Comparison of two methods for obtaining and transporting corneal samples in suspected infectious keratitis

« *Comparaison de deux méthodes d'obtention et de transport d'échantillons de cornée chez kératite infectieuse* »

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KEYWORDS: Bactec; Corneal cultures; Culture media; Corneal specimens; Infectious keratitis; Eswab

Summary

Background and purpose. — The purpose of this study is to compare two alternative methods of collecting and transporting media for the diagnosis of corneal ulcers, as not all clinical settings have conventional culture materials and transport media available.

Methods. — In this open-label, prospective, comparative, and randomized study, patients with clinical suspicion of infectious keratitis with high risk of loss of vision had corneal specimens collected using two methods and transport media: Eswab scraping with Amies transport medium and 23-gauge needle scraping in BACTEC Peds broth. The order of each collection method was randomized. The samples were processed by standard methods, comparing the positivity frequencies for both by parametric and nonparametric tests, according to normality criteria.

Results. — Corneal infiltrates from 40 eyes of 40 patients were analyzed. Culture positivity rate was 50% for Eswab and 35% for 23-gauge needle (P = 0.258). The overall growth rate of the two methods combined was not higher than with the swab alone. The results obtained with a swab was significantly higher when the sample taken with the needle was performed first (P = 0.046).

Conclusions. — The single sample Eswab method of collection and transportation for the diagnosis of high risk corneal ulcers is a valid alternative and can be used in cases in which, for various reasons, there is no access to the full set of traditional culture materials

MOTS CLÉS: Bactec; Cultures cornéennes; Milieux de culture; Spécimens cornéens; Kératite infectieuse; E-écouvillon

Résumé

Introduction. — L'objectif de cette étude est de comparer deux méthodes alternatives de col-lecte et de milieux de transport de cornées pour le diagnostic des ulcères cornéen dans lamesure où tous les établissements de soin ne disposent pas de matériel de culture et de milieuxde transport conventionnels.

Matériels et méthodes. — Dans cette étude ouverte, prospective, comparative et randomisée,des échantillons de cornée ont été recueillis chez des patients présentant une suspicion cliniquede kératite infectieuse avec un risque élevé de perte de vision, en utilisant deux méthodes etdeux milieux de transport : utilisation d'un écouvillon E avec un milieu de transport Amies etgrattage avec des aiguilles de calibre 23 avec le bouillon BACTEC Peds. L'ordre de chaque méth-ode de collecte a été randomisé. Les échantillons ont été traités selon des méthodes standard,en comparant les fréquences de positivité par des tests paramétriques et non paramétriques,en fonction de critères de normalité.

Résultats. —Les infiltrats cornéens de 40 yeux de 40 patients ont été analysés. Le taux de pos-itivité de la culture était de 50 % pour le coton-tige E et de 35 % pour les aiguilles 23 gauge(p = 0,258). Le taux de croissance global des deux méthodes combinées n'était pas supérieurà celui obtenu avec l'écouvillon seul. Les résultats obtenus avec un écouvillon n'ont pas étéinfluencés par la séquence de collecte (p = 0,122) ; cependant, le taux de positivité était signi-ficativement plus élevé lorsque l'échantillon d'aiguille avait été prélevé en premier (p = 0,046).

Conclusion. — La méthode de collecte et de transport d'un seul échantillon E-swab dans lediagnostic des ulcères de la cornée à haut risque est une alternative valable et peut êtreutilisée dans les cas où, pour différentes raisons, il n'est pas possible d'accéder à l'ensemblecomplet du matériel de culture traditionnel

Introduction

Infectious keratitis is a potentially serious condition that can result in severe vision loss. The clinical picture can vary according to the degree of inflammation and direct tissue damage due to microbial invasion. Early diagnosis and targeted treatment is necessary in order to halt progression and prevent debilitating disease. Although the response to empirical treatment with commercial third or fourth generation quinolone eye drops is favorable in most cases, the failure to detect resistant strains can invariably lead to a devastating course. More importantly, specific identification and prompt targeted treatment is essential in these cases [1—3]. Moreover, obtaining an antibiogram allows the safe addition of topical corticosteroids in order to aid in regulation of the inflammatory process. The gold standard for the diagnosis of infectious keratitisis still direct inoculation of the organism on blood, chocolate, or

