

Does the sampling instrument influence corneal culture outcome in patients with infectious keratitis? A retrospective study comparing cotton tipped applicator with knife blade

Susanna Sagerfors ,¹ Birgitta Ejdermik-Lindblad,¹ Bo Söderquist²

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¹Department of Ophthalmology, Faculty of Medicine and Health, Örebro University, SE 70182, Örebro, Sweden

²Department of Laboratory Medicine, Clinical Microbiology, Faculty of Medicine and Health, Örebro University, SE 70182, Örebro, Sweden

Correspondence to

Dr Susanna Sagerfors; susanna.sagerfors@regionorebrolan.se

ABSTRACT

Objective This study aimed to compare the efficacy of a cotton tipped applicator and a knife blade in obtaining corneal samples in patients with infectious keratitis.

Methods and analysis This is a retrospective cohort study of patients with suspected infectious keratitis during 2004–2014. Samples for corneal culture were obtained by a cotton tipped applicator and a knife blade, and directly inoculated on GC agar, blood agar and Sabouraud agar.

Results In all, 355 patients were included. Corneal sampling by cotton tipped applicator yielded a significantly higher rate of patients with positive corneal culture, 156/355 (43.9%), compared with knife blade, 111/355 (31.3%) ($p < 0.001$). On a patient level, the culture results obtained by the cotton tipped applicator and the knife blade were identical in 269/355 (76%) of the patients. The overall agreement between the two instruments on microbial level was 0.66 (Cohen's kappa 95% CI 0.60 to 0.72).

Conclusion Corneal sampling by cotton tipped applicator generated a higher rate of positive corneal cultures and a higher proportion of isolated microbes than by knife blade. Future studies with randomised sampling order are needed to establish which instrument, cotton tipped applicator or knife blade, is the most effective in sampling microbes for direct inoculation in patients with infectious keratitis.

INTRODUCTION

Non-viral infectious keratitis is an acute ophthalmological condition caused by bacteria, fungi or protozoa that in a worst case scenario can result in corneal perforation and endophthalmitis. The diagnosis is clinical, based on slit-lamp biomicroscopy and patient history, but corneal sampling for culture is required to identify the causative microbe(s) and their antibiotic susceptibility pattern. Some of the steps in the procedure of corneal culture in infectious keratitis are well studied, such as which topical anaesthesia to use prior to corneal sampling,^{1–4} appropriate inoculation media and whether to inoculate directly or via a transport medium.^{5–11} The optimal

Key messages

What is already known about the subject?

- ▶ The identification of the causal microbe and the determination of its antibiotic susceptibility pattern are vital in the management of infectious keratitis. Thus, optimising the corneal culture procedure is of importance.

What are the new findings?

- ▶ The findings of the present study indicate that the instrument used for corneal sampling in patients with infectious keratitis influences the corneal culture outcome. In our study, sampling with cotton tipped applicator generated a higher rate of positive cultures and a higher proportion of isolated microbes than the knife blade.

How might these results change the focus of research or clinical practice?

- ▶ The findings of the current study indicate that a dry cotton tipped applicator is a more efficient instrument to use for corneal sampling in patients with infectious keratitis than a knife blade.

method for obtaining a corneal sample has only been reported in cases series with a limited number of patients included.^{12–14} The instruments used can be divided into two groups: sharp or blunt metal instruments such as Kimura spatula, surgical knife blade or needle; and non-metal instruments with absorbent qualities such as calcium alginate swab, cotton tipped applicator or Mini-tip Culturette.

The aim of this study was to investigate if the sampling instruments, cotton tipped applicator and knife blade, by direct inoculation on agar plates, influenced corneal culture outcome in infectious keratitis.

MATERIALS AND METHODS

This retrospective study made use of both medical records and microbiology reports

over a period of 11 years, from 1 January 2004 to 31 December 2014.

Patients with suspected infectious keratitis from whom corneal cultures were obtained at any of the three departments of ophthalmology in the region of Örebro County were eligible for inclusion. One of these, the Department of Ophthalmology at the University Hospital in Örebro is a tertiary corneal referral centre. The other two, situated in the towns of Lindesberg and Karlskoga, respectively, provide primary outpatient ophthalmological care during office hours. Patients were included if they met at least one of two inclusion criteria¹⁵: (1) positive corneal culture (all growth of microbes was considered positive) and (2) stromal infiltration with overlying epithelial defect in combination with one or more of the following: lesion within or overlapping the central 4mm of the cornea and/or uveitis and/or pain.

