Differential between Infectious and Noninfectious Ulcerative Keratitis by Raman Spectra of Human Teardrops: A Pilot Study

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PURPOSE. The aim of this study was to use Raman spectra of tears to differentiate between infectious and noninfectious ulcerative keratitis.

METHODS. Raman microspectroscopy was applied using the drop-coating deposition method on Ti/Au-coated glass slides to obtain sample spectra from different human tear groups, including tears from healthy subjects and from patients with infectious and noninfectious ulcerative keratitis. By comparing the difference spectra of the groups, the authors identified local Raman features useful for differentiation of ulcerative keratitis. Principal components (PCs) of normal tears were used as asinical spectral coordinates. After performing projections of Raman spectra of both infectious and noninfectious tear samples, the authors compared the two groups to identify global spectral parameters with differential statistical significance.

RESULTS. Differentiation between infectious and noninfectious ulcerative keratitis might be made directly through observation of the normalized tear Raman spectra or the transformed principal scores. Spectral segments with differential statistical significance included 878–888 cm−1, 885–888 cm−1, 945–995 cm−1, 1007–1101 cm−1, 1074–1100 cm−1, 1090–1094 cm−1, 1096–1099 cm−1, 1386–1403 cm−1, 1463–1469 cm−1, 1469–1473 cm−1, 1557–1563 cm−1, 1584–1588 cm−1, and 1614–1621 cm−1. There were two PCs with statistically significant differences for the two groups of ulcerative keratitis, PC1 (P = 0.01) and PC2 (P = 0.05).

CONCLUSIONS. This novel approach using the analysis of Raman spectra of teardrop samples for differentiation of ulcerative keratitis demonstrates the potential application of Raman microspectroscopy for clinical practice. This technology should complement the conventional cytological method for rapid diagnosis in the clinician’s office. (Invest Ophthalmol Vis Sci. 2012;53:1436–1444) DOI:10.1167/iovs.11-7923

Infectious ulcerative keratitis (IUK), which manifests as painful eye redness and a cloudy cornea caused by infectious pathogens,1–4 is a vision-threatening disease worldwide.5–9 Noninfectious ulcerative keratitis (NIUK),10,11 which has a clinical presentation similar to that of IUK but no known infectious cause,12 is a diverse disease associated with systemic disorders and peripheral corneal invasion.13,14 The development of corneal ulceration in systemic autoimmune disease may represent the progression of a life-threatening disease.14,15 Contact lens wear16,17 and trauma12 are common risk factors18,19 for IUK and NIUK. Antimicrobial agents are used to treat IUK,20–23 whereas immunosuppressive agents are used to treat NIUK.11,24–26 Delayed diagnosis or misdiagnosis is possible because it can be difficult to differentiate between IUK and NIUK,27–29 and surgical management may be needed for both IUK and NIUK.30,31

Rapid diagnostic aid for IUK is important to reduce the complications caused by delayed diagnosis. However, few methods can diagnose IUK within the 2-hour time frame required in the ophthalmologist’s clinic.32–35 Cytological diagnosis uses light microscopy to observe stained samples from corneal scraping or impression techniques.34,35 However, the detection limit of bacterial density (≥105 counts/mL)36,37 of this method may cause sensitivity to be reduced to 40% and yield a false-negative rate of up to 60%.38 In vivo confocal microscopy is another rapid and less invasive technique for the diagnosis of IUK,39,40 however, its discriminative power is limited in relatively large microorganisms, such as Acanthamoeba species and large fungi.41,42 Although microbial culture is the gold standard for the diagnosis of IUK, the incubation time is at least 2 days for rapidly growing bacteria and up to 2 to 4 weeks for viruses, fungi, and mycobacteria.43 Polymerase chain reaction (PCR)-based methods can be considered a new standard for IUK diagnosis, which might shorten the diagnostic time to 4 to 8 hours,44,45 but they still do not suit the need for more rapid diagnosis in a clinic.

