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Diagnosis of *Pneumocystis jirovecii* Pneumonia by Real Time PCR: An Analysis of 10 Clinical Cases

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Abstract

Introduction: *Pneumocystis jirovecii* Pneumonia (PCP) is a life-threatening disease in immunocompromised patients. The aim of this work is to demonstrate the contribution of molecular biology in diagnosis compared to conventional methods and we propose an adapted cut-off value for differentiating *Pneumocystis* colonization from infection using real-time PCR.

Methodology: This was a prospective study enrolled from April 2015 to December 2018 at the Laboratory of parasitology of Military Hospital of Tunis. All pulmonary secretions samples were analyzed using: May-Grunwald-Giemsa (MGG) and Gomori Grocott Modified MUSTO Coloring Technology (GG). Optimization of conventional PCR and real-time PCR were accomplished.

Results: During the study period, we collected 200 samples. The prevalence of the disease was 5% (10/200). MGG coloring didn't discern vegetative forms of *Pneumocystis jirovecii* for all samples. Cysts were visualized by GG coloring for seven samples. Conventional PCR and Real-time PCR were positives for 10 samples with quantity of DNA going from one copy to 10^4 copies per milliliter.

Conclusion: Molecular biology is more sensitive than techniques of coloring. Currently real-time PCR gives at the same time a quantitative and qualitative approach with a threshold of detection very low which allows differentiating between a simple colonization and an infestation.

Keywords: *Pneumocystis jirovecii* • PCP • Real-time PCR • Diagnosis

Introduction

Database about *Pneumocystis jirovecii Pneumonia* (PJP) in humans shows an important rate of morbidity and mortality among immunocompromised patients [1-3]. This microbe affects patients with Human Immunodeficiency Virus (HIV), as well as hematological malignancies [4], solid organ transplantation, solid tumors, and immunosuppressive treatments [5]. The identification of *P. jirovecii* is mainly based on microscopic coloration methods and immunofluorescent staining [6,7]. Sensitivity of the staining techniques is acceptable for Broncho-Alveolar Lavage (BAL) specimens [8]. Various PCR protocols, including conventional PCR, nested PCR and real-time PCR [9-11], have been published and evaluated. They offer a qualitative and quantitative detection of the *P. jirovecii* DNA in a clinical sample even at low levels. Moreover, molecular detection of *P. jirovecii* in less-invasive specimens has also been shown to be cost-effective. In this study, we aimed to compare sensibility and specificity of different techniques (standard staining, conventional PCR and real-time PCR) used in the diagnosis of PCP. We propose an adapted cut-off value for differentiating Pneumocystis colonization from infection using real-time PCR.

Materials and Methods

Specimen's collection

Throughout four years (2015-2018), we collected pulmonary samples from 200 patients, including 74 (37%) broncho-alveolar lavages, 62 (31%) sputum and 64 (32%) protected tracheal sampling requiring a specific *P. jirovecii* investigation. These specimens were received from hospitalized patients in the Military Hospital of Tunis and Abdelrahmen-Mami Ariana Hospital. Patients' clinical characteristics are presented in Table 1. Suspicion of PCP was based on epidemiological, clinical and radiological findings. Symptoms considered to be common during PCP were: progressive dyspnea, non-productive cough, and low-grade fever, hypoxemia. Radiological signs considered to be associated with PCP were bilateral perihilar interstitial infiltrates. Respiratory specimens were centrifuged at 6000 g for 10 min. Part of the suspended pellet (about 200 µl) was used for microscopic examination (MGG, GG stains) and 200 µl was used for PCR (conventional PCR and real-time PCR).

Conventional techniques-microscopy

MGG and The MGG stain highlights intra-cystic bodies and/or trophozoites of *P. jirovecii*. Indeed Giemsa stains the nucleic of all *Pneumocystis* life cycle stages in purple pink but does not stain the cell wall. While the Gomori stains the wall of cysts of *P. jirovecii* in black brown on a green background.

Molecular diagnosis

DNA extraction: DNA was extracted by the Enro Gold Tissue DNA Mini Kit (EuroClone®, Italy) according to the manufacturers' recommendations. The extracts are either directly amplified or stored at -20°C for subsequent PCR.

