

# Reducing oral contamination during corneal scrapes

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## ABSTRACT

**Aims:** To identify potential contaminants of the corneal sampling procedure and examine the effect of wearing surgical face masks on the rate of contamination.

**Methods:** Ten surgeons recited out loud a 30 s standardised script for corneal scraping with blood agar plates positioned 30 cm away from them. Three groups were identified: in group 1 a surgical mask was worn; group 2 had no mask worn; and group 3 had no mask but used agar plates pretreated with 5% povidone-iodine as a negative control. Each surgeon repeated the process 10 times for all groups, totalling 30 plates per surgeon and 300 plates for the experiment. All plates were masked and incubated aerobically at 37°C for 24 hours, and the number of colony forming units (CFUs) was determined.

**Results:** At 24 hours, group 1 had a mean of 0.3 CFUs per surgeon; group 2 had 6.4 CFUs per surgeon and group 3 had 0.1 CFUs per surgeon. The difference between group 1 and group 2 was significant ( $p<0.001$ ) whereas the difference between group 1 and group 3 was non-significant ( $p=0.4$ ). Use of face masks decreased the number of plates with CFUs by 93% (from 29 to 2 plates) and decreased the total number of CFUs by 95% (from 63 to 3 CFUs). The most common microbiota identified was *Streptococcus* species.

**Conclusions:** Oral bacterial microbiota may contaminate the slides and media used to collect samples during corneal sampling. Use of a face mask can significantly decrease the rate of contamination of such samples.

## INTRODUCTION

Corneal tissue sampling for microscopy and culture is the gold standard for identifying the causative organisms in microbial keratitis.<sup>1</sup> Accurate diagnosis is needed as visual loss can occur rapidly and significant morbidity is associated with chronic disease. Further, a diversity of species has been implicated.<sup>2</sup> Corneal sampling, however, does not always yield reliable results<sup>3 4</sup>; the reported culture positivity rate ranges from 25% to 78%,<sup>5 6</sup> and false positives and negatives occur. Improved diagnosis of keratitis is needed because it is a significant and increasing

## Key messages

### What is known?

- Corneal sampling is the gold standard for diagnosing microbial keratitis, though the culture positivity rate can be highly variable with reports ranging from 25% to 78%.
- Contaminants reduce the diagnostic value of corneal sampling and can delay appropriate treatment.

### What are the new findings?

- Oral microbiota can contaminate agar plates.
- The use of face masks decreased the number of plates with colony forming units (CFUs) by 93% and decreased the total number of CFUs by 95%.

### How might these results change the focus of clinical practice?

- Wearing a face mask is a low-cost and no harm method that significantly decreased contamination rates and may potentially improve the reliability of the results obtained from corneal samples in microbial keratitis.

cause of visual loss worldwide<sup>3 7 8</sup>; it was estimated that over 1.5 million people worldwide developed corneal ulceration annually and its prevalence is rising with the increased use of contact lenses.<sup>9-11</sup>

The reliability of corneal sampling may be compromised by contamination of the specimen. This can reduce the diagnostic value of the scrape resulting in delayed or inappropriate therapy.<sup>12</sup> Methods to reduce contamination during corneal scraping have not been investigated. Recently, a simple experimental study identified that the use of surgical face masks significantly decreased the dispersion of bacteria that can contaminate the surgical field during intravitreal injections, leading to its widespread adoption to decrease contamination risk.<sup>13</sup> Currently, masks are not routinely worn for corneal scraping.

