EVALUATION OF NESTED PCR TECHNIQUE FOR DETECTION OF *PYTHIUM INSIDIOSUM* IN PATHOLOGICAL SPECIMENS FROM PATIENTS WITH SUSPECTED FUNGAL KERATITIS

Panida Kosrirukvongs[1], Angkana Chaiprasert[2], Mongkol Uiprasertkul[3], Metinee Chongcharoen[1], Ramrada Banyong[2], Theerapong Krajaejun[4] and Wanchai Wanachiwanawin[5]

[1]Department of Ophthalmology, [2]Department of Microbiology, [3]Department of Pathology, [5]Department of Medicine, Faculty of Medicine Siriraj Hospital; [4]Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Abstract. Diagnosis of *Pythium* keratitis is problematic due to the difficulty in obtaining a culture report resulting in unnecessarily prolonged usage of antimicrobial medication due to misdiagnosis. This study evaluated and compared nested PCR technique with culture and immunoperoxidase staining assays of *Pythium insidiosum* in paraffin-embedded corneal tissues from patients with suspected fungal keratitis. Six of 51 pathological reports compatible with fungal infection and 6 of 48 culture-proven fungal keratitis were identified as *Pythium*. Twenty-seven specimens were PCR-positive for *Pythium insidiosum*. In comparison with fungal culture for *P. insidiosum*, PCR had 83% sensitivity and 77% specificity with fair agreement (Kappa score of 0.227, $p = 0.001$). The mean age of PCR-positive is younger than PCR-negative group and there is a female preponderance in *Pythium*-infected group ($p = 0.002$ and $p = 0.004$, respectively). Nineteen specimens had positive results using immunoperoxidase staining assay with fair agreement to culture method (Kappa 0.340, $p < 0.001$), and 83% sensitivity, 85% specificity and 85% accuracy (95% CI: 76.7-90.7). PCR-based technique compared with culture and/or immunoperoxidase staining assay had 91.7% sensitivity, 81.8% specificity and 83% accuracy (95% CI: 74.5-89.1) with moderate agreement (Kappa 0.477, $p < 0.001$). Thus nested PCR detection of *P. insidiosum* should be employed in preliminary diagnosis of *Pythium* keratitis in order to initiate proper management.

Keywords: *Pythium insidiosum*, fungal keratitis, nested PCR, paraffin-embedded tissue, immunoperoxidase staining assay

Correspondence: Dr Panida Kosrirukvongs, Department of Ophthalmology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok Noi, Bangkok 10700, Thailand.
Tel: 66 (0) 2419 8037; Fax: 66 (0) 2411 1906
E-mail: panida.kos@mahidol.ac.th