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Simplifying Collection of Corneal Specimens in Cases of Suspected Bacterial Keratitis

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Identification of the causative organisms in suspected bacterial keratitis traditionally involves collecting multiple corneal scrapes, which are plated directly onto different solid agar culture media. Difficulties have been reported with this practice, so the development of a simpler diagnostic method in suspected bacterial keratitis would be useful. It is unclear whether a single corneal scrape sent to the microbiology laboratory in a liquid transport culture medium (indirect method) is as reliable for the diagnosis of bacterial keratitis as inoculation of multiple scrapes directly onto agar plates (direct method). To investigate this, bacterial recovery was assessed following transfer and transport of different concentrations and types of bacteria from an artificially contaminated surgical blade into brain heart infusion (BHI). Bacterial recovery rates between the proposed (indirect) and standard (direct) method were then compared after the in vitro inoculation of pig corneas and following specimen collection in patients with presumed bacterial ulcerative keratitis. Recovery of bacteria from contaminated surgical blades was found to be the same from both solid and liquid culture media. There was no significant difference in the numbers of positive cultures from solid (direct) and liquid (indirect) culture media, both in the experimental pig cornea inoculation study ($P = 0.34$) and in experiments with patients with clinical infections ($P = 0.4$), with an 85.2% agreement between methods (kappa = 0.61, $P < 0.0001$). In conclusion, therefore, the collection of two corneal scrapes, one used for Gram staining and the other transported in BHI followed by plating and subculturing in an enrichment medium, provides a simple method for the investigation of presumed bacterial keratitis.

Microbial keratitis is a sight-threatening condition with significant morbidity (1, 8, 16, 20, 23, 25). Successful treatment of this condition may depend upon accurate and rapid identification of the causative organism (1, 3, 14, 20).

The need to detect bacterial, fungal, and amoebic pathogens, coupled to the fact that there may be only a few organisms in a corneal ulcer, means that adequate clinical material must be obtained and cultures must be grown on a variety of different media (1, 3, 5, 14, 15, 20, 26). This has led to the traditional practice of taking multiple scrapes from the ulcer and directly plating the material onto several culture media (1, 3, 5, 14, 15, 18, 20, 26). Implicit in this approach are the assumptions that multiple scraping decreases sampling error which might otherwise result in false-negative cultures and that sending a tiny amount of clinical material to the laboratory in a conventional transport medium might, through dilution, lead to sampling error in the laboratory.

This approach, however, is unique in clinical microbiological practice, and there are questionable, although as yet unproved, drawbacks which might compromise accurate diagnosis. Collecting multiple scrapes, particularly from the eye of an uncooperative patient, is not always easy, and growing a minute sample in culture on an agar plate is technically difficult, even for experienced operators (especially when a sharp instrument is employed, in which case the inoculum might be deposited beneath the surface of the agar). A full range of fresh culture media is not always instantly to hand (31), and the traditional method of using multiple plates and samples is not always followed, even in a university hospital setting (31). There is also an increased risk of extraneous contamination of culture plates when these are handled outside of a controlled laboratory environment. We decided, therefore, to test the hypothesis that a single corneal scrape (placed directly into a liquid transport culture medium for subsequent detailed analysis in the microbiology laboratory) yields diagnostic information as reliable as that from the conventional multiple-scrape technique. If this were the case, the more user-friendly approach of a placing a single scrape into a single (long shelf-life) transport medium would simplify matters for all concerned: patients as well as ophthalmologists and microbiologists.

MATERIALS AND METHODS

Bacterial counts in liquid media were measured using a spiral plater (11) (Don Whitley Scientific, Shipley, West Yorkshire, England). Calculations of 95% confidence intervals for expected recoveries of viable organisms were performed in accordance with the guidelines of Meynell and Meynell for growth on solid media (21) and of the American Society for Microbiology for liquid enrichment cultures (10).

