Chapter 6

Inter-laboratory comparison of three different real-time PCR assays for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage fluid samples

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Journal of Medical Microbiology 2006;55:1229-1235

Abstract

Objective
Pneumocystis jiroveci pneumonia (PCP) is an opportunistic infection affecting immunocompromised patients. While conventional diagnosis of PCP by microscopy is cumbersome, the use of polymerase chain reaction (PCR) to diagnose PCP has great potential. Nevertheless, inter-laboratory validation and standardization of PCR assays is lacking. The aim of this study was to evaluate the inter-laboratory agreement of three independently developed real-time PCR assays for the detection of *P. jiroveci* in bronchoalveolar lavage fluid (BALF) samples.

Study design
A total of 124 samples were collected in three tertiary care laboratories (LUMC, MINC and RUNMC) and were tested by both microscopy and real-time PCR.

Results
Out of 41 samples positive for *P. jiroveci* by microscopy, 40 were positive in all three PCR assays. The remaining sample was positive in a single assay only. Out of 83 microscopy-negative samples, 69 were negative in all three PCR assays. The other 14 samples were found positive, either in all three assays (n = 5), in two (n = 2) or in one of the assays (n = 7).

Conclusion
Our data demonstrate high inter-laboratory agreement between (real-time) PCR assays for the detection of *P. jiroveci*.

Introduction

Pneumocystis carinii is an opportunistic pathogen that was classified as a fungus in 1988[1]. Although human-derived *Pneumocystis carinii* (*P. carinii f.sp. hominis*) has recently been renamed *Pneumocystis jiroveci*, the abbreviation PCP (now referring to *Pneumocystis* pneumonia) remains in use[2]. Patients at risk for *P. jiroveci* pneumonia can be divided into two categories: HIV-positive and -negative patients. Several risk factors have been identified in the HIV-negative group, such as immunosuppressive medication or an inherited or acquired immunodeficiency[3]. Since untreated PCP is associated with a high morbidity and mortality[4,5], especially in HIV-negative patients, a rapid and reliable diagnosis is mandatory. Current diagnosis of PCP relies on tinctorial and/or immunofluorescent staining of induced sputum or bronchoalveolar lavage fluid (BALF) samples[6,7]. Using these methods, sensitivity and specificity rates are reached which exceed 95%[8-11]. Major drawbacks of microscopy are, however, that it is cumbersome and requires trained microscopists. The latter makes it essential that positive samples are encountered regularly in order to ascertain an expertise in microscopy. In recent years, the incidence of PCP has declined significantly, especially in HIV-positive patients. Due to the introduction of highly active anti-retroviral therapy, PCP chemoprophylaxis, and an increase of patients receiving chemotherapy, the future trend will be towards samples with relatively low *P. jiroveci* burdens, making the diagnosis of PCP even more difficult[12,13]. Therefore, a rapid diagnostic technique, which can identify the presence of a low number of cysts, is needed.

Nucleic acid amplification tests, such as the polymerase chain reaction (PCR), play an increasing role in the detection of *P. jiroveci*[14,15]. In particular, real-time PCR is highly suitable in the diagnosis of PCP, since this technique allows the generation of quantitative results. This is crucial, as *P. jiroveci* may be present in low quantities in some asymptomatic individuals[16,17]. Consequently, it is of utmost importance to be able to discriminate between asymptomatic carrier ship and clinically relevant infection. In recent years, molecular tests for detecting *P. jiroveci* have shifted from research to diagnostic applications[18-20]. Nevertheless, quality control panels for validation and standardization of such tests are currently lacking. Therefore, a study was initiated to compare the performance of three independently developed real-time PCR assays for the detection of *P. jiroveci* in three different tertiary care centers in The Netherlands. This retrospective study assessed the routine diagnostic performance of these assays on a collection of BALF samples of which the microscopic evaluation for the presence of *P. jiroveci* had already been performed.