Sabouraud agar after deep scraping of the ulcer edge using a Kimura spatula. However, the unavailability of fresh culture media in some office settings, or the long transport and processing times involved with using outsourced microbiology laboratories, pose significant limitations for obtaining adequate culture results. Alternative methods of collecting and transporting corneal samples without the use of fresh solid culture media include, but are not limited to, corneal scraping and inoculation in a pediatric blood culture bottle (PBCB) [4—6], as well as a swab of the corneal ulcer placed in Amies transport media [7,8]. There are only few reports of the culture positivity rate of these methods. Therefore, the purpose of this study is to compare the frequency of isolation of microorganisms by means of these two methods, in an effort to determine which would be the preferred technique in the setting where the gold standard technique is not available.

Material and methods

This is a prospective, comparative, randomized, open-label study carried out in the Ophthalmology Department (Institut Clinic d'Oftalmologia, ICOF) and the Microbiology Department (Center of Biomedical Diagnostic) of the Hospital Clínic of Barcelona between January 2015 and January2018. The study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board and Ethics Committee of the Hospital Clinic of Barcelona, Spain. Adults with clinical suspicion of high-risk bacterial or fun-gal keratitis were included in the study. High-risk keratitis was identified using the ''1, 2, 3'' rule, which is a system that categorizes the keratitis into those that have a high like-lihood of visual loss. ''High risk'' is defined as having any of the following three characteristics: ≥ 1+ cell in the anterior chamber, dense infiltrate ≥ 2 mm in greatest linear dimension, or edge of infiltrate ≤ 3 mm from the center of the cornea [9]. The exclusion criteria were: peripheral ulcers, clinical suspicion of herpetic etiology, suspicion of parasitic etiology (*Acanthamoeba* spp.), neurotrophic ulcers without clinical suspicion of superinfection, and previous antibiotic treatment at therapeutic doses. After obtaining informed consent, the collection sequence of the corneal samples were set as Swab-Needleor Needle-Swab, and was randomized to a 1:1 ratio. The residents at the emergency room who were trained on the standard study procedures, performed the sampling in all cases.

Collection procedure: After eyelid antisepsis with 10% iodine, 1 drop of topical anesthetic (Oxibuprocaine hydrochloride4 mg/1 mL + Tetracaine hydrochloride 1 mg/1 mL, double anesthetic Colircusi, ALCON) was instilled and an eye speculum was subsequently placed. After being randomized to a sampling sequence, the collection of the corneal specimen was as follows.

23 gauge needle in PBCB: The bevel of a sterile 23 gauge needle attached to a 2 mL syringe was used to scrape the edge of the ulcer edge. The sample was

then inoculated into 1.5 mL of saline solution in a sterile tube of Eppi 40 (Eppendorf International) by aspirating and injecting several times to suspend the sample. After disinfection of the BACTEC rubber lid with 96% alcohol, the suspension was aspirated 2 or 3 times and inoculated into the PBCB (Bactec, Beckton Dickinson, Ca. USA) [5].