Agar culture plates. Blood agar and chocolate agar plates contained 7.5 and 10% horse blood (TCS Biosciences, Buckingham, United Kingdom) in a Columbia agar base (Oxoid, Basingstoke, United Kingdom), respectively. Sabouraud's dextrose agar (Bioconnections, Leeds, United Kingdom) contained 100 μg of chloramphenicol/ml.

Brain heart infusion [BHI (Oxoid)] medium was chosen as the liquid transport culture medium in all our studies for the following reasons. It is a standardized and widely available commercial product. It is a proven enrichment culture medium that supports the growth of yeasts and fungi as well as of fastidious

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bacteria (4, 9, 10, 17, 24, 27). The protocol used in all the studies to be described was first to place into a transport bottle containing 0.5 ml of BHI a surgical blade (no. 11 Baird Parker surgical blade; Swann Morton Ltd., Sheffield, United Kingdom) that had either been experimentally contaminated or used to gather corneal material. The rationale was that there would be no loss of viable pathogens during transport to the laboratory either from desiccation or due to inimical physicochemical factors that are encountered with water and even buffered saline. Second, the sample arrived in the laboratory in a medium and container that allowed for direct homogenization without risk of extraneous contamination. Third, aliquots of the homogenate (obtained by vortexing the sealed container with the blade still inside to create a blender effect) were used for Gram staining and growth of subcultures on appropriate solid media, while the remaining homogenate in BHI served for growth of enrichment cultures without further manipulation.

(i) Bacterial recovery from surgical blades contaminated with known inocula of bacteria. Four recognized ocular bacterial pathogens were studied: *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae* (a fastidious bacterium). All were clinical isolates (from nonocular specimens) to reflect potential pathogenicity.

BHI was initially assessed by inoculating 6×10^2 CFU of *S. pneumoniae* into water, saline and BHI, which were then subcultured onto blood agar at intervals of half an hour for 3 h (to represent clinically realistic transport times from clinic to laboratory). Using standard aseptic laboratory technique on the open bench to then compare the microbial recovery rates of BHI and direct-plate inoculation, 2 μl of a bacterial suspension of known density was inoculated onto the tips of surgical blades which were then either plated directly (direct inoculation) onto chocolate blood agar or immersed in 0.5 ml of BHI for 60 min at room temperature. A total of 40 μl was then subcultured (indirect inoculation) onto chocolate blood agar, and the remaining 460 μl was added to 10 ml of BHI with 10% added horse blood (Tissue Culture Services) for enrichment culture (enrichment) and incubated overnight (37°C, 5% carbon dioxide, 80% relative humidity) before being grown in subcultures on chocolate blood agar.

In a second experiment, two suspensions of each bacterium were prepared by diluting overnight (stationary phase) broth cultures, one intended to contain a low density of viable bacteria (as might be encountered in vivo in clinical infections) and the other intended to contain a 100-fold-higher concentration. The concentrations (in CFU per milliliter) actually achieved were measured by spiral plating and were suspensions of *S. pneumoniae* at 520 CFU/ml and 19×10^4 CFU/ml, *P. aeruginosa* at 13,000 CFU/ml and 9.3×10^4 CFU/ml, *S. aureus* at 3,200 CFU/ml and 6×10^4 CFU/ml, and *H. influenzae* at 1,600 CFU/ml and 2.6×10^4 CFU/ml. Each experiment was performed in triplicate.

(ii) Bacterial recovery from inoculated corneas of enucleated pig eyes. A total of 12 enucleated pig eyes were placed in individual sterile moist chambers (gauze soaked in 0.9% N saline) at 4°C overnight. Each eye was rinsed through a series of baths (per the United Kingdom Transplant Service eye-banking technique) consisting of immersion and agitation in 0.3% sodium thiosulfate (BDH Laboratory Supplies, Poole, United Kingdom) for 15 s followed by two rinses in 0.9% N saline for 10 s. Using a 25-gauge sterile needle (Terumo, Leuven, Belgium) in cases in which the eye was hypotonous, 0.5 to 1 ml of sterile 0.9% N saline was injected into the vitreous via the optic nerve stump to render the eye firm but not tense. The eye was held in a wet laboratory mounting block and then dunked onto a blood agar plate for growth of a negative-control culture.