Materials and methods

Design of the study

Three diagnostic medical microbiology laboratories participated in this study, *i.e.* Maastricht Infection Center (MINC), Radboud University Nijmegen Medical Center (RUNMC) and Leiden University Medical Center (LUMC). All three laboratories are university tertiary care centers located in The Netherlands. Each of these laboratories supplied *P. jirovecí*-positive and -negative samples. Diagnosis was based on microscopy. The materials were collected by one of us (C.F.M.L), encoded, and distributed to the other laboratories. Each laboratory received a set of samples and performed its own real-time PCR in a blinded fashion.

BALF sampling and conventional diagnosis of PCP

Samples obtained from patients suspected of PCP were collected over a period from August 1999 until April 2004. Bronchoscopy with BAL was performed with sterile saline, in four aliquots of 50 ml (MINC), three aliquots of 20 ml (LUMC) and three aliquots of 50 ml (RUNMC). Samples were immediately transported to the laboratory and processed upon arrival. At the LUMC and RUNMC, aliquots of 10 ml were centrifuged at 3000×*g* and smears were made from the sediment. At the MINC, cytocentrifuged monolayer preparations were made as previously described[21]. Subsequently, slides were subjected to either Giemsa and methenamine silver staining (LUMC), Giemsa and direct immunofluorescence staining (PneumoCel, CeLLabs, Brookvale, Australia) (RUNMC) or May-Grünwald Giemsa and methenamine silver staining (MINC).

Nucleic acid extraction and real-time PCR analysis

At the MINC, 200 µl BALF was used for DNA isolation using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). Purified DNA was resuspended in a final volume of 120 µl. A real-time PCR was designed that targets the major surface glycoprotein (MSG) gene[15]. Assays were performed in 96-well Optical Reaction plates (Applied Biosystems [ABI], Foster City, California) in a volume of 50 µl containing 0.6 µM of each primer PCPFor and PCPRev (Table 6.1), 0.15 µM of probe PCPProbe, 1×TaqMan® Universal Master Mix (ABI) and 20 µl of purified DNA. Thermal cycling was carried out on an ABI PRISM 7000 Sequence Detection System (ABI) as follows: two minutes at 50°C, 10 minutes at 95°C, followed by 42 cycles of 15 seconds at 95°C and one minute at 60°C. Quantification was performed using the ABI PRISM software and is based on extrapolation of data to standard curves, which were

generated by amplification of quantified dilutions of plasmid pPCP, which contains the *P. jiroveci* PCR fragment.

Table 6.1 Description of primers and probes used by the three laboratories.

Laboratory	Target		Primer [5'-3'] (Name)	GenBank accession no.	Position on gene
MINC	MSG	F[a]	CAAAAATAACAYTSACATCAACRAGG (PCPFor)	AF372980	223-248
		P[b]	FAM-TGCAAACCAACCAAGTGTACGACAGG-TAMRA (PCPProbe)		252-277
		R[c]	AAATCATGAACGAAATAACCATTGC (PCPRev)		378-354
LUMC	DHPS	F[a]	ATGATTCTATATTAATGGATGTGGAG (PJIRs)	AJ586567	148-173
		P[b]	MB-CGCGCTGGGCGACGATAATTGATATTGGTGGAGCGCG-FAM (578PJIR)		190-214
		R[c]	AGATATTTTATAGCAGGAATAACTCG (PJIRas)		290-268
RUNMC	MSG	F[a]	GAATGCAAATCYTTACAGACAACA (JKK114/15)	AF37298	135-158
		P1[b]	CAAAAATAACAYTSACATCAACRAGGC (PCMSGFRET1U)		223-248
		P2[b]	TGCAAACCAACCAAGTGTACGACAGG (PCMSGFRET1D)		252-277
		R[c]	AAATCATGAACGAAATAACCATTGC (JKK17)		378-354

[a] Forward primer, [b] Probe, [c] Reverse primer. MSG: Major surface glycoprotein, DHPS: dihydroperoate synthase gene. The sequences from primers and probes used by MINC and RUNMC were obtained from reference[15] .