Conventional PCR: PCR was performed according to Mei et al., [12]. The PCR mixture (50 µl) contained 2 mM of each primer, JKK14 (5'GAATGCAAATCCTTACAGACAACAG3') and JKK17 (5'AAATCATGAACGAAATAACCATTGC 3'), derived from the surface glycoprotein gene of *Pneumocystis jirovecii*, 0.2 mM dNTPs, 4.5 mM $MgCl_2$, 1 concentrated PCR buffer, 1.25 U Taq DNA polymerase and 100 ng of purified DNA. The reaction mixture was initially incubated for 15 min at 95°C. Amplification was performed by 40 cycles of denaturation (95°C for 30 s), annealing (56°C for 1 min), extension (72°C for 30 s) and the finally extension (72°C for 5 min). The PCR amplification products (249 bp fragments) were

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Table 1. Results of techniques used for the ten patients infected with *Pneumocystis*.

No.	Sex	Patient's history	age	Sample	Grocott and/or MGG coloring	Conventional PCR	Real time PCR (CP) copies/ml	Radiology	HIV Serology	Treatment
1	W	Renal transplant +infectious neumonia	53	Sputum	+	+	(36)/10^2	-	-	NS
2	M	Myeloma	60	BAL	-	+	(38)/10	-	-	
3	M	Lymphoma	67	Tracheal sampling	-	+	(38)/10	-	-	NS
4	W	Infectious pneumonia	66	BAL	+	+	(35)/10^2	+	-	NS
5	W	Lymphoma	51	BAL	+	+	(30)/10^4	+	-	NS
6	M	Rénal transplant+Infectious pneumonia	47	Tracheal sampling	+	+	(33)/10^3	+	-	NS
7	W	Inflamatory disease	77	Sputum	-	+	(34)/10^2	+	-	NS
8	M	Renal transplant	56	Sputum	+	+	(34)/10^2	+	-	Trimethoprim/ Sulfamethoxazole
9	W	Inflamatorydisease	48	BAL	+	+	(33)/10^3	+	-	
10	M	Infectious pneumonia	50	BAL	+	+	(29) 10^4	+	-	NS

M: Male; W: Woman; NS: Unsigned

subjected to electrophoresis in 1.5% agarose gel in Tris-borate-EDTA buffer and detected after staining with Red safe under UV illumination. We used DNA extracted from trophozoites and / or cysts of *P. jirovecii* as a positive control and the water as a negative control. All positive samples were tested twice.

Real-time PCR: Liferiver™ *Pneumocystis jirovecii*® real-time PCR kit allows specific amplification of *P. jirovecii* DNA using two specific probes labeled with different fluorochromes (FAM for *P. jirovecii* and HEX for the internal control). The reaction is carried out in a total volume of 40 µl contained 4 µl DNA, 1 µl Internal Control, 35 µl Tampon Mix and 0.4 µl Enzyme Mix. The kit has a positive control (10^7 copies ml^{-1}) that can be diluted to achieve a seven-point range (10^7 to 10^1 copies ml^{-1}). The amplification is carried out on the automat LigthCycler® 480 II (Roche) according to the recommended amplification program. The reaction Mixture was initially incubated for 2 min at 37°C (1 cycle) and 2 min at 94°C (1 cycle). Amplification was performed by 40 cycles: 15 sec at 93°C and 1min at 60°C. The results are rendered in number of Cycle Threshold (Ct) and in number of copies per millilitre when the quantization range is achieved. Clinical, biological, radiological, treatment, and prognostic data of all patients were collected. The diagnosis of pneumocystosis was considered as:

•Confirmed PCP=Positive direct examination;

•Possible PCP=Negative direct examination and PCR positive: clinical and/or para-clinical features indicative of PCP, and completer solution of symptoms following curative anti-PCP treatment.

•PCP ruled out, classified as colonization=Negative direct examination and PCR positive: Identification of another etiology responsible for the observed respiratory symptoms, and absence of curative anti-PCP treatment.

Statistical analysis

Statistical analyses were performed using SPSS 22.0. The accuracy of the real-time PCR assay was measured by the area under the Receiver Operating Characteristic (ROC) curve. The odds ratio (OR) and 95% confidence intervals (95% CI) were used to describe associated factors.

