Changi General Hospital
SingHealth

Tel: (65) 6788 8833
Fax: (65) 6788 0933
Changi General Hospital
2 Simei Street 3
Singapore 529889
www.cgh.com.sg
Reg No 198904226R

Test Name	Pneumocystis jirovecii (carinii) PCR
Specimen Type	**Preferred specimens:** Broncho-alveolar lavage (BAL) **Other specimens:** Endotracheal aspirate (ETTA) Sputum, induced Sputum, expectorated Nasopharyngeal aspirates (unvalidated sample type, the test performance characteristics are not relevant to this sample type)
Special Instructions For Laboratory	-
Specimen Storage And Transport	Collect at least 1 ml in sterile container. Sample should reach the laboratory as soon as possible, preferably within 24 hours after collection. Otherwise, store specimen at 4°C and transport to CGH laboratory as soon as possible.
Specimen Minimum Volume	1 ml
Test Method	Real-time PCR (Lab developed test) (non-FDA approved) This is an in-house developed assay based on published data, which detects the beta-tubulin gene (single copy) and mtLSU gene (multi-copy) present in Pneumocystis jirovecii. The improved assay tests for both the beta-tubulin (single copy gene) and mtLSU (multicopy gene) targets, to improve the in-vitro sensitivity of the PCR. References: 1. Brancart F, Rodriguez-Villalobos H, Fonteyne PA, Peres-Bota D, Liesnard C. Quantitative TaqMan PCR for detection of Pneumocystis jiroveci. J Microbiol Methods. 2005 Jun;61(3):381-7. PubMed PMID: 15767014. 2. Botterel F, Cabaret O, Foulet F, Cordonnier C, Costa JM, Bretagne S. Clinical significance of quantifying Pneumocystis jirovecii DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. J Clin Microbiol. 2012 Feb;50(2):227-31.
Expected Result	DNA loads will be reported as "Very Low", "Low", "Moderate" or "High". The PCR report incorporates semi-quantitative reporting based on the single copy beta-tubulin gene to provide a crude estimation of the Pneumocystis DNA load present in the sample. DNA loads will be reported as "Very Low", "Low", "Moderate" or "High". Based on samples for which parallel PCR/Microscopy testing was performed, a DNA load reported as "Moderate" or "High" would generally also be positive for PCP by microscopy, while DNA loads of "Very Low" or "Low" would almost always be negative by microscopy. It is important to recognise the performance of PCP microscopy will also depend on the microscopic testing modality that was used (e.g. immunofluorescence v.s. silver staining).

Changi General Hospital
SingHealth

Tel: (65) 6788 8833
Fax: (65) 6788 0933
Changi General Hospital
2 Simei Street 3
Singapore 529889
www.cgh.com.sg
Reg No 198904226R

Reference Ranges	TEST INTERPRETATION Low and Very Low Pneumocystis DNA loads must be interpreted in context with available clinical, radiological and other laboratory investigations. Pneumocystis colonization (without infection) is reported in various patient groups, including those with underlying lung disease (e.g. COPD). Infection in the non-HIV population is associated with lower pathogen loads. Risk factors for infection include 1) haematological malignancy, 2) solid tumours with high-dose chemotherapy or prolonged steroid therapy (e.g. >30 mg prednisolone>4 weeks) 3) solid-organ transplantation with CD4 + lymphopenia 4) autoimmune disease with low CD4+ count and receipt of immune-modulatory agents Disease in non-HIV infected patients appears to have more rapid onset and/or progression with shorter duration of symptoms. The radiographic appearances in non-HIV patients generally show diffuse infiltrates with ground-glass opacities and patchy consolidation. (ref: Exp Rev Anti-infect Ther, 17:10, 787-801)
Turn Around Time	1-3 days
Days Of Testing	Monday - Saturday
Hospital	CGH
Laboratory	Microbiology Lab
Discipline	Microbiology
Contact Details	68504935 / 68504936

Changi General Hospital
SingHealth

Tel: (65) 6788 8833
Fax: (65) 6788 0933
Changi General Hospital
2 Simei Street 3
Singapore 529889
www.cgh.com.sg
Reg No 198904226R

Clinical Information	**SUMMARY OF TEST** Pneumocystis is a fungus with worldwide distribution. Serological evidence indicates that most healthy persons have been exposed by 3-4 years of age. Pneumocystis pneumonia (PCP) occurs in immunosuppressed individuals and in premature, malnourished infants. PCR has been demonstrated to show high sensitivity and specificity when used for the detection of PCP in HIV-immunosuppressed patients, when compared to conventional microscopic diagnosis. When compared to microscopic methods, the greater detection capability of real-time PCR assays means that a proportion of samples may be positive by real-time PCR but negative by microscopy. A study in 2012 using an expanded laboratory "gold standard" (which included a second PCR assay) concluded that PCR testing showed a very high negative predictive value, thereby reliably excluding pneumocystis pneumonia. Studies using PCR for the detection of PCP usually identify a subset of patients that are microscopy (-) but PCR (+). A subset of these cases is identified as likely or confirmed cases of PCP infection, while the remainder are interpreted as colonisation. Other studies have demonstrated that Pneumocystis DNA may also be detectable in patients with chronic lung disease and in individuals with no apparent disease. The exact role of Pneumocystis colonisation remains to be clearly defined in non-immunosuppressed individuals. Crude quantitation of PCP fungal load has been attempted in some studies evaluating PCR diagnosis. A summary of the relevant conclusions is listed below: a) the fungal load during PCP can vary according to the population of the patients tested but is often higher in HIV-infected patients b) the fungal burden is lower in colonized patients compared to that in patients with PCP infection c) it is not possible to determine a semi-quantitative cut-off that reliably discriminates between colonisation and infection d) many other variables affect the quantitation of fungal load (lack of standardization of pulmonary samples, quality of tested samples, fungal polymorphism) Despite these limitations, provision of semi-quantitative information on fungal load provides additional information to the clinician when determining the significance of a positive result for PCP DNA. **LIMITATIONS** The clinical performance of this real-time PCR has only been evaluated from BAL and ETTA samples. The performance of real-time PCR has been demonstrated to be equivalent between lower and upper respiratory tract specimens for the diagnosis of PCP in children. However, no equivalent data is available for this PCR performed in CGH. In particular, nasopharyngeal aspirates should be avoided unless no other sample is available, and results from these sample types should be interpreted with caution.
Link Out For Additional Information	-

Changi General Hospital
SingHealth

Tel: (65) 6788 8833
Fax: (65) 6788 0933
Changi General Hospital
2 Simei Street 3
Singapore 529889
www.cgh.com.sg
Reg No 198904226R

Remarks	A total of 65 respiratory specimens from immunocompromised patients were tested by this real time PCR assay and by the reference method, microscopy and/or 2nd PCR method. Relative to microscopic results, this assay demonstrated sensitivity and specificity of 100% and 67%, respectively. When compared with microscopy/2nd PCR assay, this assay demonstrated 100% sensitivity and 100% specificity. The limit of detection of the assay is equivalent to 6-7 PCP organisms. The clinical performance of this real-time PCR has only been evaluated from BAL , ETTA and sputum samples. Pneumocystis may be detectable in patients with chronic lung disease and in individuals with no apparent disease. Positive results, especially those with low fungal loads, must be interpreted in the context of patient symptoms in order to be able to distinguish colonization from infection.