At the RUNMC, the MagnaPure LC Isolation station (Roche Applied Science, Mannheim, Germany) was used for nucleic acid isolation. A 100-µl aliquot was isolated using the Total Nucleic Acid isolation kit (Roche Applied Science). Nucleic acids were resuspended in 50 µl of H_2O. Real-time PCR for *P. jiroveci* was performed as described by Larsen *et al.*, using the MSG gene as target[15]. All reactions were performed in 20 µl, consisting of 1×LightCycler FastStart DNA Master Hybridization Probes reaction mixture (Roche), 4 mM $MgCl_2$, 1.0 µM of each primer JKK14/15 and JKK17 (Table 6.1), 0.2 µM of each probe PCMSGFRET1U and PCMSGFRET1D, and 5 µl template. The PCR thermal profile consisted of an initial incubation at 95°C for 10 minutes, followed by a touch-down procedure, consisting of 11 cycles of 5 seconds at 95°C and 10 seconds at temperatures decreasing from 65°C to 50°C. This was followed by 35 cycles of 5 seconds at 95°C and 10 seconds at 50°C, and a final step of 15 seconds at 72°C. Amplification, detection and data analysis was executed using the LightCycler v2.0 system (Roche).

At the LUMC, nucleic acids were extracted from 200 µl-samples using the Qiagen whole blood DNA extraction kit (Qiagen, Hilden, Germany). Each sample was eluted using 200 µl of elution buffer. Real-time PCR for *P. jiroveci* was targeted at the dihydroperoate synthase gene (DHPS), and was performed in 50 µl reaction mixtures, consisting of 1× HotstarTaq™ mastermix (Qiagen, Hilden, Germany), 3.5 mM $MgCl_2$, 0.4 µM of each primer, 0.34 µM of molecular beacon probe and 10 µl of purified DNA. The PCR thermal profile consisted of 15 minutes at 95°C, followed by 50 cycles of 30 seconds at 95°C, 30 seconds at 55°C and 30 seconds at 72°C. Amplification, detection and data analysis were performed with an iCycler IQ Real-Time Detection System (Bio-Rad, Veenendaal, The Netherlands).

Additional analyses

Since two of the three PCR assays were based on a multicopy target (MSG) and one assay on a single copy target (DHPS), comparison of quantification was performed on the basis threshold cycle (C_t) values rather than absolute quantifications. In 26 samples (positive samples from the MINC), the *P. jiroveci* burden was also microscopically quantified[21]. For these samples, microscopic quantification was compared to PCR quantification using the MINC assay (described above).

Statistical analysis

Qualitative inter-assay agreement was assessed by pair wise comparisons of test results from the three laboratories by calculating the crude percent agreement and the kappa statistic. For this comparison, the results obtained on the initial run for each sample were taken and any C_t value obtained was considered as a positive result. Further, pair wise correlations between the different laboratories' C_t values for each sample were calculated using Pearson correlation coefficient. Correlation between microscopy quantification and real-time PCR quantification was also expressed as a correlation coefficient.

Results

Origin of BALF samples

A total of 124 BALF samples collected during the period August 1999-April 2004 were included. They were recovered from either HIV-positive or HIV-negative patients with a known risk factor for PCP, such as (hematological) malignancy, bone marrow or organ transplantation, Wegener's granulomatosis, and immunosuppressive or corticosteroid therapy[7,12]. In addition, a number of

BALF samples obtained from patients with no known risk factor for PCP were included (Table 6.2). These samples were obtained from patients with newly diagnosed sarcoidosis or ventilator-associated pneumonia. The samples were obtained form the LUMC (n=20), RUNMC (n=18) and MINC (n=86). As shown in Table 6.2, 41 samples (33.1%) were found to be positive for *P. jiroveci* by microscopy.

Table 6.2 Origin of BALF samples included in this study.