Results

Patients' characteristics

From April 2015 to December 2018, 200 samples were received from patients suspected to have Pneumocystosis. A total of 200 patients were included in this study. The molecular detection of *Pneumocystis jirovicii* was confirmed for only 10 patients (5%). There were six male and four female. Their median age was 62 years (ranging from 47 to 77 years old). They suffer from Hematological malignancies (3/10; 30%), solid organ transplantation (2/10; 20%) and inflammatory diseases (2/10; 20%). Clinically, fever and dyspnea were observed in 70% of these patients and cough in 10% of them. Bilateral diffuse interstitial infiltrate was observed in 70% of cases. The mortality rate was 1%.

Biological diagnosis and results interpretation

Microscopic Examination (ME) was positive for 7/10 patients (70%), revealing cysts of *P. jirovecii* in 5broncho-alveolar lavages, and one sputum and protected tracheal sampling each. Molecular diagnosis was positive for all patients [10] (Figure 1). The sensitivity of the PCR was 6.7% for broncho-alveolar lavages (5/74); 4.8% for sputum (3/62) and 3.1% for protected tracheal sampling (2/64) and the specificity was 100%. Patients with positive PCR are suffering from at least one symptom (fever, dyspnea and cough). Thirteen percent of them present all the 3 symptoms, 20% present 2 symptoms, 30% present one symptom and 20% of patients are asymptomatic. The ME positivity was 10% for patients presenting one symptom and was about 20% for other category of patients each. The ME positivity was 20% for patient with no symptoms, two symptoms and three symptoms each and 10% for one symptom. Conventional PCR detected a 249 bp band for 10 samples (Figure 2). Similarly, the real-time PCR was positive for 10 samples and the quantity of DNA varies from one copy to 10^4 copies per ml (Figure 3 and Table 1). The sensitivity of molecular biology was 100% and the specificity was 98%. The positive predictive value was 70% and the negative predictive value was 100%. The ROC curve (Figure 4) represents the sensitivity and the 1-specificity of the different Cycle Threshold (Ct) of the real-time PCR assay compared with the ME method. The area under the ROC curve was 0.990 with a 95% confidence interval [0.997-1.000].

The best sensitivity of the real-time PCR technique was for Ct ≥ 38 (100%) and the best specificity for Ct ≤ 30(100%), using ME as a reference technique. Between these two points of cut, there was a gray area in which the results of the PCR and the ME may differ. According to the PCR cycle thresholds previously described, patients could be divided into three groups, group with "Ct ≥ 38" (two patients), group with "30 < Ct < 38"(7 patients) and group with "Ct ≤ 30" (two patients). For the two patients of the group with "Ct ≥ 38", the ME was negative with a positive PCR and the quantity of DNA was less than 10^2 copies per ml. They have developed clinical signs of PJP (fever, dyspnea and cough) with absence of an interstitial radiological syndrome. Compared to this group, the two patients including in the group with "Ct ≤ 30" presented in addition to clinical signs, interstitial syndrome in imaging. The ME was positive with a quantity of DNA greater than or equal of 10^4 copies per ml. For the five patients in the group with "30 < Ct < 38", the quantity of DNA varies between 10^2 copies per ml and 10^4 copies per ml. In conclusion, eight patients were selected for developing PJP, and two were colonized (Table 2).

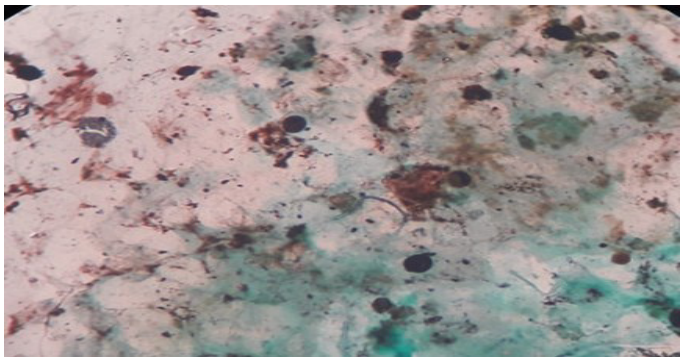

Figure 1. Cysts of *Pneumocystis jirovecii colored* by Gomori-Grocott modified by MUSTO.