Inoculation, scraping, and culture growth. Using a 27-gauge needle (Sherwood Medical, Belfast, United Kingdom), each pig eye was inoculated intrastromally with 20 μl of an *S. aureus* suspension (2.6×10^4 CFU/ml, giving an absolute inoculum of 520 CFU) to raise a 3-mm-diameter bleb in the central anterior corneal stroma. Using a new sterile no. 11 Baird Parker surgical blade for each scrape, five corneal scrapes were collected from each of 12 eyes. The bleb was scraped over its base and edges to collect visible fluid and/or material. The scrape technique was identical for each eye, but either direct or indirect inoculation was used alternately. One microbiologist (S.H.) performed all of the plating procedures. Each blade was either inoculated directly (direct method) by wiping both sides of the blade onto the surface of the blood agar plate or transferred into 0.5 ml of BHI, and after 1 h, 40 μl of the collected material was grown in a culture on a blood agar plate (indirect method). The remainder of the broth was transferred into 10 ml of BHI (with horse blood; indirect method with enrichment culture), incubated overnight (37°C, 5% CO_2, 80% relative humidity), and grown in a subculture on blood and chocolate agar. Each eye was then placed onto a second blood agar plate (positive-control culture).

(iii) In vivo bacterial recovery from corneal scrapes in clinical practice. Patients presenting to the external eye disease service with suspected microbial keratitis were recruited to the study. Local ethics committee approval was obtained, and informed consent was obtained in accordance with the declaration of Helsinki. Suspected infectious ulcerative keratitis was considered when there was an epithelial defect with infiltration of the underlying stroma (1, 8, 14). Ulcer details (location, major and minor axial diameters, and percentage of depth) were recorded. Risk factors such as contact lens wear (6), previous infection or scarring, surgery, exposure to waterborne organisms, and the presence of ocular surface disease (1, 6) were also documented. Corneal specimens for microbiological diagnosis were collected by two of the authors (P.G.R. and S.B.K.) as detailed below. Sterile gloves and an aseptic technique for handling of specimens were used in all cases. Care was taken to avoid touching the lids and conjunctiva. Patients were sequentially and alternatively allocated to either group A or B (see below) to determine any effect from the order in which the specimens were collected. Each patient had conjunctival swabs taken from both eyes for both viral and bacterial cultures.

Following a single application of topical benoxinate (0.4%) to the cornea, six corneal scrapes were obtained from the affected area with separate sterile blades handled with sterile gloves. For patients in group A, the blade used for the first scrape was dropped into 0.5 ml of BHI and a new blade used for the second scrape was inoculated on the surface of a chocolate agar plate. For patients in group B, the first scrape was placed onto chocolate agar and the second was dropped into 0.5 ml of BHI. For the patients in both groups, the third, fourth, fifth, and sixth scrapes were placed onto a slide (for Gram staining), Sabouraud's medium (for fungal culture), blood agar (as a second check for bacterial growth), and *Acanthamoeba* medium (nonnutrient agar with a lawn of dead *Escherichia coli*), respectively. In the laboratory (typically ~1 h after the scrapes had been taken), the BHI was vortexed, a smear was prepared from the homogenate for Gram staining, and subcultures were grown on each of the four agar media listed above. The remainder of the BHI was incubated (37°C, 5% CO_2, 80% humidity) overnight in enrichment medium before further subculture growth. Treatment of all patients with topical teicoplanin (1%) and ciprofloxacin (3%) was commenced.

RESULTS

In vitro. (i) Bacterial recovery from surgical blades contaminated with known inocula of bacteria. BHI was able to support the growth of a fastidious organism such as *S. pneumoniae*. That is, after 3 h, there was no loss of *S. pneumoniae* in BHI (6.2×10^2 CFU), while there was a slight reduction following inoculation in saline (1.6×10^2 CFU) and an almost complete loss in water (<20 CFU).