	Patients at risk[a]		Patients not at risk		
Microscopy result for *P. jiroveci*	HIV-positive	HIV-negative	Ventilator-associated pneumonia	Sarcoidosis[b]	Total
Positive	21	20	0	0	41
Negative	10	33	20	20	83

[a] HIV-infected patients, or patients with a known risk factor for PCP, such as hematological malignancy, bone marrow or organ transplantation, Wegener's granulomatosis, and immunosuppressive or corticosteroid therapy; [b] Sarcoidosis patients were not receiving immunosuppressive therapy or corticosteroids.

Qualitative agreement between the three *P. jiroveci* real-time PCR assays

All 124 samples were subjected to real-time PCR at each of the three participating laboratories. For 114 (91.9%) of the 124 samples, the three laboratories obtained identical qualitative results (Table 6.3). Forty out of 41 (97.6%) microscopy-positive samples were found positive in all three PCR assays. The remaining microscopy-positive sample was only positive in a single assay, but showed a relatively high C_t value (C_t = 36.6). This sample was obtained from a patient with a low parasite burden (one cyst in one out of three investigated microscopic slides). This patient was diagnosed with PCP a week earlier and had been treated with cotrimoxazole during the week previous to the bronchoscopy.

Table 6.3 Comparison of the three real-time PCR assays for the detection of *P. jiroveci*.

	Real-time PCR positive (+) or negative (-)		
Number of samples	MINC	LUMC	RUNMC
Microcopy-positive samples (n = 41)			
40	+	+	+
1	+	-	-
Microscopy-negative samples (n = 83)			
69	-	-	-
5	+	+	+
4	-	-	+
2	+	-	-
1	+	+	-
1	+	-	+
1	-	+	-

Of the 83 microscopy-negative samples, 69 (83.1%) were found negative in all three PCR assays (Table 6.3). The remaining 14 samples were PCR-positive in at least one of the three assays. Five of these were positive in all three assays, two samples were positive in two of the assays, and seven samples were positive in a single assay only. The majority (12/14) of the patients with microscopy-negative/PCR-positive results were HIV-negative patients with one or more risk factors for PCP (data not shown). An additional patient was HIV-positive and the remaining patient did not have any known risk factors. The most likely explanation for the microscopy-negative/PCR-positive results is the higher sensitivity of PCR as opposed to microscopy.

The agreement between the three *P. jiroveci* real-time PCR assays was assessed by pair wise comparisons of the qualitative test results. Highest agreement was found between the MINC and LUMC assays, with a percentage of agreement of 96.8% and a Kappa value of 0.93. The agreement between the other assays was also excellent, with a percentage of agreement of 94.4% and a Kappa value of 0.88, between both the MINC and RUNMC assays and the RUNMC and LUMC assays.

Quantitative comparison between the three real-time PCR assays

To compare the quantitative performance of the three real-time PCR assays, pair wise correlations between C_t values generated on samples that were scored positive in all three assays (n=45; Table 6.3) were calculated. As shown in Figure 6.1, there was a good correlation between the C_t values produced by each of the three assays. The correlation coefficients were 0.84, 0.90 and 0.99 between the LUMC and MINC assays, the MINC and RUNMC assays, and the LUMC and RUNMC assays, respectively. While the RUNMC and LUMC assays generated similar C_t values (Figure 6.1A), the C_t values produced by the MINC assay were somewhat lower than those produced by the other assays (Figure 6.1B and C).

Quantitative comparison between microscopy and real-time PCR

To examine the correlation between microscopy and real-time PCR in quantitative detection, the results from these assays were compared for a selection of positive samples (n=26). Since a comparison to C_t values may be difficult to interpret, these values were first converted to copy number equivalents of a plasmid (pPCP) containing the *P. jiroveci* amplicon. This was achieved in the MINC real-time PCR assay by generating a standard curve using this plasmid (Figure 6.2A en B). Subsequently, the copy number equivalents of the 26 *P. jiroveci*-positive samples were plotted against the microscopically quantified *P. jiroveci* burden (expressed as clusters per

cytospin spot). As shown in Figure 6.2C, there was a good correlation between both methods, which was expressed as a correlation coefficient of 0.83.