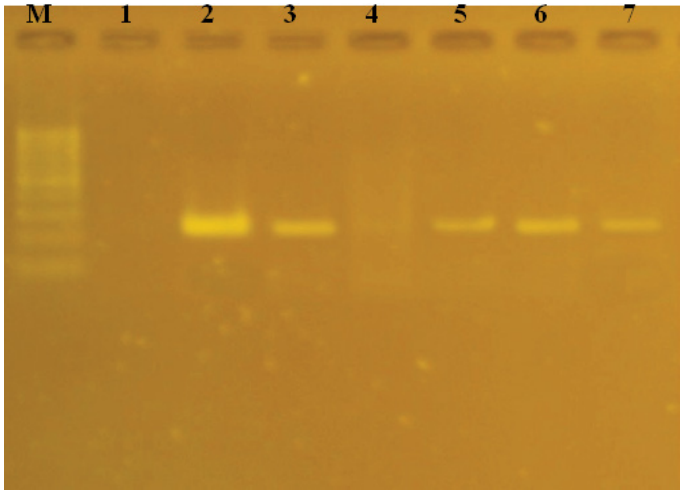

Figure 2. Agarose gel electrophoresis of conventional PCR products of *P. jirovecii MSG* gene.

Lanes: M, 100 bp DNA size marker.; 1, negative control ; 2, positive control ; 3, 5, 6 and 7, positive clinical samples having specific 249 bp bands representative of *P. jirovecii* positive; 4, negative clinical samples.

Discussion

PJP is an opportunistic infection responsible for severe interstitial lung disease in immunocom promised patients. Pneumocystis, which was initially very rare, saw its incidence explode since 1980 with the onset of the epidemic caused by HIV. Currently, cases of pneumocystosis in HIV-infected patients tend to decrease due to the use of anti-*Pneumocystis jirovecii* chemoprophylaxis on the one hand and antiretroviral treatments which allow a better control of immune depression on the other hand [13,14]. The prevalence of PJP in our study is 5%. For Issa et *al.,* 2018 7% have been developed pneumocystosis, of which 25% were HIV+ (38/150 patients) [15]. Dieng et al., founded that of 20 patients with pneumocystosis (9%) only 4 are HIV+ [16]. This frequency is less than reported in another Tunisian study of kaouech et al., 2009 (29.6%) where the majority of cases are HIV+ (11/15) [17]. Furthermore in Ethiopia, the prevalence of the PJP was 42.7% and all patients were HIV+ [18]. This difference could be due to a difference in the virulence of genotypes circulating in these countries and the improvement of identification techniques. In our study, the median age was 62 years and 59 years as reported by Issa et al., in 2018 and Teh et al., in 2014 respectively [19]. Similarly, we noted a male predominance in 60% of cases with a sex-ratio of 1.5. This masculine predominance was found in 68.9% and 70% of the considered populations reported by Teh et al., [20], Issa et al., respectively. At present, most of our patients (70%) present dyspnea and fever. Similarly, for Tech et al., clinical symptoms included shortness of breath (64.4%), cough (48.9%) and fever (42.2%). Issa et al., 2018 reported that the most common symptoms were dyspnea (85%), cough (83%), hyperthermia (85%), and deterioration of general status (82%).