The results regarding expected and observed recoveries for direct, indirect, and enrichment cultures are shown in Table 1. The results are consistent with no loss or gain of bacteria from the procedure. In particular, the enrichment cultures gave positive results in all cases where this was expected. For example, when a small inoculum of *S. aureus* (6 CFU) was used, the observed counts using direct inoculation of 7, 12, and 19 CFU were all within the 95% confidence interval (CI) (2 to 20 CFU). Similarly, the 95% CI after dilution in BHI was 0 to 2 CFU, with observed counts of 0, 1, and 1 CFU. The only discrepancy was the observation of slightly higher than expected recovery following indirect inoculation of a large dose of *P. aeruginosa*.

(ii) Bacterial recovery from inoculated corneas of enucleated pig eyes. No organisms were grown from the eyes before inoculation. There was on average 1 to 3 μl of fluid (20 to 60 CFU expected) collected from each scrape, so approximately 100 to 300 CFU in total would have been expected. For direct inoculation, the average total colony count after five scrapes was ~120, which represents a recovery of between 50 and 100%. Similarly, the expected yield from indirect inoculation in BHI (10 to 30 CFU per eye) was similar to the actual total count per eye (20 CFU) after five scrapes. The means of viable counts per eye for indirect and direct inoculation were 20 and 144 CFU, respectively, representing a ratio of 0.13 between

TABLE 1. Expected and observed recovery of bacterial inocula from experimentally contaminated blades[a]

Inoculum level and organism	No. of recovered organisms (CFU):				
	Calculated[c]	After direct inoculation on solid medium		After indirect inoculation (BHI)[b]	
		Expected[d] (95% CI)	Observed[e]	Expected[d] (95% CI)	Observed[e]
Low					
S. pneumoniae	1	0–5	0, 0, 1	0	0, 0, 0
H. influenzae	3	0–10	0, 0, 1	0–1	0, 1, 1
S. aureus	6	2–20	7, 12, 19	0–2	0, 0, 0
P. aeruginosa	26	5–50	6, 24, 29	0–6	0, 0, 1
High					
S. pneumoniae	380	>250[f]	>250[f]	20–40	34, 45, 50–100
H. influenzae	52	20–100	50–100[f]	1–8	4, 5, 5
S. aureus	120	50–200	18, 25, 50–100	4–17	5, 5, 20
P. aeruginosa	186	100–250	50–100	8–20	44, 50–100[g]

[a] Results represent recoveries in triplicate experiments.
[b] Indirect inoculation, dilution in BHI followed by plating on solid medium.
[c] Calculated results differed according to the inoculum used.
[d] Expected results represent 95% CI for 100% CFU recovery.
[e] Observed results correspond to actual colony counts.
[f] The same numbers of CFU were expected and/or observed in triplicate experiments.
[g] The same numbers of CFU were observed in duplicate experiments.

indirect and direct inoculation, which was similar to the expected ratio of 2/25 or 0.08. There was no significant difference between indirect (BHI) and direct inoculation when the dilution effect of the BHI was considered ($P = 0.48$) (Table 2). There were also no significant differences between scrapes for either indirect (BHI) or direct inoculation ($P = 0.34$ or 0.85, respectively). The viable counts did not increase or decrease between the first and fifth scrape ($P = 0.47$). In all cases (30) of enrichment culture, there was recovery of the inoculated bacteria from each of the five scrapes from each of six pig eyes. Comparing the variance between scrapes and between eyes for direct and indirect inoculation, there was a lower between-eye and between-scrape variance for indirect than direct inoculation (F test; $P < 0.001$).