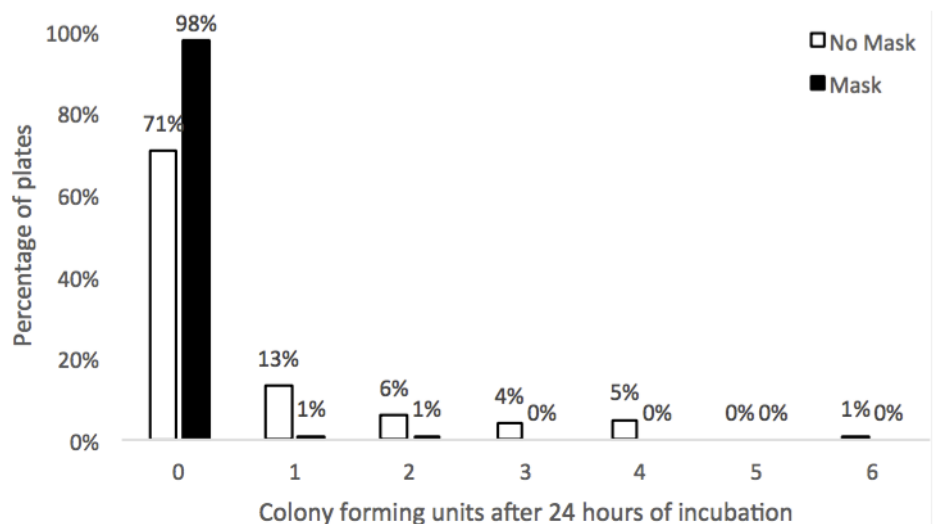
Because of the lack of evidence on the role of face masks in reducing contamination of the collected samples in microbial keratitis, we performed a prospective experimental study. In this study, ophthalmologists and ophthalmology trainees reenacted the corneal scraping procedure with and without a face mask. The purpose was to examine the effect of wearing a face mask on bacterial microbiota counts cultured on agar plates during an experimental corneal scrape procedure.

## METHODS

### Experimental structure

A total of 10 ophthalmologists or ophthalmology trainees participated in the experiment. Each surgeon read out loud a standardised script lasting 30 seconds with a horizontally positioned blood agar plate 30 cm from their mouth. The script and position of the agar plates were determined via consultation with ophthalmologists and trainees who regularly perform corneal scrapes and reflect the length of dialogue required to give instructions to patients and location of the slides and media used to collect samples during the procedure. To mimic clinical practice during a scrape, plate lids were removed just prior to inoculation and replaced immediately afterwards. The experiment was conducted in our ophthalmology emergency department where corneal scrapes are routinely performed. Each surgeon repeated the procedure 10 times for all groups. In group 1, a standard surgical mask (Livingstone Premium Surgical Face Mask, Livingstone International, Australia) was worn. In group 2 no mask was worn. Group 3 had no mask and used agar plates pretreated with 5% povidone-iodine (Betadine 5% Sterile Ophthalmic Prep Solution, Alcon, USA) to act as a negative control. In total, 300 plates (100 for each group) were collected. The plates were incubated aerobically at 37°C for 24 hours, after which the number of bacterial colony forming units (CFUs) on each plate was counted.

**Figure 1** Histogram of number of colony forming units (CFUs) after 24 hours of incubation between group 1 and group 2. There was a 95% reduction in CFUs with the use of a surgical face mask.



**Table 1** Characteristics between groups

|                                     | Group 1 (Mask) | Group 2 (No mask) | Group 3 (Povidone-iodine) |
|-------------------------------------|----------------|-------------------|---------------------------|
| Mean CFUs per surgeon               | 0.3            | 6.4               | 0.1                       |
| SD                                  | 0.3            | 1.2               | 0.1                       |
| Statistical comparison with group 1 |                | p<0.001           | p=0.4                     |

CFUs, colony forming units.

The plates were labelled numerically and laboratory staff were masked to prevent bias.

### Identification of organisms

See online [supplementary material](#). In brief, different colony morphotypes from the agar plates were isolated and the identification of bacteria was conducted by sequencing the 16S rRNA gene from each colony morphotype.

### Power calculation and statistical analysis

A power calculation was performed prior to the experiment that determined that 100 plates per category exceeded the number needed to detect a difference in mean CFUs per group of 0.5 at a power of 0.95. The Mann-Whitney U test for non-parametric testing was used to determine the significance of the difference between means per group.