Confirmation of PJP diagnosis relies on the detection of cysts and/or

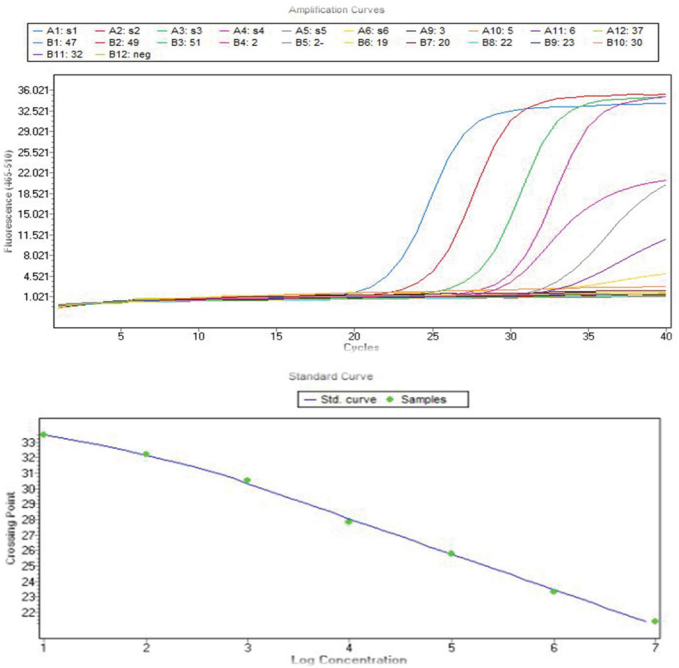

Figure 3. Efficiency of the real-time PCR assay for the identification of *P. jirovecii.*

Positive control dilution series were used to create calibration curves for the calculation of DNA concentrations of *Pneumocystis jirovecii* in positive samples. The upper curve shows the amplification curves; A1 to A6: standards with a DNA concentration of 10^7 to 10^2 copies/µl; A9 to B11: The samples tested; B12: Negative control. The lower curve shows the standard curve constructed from the values of Cp (crossing point) versus the log DNA copy number / (range = 10^7 to 10^2 copies/µl).

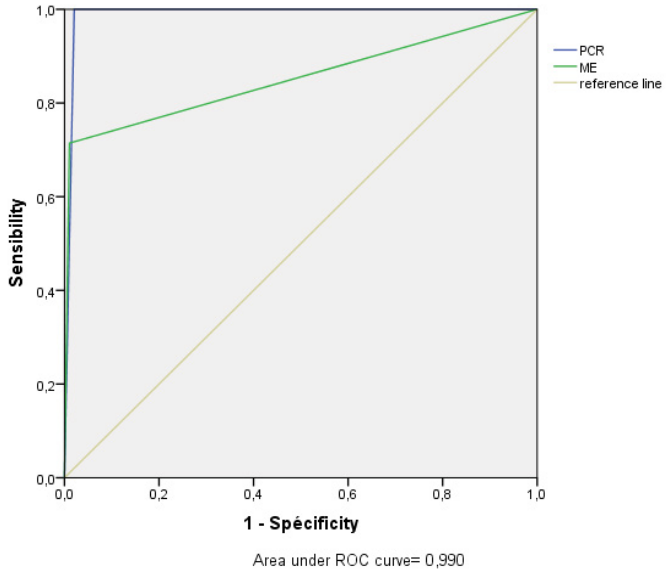

Figure 4. ROC curve of the real-time PCR assay for the diagnosis of *P. jirovecii* compared with the ME.

trophozoites (GG and/or MGG staining) of *P. jirovecii* at direct examination of respiratory secretions. The sensitivity of this usual examination in case of PCP in HIV-infected patients ranges from 80% to 100%, irrespective of the type of sample collection method [21]. The sensitivity of the direct examination in non-HIV-infected patients ranges from 50% to 70% when BAL is performed [22,23]. At present, to ameliorate the diagnosis of PJP many molecular tests have been described and recently real-time PCR was used. Kaouech revealed a positive PCR in 14 cases in which only 5 cases were positive by conventional methods. This can be explained by the lack of sensitivity of the microscopic methods and by the difficulty of reading when the samples are non-invasive or superficial. Currently, several studies have shown that molecular biology techniques are more sensitive than conventional techniques for diagnosing PCP [24,25].

Table 2. Description and bivariate analysis of the 10 included patients.

		PCR results			OR[a] [95 CI]	OR[b] [95 CI]
		Ct ≥ 38	30<Ct<38	Ct ≤ 30		
		N=2	N=6	N=2		
	Male	1	3	1	NS	NS
Sex	Woman	1	3	1	NS	NS
Age	>47	2	5	2		
	Myéloma	1	0	0	NS	NS
	Lymphoma	1	1	0	NS	NS
Chronic Disorders	Organ transplantation	0	0	2	NS	NS
	Inflammatory disease	0	2	0	NS	NS
	Sputum	0	2	1	NS	NS
Sample type	PTP	1	1	0	NS	NS
	LBA	1	3	1	NS	NS
Fever (n=)	>39°C	2	3	2	NS	NS
Cough (n=)	Yes	0	0	1	NS	NS
Dyspnea (n=)	Yes	1	5	1	NC	NC
Interstitial Syndrome (n=)	Yes	0	3	2	NS	NS
Interpretation		Colonized	PJP / Probable	PJP		

NS: No Significant, NC: No Calculable,
[a]Odds Ratio [95% Confidence Interval] of "30<Ct<38" group versus Ct ≥ 38" group.,
[b]Odds Ratio [95% Confidence Interval] of "30<Ct<38" group versus "Ct ≤ 30" group.