TABLE 2. Pig eye model[a]

Method and eye	Result for:				
	Scrape 1	Scrape 2	Scrape 3	Scrape 4	Scrape 5
Direct[b]					
1	$+^{4}$	$+^{26}$	$+^{9}$	$+^{12}$	$+^{>50}$
3	$+^{3}$	$+^{16}$	$+^{6}$	$+^{9}$	$+^{13}$
5	$+^{18}$	$+^{>50}$	$+^{48}$	$+^{>50}$	$+^{25}$
7	$+^{27}$	$+^{11}$	$+^{14}$	$+^{12}$	$+^{4}$
9	$+^{3}$	$+^{11}$	$+^{5}$	$+^{17}$	$+^{15}$
11	$+^{30}$	$+^{34}$	$+^{22}$	$+^{>50}$	$+^{>50}$
Indirect[c]					
2	−	−	−	−	−
4	−	$+^{1}$	−	$+^{1}$	$+^{3}$
6	$+^{1}$	$+^{5}$	$+^{9}$	$+^{4}$	$+^{7}$
8	$+^{2}$	$+^{3}$	$+^{1}$	$+^{1}$	$+^{2}$
10	$+^{11}$	$+^{2}$	$+^{6}$	$+^{6}$	$+^{5}$
12	$+^{4}$	$+^{10}$	$+^{4}$	$+^{4}$	$+^{8}$

[a] Plus signs indicate a positive culture result; minus signs indicate the absence of an isolate. Numbers in superscript represent colony counts.
[b] Direct plate inoculation data represent results following inoculation with *S. aureus* (5.2×10^2 CFU).
[c] Indirect method data represent culture results following inoculation with *S. aureus* (5.2×10^2 CFU) and transport in BHI broth.

In vivo. (i) Clinical evaluation. A total of 68 patients were recruited. Seven patients were excluded because of missing or incomplete specimens received by the laboratory. The results for 61 patients were available for analysis. There were 37 males and 24 females, with an age range of 14 years to 94 years (mean, 56.5 years). Of these patients, 37 (61%) were on topical antimicrobials at presentation, 25 were receiving chloromycetin, 5 were receiving gentamicin, 5 were receiving fusidic acid, and 2 were receiving cefuroxime. Four patients were also on topical steroid eye drops. In addition, 52% had evidence of blepharitis, 31% had previous corneal disease, 26% had previous ocular surgery, 24% were contact lens wearers, 7% were on topical steroids, and 5% had history of trauma. Of all the patients, 93% had at least one of these risk factors, 24% had two risk factors, and 10% had three or more risk factors. The risk factor-to-patient ratio for those patients who had an isolate on corneal culture was similar to the ratio for those who had a negative culture result (1.3 and 1.37, respectively). There was no significant difference in ulcer size (product of major and minor axial lengths of ulcer) either between groups A and B ($P = 0.54$) or between patients who had positive or negative results with isolates from the corneal scrapes ($P = 0.32$).

(ii) Isolation rates. An organism was isolated from 33% (20/61) of patients. The overall isolation rate in those patients receiving treatment within 14 days or up to presentation was 27% (10/37), compared to 42% (10/24) for those not on treatment at presentation ($P = 0.23$). In essence, 66% (27) of patients with negative cultures were on antimicrobial treatment compared to 34% (14) of patients who were not receiving treatment. For 11 out of 20 culture-positive patients, the same organism was isolated by both methods (direct and indirect inoculation) (Table 3). For five patients, an organism was isolated following indirect but not direct inoculation, and for

TABLE 3. Clinical results: isolation of organisms by direct and indirect methods

Organism isolated by:[a]		
Both methods	Indirect method only	Direct method only
G. morbillorum	*Neisseria* sp.[b]	CNS, AHS
S. pneumoniae	LFC[b]	*S. aureus*
Pseudomonas sp.	CNS[b]	*Paecilomyces* sp.
S. aureus	CNS	
C. xerosis	Yeast	
S. pneumoniae		
Bacillus sp.		

[a] CNS, coagulase-negative staphylococcus; AHS, α-hemolytic streptococcus; LFC, lactose-fermenting coliforms.
[b] Organisms grown after enrichment culture.

another four patients, an organism was isolated from direct but not indirect inoculation. There were, therefore, 15 isolates from direct inoculation and 16 isolates from indirect inoculation, 11 of which were common to both methods. Out of the 16 isolates following indirect inoculation, 3 were detected after the use of an enrichment culture. That is, the use of an enrichment culture improved the isolation rate by 19%.