## RESULTS

All 300 plates were successfully incubated. *Streptococcus* species were the most frequently identified bacteria. [Table 1](#) demonstrates the difference in means between groups. Group 1 (mask) had a mean

**Table 2** Bacterial isolates from each group

|                               | Group 1<br>(Mask) | Group 2<br>(No mask) | Group 3<br>(Povidone-iodine) |
|-------------------------------|-------------------|----------------------|------------------------------|
| <b>Respiratory microbiota</b> |                   |                      |                              |
| <i>Streptococci</i> spp       | 2                 | 51                   | 1                            |
| <b>Skin microbiota</b>        |                   |                      |                              |
| <i>Micrococcus</i> spp        | 1                 | 6                    | 0                            |
| <i>Staphylococcus</i> spp     | 0                 | 4                    | 0                            |
| <i>Bacillus</i> spp           | 0                 | 2                    | 0                            |

of 0.3 CFUs per surgeon; group 2 (no mask) had a mean of 6.4 CFUs; and group 3 (povidone-iodine) had a mean of 0.1 CFUs. There was a significant difference between group 2 and the other groups ( $p < 0.001$ ), and no difference between group 1 and group 3 ( $p = 0.4$ ).

Figure 1 demonstrates the difference in the number of CFUs isolated after 24 hours between group 1 and group 2. Use of a face mask decreased the number of plates with CFUs by 93% (from 29 to 2 plates) and decreased the total number of CFUs by 95% (from 63 to 3 CFUs).

Table 2 shows the different bacterial organisms isolated. Group 1 had 3 bacterial CFUs identified whereas group 3 had only 1 isolate. In contrast, group 2 had 63 bacterial CFUs identified, predominantly from respiratory microbiota.

## DISCUSSION

Our study shows that wearing a surgical mask decreases the risk of agar plate contamination during an experimental corneal scrape scenario. We found that the spread of respiratory and skin microbiota was reduced by a factor of 21; the number of plates contaminated was reduced by 93% and the total number of CFUs was reduced by 95%. The use of a face mask was found to approximate the antiseptic properties of povidone-iodine in its effect on bacterial CFUs.

Recognising organisms that are potential contaminants is clinically important. Bacterial keratitis is most commonly caused by members of the coagulase negative staphylococci, *Staphylococcus aureus*, or if contact lenses have been worn, *Pseudomonas aeruginosa*.<sup>11 14 15</sup> Microbiology laboratories use various clues to identify possible contaminant bacteria. The normal ocular microbiota is composed predominantly of coagulase negative staphylococci, *Corynebacterium* sp and *Propionibacterium* sp. *Streptococci* are very occasionally isolated from normal eyes (<1%–3.7%) and can be isolated from cases of keratitis, but their presence in clinical samples is not common and so should be treated as potential contaminants unless in high numbers and isolated on most media used.<sup>16–19</sup>

In the scenario of progressive keratitis, differentiating pathogenic organisms from contaminants will potentially alter treatment. Our study identified *Streptococcus*, *Micrococcus*, *Staphylococcus* and *Bacillus* species as potential contaminants. This correlates with another study that identified these species as more likely to be cultured from broth than agar media, which is a common finding in contaminants.<sup>19</sup> Our results highlight that oral microbiota when identified from corneal samples could be contaminants and should be considered in progressive keratitis, triggering the clinician to repeat the corneal sampling to identify the causal organism. Further, we identified *Streptococcus* species as the most common bacteria cultured. This is clinically significant as these bacteria have a different antibiotic resistance profile to many Gram-positive organisms,<sup>19 20</sup> meaning that an incorrect diagnosis may delay treatment and potentially lead to avoidable loss of visual acuity.

Our study is a prospective masked study with large numbers. However, it is limited by the standardised techniques used. In clinical practice, an individual surgeon would hold the agar plate at a variable distance and would talk for a variable duration, if any. We obtained consensus from clinicians at our hospital in designing this study to align the study procedures with those in clinical practice.

In conclusion, our study confirmed that oral microbiota can contaminate agar plates placed in the field during a re-enactment of the corneal sampling procedures. In a busy clinical setting where not speaking cannot be guaranteed, wearing a face mask is a low-cost and no harm method that significantly decreased contamination rates and may potentially improve the reliability of the results obtained from corneal samples in microbial keratitis.

