did not suggest this, given the similar values for these “false negatives” between the 2 randomized groups (Results). Furthermore, although Gram stains can be and were successfully performed in this study from ESwab samples, they are ideally made from a direct corneal sample and not from a diluted medium,\textsuperscript{14} as reflected by the lower Gram stain sensitivity of the ESwab compared with the traditional method in this study. Finally, with only 2 cases of fungal keratitis, reflecting the predominance of bacterial keratitis and paucity of fungal keratitis in our region, we cannot generalize our results to keratitis caused by fungi. We are currently collaborating with a partner site with a higher prevalence of fungal keratitis to test the clinical effectiveness of ESwab in this context, but at this point we recommend traditional fungal studies if clinical suspicion exists. Further study with the ESwab will also be required for Acanthamoeba and biofilm-associated keratitis.

The single-sample ESwab method offers the opportunity for a cost-effective and less cumbersome approach to corneal culturing for ophthalmologists, particularly those in the community setting, who do not have access to the full set of traditional culture materials. With comparable results to the multi-sample approach and, potentially, a lower risk of contamination, it offers both the treating and consulting ophthalmologist an initial pretreated impression of the etiology of microbial keratitis.

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REFERENCES


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