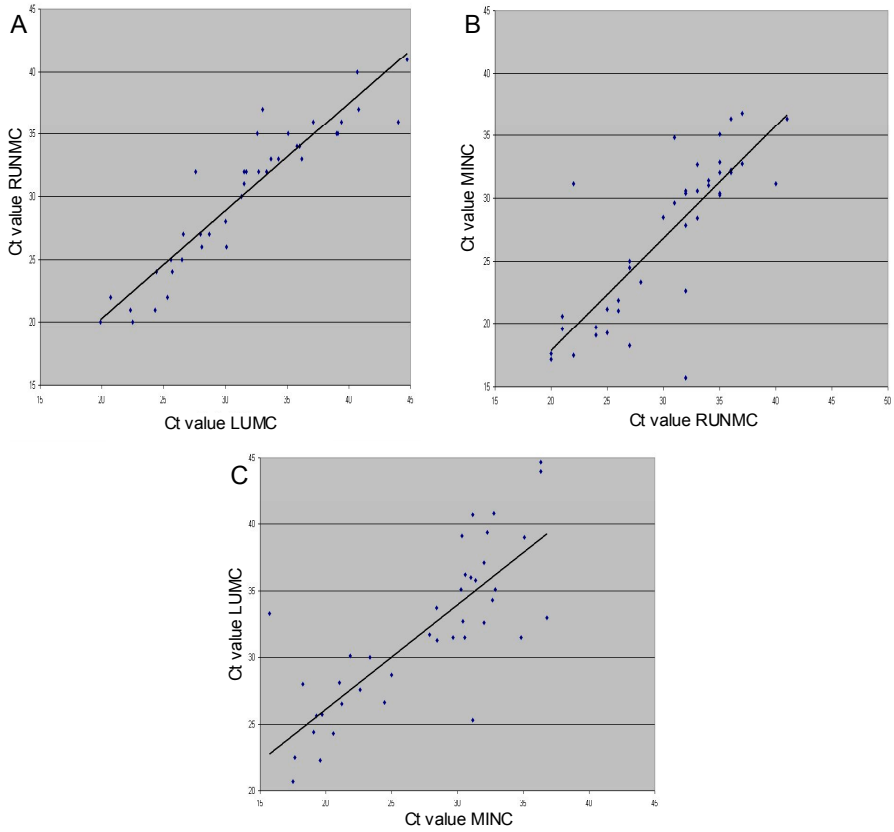

Figure 6.1 Pair wise comparison between the different C_t values for all samples (n=45) that were found to be positive in all three real-time PCR assays. (A) Comparison of C_t values from the RUNMC assay (y-axis) and the LUMC assay (x-axis). (B) Comparison of the C_t values from the MINC assay (y-axis) and the RUNMC assay (x-axis). (C) Comparison of the C_t values from the LUMC assay (y-axis)and the MINC assay (x-axis).

P. jiroveci carrier ship versus clinically relevant infection

An important issue in the diagnosis of PCP is the distinction between apparent asymptomatic *P. jiroveci*-carriers and patients with clinically obvious PCP. In most studies, carriers have been defined as patients in which *P. jiroveci* DNA could be detected in the absence of clinical signs of *P. jiroveci* infection, and without microscopically detectable *P. jiroveci* cysts in BALF samples.