Endpoint PCR is a qualitative approach which had a very high sensitivity compared to GG staining. It is faster and easier than conventional techniques but it is a long technique and susceptible to contamination comparing to real time PCR. Our study presents the first real-time PCR assessment in the routine diagnosis of *P. jirovecii* in Tunisia. The Light Cycler 480 real-time PCR can be used with a large number of samples with a well-defined standard range. The results can be obtained within 1.5 hours. The sensitivity of real time PCR reaches 100% whereas it is only 85.17% for microscopy techniques [15,22]. In addition, real-time PCR used an internal control for the detection of PCR inhibitors. The main advantage of real-time PCR is the simultaneous completion of the amplification and detection steps in a closed system, which reduces the risk of contamination. The positive controls of the real-time PCR also have a well-defined quantification (10^7 copies ml^{-1}). In addition, it allows detecting the DNA even in a small amount so we can clearly specify whether it is a simple colonization or an infection [26-28]. One of the disadvantages of this technique is the realization of the quantization range by successive dilutions of the positive control. This manipulation can generate dilution errors as well as contaminations at the origin of an erroneous quantification range. Our study can separate the population in to three groups and to propose three interpretations to clinicians:

Negative PCR results: "Absence of *P. jirovecii*. PJP diagnosis excluded".

Positive PCR with Ct ≥ 38: "Low fungal burden: Pneumocystis pneumonia improbable. Prophylactic treatment could be necessary in case of an immunocompromised patient"

Positive PCR with 30 < Ct < 38: PJP probable. The diagnosis of PJP or colonization depends on patient history, biological and radiological, treatment, and patient clinical evolution.

Positive PCR with Ct ≤ 30: PJP.

This study showed that *P. jirovecii* was detected by PCR in all cases where the ME was positive. The sensitivity and the specificity of the real-time PCR depended on the Cycle Threshold (Ct) of detection. If the Ct ≥ 38 the sensibility was 100%, on the other hand if the Ct ≤ 30 the specificity was 100%. Between these two points, there was a grey zone in which the PCR value and the ME result could be different. Several studies have shown that PCR to be more sensitive than the standard diagnostic methods ME [28]. In our study, *P. jirovecii* was detected in Tracheal sampling of patient No. 3 (Ct 38). Therefore, real-time PCR offers evidence of *Pneumocystis* carriers (simple colonization), especially since conventional techniques in this patient were negative but with a mycological culture positive to *Aspergillus fumigatus* and *Candida albicans*. Another sample of patient No. 1 was positive in GG staining and real time PCR (Ct 36) with mycological culture positive to *Aspergillus fumigatus* and *Candida albicans*. Despite the fact that the quantification of the DNA was in favor of colonization, the patient received treatment against *Pneumocystis*, taking into account his history. But he was resistant to treatment and the evolution was quickly fatal. Indeed, the curative management of PJP is based on a consensus, but the management of colonized patients is not; the benefit of primary prophylaxis for PCP in non-HIV-infected immunosuppressed patients is therefore not confirmed. As for the non-HIV-infected patients, the risk of PJP depends on the type of immunosuppressant. The expected benefit of a cotrimoxazole prophylaxis must therefore be balanced against the risk of severe adverse [28].

Conclusion

The use of molecular biology in addition to microscopy techniques is of real interest for the diagnosis of PJP. The development of real-time PCR has emerged as a major breakthrough in molecular biology. It allows a gain of sensitivity in the detection of *P. jirovecii* and a better estimation of PJP, making it a technique of choice for routine use within the laboratory.

Declarations

Funding: Not applicable.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: For this type of study formal consent is not required.