There was no significant difference in the isolation rates between groups whether direct or indirect (including enrichment) inoculation was used (binomial paired proportion; $P = 0.75$). In particular, there was 85.25% agreement between the direct method and indirect with enrichment method ($\kappa = 0.61$; $P < 0.0001$; 95% CI, 0.36 to 0.86) (Table 4).

(iii) Order of scrapes. There was no significant difference in the numbers of isolates between group A (first scrape into BHI followed by second scrape directly plated on chocolate agar) and group B (first scrape directly plated onto chocolate agar followed by second scrape into BHI) ($P = 0.65$) (Table 4). There was no significant difference in the overall isolation rates between the first and second scrapes ($P = 0.53$) for group A ($P = 0.58$) and group B ($P = 0.74$) (Table 4). That is, there was no difference in the yields from the first scrape compared to the second scrape, and the order of scrapes did not determine whether the isolation rate was higher or lower.

(iv) Type and sensitivity of organism isolated. *S. pneumoniae* was isolated from four patients, coagulase-negative staphylococci (CNS) from four, *S. aureus* from three, and *Bacillus* spp. from two patients (Table 3). The following were isolated from one patient each: a lactose-fermenting coliform (LFC), a *Pseudomonas* sp., a *Neisseria* sp., *Corynebacterium xerosis*, *Gemella morbillorum*, a *Paecilomyces* sp., and a yeast. No viruses were isolated from the conjunctival swabs. One patient isolate grew both a CNS and an alpha hemolytic streptococcus, giving a polymicrobial rate of 5%. Of the bacterial isolates, four were resistant to ciprofloxacin (two *S. pneumoniae*, a *Bacillus* sp., and *G. morbillorum*) but sensitive to teicoplanin.

TABLE 4. Results for patients with suspected microbial keratitis

Group or scrape	No. of isolates that were[a]:	
	Positive	Negative
Groups[b]		
A	11	20
B	9	21
Scrapes[c]		
First	17 (10, 7)	44 (21, 23)
Second	14 (8, 6)	47 (23, 24)

[a] Numbers in parentheses represent results for groups A and B, respectively.
[b] $P = 0.65$ for group A (first scrape into BHI, second scrape onto chocolate agar) versus group B (first scrape onto chocolate agar, second scrape into BHI).
[c] $P = 0.65$ for groups A and B for first scrape versus second scrape ($P = 0.58$ for group A; $P = 0.74$ for group B).

(v) Conjunctival swabs. Of 10 patients, 8 with positive conjunctival cultures from the affected eye and 6 with positive conjunctival cultures from the fellow eye grew the same bacteria as were isolated from their corneal scrapes. For subjects with negative cultures from the corneal scrape, bacteria were isolated from conjunctival swabs in 4 affected and 10 fellow eyes. For two of those patients with negative corneal scrapes, methicillin-resistant *S. aureus* was isolated from the fellow eye of one patient and *S. aureus* from the affected eye of the other patient. CNS and lactose-fermenting coliform were cultured in the remainder.

(vi) Gram staining. Organisms were seen on Gram stains from four patients: gram-positive diplococci from the corneas of three patients and a gram-positive bacillus from one patient. Isolates from all of these patients grew the same organism on cultures of the corneal scrape. All these organisms were identified from slides prepared in the clinic, and when Gram's staining was used, none of the slides made from the BHI showed an organism.