Cotton swab with Amies transport medium: The dry sterile cotton swab that came with the kit was swept around the base and edges of the ulcer and inoculated immediately in the Amies transport medium provided in the pack (Sterile liquid sterilization transport brush, Deltalab, Barcelona, Spain) [7,8].The samples were kept at room temperature and were sent to the microbiology laboratory in less than 24 hours for processing. The PBCB was then incubated at 37ºC for 5 days. In case of a positive result using the BacT/ALERT 3D (OrganonTeknika Corp., Durham, N.C.) microbial detection system, the specimen was subjected to gram staining and subculture in solid media for subsequent biochemical identification by mass spectrometry (MALDI Biotyper CA System, BRUKER Daltonics Inc. USA). At the same time, the swab with Amies Transport Medium was inoculated on blood agar, chocolate agar, and thioglycolate broth. The blood and chocolate agar plates were incubated at 37ºC with 10% $CO^2$for 2 days. If fungal etiology was suspected, the swab was also inoculated in a tube of Sabouraud agar and incubated for 4 weeks. Antibiotic sensitivity tests were performed using the Kirby-Bauer method on Müller-Hinton agar with or without blood, depending on the isolated microorganism. Age, sex, laterality, sampling sequence, local risk factors such as: use of contact lenses, trauma, corneal foreign body, eye lid surgeries, refractive surgery or keratoplasty, were all collected in an anonymized digital database. The dataset was completed with microbiological results from the two sampling methods.

Statistic analysis: The results are shown in absolute and relative percentage frequencies for the qualitative variables and by the mean and standard deviation (SD) for the continuous quantitative variables. The results were compared by Fisher exact test or the Chi-square test for the qualitative variables and by the Student's t-test or the Mann-Whitney U test in the case of quantitative or ordinal variables, according to criteria of normality (Kolmogorov-Smirnov). Significant results with avalue of $P < 0.05$ were considered. For the statistical analysis, the MedCalc program (MedCalc®byba., Version 17.9.7,Ostend, Belgium) was used.

Results

The patient demographic and clinical characteristics along with the results are summarized in Table 1. Forty eyes of40 consecutive patients, 21 (52.5%) male and 19 (47.5%) female, were included. Average age was 46 ± 21 years. Of the 40 eyes with infectious keratitis, thirty-one (77%) had local risk factors with the

use of contact lenses being the most frequent (24 patients, 60%). Three patients (7.5%) had diabetes, as a systemic risk factor of infection. Eleven patients (27%) have received some type of topical antibiotic treatment prior to taking the sample. The samples taken with a swab and transported on Amies media presented a positivity rate of 50%. The samples taken by scraping and inoculated in PBCB obtained a positivity rate of 35%. The difference in positivity between both methods was not statistically significant (P = 0.258) (Table 2). Gram-negative bacteria were isolated in 26 (66%) of the total cases and Gram-positive in 14 (34%). *Pseudomonas aeruginosa* was the most frequently isolated microorganism in both samples. There was no case of polymicrobial or fungal etiology. The agreement between both methods was 80%, being discrepant in the rest (2 cases with different isolations for each method and 6 cases with positive swab and negative scraping). The order of the sampling method did not significantly influence the yield from swabbing (positivity of 35%versus 65%, P = 0.112, depending on whether it was done in first or second place, respectively). On the other hand, there was a significant difference in positivity rate in scraping when it was done first (50%) versus second (20%, P = 0.046).

The prior use of antibiotics significantly decreased the positivity rate of both methods, from 65% to 9% (P = 0.001) in the case of swabbing and from 44% to 9% (P = 0.034), in the case needle scraping. The results of the sample collection with both methods are presented in Table 3.

Discussion

The positivity rate reported in literature for specimens collected with a swab in Amies transport medium are comparable to the gold standard technique and can reach up to 69% [6,7]. In our study, we obtained an isolation rate of 50%, which is comparable to the series of 30 cases of corneal ulcers described by Kratz et al. However, the yield by scraping with a 23G needle with PBCB inoculation was 35%, lower than the 53% positivity in the study of Kratz et al. Further-more, the cross design of our study shows that the microbial yield is significantly lower when the scraping procedure is done after the swabbing. Similar to other studies, we get a 50% positivity rate when the scraping with a 23 G needle was performed first [5,6]. As expected, the use of antibiotic eye drops significantly decreased the frequency of microbial isolation with both methods. This also highlights the importance of collecting corneal samples before instilling empirical treatment in order to increase the likelihood of obtaining a positive microbiology result. The microorganisms isolated by both methods were similar, with a clear predominance of Gram-negative organisms (66%), most notably, *P. aeruginosa.* This is in contrast with other series in which Gram-positive cocci are the predominant microorganisms [10—13]. The high incidence of *P. aeruginosa* is related to the use of contact lenses, which is the main local risk factor in many of our cases[14]. The absence of fungal isolates