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References

1. Judith, Fillaux, Sonia Malvy, Muriel Alvarez and Richard Fabre. "Accuracy of a routine real-time PCR assay for the diagnosis of *Pneumocystis jirovecii* pneumonia." *J Microb Methods* 75 (2008): 258–261.

2. Lisa, R. McTaggart, Nancy L. Wengenack and Susan E. Richardson, et al. "Validation of the MycAssay Pneumocystis Kit for Detection of *Pneumocystis jirovecii* in Bronchoalveolar Lavage Specimens by Comparison to a Laboratory Standard of Direct Immunofluorescence Microscopy, Real-Time PCR, or Conventional PCR." *J Clinic Microb* 50 (2012): 1856–1859.

3. E. X. Monroy-Vaca, Y. De Armas, M. T. Illnait-Zaragozi and G. Torano, et al. "Stensvold. Prevalence and Genotype Distribution of Pneumocystis *jirovecii* in Cuban Infants and Toddlers with Whooping Cough." *J Clinic Microb* 52 (2014): 45–51.

4. Inhye, E. Ahn, Theresa Jerussi, Mohammed Farooqui and Xin Tian, et al. "Atypical *Pneumocystis jirovecii* pneumonia in previously untreated patients with CLL on single-agent ibrutinib." 128 (2016): 1940-1943.

5. Alistair, B. Reid, Sharon C.-A. Chen and Leon J. "*Pneumocystis jirovecii* pneumonia in non-HIV-infected patients: New risks and diagnostic tools." *Current Opinion in Infectious Diseases* 24 (2011): 534-544.

6. Milène, Sasso, Elsa Chastang-Dumas, Sophie Bastide and Sandrine Alonso, et al. "Performances of four Real-Time PCR assays for diagnosis of *Pneumocystis jirovecii* pneumonia." *J Clinic Microb* 54 (2016): 625-630.

7. Gomes, Ana, Ricardo Ferraz, Lauren Ficke and Margaret S. Collins, et al. "Chloroquine Analogues as Leads against *Pneumocystis* Lung Pathogens." *Antimicrobial Agents Chemo*; 62 (2018): 1-8.

8. Ludovic, Tapparel, Pierre-Auguste Petignat and Gérard Praz. "Diagnostic de la pneumonie à *Pneumocystisjirovecii* chez le patient non VIH." 2010.

9. Li, Ming-Chi, Nan-Yao Lee, Ching-Chi Lee and Hsin-Chun Lee, et al. "*Pneumocystis jiroveci* pneumonia in immunocompromised patients: Delayed diagnosis and poor outcomes in non-HIV infected individuals." *J Microb Immune Infection* 47 (2014): 42-47.

10. T. Fauchier, L. Hasseine, M. Gari-Toussaint and V. Casanova, et al. "qPCR*Pneumocystis jirovecii* to differentiate colonisation and pneumonia in immunocompromised HIV positive and HIV negative patients." *J American Society Microb* 54 (2016): 1487-1495.

11. Thibaud, Guillaud-Saumur, Gilles Nevez, Amélie Bazire and Michèle Virmaux, et al. "Comparison of a commercial real-time PCR assay, RealCycler® PJIR kit, progenie molecular, to an in-house real-time PCR assay for the diagnosis of *Pneumocystis jirovecii* infections." *Diagnos Microb Infectious Dis* 87 (2017): 335–337.

12. Qin, Mei, Ross E. Turner, Vivian Sorial and Diane Klivington, et al. "Characterization of Major Surface Glycoprotein Genes of Human *Pneumocystis carinii* and High-Level Expression of a Conserved Region." *Infection and Immunity* 66 (1998): 4268–4273.

13. Colleen, F. Kelley, William Checkley, David M. Mannino and Carlos Franco-Paredes, et al. "Trends in Hospitalizations for AIDS-Associated *Pneumocystis jirovecii* Pneumonia in the United States (1986 to 2005)." *Chest Journal* 136 (2009): 190–197.

14. Alexandre, Alanio, Philippe M. Hauser, Katrien Lagrou and Willem J. G. Melchers, et al. "ECIL guidelines for the diagnosis of *Pneumocystis jirovecii* pneumonia in patients with haematological malignancies and stem cell transplant recipients." *J Antimicrobiol Chemo* 71 (2016): 2386–2396.