Eligible patients were searched for by two pathways to minimise losses due to failure to identify cases: first by diagnostic code for keratitis in the patient records (code H16.9 in version 10 of the International Classification of Disease) and second by searching for corneal cultures in the database at the Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital. Of the 392 patients with keratitis that were identified, 2 were excluded due to lack of medical charts, 2 due to inadequately handled cultures and 33 due to cultures not performed in accordance with the written instructions on corneal culture described in detail in the "microbiological culture" section below.

Patient involvement

Patients were not directly involved in the design of this study.

Microbiological cultures

Corneal samples were obtained by either an ophthalmologist or a resident in ophthalmology according to written instructions available at the relevant time period. These written instructions were in accordance with the Swedish State of the Art Document on Infectious Keratitis Caused by Bacteria, Yeast and Protozoa,¹⁶ and can be considered a routine procedure. This document recommended both a so-called direct and indirect inoculation of the corneal specimen. When directly inoculated, the corneal specimen was directly transferred onto agar plates. When indirectly inoculated, an enrichment media, fastidious anaerobic broth, was used and later subcultured onto agar plates for both anaerobic and aerobic incubation at the laboratory. In this study, only the culture results from direct inoculation on agar plates are examined.

Corneal samples were collected according to the following standard procedures. Samples from the infiltrates were first collected with sterile cotton tipped applicators, one for each plate, and streaked onto one half of each of the three agar plates. The cotton tipped applicators were not moistened with any liquid prior to corneal sampling, that is, they were used dry without any

modifications. This was followed by samples collected from the infiltrate by scraping with a sterile knife blade held with [table 1](#) a sterile glove or sterile forceps and streaked onto the other half of the same three agar plates in the same order. The plates used were GC agar (GC Medium Base, Becton Dickinson (BD), Sparks, Maryland, USA, supplemented with 1% BBL IsoVitaleX enrichment), blood agar (3.9% Columbia Blood Agar Base, Oxoid, Basingstoke, Hampshire, UK, supplemented with 6% defibrinated horse blood) and Sabouraud (SAB) agar (1.3% Agar No2, Lab M, Heywood, Bury, UK; 4% D-Glucose, VWR, Leuven, Belgium; 1% Peptone BD) for isolation of fungi.

There is only one clinical microbiological facility in the region of Örebro County, the Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital. Thus, all corneal cultures obtained at any of the three departments of ophthalmology were registered and handled at the same microbiological laboratory as follows: the GC agar plate was incubated in CO₂ at 36°C, the blood agar plate was incubated in air at 36°C and the SAB plate was incubated in air at 30°C.

All plates were checked for growth on days 1 and 2, and if no growth was detected at day 2, the plates were incubated for another 5 days for a final evaluation. The SAB plate was checked every day and discarded after 7 days if no growth was detected.

If bacterial growth was detected, further typing to species level was carried out in one of two ways. Before January 2014, typing was carried out with currently available routine methods including API kits. From January 2014 onwards, it was carried out with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (Microflex LT and Biotyper 3.1, Bruker Daltonik, Bremen, Germany).

For the purpose of further analysis, microbes isolated by direct inoculation were divided into two groups according to presumed pathogenicity. We used two different sets of definitions: Fleiszig and Efron's classifying coagulase-negative staphylococci, *Corynebacterium* spp. (except *C. diphtheriae*), *Cutibacterium acnes*, *Micrococcus* spp., *Bacillus* spp. and *Peptostreptococcus* spp. as normal flora and other microorganisms as potentially pathogenic¹⁷; second, our own classification based on microbial virulence and clinical experience (see footnote in [table 1](#)).

