DEVELOPMENT OF IN-HOUSE MULTIPLEX AND QUANTITATIVE PCR ASSAYS FOR DETECTION OF *PNEUMOCYSTIS JIROVECII* IN BRONCHOALVEOLAR LAVAGE AND SPUTUM SPECIMENS

Patsharaporn Techasintana Sarasombath[1], Sirirat Wijit[1], Pisith Chinabut[1], Jerawan Ongrotchanakun[1], Ladawan Khowawisetsut[1], Suporn Foongladda[2], Patimaporn Wongprompitak[3] and Darawan Wanachiwanawin[1]

[1]Department of Parasitology, [2]Department of Microbiology, [3]Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

Abstract. A definite diagnosis of *Pneumocystis* pneumonia requires laboratory confirmation of the presence of *Pneumocystis jirovecii* in clinical specimens. In-house multiplex and quantitative (q)PCR assays were developed to detect *P. jirovecii mtLSU rRNA* in bronchoalveolar lavage (BAL) and sputum specimens in comparison with three standard cytological assays [Gomori's methenamine silver (GMS) staining, Giemsa staining and immunofluorescence assay (IFA)] and conventional PCR. Both in-house multiplex and qPCR assays demonstrated specific of *P. jirovecii* without cross-amplification with other organisms. In BAL samples (n = 131), median copies/μl of *P. jirovecii mtLSU rRNA* DNA (measured by in-house qPCR) in samples positive by GMS staining (37%), Giemsa staining (37%) and IFA samples (42%) were $4.5x10^5$, $3.8x10^5$ and $3.8x10^5$, respectively; and in sputum samples (n = 54), median value in 4, 4 and 7 % positives was $5.5x10^5$, $5.5x10^5$ and $2.2x10^5$ copies/μl, respectively. Using conventional PCR median copies/μl *P. jirovecii mtLSU rRNA* DNA in BAL (49% positive) samples compared to in-house multiplex PCR (43% positive) were $2.5x10^5$ and $3.1x10^5$ respectively, while in sputum samples (17 and 13% positives) were $4.3x10^4$ and $4.3x10^4$ respectively. PCR methods showed higher sensitivity in *P. jirovecii* detection in both type of specimens compared to the classical staining methods. Overall, qPCR was considered the most promising diagnostic method due to its sensitivity and quantitative ability.

Keywords: *Pneumocystis carinii*, *Pneumocystis jirovecii*, *Pneumocystis* pneumonia, cytological staining, immunofluorescence, multiplex PCR, qPCR

Correspondence: Darawan Wanachiwanawin, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
Tel: +66 (0) 2419 6468; Fax: +66 (0) 2419 6470
E-mail: darawan.wan@mahidol.edu