

**TITLE OF THE STUDY:** Evaluation of a real-time quantitative PCR assay for diagnosis of *Pneumocystis jirovecii* pneumonia in immunocompromised patients – a pilot study.

**DEPARTMENT:** Clinical Microbiology

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**DEGREE AND SUBJECT:** MD, Microbiology

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### **INTRODUCTION:**

*Pneumocystis jirovecii* is an important opportunistic pathogen causing pneumocystis pneumonia (PCP) in both HIV infected and non-HIV infected immunosuppressed individuals. Since this cannot be cultured in vitro, visualisation of organism by microscopic method using various staining techniques is considered as a gold standard test for diagnosis of PCP. On account of microscopic methods having low sensitivity, molecular method is a way forward technique for diagnosis.

### **OBJECTIVES:**

To evaluate a real-time quantitative PCR (qPCR) assay for diagnosis of Pneumocystis pneumonia (PCP) in immunocompromised patients and compare with conventional microscopic methods such as Giemsa staining and direct fluorescent antibody (DFA) test as well as correlate with clinical stratification of the disease.

To evaluate cost effectiveness of qPCR assay when compared to the conventional microscopic methods currently used for routine diagnostics

### **METHODOLOGY:**

This is a prospective study done over a period of one year, in which 100 respiratory samples from immunocompromised patients who suffered from respiratory illness with PCP as one of

the differential diagnosis was collected. A qPCR assay was evaluated based on previously described method. The samples were subjected to both molecular (qPCR) and conventional microscopic methods. Patients were categorized into definite PCP, probable PCP and non PCP according to European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) clinical criteria, based on symptoms, signs, radiological features and response to anti-pneumocystis treatment. Results were compared with this clinical stratification of the disease.

Data analysis was done using statistical software STATA 13.1.

## **RESULTS:**

Among 100 patients, 30 were HIV infected and 70 were non-HIV infected immunocompromised patients. By EORTC criteria, five patients were categorized into definite PCP, 20 in probable PCP and 74 patients in non PCP group. Giemsa staining was negative in all samples. DFA was positive in all five patients of definite PCP (100%) but negative in probable and non-PCP group. The qPCR was positive in three patients (60%) from the definite PCP, four from (20%) the probable PCP and one (1.33%) from the non-PCP group. Over all sensitivity, specificity of DFA and qPCR were 20%, 100% and 28%, 98.67% respectively. Cost effectiveness analysis found that qPCR assay is cheaper than DFA test.

## **CONCLUSION**

Real time PCR is a cost effective and highly specific test for routine diagnosis of PCP

**Keywords:** PCP, *Pneumocystis jirovecii*, EORTC criteria, HIV infected, non-HIV infected immunocompromised patients.