In a recently published study, Kaye et al. (2) compared two approaches to evaluating bacterial keratitis: the conventional method, in which samples are obtained by taking several corneal scrapes and placing them on different media and slides (direct method, as described by Kaye et al.), and a proposed method (indirect), in which only one scrape is placed into a tube containing brain heart broth for ulterior processing at the laboratory. Their experiments with in vitro suspensions of ocular pathogens and with enucleated pig eyes inoculated with the same organisms showed that both methods led to similar bacterial recoveries. However, the authors apparently underestimated the fact that, with a low inoculum, a variation in the results was found by both techniques due to sampling problems, as bacteria were recovered in some cases but not in others. Even more important are results obtained with real corneal specimens. Kaye et al. found no differences in the percentages of positive results obtained by both techniques. However, it seems that they overlooked the fact that around 20 to 25% of diagnoses would be missed if only one of the methods was used (the numbers of positive cultures were 16, 15, and 20 for the indirect method, the direct method, and both methods, respectively). Indeed, this rate of missed diagnoses can also be attributed to sampling, and it argues in favor of making several corneal scrapes, since sampling enhances microbial detection in infectious keratitis. This fact is remarkable, and it is in concordance with my experience of attending patients from all across Argentina in a private clinic of Buenos Aires (Centro Oftalmológico “Dr. Brunzini”) that specializes in ocular infection. At this center, both ophthalmologists and microbiologists work in a close fashion with suffering patients. Samples are obtained by taking multiple corneal scrapes with a Kimura spatula and are directly seeded onto several media (chocolate and blood agar, brain heart broth, Sabouraud agar, nonnutrient agar for acanthamoebas, and occasionally Löwenstein-Jensen medium). In addition, at a minimum, two to three smears are made for stains (Gram, calcofluor white, and occasionally Ziehl-Neelsen or Giemsa). An enormous variety of etiologic agents must be investigated, and neither clinical signs nor risk factors, although helpful, can replace a definitive diagnosis (1, 3). An etiologic agent was identified in 178 out of 253 patients (70.4%); 71.4% of the agents were bacterial (including mycobacterial), 17.1% were fungal, and 11.5% were Acanthamoeba spp. (F. Nicola, M. Maltagliatti, R. Brunzini, and M. Brunzini, J. Argent. Microbiol., abstr. 070, 2003). Similarly to Kaye et al. (2), Nicola et al. found that 55% of patients had received an antibiotic treatment before being evaluated in our center, and this can clearly affect the recovery of an etiologic agent (45% versus 80%, with and without previous treatment, respectively [P < 0.01]). Moreover, in some cases, the pathogens were detected in only one of the inoculated media, and it was not unusual that very few colonies (fewer than five) grew on agar plates. It could be inferred that bacterial load in naturally infected corneas is usually low, and in addition, significant variations in microorganism distribution in corneal abscesses occur. Finally, it is believed that expertise in both taking representative samples by firm corneal scraping and microbiologic processing of the samples, in a direct and rapid manner, is invaluable for a good diagnostic performance. Although this approach may be difficult to implement in all ophthalmologic settings, the recommendation is to make a special effort. If no laboratory-skilled assistant can be present at the time of sampling, basic microbiologic training should be given to the ophthalmologist, and fresh media should be provided as required. Data showing a rate of definitive diagnosis (70.4%) that is among the highest of those published (1, 2, 3) are the basis for this encouragement.

REFERENCE


Authors’ Reply

We are impressed with the total microbiological yield reported by Nicola from his national tertiary referral center, but as has been reported, we remain skeptical that this can be achieved in the majority of ophthalmology centers. Our clinical yields (both from multiple scrapes and direct plating and from a single scrape transported in brain heart infusion [BHI]) are, we suspect, the result of our approach significantly reducing the occurrence of contaminants and polymicrobial infections. We would be interested to learn more details of the nature of the bacteria isolated by Nicola from 125 of his patients and what clinical significance was attached to them.

The debate as to the “best approach” needs to address two basic issues. First, does BHI transport medium that also serves for enrichment culture reliably yield growth of viable organisms that are inoculated therein? As long as subcultures are made onto appropriate solid media in the laboratory, the pathogen will be recovered; sensitivity, sensu stricto, is not an issue precisely because the primary inoculum is made into an enrichment medium expressly designed to detect a single viable organism. Our experimental data confirmed this. Furthermore, Nicola is incorrect in his assertion regarding the use of low inocula and variation in sampling. A low inoculum (as well as a high one) was intentionally used in order to approach the threshold of isolation, that is, where there is variation in the presence or absence of an isolate.

Second, probably the most important issue concerns sampling error in terms of the corneal scrapes themselves. Our proposed approach is to take a single but generous scrape, which includes the leading edge of the ulcer. Nicola takes at least seven scrapes from each patient. Apart from the difficulties of achieving this in practice and the risk of contamination, the piecemeal approach necessarily involved in taking multiple samples may well introduce significant sampling error giving...
rise to negative laboratory findings if there is a mismatch between the pathogen and the culture medium. Furthermore, we found no evidence to support a significant increase in the isolation of potential corneal pathogens with an increase in scrape number, nor was there a wide variation in the distribution of organisms across the ulcer. In particular, the lower between-scrape variation found with the indirect method improves the probability of inoculating the appropriate culture medium in the laboratory, especially when there are few organisms present. Indeed, if more than one scrape is intended, it may in fact be more appropriate to place any additional scrapes into BHI for subsequent plating in the laboratory than it is to place them directly onto plates in the clinic. Again, Nicola’s own data should give some evidence regarding the use of BHI in terms of its sensitivity in picking up positive cultures.

Stephen B. Kaye*
Godfrey Smith
St. Paul’s Eye Unit
8 Z Link
Royal Liverpool University Hospital
Prescot St.
Liverpool L7 8XP, United Kingdom

*Phone: 44 151 7062134
Fax: 44 151 7065861
E-mail: Stephen.kaye@rlbuh-tr.nwest.nhs.uk