Teardrops, which contain shed microbes and their metabolites,46–49 may be a good target by a scraping-free and less invasive collection way to differentiate IUK from NIUK. Changes in the intrinsic tear components and their concentrations may also reflect the defense mechanisms of the ocular surface against microbes or other stimuli.50–58 Lysozyme, a protective protein in tears, has been used to distinguish patients with herpes simplex virus (HSV)-caused keratitis from healthy subjects.50 Levels of leukotriene B4 and platelet-activating factor in the tears of patients with contact lens–induced peripheral ulcers were also reported to be higher than those in the tears from the control subjects.58

Raman spectra were first discovered by Raman and Krishnan in 1928.59 Raman microspectroscopy is a novel technology that
applies the inelastic scattering of light to qualitatively and quantitatively detect the molecular composition of matter in real time. Raman microspectroscopy can detect the scattering photons and provides molecular information through changes in the levels of vibrating energy following the monochromatic laser photons in the visible light spectrum, impacting the detected sample. Many studies have shown its potential application in different biomedical investigations, including distinguishing microbes and their metabolites. Among these, Raman microspectroscopy was recently determined to be a powerful tool for detecting tear components. Zhang et al. have shown that a high efficiency of drop-coating deposition Raman (DCDR) can be achieved by the deposition of aqueous samples on substrates with specific surface properties. Filink and Stone demonstrated that a Raman signal with a high signal-to-noise ratio was obtained from 1.5 μL dried teardrop for the detection of major tear components using the DCDR technique.

Principal component analysis (PCA) is a multivariate statistical technique used in almost all scientific disciplines. PCA analyzes a data matrix that represents the observations recorded by several dependent or intercorrelated variables. Its goal is to extract dominant information from the complicated data matrix and to express the features as a set of new orthogonal variables called principal components. In other words, PCA greatly concentrates many less informative dependent variables (dimension n) of a data matrix to few more informative principal components (dimension m), which may represent the important features (number of features, m ≪ number of variables, n) of this matrix. Therefore, PCA is widely used in feature extraction.

The aim of this study was to discover the differentiation potential of human tear DCDR to identify optical fingerprints to further aid the differentiation between IUK and NIUK; this will be useful in the future verification study for clinical application.

**METHODS**

**Patients**

Tear samples were prospectively collected from August 1, 2009, to December 1, 2011, from patients in the Chang Gung Memorial Hospital (CGMH), Kaohsiung Medical Center, including tears from healthy eyes (Norm group) and tears from diseased eyes with acute ulcerative keratitis (UK). All procedures involving human subjects adhered to the Declaration of Helsinki and were approved by the Committee of Medical Ethics and Human Experiments of CGMH. Informed consent was obtained from each subject in the Kaohsiung Medical Center of CGMH.

**Collection of Clinical Data**

The eyes of the Norm group were healthy on the ocular surface based on biomicroscopy evaluation. For each patient, local and systemic risk factors of UK were recorded after considering the patient’s history and findings from ocular examination. Risk factors were contact lens wear, corneal trauma (foreign body trauma, previous corneal surgery), systemic disease (such as autoimmune, diabetes, and immunosuppression), ocular surface and corneal diseases (such as blepharitis, trichiasis, limbal stem cell deficiency, and allergic keratoconjunctivitis), and previous microbial keratitis (such as herpetic keratitis and bacterial keratitis). We included patients with UK, whose clinical presentation included corneal epithelial defects with stromal inflammation and infiltrates. Patients younger than 9 years of age were excluded, as were patients who had received topical fluorescein staining before the collection of tears.

**Definition of Ulcerative Keratitis with or without Infection**

We classified UK into two groups: infectious UK (IUK group) and noninfectious UK (NIUK group). UK with infection is defined as any positive finding identified in the microbial survey protocol for confirmation of infectious keratitis for originally suspected infectious keratitis. This protocol includes cytological evaluation, microbial culture, and HSV PCR of the corneal scraping samples. For cytological evaluation, the Gram and acid-fast stains were used for immediate microscopic evaluation. If the diameter or the maximal length of the ulcer was <2 mm, the cytological evaluation was omitted to preserve the samples for microbial culture or HSV PCR (in-house real-time PCR for HSV types 1 and 2). Routine microbial culture included aerobic culture (blood agar and chocolate blood agar), anaerobic culture (CDC anaerobe 5% sheep blood agar, phenylethyl alcohol blood agar, and bacteroides bile esculin agar), mycobacterial culture (LowensteinJensen slant), and fungal culture (Sabouraud’s dextrose agar). On any specific suspicion of atypical corneal ulceration at the initial visit, we also performed Acanthamoeba culture (nonnutrient agar with Escherichia coli overlay) and HSV PCR. If treatment took an unexpected clinical course, we repeated the standard microbial survey and added Acanthamoeba culture or HSV PCR. UK without infection is defined as any positive findings by the microbial confirmation protocol for presumed microbial keratitis at the initial visit, smooth tapering of antimicrobial agents, and no occurrence of unexpected events until smoothing of the ocular surface.