15. N. Issa, F. Gabriel, G. Baulier and G. Mourissoux, et al. "Pneumocystosis and quantitative PCR." *Med Mal Infect* 48 (2018): 474-80.

16. Y. Dieng, T. Dieng, D. Sow and S. Wlouhou, et al. "Diagnostic biologique de la pneumonie à *Pneumocystis* au centre hospitalier universitaire de Fann, Dakar, Sénégal." *J Mycologie Médicale* 26 (2016): 56-60.

17. E. Kaouech, K. Kallel, S. Anane and S. Belhadj, et al. "Pneumocystose à *Pneumocystisjiroveci*: étude comparée de la PCR et des techniques de coloration." *Pathologie Biologie* 57(2009): 373-377.

18. G. Aderaye, Y. Woldeamanuel, D. Asrat, and M. Lebbad, et al. "Evaluation of Toluidine Blue O Staining for the Diagnosis of *Pneumocystis jiroveci* in Expectorated Sputum Sample and Bronchoalveolar Lavage from HIV-infected Patients in a Tertiary Care Referral Center in Ethiopia." *Infection* 36 (2008): 237-243.

19. W. Teh Benjamin, Francesca A. Azzato, Senthil M. Lingaratnam and Karin A. Thursky, et al. "Molecular diagnosis of *Pneumocystis jirovecii* in patients with malignancy: Clinical significance of quantitative polymerase chain reaction." *International Society for Human and Animal Mycology* 54 (2014): 427-432.

20. Frans J.L. Robberts, Lynne D. Liebowitz and Lynda J. Chalkley. "Polymerase chain reaction detection of *Pneumocystis jiroveci*: Evaluation of 9 assays." *Diagnostic Microbiology and Infectious Disease*. 58 (2007): 385-92.

21. Pierre Flori, Bahrie Bellete, Fabrice Durand and Hélène Raberin, et al. "Comparison between real-time PCR, conventional PCR and different staining techniques for diagnosing *Pneumocystis jiroveci* pneumonia from bronchoalveolar lavage specimens." *J Med Microb* 53 (2004): 603–607.

22. Li-Chao Fan, Hai-Wen Lu, Ke-Bin Cheng and Hui-Ping Li, et al. "Evaluation of PCR in Bronchoalveolar Lavage Fluid for Diagnosis of *Pneumocystis jirovecii* Pneumonia: A Bivariate Meta-Analysis and Systematic Review." *PLOS One* 8 (2013): 9.

23. H. H. Larsen, H. Masur, J. A. Kovacs and V. J. Gill, et al. "Development and Evaluation of a Quantitative, Touch-Down, Real-Time PCR Assay for Diagnosing *Pneumocystis carinii* Pneumonia." *J Clinic Microb*. 40 (2002): 490–494.

24. Rodney, C. Arcenas, James R. Uhl, Seanne P. Buckwalter and Andrew H. Limper, et al. "A real-time polymerase chain reaction assay for detection of *Pneumocystis* from bronchoalveolar lavage fluid." D*iagnos Microb Infectious Disease* 54 (2006): 169–75.

25. P. Rohner, V. Jacomo, R. Studer and J. Schrenzel, et al. "Detection of *Pneumocystis jirovecii* by Two Staining Methods and Two Quantitative PCR Assays." 37 (2009): 261-265.

26. M. Maillet, D. Maubon, J. P. Brion and P. François, et al. *"Pneumocystis jirovecii* (Pj) quantitative PCR to differentiate Pj pneumonia from Pj colonization in immunocompromised patients." *European J Clinic Microb Infectious Disease* 33 (2014): 331-6.

27. M.A. Jarboui, F. Mseddi, H. Sellami and A. Sellami, et al. *"Pneumocystis*: épidémiologie et approches moléculaires *Pneumocystis: Epidemiology and Molecular Approaches*." 61 (2009): 239-244.

28. Hefziba, Green, Mical Paul, Liat Vidal and Leonard Leibovici. "Prophylaxis of *Pneumocystis* pneumonia in immunocompromised non-HIV-infected patients: A systematic review and meta-analysis of randomized controlled trials." *Mayo Clinical Proc* 82 (2007): 1052-1059.

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