**Contributors** CS, NC and SW designed the study. CS collected the data. DD and MW conducted the laboratory work. YCL performed the statistical analysis. CS wrote the first draft. All authors were involved with editing the manuscript and subsequent revisions.

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## REFERENCES

- Allan BD, Dart JK. Strategies for the management of microbial keratitis. *Br J Ophthalmol* 1995;79:777–86.
- Karsten E, Watson SL, Foster LJ. Diversity of microbial species implicated in keratitis: a review. *Open Ophthalmol J* 2012;6:110–24.
- Ng AL, To KK, Choi CC, et al. Predisposing factors, microbial characteristics, and clinical outcome of microbial keratitis in a tertiary centre in Hong Kong: a 10-year experience. *J Ophthalmol* 2015;2015:769436.
- Bhadange Y, Das S, Kasav MK, et al. Comparison of culture-negative and culture-positive microbial keratitis: cause of culture negativity, clinical features and final outcome. *Br J Ophthalmol* 2015;99:1498–502.
- Tananuvat N, Punyakhum O, Ausayakhun S, et al. Etiology and clinical outcomes of microbial keratitis at a tertiary eye-care center in northern Thailand. *J Med Assoc Thai* 2012;95(Suppl 4):S8–17.
- Edwards K, Keay L, Naduvilath T, et al. Characteristics of and risk factors for contact lens-related microbial keratitis in a tertiary referral hospital. *Eye* 2009;23:153–60.
- Shah A, Sachdev A, Coggon D, et al. Geographic variations in microbial keratitis: an analysis of the peer-reviewed literature. *Br J Ophthalmol* 2011;95:762–7.
- Wong T, Ormonde S, Gamble G, et al. Severe infective keratitis leading to hospital admission in New Zealand. *Br J Ophthalmol* 2003;87:1103–8.
- Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. *Bull World Health Organ* 2001;79:214–21.
- Al-Mujaini A, Al-Kharusi N, Thakral A, et al. Bacterial keratitis: perspective on epidemiology, clinico-pathogenesis, diagnosis and treatment. *Sultan Qaboos Univ Med J* 2009;9:184–95.
- Stapleton F, Carnit N. Contact lens-related microbial keratitis: how have epidemiology and genetics helped Us with pathogenesis and prophylaxis. *Eye* 2012;26:185–93.
- Kaye SB, Rao PG, Smith G, et al. Simplifying collection of corneal specimens in cases of suspected bacterial keratitis. *J Clin Microbiol* 2003;41:3192–7.
- Doshi RR, Leng T, Fung AE. Reducing oral flora contamination of intravitreal injections with face mask or silence. *Retina* 2012;32:473–6.
- Gopinathan U, Sharma S, Garg P, et al. Review of epidemiological features, microbiological diagnosis and treatment outcome of microbial keratitis: experience of over a decade. *Indian J Ophthalmol* 2009;57:273–9.
- Kaliyamurthy J, Kalavathy CM, Parmar P, et al. Spectrum of bacterial keratitis at a tertiary eye care centre in India. *Biomed Res Int* 2013;2013:1–8.
- Ozkan J, Willcox MD, Rathi VM, et al. Effect of antibiotic drops on adverse events during extended lens wear. *Optom Vis Sci* 2014;91:13–23.
- Sankaridurg PR, Markoulli M, de la Jara PL, et al. Lid and conjunctival micro biota during contact lens wear in children. *Optom Vis Sci* 2009;86:312–7.
- Willcox MD. Characterization of the normal microbiota of the ocular surface. *Exp Eye Res* 2013;117:99–105.
- Samarawickrama C, Chan E, Daniell M. Rising fluoroquinolone resistance rates in corneal isolates: implications for the wider use of antibiotics within the community. *Healthc Infect* 2015;20:128–33.
- Tuft SJ, Matheson M. In vitro antibiotic resistance in bacterial keratitis in London. *Br J Ophthalmol* 2000;84:687–91.