MycoReal® Kit *Pneumocystis*

Kit version 1.1

For *in vitro* diagnostic use only

MycoReal® Kit *Pneumocystis*			
Order no.	**Reactions**	**Pathogen**	**Internal positive control**
DHUF00353	50	FAM channel	Cy5 channel

Kit contents:

- Assay for detection of *P. jirovecii* and of internal DNA positive control (IPC)
- IPC-Target DNA (control of DNA extraction and of PCR amplification)
- DNA reaction mix (contains a highly purified Taq Polymerase for rapid hot-start PCR, dNTPs with dUTP and Uracil-N glycosylase (UNG) to eliminate amplicon carryover, ROX™ dye (passive reference) and buffer components – additives optimized to handle PCR inhibitors)
- DNA positive control for *P. jirovecii*
- Nuclease-free water

Pathogen information: *Pneumocystis jirovecii* (formerly *Pneumocystis carinii*) is a yeast-like fungus which can be found worldwide. *Pneumocystis jirovecii* is a distinct species that only infects humans, while the related species *P. carinii* can be found in rodents and other mammals. Airborne transmission of *Pneumocystis* from host to host has been demonstrated in rodent models and several observations suggest that interindividual transmission occurs in humans. Both healthy and immunocompromised people can be colonised with *P. jirovecii*. While it does not affect healthy people, *P. jirovecii* can cause an interstitial Pneumocystis-pneumonia (PCP) in HIV-patients, persons with primary immune deficiencies, including hypogammaglobulinemia and severe combined immunodeficiency (SCID), patients receiving long-term immunosuppressive regimens for connective-tissue disorders, vasculitides, or solid-organ transplantation, patients with hematologic and nonhematologic malignancies, including solid tumors and lymphomas, and persons with severe malnutrition. Currently the diagnosis of PCP relies on microscopic methods or PCR, as *P. jirovecii* cannot be cultured in routine microbiology laboratories. The detection of *P. jirovecii* in high-risk patients indicates a Pneumocystis-pneumonia.

Intended purpose: MycoReal® Kit *Pneumocystis* is a non-automated CE-certified IVD real-time PCR test for the qualitative detection of DNA (mt LSU gene) of *P. jirovecii.* Proper specimens are DNA extracts isolated from samples of the human respiratory tract (bronchoalveolar lavage, BAL).
This test is suitable for patients of all ages with suspected infection with *P. jirovecii* and is intended as an aid in the diagnosis of infection with this pathogen in combination with patient history and additional clinical information. The test is intended for professional use and is limited to qualified personnel instructed in the procedures of real-time PCR and *in vitro* diagnostic procedures.
A probe-specific amplification-curve in the FAM channel indicates the amplification of *P. jirovecii* specific DNA. An internal DNA positive control (IPC) is detected in Cy5 channel and serves as a control for DNA extraction and possible real-time PCR inhibition. The target for the DNA IPC (artificial target DNA) is added during sample extraction.

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PCR-platforms: This test has been validated with the ABI® 7500 Fast instrument (fast cycle parameters are not supported, Thermo Fisher Scientific) and was also tested with QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), LightCycler® 480 II (Roche Diagnostics) and Mic instrument (bio molecular systems).
It is also compatible with other real-time PCR instruments which detect and differentiate fluorescence in FAM and Cy5 channel (e.g., QuantStudio™ 5 (Thermo Fisher Scientific), qTOWER^{3}G (Analytik Jena), cobas z 480 Analyzer (Roche), Mx3005P® (Agilent)).

Performance data: The LoD95% (smallest number of copies of target DNA which can be detected in 95% of cases) is 5 target copies/reaction. The mt LSU gene of *P. jirovecii* is a multicopy gene and is present up to 15 times in the genome of *P. jirovecii.* The test is specific for *P. jirovecii,* but possible cross-reactions with expected lower sensitivity with some *Pneumocystis carinii* strains isolated from macaques might occur. Clinical validation was performed with 200 clinical samples (Table 1).