Statistical analysis

Agreement between cotton tipped applicator and knife blade on a microbial level was calculated as Cohen's kappa and as positive and negative agreement, 95% CI. Differences in culture outcome and in isolation of the different microbes were calculated with McNemar's test. When analysing the isolated microbes in total and in the two subgroups according to pathogenicity, we used the same procedure of summarising the culture negative readings by both methods as Pakzad-Vaezi *et al.*,⁹ that is, the negative plate readings for each species were totalled. All these statistical calculations were performed through the Vassarstat.net website.¹⁸ Additional statistical analysis concerning the

Table 1 Agreement and differences between cotton tipped applicator and knife blade in isolated microbes subdivided according to presumed pathogenicity in the study population 2004–2014

	Agreement (95% CI)				Method difference					
	Microbes isolated by both cotton tip and knife blade	Microbes isolated by cotton tip only	Microbes isolated by knife blade only	Total sum of plate readings negative by both cotton tip and knife blade	Kappa	Positive agreement	Negative agreement	Proportion positive by cotton tip	Proportion positive by knife blade	P value*
Total	99	81	18	7257	0.66 (0.60 to 0.72)	0.66 (0.59 to 0.74)	0.99 (0.99 to 0.99)	2.4%	1.6%	0.000001
Probably pathogenic, authors' definition†	59	29	4	4878	0.78 (0.70 to 0.85)	0.78 (0.67 to 0.86)	1 (0.99 to 1)	1.8%	1.3%	0.000011
Commensals, authors' definition‡	40	52	14	2379	0.54 (0.44 to 0.63)	0.55 (0.43 to 0.66)	0.99 (0.98 to 0.99)	3.7%	2.2%	0.000003
Potential pathogens§	70	37	7	5566	0.76 (0.69 to 0.83)	0.76 (0.66 to 0.84)	1 (0.99 to 1)	1.9%	1.4%	0.000005
Normal flora¶	29	44	11	1691	0.50 (0.38 to 0.61)	0.51 (0.38 to 0.63)	0.98 (0.98 to 0.99)	4.1%	2.3%	0.000009

*McNemar's test.

†Probably pathogenic: *Aggregatibacter segnis*, *Candida albicans*, *Corynebacterium macginleyi*, *Escherichia coli*, *Haemophilus influenzae*, beta-hemolytic streptococci, *Klebsiella oxytoca*, *Neisseria meningitidis*, *Moraxella catarrhalis*, *Fusarium* spp., *Pasteurella multocida*, *Streptococcus pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Serratia* spp., *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Stenotrophomonas maltophilia*.

‡Commensals: *Corynebacterium* spp., except *C. macginleyi*, Gram-negative rod, coagulase-negative staphylococci, except *S. lugdunensis*, *Micrococcus* spp., *Moraxella* spp., mould unspecified, *Pseudomonas* spp., alpha-hemolytic streptococci.

§Potential pathogens¹⁷: *A. segnis*, *C. albicans*, *C. macginleyi*, *E. coli*, *H. influenzae*, beta-hemolytic streptococci, *K. oxytoca*, *N. meningitidis*, *M. catarrhalis*, *Fusarium* spp., *P. multocida*, *S. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. marcescens*, *Serratia* spp., *S. aureus*, *S. lugdunensis*, *S. maltophilia*, Gram-negative rod, *Moraxella* spp., *Pseudomonas* spp., alpha-hemolytic streptococci, mould unspecified.

¶Normal flora¹⁷: *Corynebacterium* spp. includes difteroides non-specified and *C. macginleyi*, *Micrococcus* spp., coagulase-negative staphylococci.

**Table 2** Baseline characteristics in the study population with suspected infectious keratitis (2004–2014), in total and according to outcome of corneal culture by direct plating under aerobic conditions

	All included patients, both culture positive and culture negative (n=355)	Culture positive patients (n=165)	Culture negative patients (n=190)	P value
Mean age at episode onset (SD; range)	50.5 (21.3; 5–98)	57.1 (21.5; 7–98)	44.7 (19.4; 5–91)	<0.001*
Sex (Female)	202 (57%)	90 (55%)	112 (59%)	0.404†
Laterality (Right)	178 (50%)	73 (44%)	105 (55%)	0.038†
Monomicrobial growth of aerobic microbe isolated by direct plating	136	136	–	–
Polymicrobial growth of 2 aerobic microbes isolated by direct plating	26	26	–	–
Polymicrobial growth of 3 aerobic microbes isolated by direct plating	2	2	–	–
Polymicrobial growth of 4 aerobic microbes isolated by direct plating	1	1	–	–
Topical antibiotic treatment prior to corneal culture	65 (18%)	24 (14%)	41 (22%)	0.087†
Fucithalamic	16	8	8	–
Chloramphenicol	15	6	9	–
Fluoroquinolones	18	5	13	–
Polymyxin B	2	2	0	–
Combination of 2	13	3	10	–
Combination of 3	1	0	1	–
Contact lens wear	155 (44%)	57 (34%)	98 (52%)	0.001†
No known risk factor	70 (20%)	34 (21%)	36 (19%)	0.695†
Ocular surface disease including eyelid disorders	57 (16%)	34 (21%)	23 (12%)	0.030†
Prior ocular surgery and trauma	46 (13%)	23 (14%)	23 (12%)	0.608†
Blind/severely ill eye prior to episode	8 (2%)	4 (2%)	4 (2%)	1.000‡
Combination of 2 of above risk factors	19 (5%)	13 (8%)	6 (3%)	0.049†
Median best corrected visual acuity at first visit with keratitis episode (SE)	0.6	0.2	0.8	<0.001§
25th percentile	0.06	0.01	0.3	–
75th percentile	1.0	0.85	1.0	–