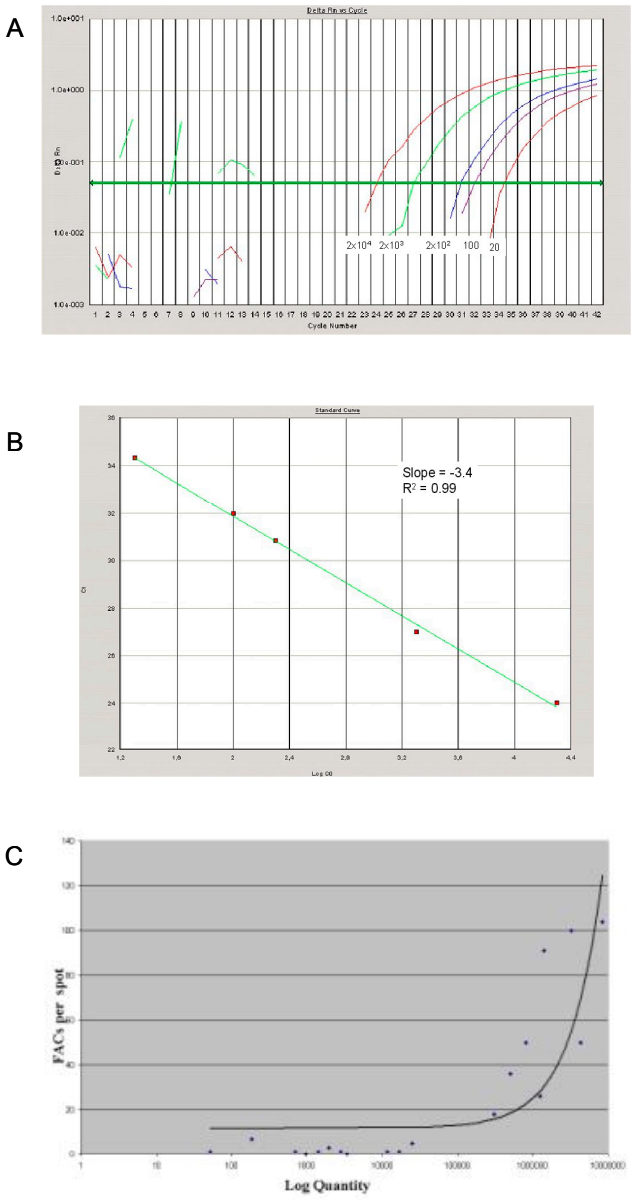

Figure 6.2 Correlation between microscopy and the MINC real-time PCR assay in quantification of *P. jiroveci*. (A) Amplification plot (Delta Rn versus cycle number) generated with dilutions of plasmid pPCP (2×10^4, 2×10^3, 2×10^2, 100, and 20 copies per reaction), which contains the *P. jiroveci* amplicon. (B) Standard curve (C_t versus log copy number [CO]) generated from the amplification plot shown in (A). (C) Correlation between the number of *P. jiroveci* clusters per cytospin spot (foamy alveolar casts (FACs) per spot) and the log of the *P. jiroveci* copy number (Log Quantity) for 26 PCR-positive BALF samples.

Discussion

In the present study, we found an overlap between C_t-values in samples obtained from potential carriers of *P. jiroveci* on the one hand and some of the samples from patients with clinically and microscopically proven PCP on the other hand. This is in line with previous reports, in which a reliable cut-off value for the differentiation between disease and carrier state could not be firmly established[18,19]. Nevertheless, it is possible to divide patients into three categories. In the first category, patients are included with clinical symptoms suspect for PCP with a positive PCR result and/or a positive microscopy result; these patients are diagnosed with PCP. In the second category, patients are included who have no clinical symptoms and do not have any indication for infection with *P. jiroveci*, as indicated by negative results in both microscopy and PCR. The third group is more complex, and consists of patients who do not have typical clinical symptoms of PCP, but show a positive PCR result (usually with high C_t values). We hypothesize that the patients without risk factors for PCP should be regarded as asymptomatic carriers and do not require treatment for PCP. In case of patients belonging to the at-risk group with negative microscopy results, the microscopy should be re-evaluated and the patient should be clinically followed and receive therapy with the appearance of any clinical symptoms suspect of PCP.