**Collection of Human Tears**

To minimize interference from topical compounds in case of pretreated patients, tear collection was performed at least 1 hour after topical medications were stopped. Tears were gently collected from all participants under 16× magnification with a slit lamp using disinfected micropipettes (Hirschmann Laborgeräte GmbH & Co. KG, Eberstadt, Germany) with modified head curvature. Tear samples (5–10 μL) were collected and stored at 4°C immediately before spectral acquisition using Raman microspectroscopy within 2 weeks.

**Dried Teardrop Preparation for Raman Spectral Acquisition**

We applied Raman microspectroscopy (Thermo Fisher Scientific Inc., Waltham, MA) based on the DCDR method with Ti/Au-coated glass slides with slight modification, as described in our previous study. In brief, 1.5 μL tear sample was dropped onto a clean glass slide coated with 30/150 nm Ti/Au. The slide was placed in a closed chamber and allowed to dry at room temperature with 48% relative humidity. Raman spectra in the central zone of the dried teardrop were sampled by a line-mapping procedure. Poorly formed dried teardrops resulting from inappropriate pipette control on the Ti/Au-coated glass slide were excluded from further spectral collection. Raman spectra of the tear samples were collected under the following experimental conditions: 633 nm He-Ne laser excitation, laser power at the sample position of approximately 7 mW, and a 50× objective lens with a connected charge-coupled device for scattered light collection. The Raman shift was recorded using a signal of 520 cm⁻¹ generated from a silicon wafer. A laser exposure time of 10 seconds with a signal accumulation of three times was applied for each spectral acquisition point. Post-processing of the spectra was conducted as described elsewhere.

**Statistical Analysis**

For each subject, 10 Raman spectra in the central zone of a dried teardrop were collected for analysis. We collected Raman signals in the range of 700 to 1800 cm⁻¹ for analysis and used a spreadsheet (Excel 2007; Microsoft Corporation, Redmond, WA) as a graphic tool. Mean spectra of tear samples from the three groups (NIUK-Norm, IUK-Norm, and IUK-NIUK) and difference spectra were visually compared to determine the candidate parameters of differential Raman shifts and those of differential segments of the Raman spectra. Integ
didate segments were formatted as new parameters for differential purposes. These local spectral parameters were then univariantly tested with the Mann-Whitney U test for the two diseased groups. We used technical computing software (MatLab 2008a; The MathWorks Inc., Natick, MA) as the multivariable analytical tool for PCA to extract the global spectral features of tears from the normal tear group. The determined principal vectors were used as the transfer function to determine the principal component (PC) scores for each spectrum of each subject. The first few PCs for which the relevance cumulated to 80% were chosen as candidate global parameters. The mean scores of these candidates in the two diseased groups were compared univariately by one-tailed t-test to evaluate their statistical significance. Analysis of subgroups in IUK samples were based on candidate global parameters with statistical significance.

RESULTS

Clinical Data Analysis

There were 30 subjects in the Norm tear group, 26 patients in the IUK group, and 26 patients in the NIUK group (Table 1). There was no statistically significant difference with respect to sex (χ² = 0.67), age (one-way ANOVA, P = 0.68), or laterality of the eye (χ²; P = 0.85) among the three groups. Comparison of the two groups indicated that contact lens wear and corneal trauma were the most common risk factors for both. Although there was no statistical significant difference for the overall frequencies of these risk factors (χ²; P = 0.78), ocular surface diseases were more frequent in the NIUK group and previous UK was more frequent in the IUK group. Because CGMH is a tertiary medical center, several patients had received antimicrobial medication before they participated in this study. However, there was no statistically significant difference for both groups (χ²; P = 0.97). Analyzing the morphologic factors for UK indicated a tendency for ulceration than the NIUK group. There was a tendency for boundary pattern because the IUK group had more elliptical shape (P = 0.97). There was no statistically significant difference with respect to age (one-way ANOVA, P = 0.68), or location (χ²; P = 0.27). The identified pathogens and risk factors for the IUK group are summarized in Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7923/-/DCSupplemental.