Thirty-six cases were excluded from calculations on median visual acuity, since visual acuity was not measured at first visit. Of these, 16 cases were culture negative for aerobic microbes on direct plating and 20 were culture positive.

*t-test.

†Pearson's χ^2 test.

‡Fisher's exact test.

§Mann-Whitney U-test.

SE, Snellen equivalents.

baseline characteristics (table 2) were calculated in using IBM SPSS software package V.25, using Pearson's χ test or Fischer's exact test when appropriate, t-test for comparing mean age and the Mann-Whitney U-test for comparing median visual acuity at first visit.

RESULTS

In all, 355 patients with infectious keratitis were included. Antimicrobial treatment had already started at the time of sampling in 65 cases. Mean age at episode onset was 50.5 years, median BCVA in Snellen equivalents (SE) was 0.6 and the most common risk factor for keratitis was contact lens wear (n=155) (table 2).

When the study population was divided into culture positive and culture negative outcome, based on direct inoculation, the culture positive group displayed a significantly higher mean age ($p<0.001$), a significantly higher proportion of left eyes ($p=0.038$) and a significantly lower median BCVA (SE) at first visit ($p<0.001$) (table 2).

Positive corneal cultures by direct inoculation were seen in 46.5% (165/355) of the cases. Cotton tipped applicator generated a culture positive rate of 43.9% (156/355) and knife blade a culture positive rate of 31.3% (111/355) ($p<0.001$).

Polymicrobial growth on direct inoculation was seen in 29 cases: 26 cases with two microbes, 2 cases with three microbes and 1 case with four different aerobic microbes (table 2). In total, 198 isolates (194 bacterial and four fungal) were isolated by direct plating. Of these, 99

(50%) microbes were isolated by both instruments, 81 (41%) by cotton tip only and 18 (9%) by knife blade only (table 3).

On the patient level, cultures by cotton tipped applicator and knife blade were identical in 76% (269/355)

Table 3 Agreement between cotton tipped applicator and knife blade on isolated microbes after direct plating in patients with infectious keratitis

	No. of isolates obtained by both instruments (n=99)	No. of isolates obtained by cotton tipped applicator only (n=81)	No. of isolates obtained by knife blade only (n=18)	Negative by both instruments	Cohen's kappa (95% CI)
<i>Staphylococcus aureus</i>	18	15	3	319	0.64 (0.49 to 0.79)
<i>Staphylococcus lugdunensis</i>	1	0	0	354	1 (1 to 1)
Coagulase-negative staphylococci	11	30	9	305	0.31 (0.15 to 0.47)
<i>Streptococcus pneumoniae</i>	7	0	0	348	1 (1 to 1)
Beta-hemolytic streptococci	2	1	0	352	0.8 (0.41 to 1)
Alpha-hemolytic streptococci	7	6	1	341	0.66 (0.42 to 0.89)
<i>Corynebacterium macginleyi</i>	8	3	0	344	0.84 (0.66 to 1)
<i>Corynebacterium</i> spp.	8	11	1	335	0.56 (0.33 to 0.78)
<i>Micrococcus</i> spp.	1	0	1	353	0.67 (0.05 to 1)
<i>Haemophilus influenzae</i>	3	1	0	351	0.86 (0.58 to 1)
Enterobacteriaceae	2	3	0	350	0.57 (0.13 to 1)
<i>Pseudomonas aeruginosa</i>	11	3	0	341	0.88 (0.74 to 1)
<i>Stenotrophomonas maltophilia</i>	0	1	0	354	0 (0 to 0)
<i>Pseudomonas</i> spp.	1	0	0	354	1 (1 to 1)
<i>Neisseria meningitidis</i>	3	0	0	352	1 (1 to 19)
<i>Moraxella catarrhalis</i>	2	1	0	352	0.8 (0.41 to 1)
<i>Moraxella</i> spp.	12	5	0	338	0.82 (0.67 to 0.97)
*Miscellaneous Gram-negative	0	1	1	353	x
<i>Candida albicans</i>	1	0	0	354	1 (1 to 1)
<i>Fusarium</i> spp.	1	0	0	354	1 (1 to 1)
Mould unspecified	0	0	2	353	0 (0 to 0)
Total	99	81	18	7257	0.66 (0.60 to 0.72)