in our cohort goes in pair with the low incidence of such infections in developed countries (in an Ireland hospital, 3% of cases were found to be fungal keratitis [15]). It could be assumed that the needle scraping at the ulcer edges would allow us to obtain more reliable samples since the swab can be contaminated by adjacent structures. However, the swab allowed us to detect 3 isolates of *P.aeruginosa* that were not detected by scraping and inoculation in PBCB. Naturally, it is unlikely that *P. aeruginosa* is a contaminant of the conjunctival commensal flora. On the other hand, the isolation of opportunistic pathogens such as *Moraxella* spp. or coagulase negative staphylococci in high risk corneal ulcers should always be taken into account[16,17].

According to the results, combining both methods did not increase the sensitivity, as performing one method did not isolate additional microorganisms to the ones obtained with the other method. In conclusion, the sampling of high-risk corneal ulcers using a cotton swab and transport in Amies medium is an acceptable method comparable to 23G needle scraping and inoculation in PBCB. However, both methods are alternatives to the gold standard technique, when the plates are not available.

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Table 1 Results summary.

Patient number	Patient characteristics						Randomization	Results	
	Age (years), Sex	Laterality	High risk criteria	Previous antibiotics	Local risk factors	Systemic risk factors	Collection sequence of corneal samples (1: Swab/Needle, 2:Needle/Swab)	Results with the swab[a]	Results with the needle[b]
1	26, M	L	i, d	+	–	–	1	n	n
2	97, F	R	c, d	–	Distichiasis	–	2	*M. monlique-fasciens*	*M. monliquefasciens*
3	29, F	R	c, d	–	CL	–	1	n	n
4	55, M	L	i, c, d	–	–	DM	2	*C. gingivalis*	*Staphilococcus CN*
5	27, M	L	c, d	–	CL	–	1	*P. aeruginosa*	n
6	34, F	R	c	–	CL	–	2	*P. aeruginosa*	n
7	39, F	L	c, d	+		–	1	n	n
8	27, F	L	c	+	CL	–	2	n	n
9	24, M	L	i, c, d	–	CL	–	1	*A. pitti, Corinebac-terium sp*	n
10	30, M	L	d	–	CL	–	2	n	n
11	58, F	L	c, d	–	CL	DM	1	n	n
12	33, F	R	c, d	–	CL	–	2	n	n
13	41, M	R	c, d	–	CL	–	1	n	n
14	91, F	L	c, d	–	–	–	2	*M. lacunata*	*M. lacunata*
15	29, F	L	c	–	CL	–	1	n	n
16	24, F	L	c	–	CL	–	2	*Staphilococcus CN*	n
17	57, F	R	i, c	–	CL	–	1	*P. aeruginosa*	*P. aeruginosa*
18	25, M	R	c	–	–	–	2	*Staphilococcus CN*	*Staphilococcus CN*
19	55, M	R	i, c, d	–	CL	–	1	n	n
20	55, F	L	c	–	CL	–	2	*P. acnes, Staphilococcus epidermidis*	n
21	42, M	R	i, d	–	Hordeolum	–	1	n	n
22	58, M	L	i, c, d	–	–	GVHD	2	*S. pneumoniae*	*S. pneumoniae*

Table 1 (*Continued*)

