R-Biopharm AG

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

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. The mortality from Pneumocystis jirovecii in HIV-uninfected patients can be as high as 40 %. 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.

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).

References:

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.