*Miscellaneous Gram-negative: *Pasteurella multocida*, *Aggregatibacter segnis*.

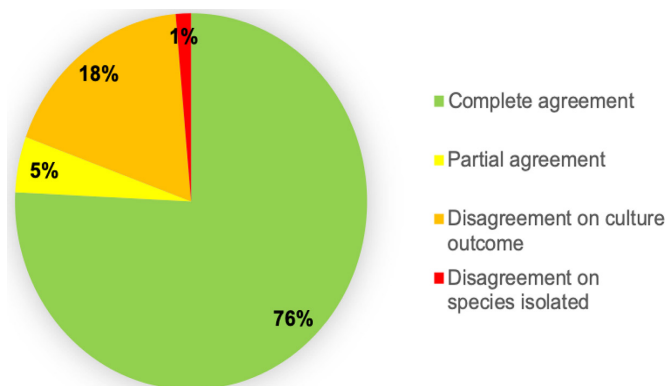


Figure 1 Agreement on the patient level in corneal culture results between sampling with cotton tipped applicator and knife blade in patients with infectious keratitis 2004–2014 (n=355). Complete agreement (■): both methods culture negative (n=190) or both methods culture positive displaying the same species (n=79), partial agreement (■): of one isolated species, in samples displaying polymicrobial growth (n=17), disagreement on culture outcome (■): culture positive by cotton tipped applicator only (n=55) or by knife blade only (n=9), disagreement (■) on species isolated (n=5).

of the patients, either culture negative (190 patients) or culture positive with identical isolated microbe(s) (79 patients) (figure 1). Of the 79 patients with positive corneal culture, 76 cases showed monomicrobial growth and 3 cases showed polymicrobial growth with two microbes. Cotton tipped applicator and knife blade disagreed in 19% of the patients (69/355), either on culture outcome (n=64 (18%)) or on the identity of the microbe isolated (n=5 (1%)). The isolates from these five patients are shown in table 4.

Of the 64 patients who differed in culture outcome, 60 showed monomicrobial growth and 4 showed polymicrobial growth of two microbes; 55 were culture positive by cotton tipped applicator only and 9 by knife blade only.

In the remaining 5% (17/355), cotton tipped applicator and knife blade agreed partially; that is, on the identity of one of two to four isolated microbes in patients displaying polymicrobial growth (figure 1). Of these

Table 4 Microbes isolated in patients with inconsistent corneal culture result between cotton tipped applicator and knife blade

Patient	Cotton tip	Knife blade
1	<i>Staphylococcus aureus</i>	Coagulase-negative staphylococci
2	<i>S. aureus</i>	Coagulase-negative staphylococci
3	Alpha-hemolytic streptococci	Coagulase-negative staphylococci
4	Coagulase-negative staphylococci	<i>S. aureus</i>
5	<i>S. aureus</i>	<i>Pasteurella multocida</i> , <i>Micrococcus</i> spp.

patients, 15 had two microbes, 1 had three microbes and 1 had four microbes.

On the microbial level, the overall agreement between cotton tipped applicator and knife blade as calculated with Cohen's kappa was 0.66, 95% CI 0.60 to 0.72. When isolated microbes were classified as either potential pathogens or normal flora according to Fleiszig and Efron,¹⁷ the agreement was 0.76 for potential pathogens and 0.50 for normal flora. When our own classification of 'probably pathogenic' versus 'commensal' was applied (see footnote in table 1), Cohen's kappa was 0.78 and 0.54, respectively (table 1).