DISCUSSION

There are various approaches to the microbiological investigation of patients with suspected bacterial ulcerative keratitis. While conventional methods include the use of multiple corneal scrapes with direct inoculation onto different enrichment media (1, 5, 14, 18, 19, 26), a variety of techniques have also been used to obtain and process materials from corneal ulcers (3, 5, 7, 13, 28) and to simplify the process (31). McDonnell et al. found that 49% of ophthalmologists treated corneal ulcers empirically without attempting to identify the causative organism (18, 19). This may have been due to difficulties encountered obtaining multiple corneal scrapes and maintaining up-to-date media and to inexperience with direct plating (18, 19). Even in a university hospital setting, fresh medium may not always be readily available and traditional methodology may not be followed (31). Clearly, deployment of a simpler but efficient method might avoid these problems (18, 19, 31) and lessen the need for empirical treatment of microbial keratitis.

The use of BHI for transport and culture resulted in detection rates similar to those of direct plating (Table 1). There was no significant loss or gain of organisms, suggesting that the sensitivity (no significant loss of bacteria) and specificity (no significant gain of either the original or contaminating bacteria) were similar for both methods. Due to the small amount of material that is usually obtained from a corneal scrape and the relatively low numbers of infecting bacteria, a very small inoculum (e.g., 1 CFU/ml for *S. pneumoniae*) was also used in the experiments. It is apparent that the method of direct plating and that of transport in BHI followed by use of an enrichment medium are both highly sensitive methods. The lack of a sig-

nificant difference between the expected and observed yields for direct plating indicates that bacteria can be transferred from a surgical blade without loss and probably also reflects the expertise of a laboratory-trained person. This is important in the clinical situation, where with fewer organisms available from a corneal scrape, processing is best done in the laboratory by a person trained in microbiological methods. The absence of a trend for the pig's eye model regarding the number of colonies grown in cultures between corneal scrapes supports the clinical data indicating that the isolation rate does not depend on the order of the corneal scrapes. Importantly, the lower between-scrape variation seen using the indirect method improves the probability of inoculating the appropriate culture medium in the laboratory, particularly when there are few organisms present, such as in microbial keratitis. The characteristics of the referral population are a confounding factor in comparisons of studies. In the present study, 61% of patients were on treatment with topical antimicrobials at presentation; although the overall isolation rate was 33%, the isolation rate was 27% for those on treatment and 42% for those not on treatment. This effect of pretreatment may account in part for the isolation rate being lower than that reported in previous studies (20, 22). For example, Morlet et al. (22) reported an isolation rate of 41.5% for a group of patients of whom only 22% were receiving topical antimicrobial medications, while McCleod et al. (20), having excluded patients who had had prior treatment, obtained an isolation rate of 69.1% (56/81).

Smears are useful for providing information on the presence of inflammatory cells, for the rapid diagnosis of bacterial and fungal infections, and to corroborate culture results (1, 5, 14). In the present study, organisms were seen after Gram staining in four patient isolates (7%), and the same Gram stain organism morphotype was detected on cultures from the corneal scrapings. However, all of these organisms were identified from Gram stains of smears prepared in the clinic and not from smears made from the transport medium. This is likely to reflect dilution of organisms in the transport medium. A smear for a Gram stain needs to be made directly from the corneal scrape and not indirectly from the transport medium.

As with most published studies, there was a high prevalence of gram-positive bacteria (1, 14, 15, 20, 21, 23, 31), with *S. pneumoniae* (20%), CNS (20%), and *S. aureus* (15%) being the most common ocular pathogens. We did not routinely grow cultures to detect anaerobes, and this may have contributed to the lower isolation rate (26). The low polymicrobial isolation rate (5%) may have reflected the level of expertise of the persons collecting the scrapes, who were meticulous in avoiding contamination. Importantly, however, the low polymicrobial rates achieved by both direct and indirect inoculation would make it unlikely that other organisms were missed. CNS tend to comprise a high proportion of bacteria reported in polymicrobial infections. Because they occur as a commensal in the conjunctiva and lid margin, it is not always clear whether they represent a contaminant or a significant pathogen. Concern that BHI is susceptible to extrinsic contamination was not substantiated in the present study.