Previously, only immunocompromised patients, such as HIV-positive patients, were considered to be potential carriers of *P. jiroveci*[22,23]. More recently, however, immunocompetent individuals were also found to be putative carriers[24,25]. Miller and coworkers investigated health care workers who came into contact with patients with PCP and found among them a carrier rate of 30.5%[12]. In particular, health care workers taking BALF or induced sputum samples were found to be at risk for developing a carrier status, which in one case even persisted for 27 months[26]. All *P. jiroveci*-DNA positive patients from our study belonged to the group at risk, except for a single patient who was admitted to the intensive care unit and diagnosed with a community-acquired pneumonia caused by *Streptococcus pneumoniae*.

In conclusion, we have compared the performance of three different in-house developed real-time PCR assays for *P. jiroveci*. Interestingly, while these three assays employ different methods for nucleic acid isolation, amplification as well as detection, an excellent agreement in performance was found between the assays, both qualitatively (the diagnosis of PCP) and quantitatively (the *P. jiroveci* burden). Likewise, a good correlation was found between the *P. jiroveci* quantity determined by real-time PCR and microscopic quantification. A cut-off value to discriminate between disease and carrier status for *P. jiroveci* could

not be established in the present, retrospective study. A future prospective study is needed to investigate whether quantitative PCR results can be employed to differentiate between PCP and carrier ship of *P. jiroveci.* Finally, in order to monitor the performance of the different in-house PCR assays currently used in microbiology laboratories, the availability of quality control panels is of utmost importance. The assays described here could serve as reference assays in the development and maintenance of such panels.

References

1. Edman JC, Kovacs JA, Masur H, Santi DV, Elwood HJ, Sogin ML. Ribosomal RNA sequence shows Pneumocystis carinii to be a member of the fungi. Nature 1988;334:519-22.
2. Stringer JR, Cushion MT, Wakefield AE. New nomenclature for the genus Pneumocystis. J Eukaryot Microbiol. 2001;Suppl:184S-9S.
3. Kovacs JA, Gill VJ, Meshnick S, Masur H. New insights into transmission, diagnosis, and drug treatment of Pneumocystis carinii pneumonia. Jama. 2001;286:2450-60.
4. Sepkowitz KA. Opportunistic infections in patients with and patients without Acquired Immunodeficiency Syndrome. Clin Infect Dis. 2002;34:1098-107.
5. Yale SH, Limper AH. Pneumocystis carinii pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. Mayo Clin Proc. 1996;71:5-13.
6. Djamin RS, Drent M, Schreurs AJ, Groen EA, Wagenaar SS. Diagnosis of Pneumocystis carinii pneumonia in HIV-positive patients. Bronchoalveolar lavage vs. bronchial brushing. Acta Cytol. 1998;42:933-8.
7. Thomas CF, Jr., Limper AH. Pneumocystis pneumonia. N Engl J Med. 2004;350:2487-98.
8. Kovacs JA, Ng VL, Masur H, Leoung G, Hadley WK, Evans G, Lane HC, Ognibene FP, Shelhamer J, Parrillo JE., et al. Diagnosis of Pneumocystis carinii pneumonia: improved detection in sputum with use of monoclonal antibodies. N Engl J Med. 1988;318:589-93.
9. Elvin KM, Bjorkman A, Linder E, Heurlin N, Hjerpe A. Pneumocystis carinii pneumonia: detection of parasites in sputum and bronchoalveolar lavage fluid by monoclonal antibodies. BMJ. 1988;297:381-4.
10. Amin MB, Mezger E, Zarbo RJ. Detection of Pneumocystis carinii. Comparative study of monoclonal antibody and silver staining. Am J Clin Pathol. 1992;98:13-8.
11. Chandra P, Delaney MD, Tuazon CU. Role of special stains in the diagnosis of Pneumocystis carinii infection from bronchial washing specimens in patients with the acquired immune deficiency syndrome. Acta Cytol. 1988;32:105-8.
12. Miller RF. Pneumocystis carinii infection in non-AIDS patients. Curr Opin Infect Dis. 1999; 12:371-7.
13. Limper AH, Offord KP, Smith TF, Martin WJ, 2nd. Pneumocystis carinii pneumonia. Differences in lung parasite number and inflammation in patients with and without AIDS. Am Rev Respir Dis. 1989;140:1204-9.
14. Wakefield AE, Pixley FJ, Banerji S, Sinclair K, Miller RF, Moxon ER, Hopkin JM. Detection of Pneumocystis carinii with DNA amplification. Lancet. 1990;336:451-3.
15. Larsen HH, Masur H, Kovacs JA, Gill VJ, Silcott VA, Kogulan P, Maenza J, Smith M, Lucey DR, Fischer SH. Development and evaluation of a quantitative, touch-down, real-time PCR assay for diagnosing Pneumocystis carinii pneumonia. J Clin Microbiol. 2002;40:490-4.
16. Maskell NA, Waine DJ, Lindley A, Pepperell JC, Wakefield AE, Miller RF, Davies RJ. Asymptomatic carriage of Pneumocystis jiroveci in subjects undergoing bronchoscopy: a prospective study. Thorax. 2003;58:594-7.
17. Nevez G, Guyot K, Totet A, Raccurt C, Dei-Cas E. Pulmonary colonisation with Pneumocystis carinii in an immunosuppressed HIV-negative patient: detection and typing of the fungus by PCR. J Med Microbiol. 2001;50:198-200.
18. Flori P, Bellete B, Durand F, Raberin H, Cazorla C, Hafid J, Lucht F, Sung RT. Comparison between real-time PCR, conventional PCR and different staining techniques for diagnosing Pneumocystis jiroveci pneumonia from bronchoalveolar lavage specimens. J Med Microbiol. 2004;53:603-7.
19. Larsen HH, Huang L, Kovacs JA, Crothers K, Silcott VA, Morris A, Turner JR, Beard CB, Masur H, Fischer SH. A prospective, blinded study of quantitative touch-down polymerase chain reaction using oral-wash samples for diagnosis of Pneumocystis pneumonia in HIV-infected patients. J Infect Dis. 2004;189:1679-83.