DISCUSSION

In the present study, we investigated two instruments used for obtaining corneal material for direct inoculation in patients with suspected infectious keratitis: a blunt instrument with absorbent properties, that is, a dry sterile cotton tipped applicator and a sharp, sterile, non-absorbent knife blade.

The cotton tipped applicator yielded a statistically significant higher rate of both positive corneal cultures and a higher proportion of isolates than the knife blade. In a previous study by Benson *et al*¹² where samples were inoculated directly on agar plates, they observed that a moistened calcium alginate swab gave a significantly higher retrieval of organisms from bacterial keratitis than a platinum spatula.¹² Jacob *et al* compared knife blade with a moistened calcium alginate swab, and they did not find any difference between the two instruments used on bacterial and mixed ulcers, but the swab retrieved a significantly greater number of organisms in cases of mycotic ulcers. However, they inoculated the specimens indirectly through a Calgon solution.

We chose the dry cotton tip approach since moistening the cotton tip with any liquid may reduce its absorbent qualities. However, in an animal study by Epley *et al*, there were no difference in yield between a dry and a saline moistened Dacron tipped applicators (Mini-tip Culturette).¹³

There have been concerns that a cotton tipped applicator may favour the uptake of transient commensals and not only representative infectious material from the infiltrate.¹⁹ We therefore divided the findings of isolated microbes into two groups according to presumed pathogenicity. When grouping the isolated microbes into more or less pathogenic, the cotton tip yielded a statistically higher proportion of isolated microbes in both groups no matter which pathogenicity classifications we used.

There may be several explanations as to why the cotton tip generates more isolates and a higher rate of positive cultures. The ophthalmologists may be more confident in handling a cotton tipped applicator compared with a knife blade, which can potentially induce more harm to the patient. In addition, the risk of perforating the surface of the agar when transferring the obtained sample from the instrument to the agar plate is probably less with a cotton tip than a knife blade, which facilitates a correct interpretation

of the agar plate. The absorbent qualities of the cotton tip compared with the knife blade may also play a role.¹²

This study showed an overall agreement of 76% between the instruments on a patient level and an agreement of 0.66 (Cohen's kappa) on microbial level, which can be considered as good²⁰ or substantial.²¹ The major difference in the patient level was not on the identity of the isolated microbe, but on culture outcome, that is, if the culture was positive or not, with a majority of the cultures (55/64) positive by cotton tipped applicator only. This could indicate that sampling by cotton tip is more effective at obtaining microbes in patients with suspected infectious keratitis. Since 18% of the culture positive cases in our study displayed polymicrobial growth, and polymicrobial growth has been reported in even higher rates, 32%,²² we believe that agreement on the patient level is relevant from a clinical point of view.

One strength of our study is the number of patients included and the likelihood of a low number of missing cases, since we applied two separate approaches to identifying patients with suspected infectious keratitis.

A major limitation of the study is the retrospective design. Medical charts and culture procedures were not always complete, which resulted in exclusion of some cases. The order of obtaining corneal material for culture was not randomised, and this may have favoured the cotton tipped applicator. There is, however, evidence in the literature that sampling order does not influence the number of different species recovered by direct plating⁹ or the amount of microbial growth.¹²⁻¹⁴ Furthermore, in an in vitro study by Kaye *et al*, there was evidence that up to five scrapings could be obtained from pig corneas without affecting viable counts of *S. aureus*.⁶

In our region, fungi as an infectious agent in keratitis are rare compared with bacteria, and therefore, the sample size of this subgroup was very small.

CONCLUSION

The overall agreement on microbial level between cotton tipped applicator and knife blade was good. Corneal sampling for direct inoculation by cotton tipped applicator yielded a significantly higher proportion of positive cultures and higher proportions of isolates, both those considered pathogenic and those considered not. In our study population, which consisted of mainly bacterial ulcers, very few probably pathogenic microbes would have been missed if sampling for direct inoculation was performed by using solely a cotton tipped applicator. Further prospective studies with randomised sampling order are needed to establish which instrument is the most effective in sampling microbes for direct inoculation in patients with infectious keratitis.

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Competing interests None declared.

Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article.

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ORCID iD

Susanna Sagerfors <http://orcid.org/0000-0003-1150-1751>

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