Benson and Lanier (5) found that a calcium alginate swab moistened with soy broth was superior to a platinum spatula for bacterial isolation from a corneal ulcer. Calcium alginate swabs have been shown to be effective for isolation of bacteria from the nasopharynx and for preserving bacterial metabolic activity (12, 29). Similarly, Applebaum et al. (2) reported that a Dacron swab placed in modified Stuart's medium minimized bacterial death during transport and reported sensitivity of 83.3% and specificity of 100%. They advocate that specimens be transported in the culturette's culture medium to allow processing in the laboratory and simplification of the process. Levey et al. (15) used a Kimura spatula and obtained positive cultures in 47.1%. Using a mini-tip Culturette swab, Epley et al. (7) found a culture-positive rate of 42%. As with a platinum spatula, a blade would seemingly rely on the adhesive properties of the organisms and suppurative material (5). However, use of a blade or a needle (1) allows material to be obtained from the leading edge of the ulcer and, by debriding the surface layer, may allow detection of organisms from the deeper layer, which a swab may not achieve. Although we did not compare a blade to a moistened alginate swab, we found no loss of bacteria from the blade and no difficulty in eluting the organisms. This was not necessarily because the blade was placed and left in the BHI, as direct inoculation with the blade achieved similar results. Benson and Lanier (5) advocate use of a platinum spatula to scrape the ulcer (for retrieval of filamentous bacteria and fungi) followed by two swabs moistened with Trypticase soy broth both to inoculate different media. Although we did not specifically assess the yield for fungal isolation, a yeast was isolated with BHI and with direct inoculation. Furthermore, BHI is used for the primary recovery of both saprobic and pathogenic yeasts and molds (4, 24). In our study, we did not transfer subcultures from BHI to anaerobic media. The question arises whether BHI used as a single transport and/or enrichment medium meets the requirement for effective isolation of anaerobic bacteria. Although we did not evaluate this, there is evidence that BHI directly supports the growth of a range of anaerobes (9, 17, 27). In addition, local preference could dictate the precise formulation of the BHI, such as the addition of 0.1% agar to the medium (27), to overcome any potential shortcomings revealed by in-house quality control for anaerobic bacteria. Also, although we did not test the system using *Acanthamoeba* sp., if BHI were in any way unfavorable for trophozoites, these would encyst and could be recovered when subcultured onto nonnutrient agar containing *E. coli*.

Allen and Dart advocate direct plate inoculation rather than transport or storage media because of the small amount of material available (1). In the first part of the study, a tiny inoculum was used deliberately without loss of sensitivity. Results showing similar yields from direct and indirect inoculation support the use of BHI. Importantly, in cases in which there is only a finite yield from an ulcer, employing BHI may avoid the use of an inappropriate medium for the causative organism. The proposed system for collecting corneal specimens removes the difficulty (highlighted by McLeod et al. [20]) for ophthalmologists of collecting and plating material in the community and the difficulty and expense of storing laboratory plates under the right conditions. Agar plates need to be stored in a refrigerator, have a short shelf life, and are prone to contamination which is not always evident at the point of use. BHI has a shelf life of at least 3 months at room temperature. When it is stored at room temperature, contamination of BHI is easily recognized before use by cloudiness of the medium. Although

we did not test the system beyond 3 h, BHI may be regarded a priori as a suitable transport medium. First, it provides excellent recovery of a range of fastidious organisms (which would not be the case if it were in any way inhibitory); second, it is an established medium for holding stock cultures in the laboratory at room temperature (4, 9, 10). A possible disadvantage of BHI as a transport medium is that semiquantitative enumeration of organisms in the original sample is not possible when growth occurs in transit. Transporting the sample in BHI, however, does allow further subculturing in an added enrichment medium, such as with 10% horse blood used in this study. This is not, however, possible with direct plating unless an additional scrape is taken, so the potential sensitivity may be less. Whatever approach is selected for isolation of the offending organisms in ulcerative keratitis, there is still the need to obtain immediate information from Gram-stained smears. In conclusion, therefore, two scrapes may be taken from a corneal ulcer (one for a Gram stain and the other placed in BHI) and transported to the laboratory to be plated onto appropriate culture media.

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