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Evaluation of a novel RIDA®GENE Pneumocystis jirovecii real-time PCR assay for the diagnosis of Pneumcystis jirovecii pneumonia

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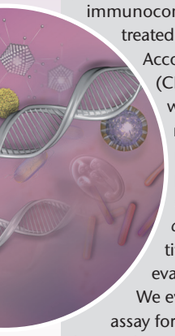
Objectives



Pneumocystis pneumonia (PCP), caused by the fungus *Pneumocystis jirovecii* (former *P. carinii*), is an important opportunistic infection in immunocompromised patients like HIV/AIDS patients, chemotherapy-treated patients and patients receiving an organ transplant.

According to the Centers for Disease Control and Prevention (CDC), *Pneumocystis jirovecii* causes 100 % mortality in patients without treatment and the mortality rate in immunocompromised patients is between 5 - 40 % in treated patients.[1] The mortality from *Pneumocystis jirovecii* in HIV-uninfected patients can be as high as 40 %.[2] Diagnosis of pneumocystosis usually relies on microscopic demonstration of *Pneumocystis jirovecii* in respiratory samples. Detection by quantitative PCR is faster and more sensitive than microscopic evaluation.

We evaluated the RIDA®GENE Pneumocystis jirovecii real-time PCR assay for the qualitative, direct detection of *Pneumocystis jirovecii* from human bronchoalveolar lavage fluid (BAL).

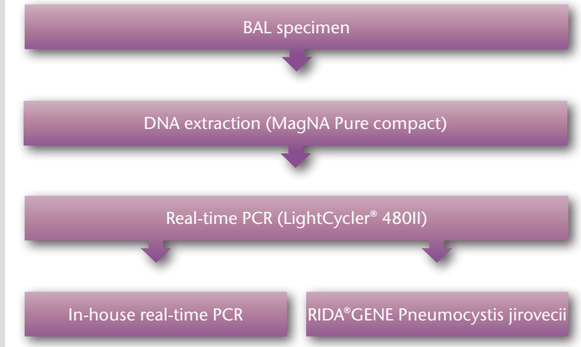
Methods

RIDA®GENE Pneumocystis jirovecii is a quantitative assay targeting the mitochondrial large subunit (mLSU) of *Pneumocystis jirovecii* with fluorogenic target-specific hydrolysis probes. An included Internal Control DNA (ICD) detects PCR inhibition, monitors reagent integrity and confirms that nucleic acid extraction was sufficient and hence ensures reliable results.

RIDA®GENE Pneumocystis jirovecii real-time PCR contains three DNA standards to quantify the amount of Pneumocystis jirovecii present in a positive sample.

Evaluation of the RIDA®GENE Pneumocystis jirovecii assay was performed retrospectively on 154 extracted BAL specimens against a routine in-house real-time PCR assay on the LightCycler® 480II (Roche). DNA extraction was performed on the MagNA Pure compact (Roche) with the MagNA Pure compact Nucleic Acid Isolation Kit I (Figure 1). In a second study we also compared the RIDA®GENE Pneumocystis jirovecii assay with an immunofluorescence assay (IFA) on 10 BAL samples.

Figure 1 Study design



References:
1. *Centers for Disease Control and Prevention*. Pneumocystis pneumonia Statistics 2012.
2. *Krajicek BJ et al.* Pneumocystis pneumonia: current concepts in pathogenesis, diagnosis, and treatment. Clin Chest Med 2009, 30: 265-278.

Results

Of the 154 samples, 149 were concordant (96.8 %). 31/154 (20.1 %) samples were positive by at least one real-time PCR assay (Figure 2). Of the 5 positive samples that were not detected by the RIDA®GENE Pneumocystis jirovecii assay, all 5 had low copy numbers below the Limit of Detection (LoD).

Compared to the in-house real-time PCR, sensitivity and specificity of the RIDA®GENE Pneumocystis jirovecii assay were 83.9 % and 100 %, respectively (Table 1). In the second study, 5 out of 10 samples were positive by IFA and the RIDA®GENE Pneumocystis jirovecii assay and 4 samples were negative by both methods. One positive RIDA®GENE Pneumocystis jirovecii sample was negative by IFA (Figure 4).

Picture 1 Pneumocystis jirovecii real-time PCR

Figure 2 In-house RIDA®GENE Pneumocystis jirovecii *vs.* real-time PCR

		In-house real-time PCR		
		+	-	Total
RIDA®GENE Pneumocystis jirovecii	+	26	0	26
	-	5[a)]	123	128
	Total	31	123	154

[a)] Five (5) samples are below the limit of the detection (LOD) of the RIDA®GENE Pneumocystis jirovecii assay with a Ct value >35 in the reference in-house real-time PCR assay.

Sensitivity:	83.9 %
Specificity:	100.0 %
PPV:	100.0 %
NPV:	96.1 %

Figure 3 Example of Pneumocystis jirovecii run on the LightCycler® 480II

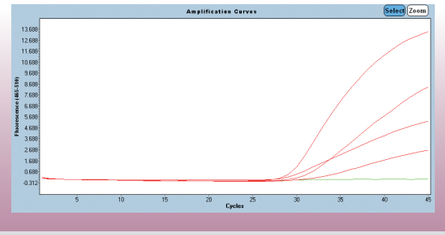


Figure 4 RIDA®GENE Pneumocystis jirovecii *vs.* IFA

		IFA		
		+	-	Total
RIDA®GENE Pneumocystis jirovecii	+	5	1	6
	-	0	4	4
	Total	5	5	10

Conclusion

RIDA®GENE Pneumocystis jirovecii real-time PCR shows good correlation with an established real-time PCR and is more sensitive than IFA for the detection of *Pneumocystis jirovecii*.

The new RIDA®GENE Pneumocystis jirovecii assay proved to be a sensitive and specific real-time PCR assay for the diagnosis of pneumocystosis.

Results are available in less than 2 hours.

The RIDA®GENE Pneumocystis jirovecii real-time PCR kit contains three DNA standards for quantification of the amount of *Pneumocystis jirovecii* present in a positive sample.

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