20. Olsson M, Stralin K, Holmberg H. Clinical significance of nested polymerase chain reaction and immunofluorescence for detection of Pneumocystis carinii pneumonia. Clin Microbiol Infect. 2001;7:492-7.
21. Jacobs JA, Dieleman MM, Cornelissen EI, Groen EA, Wagenaar SS, Drent M. Bronchoalveolar lavage fluid cytology in patients with Pneumocystis carinii pneumonia. Acta Cytol. 2001;45:317-26.
22. Weig M, Klinker H, Wilhelm M, Lemmer K, Gross U. Correlation of Pneumocystis carinii PCR with clinical diagnosis in immunocompromised patients. Lancet. 1996;347:1266.
23. Weig M, Klinker H, Bogner BH, Meier A, Gross U. Usefulness of PCR for diagnosis of Pneumocystis carinii pneumonia in different patient groups. J Clin Microbiol. 1997;35:1445-9.
24. Sing A, Roggenkamp A, Autenrieth IB, Heesemann J. Pneumocystis carinii carriage in immunocompetent patients with primary pulmonary disorders as detected by single or nested PCR. J Clin Microbiol. 1999;37:3409-10.
25. Visconti E, Marinaci S, Zolfo M, Mencarini P, Tamburrini E, Pagliari G, Ortona E, Siracusano A. Very low frequence of Pneumocystis carinii DNA detection by PCR in specimens from patients with lung damage. J Clin Microbiol. 2000;38:1307-8.
26. Miller RF, Ambrose HE, Wakefield AE. Pneumocystis carinii f. sp. hominis DNA in immunocompetent health care workers in contact with patients with P. carinii pneumonia. J Clin Microbiol. 2001;39:3877-82.