Evaluation of Amplicor® Chlamydia PCR and LCX® Chlamydia LCR to detect *Chlamydia trachomatis* in synovial fluid

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Abstract

Objectives

PCR has been successfully used in research for the detection of C. trachomatis *DNA in synovial samples. However, each research laboratory has developed its own PCR, making inter-laboratory comparisons difficult. To allow for standardization we evaluated two commercially available amplification systems originally designed for the examination of urogenital samples (Roche Amplicor® Chlamydia PCR and Abbott LCX® Chlamydia LCR), using them to analyse spiked and clinical synovial fluid (SF) samples from reactive arthritis (ReA), undifferentiated arthritis (UA), and rheumatoid arthritis (RA) patients. We compared their sensitivity in assays of clinical SF samples with our in-house developed* C. trachomatis *specific nested PCR.*

Methods

SF was spiked with purified C. trachomatis *elementary bodies (EB) and analyzed by the commercial assays. Clinical SF samples from ReA (n=21), UA (n=79) and RA (n=50) patients were examined by the two commercial assays and our in-house PCR.*

Results

Using SF samples spiked with defined numbers of C. trachomatis *EB, the sensitivity of the commercial tests was high and similar to published PCR sensitivity. In clinical SF specimens the commercial assays was also able to detect CT; however, the in-house PCR was more sensitive. Out of 10 PCR-positive SF samples Amplicor tested positive in only 4/10 and LCX in only 3/10. The in-house PCR detected chlamydial DNA in synovial fluid from 5/21 ReA (24%), 5/79 UA (6%) and in none of the 50 RA patients.*

Conclusion

Commercial amplification assays allow the detection of C. trachomatis *in clinical specimens, although with a lower sensitivity than optimized PCR. Potential explanations are discussed.*

Key words

Chlamydia trachomatis, PCR, LCR, transcription mediated amplification, synovial fluid, reactive arthritis.

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Introduction

Chlamydia trachomatis is an obligate intracellular bacterium and a frequent cause of sexually acquired urethritis, cervicitis, salpingitis, and tubar infertility (1, 2). It has also been recognized as a major cause of reactive arthritis developing after symptomatic as well as clinically silent chlamydial infection of the urogenital tract (3-7). Although the microorganism cannot be cultured from synovial fluid specimens, chlamydial antigens, DNA, rRNA and highly unstable primary rRNA transcripts have been demonstrated in synovial fluid (SF) and synovial membrane, suggesting that viable, but non-culturable *Chlamydia* cause this type of reactive arthritis (8-12).

Consequently, the polymerase chain reaction (PCR) has been used to detect chlamydial DNA in joint material from arthritis patients. Using primers specific for the *Chlamydia* cryptic plasmid, the chromosomal major outer membrane protein (MOMP) gene, or the 16S rRNA gene, chlamydial nucleic acids were demonstrated in a considerable percentage of arthritis patients (9, 11-13). From these studies it was concluded that PCR is a sensitive tool to diagnose *Chlamydia*-induced reactive arthritis. However, the amplification systems used in those investigations were individually developed in each laboratory, making it difficult to compare the results of the different studies and to adopt the molecular assays in other laboratories.

Recently, molecular amplification systems for the detection of *C. trachomatis* nucleic acids became commercially available. These include amongst others the Amplicor® Chlamydia PCR targeting at a 207 bp fragment of the cryptic *C. trachomatis* plasmid (Roche Molecular Systems) and the LCX® Chlamydia ligase chain reaction (LCR), targeting at a 48 bp fragment of the *C. trachomatis* plasmid (Abbott Diagnostics) (14-18). These test systems were shown to have high diagnostic sensitivity and specificity to detect *C. trachomatis* in urogenital smears and urine specimens (18-24).

The aim of the study presented herein was to evaluate these commmcercially available molecular amplification assays to detect *C. trachomatis* DNA in SF. The sensitivity of these detection assays was determined using SF spiked with defined numbers of purified *C. trachomatis* elementary bodies. Then clinical SF specimens from patients with reactive arthritis (ReA), undifferentiated arthritis (UA), and rheumatoid arthritis (RA) were examined for the presence of *C. trachomatis* DNA using these commercially available tests compared to the in-house developed *C. trachomatis* omp1-gene specific PCR.

Materials and methods

Patients

The clinical synovial fluid (SF) specimens were obtained from 21 patients with reactive arthritis and 79 patients with undifferentiated arthritis. SF from 50 patients with rheumatoid arthritis as defined by the 1987 revised ACR criteria served as controls (25). Reactive arthritis was diagnosed based on the proposed diagnostic criteria for reactive arthritis, with the exception that polyarticular arthritis was also considered as reactive arthritis provided that other definite rheumatic diseases were excluded and that there was evidence of preceding infection as suggested by the Third International Workshop on Reactive Arthritis (26). Undifferentiated arthritis was defined by synovitis without psoriatic skin lesion, not meeting any criteria for other defined rheumatic diseases, especially the 1987 revised ACR criteria for rheumatoid arthritis (25), the New York criteria for ankylosing spondylitis (27), the CDC criteria of Lyme arthritis (28), and the above mentioned criteria for reactive arthritis (26).