Comparison of Raman Spectra of Samples from the Normal Tear Group, NIUK Group, and IUK Group to Select Local Features in Spectra for IUK Identification

The mean Raman spectra from the Norm, NIUK, and IUK tear groups showed similar patterns, but several different features can be pointed out clearly (Fig. 1a). There were two small peaks on the segment 753–757 cm⁻¹ in the IUK group, whereas there was only one small peak on the same band in the NIUK and Norm groups. Upward deviation was revealed from the end of segment 1074–1100 cm⁻¹ to the start of segment 1176–1182 cm⁻¹ in the IUK group, whereas negative deviation was observed on the same region in the other two groups. There were two characteristic peaks on the segments 1227–1241 cm⁻¹ and 1275–1291 cm⁻¹ in the IUK group. Although there were other local features among the three groups, we did not describe them one by one to avoid redundancy.

The patterns of difference spectra in Figure 1b were similar between the IUK-Norm and IUK-Norm spectra, whereas the oscillating pattern around the baseline was shown in the NIUK-Norm spectrum. These difference spectra indicated the IUK group is clearly different, either from the NIUK group or from the Norm group. Based on Figure 1b, we plotted the red and blue vertical bands for all spectral figures: the red vertical bands indicate that there were visible differences in any two groups. The blue vertical bands indicate that there were visible differences only between the IUK group and the NIUK group and between the IUK group and the Norm group. We statistically tested these selected local features between the IUK group and the NIUK group and found many features with significant differences (Sup-

### Table 1. Clinical Data of the Three Participant Groups

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Norm Group</th>
<th>IUK Group</th>
<th>NIUK Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case number</td>
<td>30</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Age, y</td>
<td>42.7 ± 13.5</td>
<td>46.5 ± 17.8</td>
<td>46.1 ± 21.5</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>15/15</td>
<td>9/17</td>
<td>12/14</td>
</tr>
<tr>
<td>Laterality, OD/OS</td>
<td>16/14</td>
<td>11/15</td>
<td>12/14</td>
</tr>
<tr>
<td>Risk factors for keratitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact lens wear</td>
<td>9</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Corneal trauma</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Systemic disease</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ocular surface and corneal disease</td>
<td>2</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Previous ulcerative keratitis</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Topical antimicrobials before visit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Yes, ≤3 days</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Yes, &gt;3 days</td>
<td>12</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Boundary pattern of ulcer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near elliptical shape</td>
<td>19</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Nonelliptical shape</td>
<td>7</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Area of ulcer, mm²</td>
<td>10.2 ± 9.3</td>
<td>6.5 ± 10.8</td>
<td></td>
</tr>
<tr>
<td>Depth of ulcer (anterior one-third/posterior two-thirds)</td>
<td>17/9</td>
<td>21/5</td>
<td></td>
</tr>
<tr>
<td>Location of ulcer (central/paracentral/peripheral)</td>
<td>12/11/3</td>
<td>8/9/9</td>
<td></td>
</tr>
</tbody>
</table>

Anterior one-third, infiltrates involved within anterior one-third corneal thickness by slit-lamp evaluation; posterior two-thirds, infiltrates deeper than anterior one-third corneal thickness; central, stromal infiltrates involving the central 3-mm diameter; paracentral, stromal infiltrates involving the central 6-mm diameter without involving the central 3-mm diameter; peripheral, stromal infiltrates involving only the corneal region outside the central 6-mm diameter of the cornea.
The tested segments with differential potential included 878/11011, 888 cm⁻¹/11002, 885/11011, 888 cm⁻¹/11002, 945/11011, 993 cm⁻¹/11002, 1007/11011, 1015 cm⁻¹/11002, 1074/11011, 1100 cm⁻¹/11002, 1176/11011, 1182 cm⁻¹/11002, 1227/11011, 1241 cm⁻¹/11002, 1386/11011, 1403 cm⁻¹/11002, 1463/11011, 1469 cm⁻¹/11002, 1469/11011, 1473 cm⁻¹/11002, 1557/11011, 1563 cm⁻¹/11002, 1584/11011, 1588 cm⁻¹/11002, 1614/11011, 1621 cm⁻¹/11002, and 1690/11011, 1094 cm⁻¹/11002, 1094/11011, 1100 cm⁻¹/11002, 1096/11011, 1099 cm⁻¹/11002, 1138/11011, 1140 cm⁻¹/11002, 1146/11011, 1149 cm⁻¹/11002, 1469/11011, 1473 cm⁻¹/11002, 1557/11011, 1563 cm⁻¹/11002, 1614/11011, 1621 cm⁻¹/11002.