Patient number	Patient characteristics						Randomization	Results	
	Age (years), Sex	Laterality	High risk criteria	Previous antibiotics	Local risk factors	Systemic risk factors	Collection sequence of corneal samples (1: Swab/Needle, 2:Needle/Swab)	Results with the swab[a]	Results with the needle[b]
23	42, M	L	i, c, d	+	CL	–	1	n	n
24	34, M	L	i, d	–	CL	–	2	*Staphilococcus aureus*	*Staphilococcus aureus*
25	51, M	R	i, d	+	CL	–	1	n	n
26	31, M	L	i, c, d	+	CL	–	2	n	n
27	88, M	R	c, d	–	–	–	1	*P. aeruginosa*	n
28	17, M	L	c	–	CL	–	1	*K. Pneumoniae*	*Klebsiella pneumoniae*
29	49, F	L	i, c	+	CL	–	2	*P. aeruginosa*	*P. aeruginosa*
30	25, F	R	i, c, d	+	Actinic keratitis	–	2	n	n
31	26, M	R	c, d	+	CL	–	2	*P. aeruginosa*	*P. aeruginosa, Staphilococcus CN*
32	77, F	R	c	–	CL	–	1	*P. aeruginosa*	*Staphilococcus hominis hominis*
33	41, F	R	c, d	–	CL	–	1	n	n
34	42, M	R	c, d	–	Subtarsal foreign body	–	2	n	n
35	83, M	L	i, d	+	–	–	1	*n*	*n*
36	34, M	R	i, c, d	+	–	–	1	n	n
37	61, M	R	i, c, d	–	III nerve palsy	–	1	*P. aeruginosa*	*P. aeruginosa*
38	77, F	L	i, d	+	–	–	2	n	n
39	22, F	R	i, c	–	CL	–	2	*P. aeruginosa*	*P. aeruginosa*
40	84, F	L	i, c, d	–	Palpebral surgery	–	2	*S. pneumoniae*	*S. pneumoniae*

Topical antibiotics at infraterapeutic doses. F: female; M: male; R: right eye; L: left eye; c: cells > 1+; I: infiltrate > 2 mm; d: distance from center < 3 mm; CL: contact lens; GVHD: graft vs. host disease; DM: diabetes mellitus; n: negative.

[a] Using a 23G needle bevel and inoculation into a pediatric blood culture bottle.

[b] By smear with cotton swab and transport in the middle of Amies.

Table 2 Bacterial isolation using two types of sample recollection in high-risk infectious keratitis.

Method	Scraping[a]	Smear[b]	Total	*P*
Eyes	40	40	40	
Samples (*n*)	40	40	80	
Positive culture sample	14 (35%)	20 (50%)	34	0.258

[a] Using a 23G needle bevel and inoculation into a pediatric blood culture bottle.
[b] By smear with cotton swab and transport in the Amies medium.

Table 3 Types of bacteria isolated from patients with high-risk infectious keratitis.

Name of bacteria	Scraping[a] *n* = 14 Nº (%)	Smear[b] *n* = 20 Nº (%)	Total *n* = 34 Nº (%)	*P*
Gram-negative	8 (57.14%)	14 (70%)	22 (64.70%)	0.487
P. aeruginosa	5 (41.66%)	9 (45%)	14 (41.17%)	
K. pneumoniae	1 (7.14%)	1 (5%)	2 (5.88%)	
A. pitti	–	1 (5%)	1 (2.94%)	
C. gingivalis	–	1 (5%)	1 (2.94%)	
M. lacunata	1 (7.14%)	1 (5%)	2 (5.88%)	
M. moliquefasciens	1 (7.14%)	1 (5%)	2 (5.88%)	
Gram-positive	6 (42.85%)	6 (30%	12 (35%)	1.000
Staphylococcus CN	2(14.28%)	2 (10%)	4 (11.76%)	
S. aureus	1 (7.14%)	1 (5%)	2 (5.88%)	
S. epidermidis	–	1 (5%)	1 (2.94%)	
S. hominis	1 (7.14%)	–	1 (2.94%)	
S. pneumoniae	2 (14.28%)	2 (10%)	4 (11.76%)	

[a] Using a 23G needle bevel and inoculation into a pediatric blood culture bottle.
[b] By smear with cotton swab and transport in the middle of Amies.