Table 1 Results of clinical validation

	Value	95% CI
Sensitivity	97.96%	92.82% to 99.75%
Specificity	100.00%	96.41% to 100.00%
NPV	98.08%	92.82% to 99.51%
PPV	100.00%	-
Prevalence	49.00%	
Accuracy	99.00%	96.43% to 99.88%

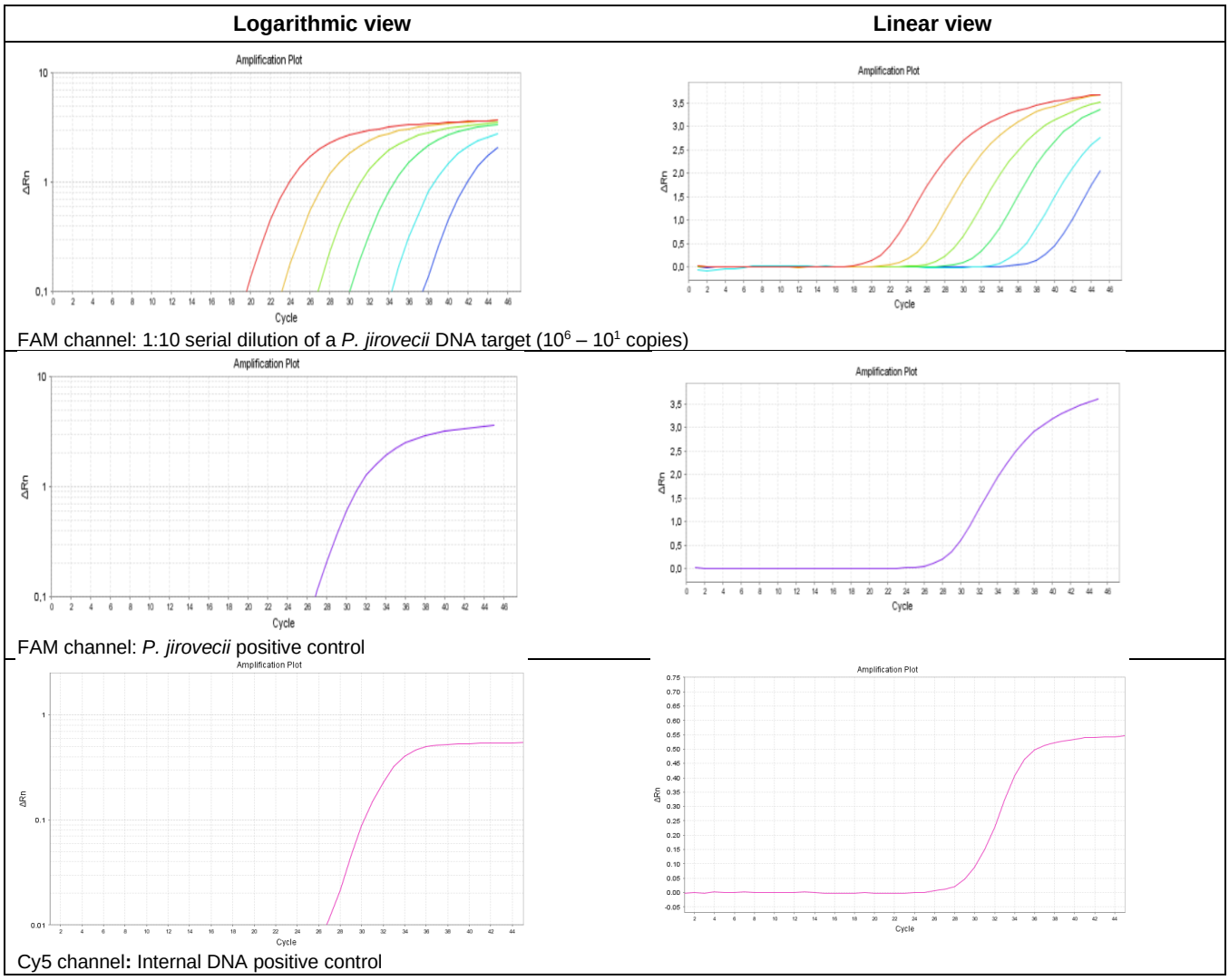

Figure 1 Performance of MycoReal® Kit *Pneumocystis*