Extraction of Global Features of Raman Spectra from Normal Tears by PC Transformation

Ten Raman spectra in the central zone of a dried teardrop for each subject in the normal control group were collected (300 spectra sampled from 30 normal teardrops) to establish the transfer function of Raman spectra of tear samples (Fig. 2a). Because the relevance cumulated from PC1 to PC6 was approximately 80%, as indicated in Figure 2b, we recognized the six new variables as global features of Raman spectra of tear samples with 95% confidence intervals of different tear groups (b).

Comparison of Infectious and Noninfectious UK by Raman PCs of Tear Samples

After transformation of the mean spectra (N = 10 for each case) to the PC domain, we compared the Norm-based principal scores of the first six PCs between the IUK and NIUK groups (Fig. 3). There were two PCs with statistically significant differences for the two groups, PC1 (P = 0.01, power = 0.98) and PC2 (P = 0.05, power = 0.98). There was a greater trend in the IUK group than in the NIUK group for PC4 (P = 0.09, power = 0.87), but there was no statistically significant difference for PC3, PC5, and PC6. We focused further on the three potential PCs (corresponding principal vectors shown in Supplementary Fig. S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-7923/-/DCSupplemental). The transformed scores of the six PCs were used for the subsequent comparative analysis.

**FIGURE 1.** Mean Raman spectra of tear samples with 95% confidence intervals of different tear groups, including healthy subjects (300 spectra of 30 subjects), noninfectious keratitis (260 spectra of 26 patients), and infectious keratitis (260 spectra of 26 patients) (a). Difference in the Raman spectra of tear samples with 95% confidence intervals of different tear groups (b).
doi:10.1167/iovs.11-7923/-/DCSupplemental) to analyze the subgroups in the IUK group.

**Estimation of Mean Scores of Significant PCs for Subgroups of IUK**

In Figures 4a, 4c, and 4e, indicate the score difference in the selected PCs for the classification of the pathogens in this study. Because there were only two cases of *Acanthamoeba* spp. and two cases of HSV, we focused on determining score deviations from the Norm group in the fungal and bacterial groups. For the PC1 and PC4 scores, there were stronger positive deviations from the normal mean score (zero score) in the bacterial and fungal groups. In contrast, there was no apparent deviation from the zero score in the bacterial and fungal groups for the PC2 score. For plots Figures 4b, 4d, and 4f, we focused on the subgroup analysis for the bacteria group. There were similar positive deviations for PC1 and PC4 scores for the Gram-positive cocci (GPC) group and Gram-negative bacilli (GNB) group. For the PC2 score, both the GPC and GNB groups did not show apparent deviation from the zero score. Therefore, both the PC1 and the PC4 scores might be useful indicators to differentiate bacterial and fungal tears from normal tears. Joining the result in the differentiation between the IUK group and the NIUK group, the PC1 score might be a bifunctional indicator to differentiate IUK tears either from NIUK tears or from normal tears.

**DISCUSSION**

It is critical to determine whether UK is caused by infection. In addition to different management strategies for IUK and NIUK, a rapid diagnosis to minimize ulcerative complications such as central, deep corneal involvement, scarring severity, and irregular astigmatism is critical to avoid vision loss. Conventional cytological examination is quick but limited by microscope levels and invasiveness in the tissue scrapings for use in routine clinical practice. In this study, we propose a novel noninvasive approach using analysis of Raman spectra of dried teardrops to complement the cytological approach. The differentiation between IUK and NIUK might be directly made through observation of the normalized tear Raman spectra or the transformed principal scores. Global spectral parameters of the tear samples were transformed from PCA, and the local spectral parameters were selected from difference spectra by visualization methods. We found some of these candidate parameters had statistical differences between the two disease groups. This approach may be a good adjunct diagnostic technique to promote diagnostic quality for UK.

Willemse-Erix et al.95 showed that Raman spectroscopy is a real-time typing method for classifying clinical isolates of methicillin-resistant *Staphylococcus aureus* with an accuracy of up to 95%. A 1.5-µL teardrop sample from a healthy human subject is sufficient for Raman signal detection of major tear components with a high signal-to-noise ratio and for reproducibility, as shown by Filik and Stone.83 Reyes-Goddard et al.96 demonstrated the discrimination potential of Raman spectra of tear samples for HSV by using a synthetic tear model on silver mirror reaction glass slides and gold thin film. The mean diagnostic sensitivity and specificity were estimated between 75% and 80%. Rossi et al.92 demonstrated that Raman spectroscopy can be used to differentiate endophthalmitis from uveitis in a rabbit model. In adjoining principal components PC3 and PC4 using Mahalanobis distance as a discriminator, the diagnostic model showed sensitivity of 89%, specificity of 100%, and accuracy of 92% by a cross-validation procedure. In our study, we used gold thin film as a substrate for a 1.5-µL dried teardrop sample...
to differentiate IUK from NIUK by Raman spectroscopy, which supported the potential clinical application of this optical finger printing technology. We identified a set of global spectral features for the teardrop samples based on principal components and several local spectral features with differential potential shown in statistics.

This study focused on the discovery of clinical application of Raman spectra analysis of tear samples to differentiate UK with and without infection. We hypothesized that several low-concentration products may be released because of interactions between tears and microbes, which may be different between IUK and NIUK. Although there might be microbial involvement in the pathogenesis of NIUK, the quorum-sensing effect likely dominates in IUK.

There are some limitations to this pilot study. The number of cases may be too few; hence, some spectral parametric candidates might not show statistical significance. We objectively defined IUK and NIUK based on the strict results of a conventional microbial workup protocol. However, presumed microbially driven UK is often only culture-positive in 40% to 60% of cases. UK without infection as defined might be caused by infectious entities, such as those seen in a case report of microbial keratitis initially diagnosed as a contact lens peripheral ulcer. This study took place at a tertiary medical center; only approximately one-fourth of the patients had not yet received treatment before visiting our hospital. Some patients in the NIUK group might initially have had microbial infection, which might make the validity in clinical human

**FIGURE 4.** Comparison of 95% confidence intervals of the significant scores between the IUK and Norm groups. Exclusive comparisons among different microbial subgroups of IUK for the scores of PC1 (a), PC2 (c), and PC4 (e). Inclusive comparisons among overlapping bacterial subgroups for the scores of PC1 (b), PC2 (d), and PC4 (f).
tears lower than those shown in synthetic tears\textsuperscript{46} and in animal vitreous fluid\textsuperscript{12} for differentiation. In spite of the natural limitations mentioned, by combining concepts from quorum sensing with those from flora adaptation on the ocular surface,\textsuperscript{100,103} the true classification of IUK and NIUK might overlap in the same spectrum. The summation influence from different arms and different levels controlled by microbial cross-talk, host factors, and premedications determines UK of infectious or noninfectious status. The value of differentiation into the two groups may be a useful aid for diagnosis of different stages of microbial contact and for the rapid justification of systemic survey of life-threatening disorders. As such, Raman spectra analysis of teardrops may improve diagnostic quality, rather than replace cytology, culture techniques, and PCR-based methods. In the future, this technique may be further developed as a diagnostic tool for pathogens through the accumulation of more cases with the same infectious entities, such as \textit{Pseudomonas} keratitis or \textit{Staphylococcus} keratitis.

In conclusion, this novel approach of Raman spectra analysis of tear samples for differentiation of UK showed the potential application of Raman microspectroscopy for clinical practice. We suggest that the resolution of tear Raman spectra for pathogenic diagnosis will be gradually improved in the near future through the study of more cases in the IUK subgroups.

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\textbf{References}