The patients were seen at our university-based tertiary care center or by rheumatologists in private practice. Seventeen of the patients with undifferentiated arthritis were included in the study reported by Schnarr *et al.* recently (29). The synovial fluid in one of these patients was *C. trachomatis* PCR positive (Patient #5 in Table III). For the study by Schnarr *et al*. and the study reported herein different aliquots of synovial fluid were analyzed by PCR. Synovial fluid (SF) was obtained by

Table I. Demographic and clinical characteristics of the patients with reactive arthritis (ReA) and undifferentiated arthritis (UA) included in this study.

Characteristics	ReA	UA
Number of patients	21	79
Gender (male/female): no. of patients (%)	9 (43) / 12 (57)	43 (54) / 36 (46)
Age (Mean ± SD) [years]	37 ± 14	38 ± 13
Disease duration [months]	24 ± 31	12 ± 8
Monoarthritis: no. of patients (%)	6 (29)	33 (42)
Oligoarthritis: no. of patients (%)	13 (62)	40 (51)
Polyarthritis: no. of patients (%)	2 (9)	6 (7)
UGT-infection[1]: no. of patients (%)	7 (33)	3 (4)
GI-infection[1]: no. of patients (%)	5 (24)	2 (3)
IgG-Chlamydia antibody positive[2]: no. of patients (%)	8 (38)	12 (15)
IgA-Chlamydia antibody positive[2]: no. of patients (%)	7 (33)	4 (5)
HLA-B27 positive: no. of patients (%)[3]	7 (33)	11 (14)

[1]Signs and/or symptoms of UGT (urogenitaltract) and GI (gastrointestinaltract) infection, respectively, within one month prior to onset of the arthritis.
[2]Chlamydia serology by r-ELISA (Medac, Hamburg, Germany); 3 patients in the ReA and 2 patients in the UA group were not tested.
[3]Three patients in both groups were not tested for HLA-B27.

standard arthrocentesis for diagnostic purposes after the patients had given their informed consent. The study had been approved by the local ethics committee. Table I summarizes the demographic and clinical characteristics of the study cohorts. SF specimens were stored at -70°C after collection and thawed only once before examination by the molecular amplifications assays.

Preparation of purified elementary bodies of C. trachomatis
Infectious EB of *C. trachomatis* serovar K (UW/31/Cc Washington Research Foundation, Seattle, WA) were grown in HEp2-cells in RPMI 1640 medium supplemented with fetal calf serum (Biochrome, Berlin, Germany) 10% v/v, L-glutamine 1% w/v, vancomycin 0.1% w/v, and gentamycin 0.1% w/v. Cultures were incubated for 48 h at 37°C in an atmosphere of CO_2 5% w/v. The organisms were purified in a discontinuous gradient of 33% v/v Urografin (Schering, Berlin, Germany) by ultracentrifugation as described by Caldwell (30). Purified EB were resuspended in sucrose phosphate buffer (0.01 M sodium phosphate, 0.25 M sucrose, 5 m L-glutamine acid (pH 7.2) (all from Sigma, St. Louis, Missouri). A stock solution of 7.5 x 10^6 inclusion-forming units (IFU) was prepared by

titration of purified EB on HEp2-cells and a subsequent immunoperoxidase assay as described (31), divided into aliquots and stored at -80°C. To determine the number of chlamydial elementary bodies per IFU, three randomly selected aliquots of purified IFU were diluted to 20, 30, 40, 50, 60, 70, and 80 IFU/10 l. Slides were prepared from these samples as has been described (32) for further investigation by direct immunofluorescence for MOMP-positive EB (MicroTrak, Syva, Darmstadt, Germany). On average, 8 elementary bodies corresponded to 1 IFU. One aliquot of this solution of purified EB was used in each of the following experiments.

Preparation of C. trachomatis *serial dilutions in synovial fluid*
Synovial fluid was obtained from patients with rheumatoid arthritis who had given informed consent and who had no clinical and serological evidence of active or prior chlamydial infection. The synovial samples used for spiking contained 2,000 to 7,000 WBC per mm[3]. They were collected without additives, transported to the laboratory immediately after arthrocentesis and further processed within 3 hours. Purified EB were serially diluted 10-fold in these synovial samples and were stored

at -80°C until used in the experiments. Five different series of SF spiked with defined numbers of EBs were analyzed by Amplicor-PCR and LCX, respectively, at different times. Three negative controls with no EB added were included in each experiment. Prior to DNA extraction SF-samples with 1 ml each were centrifuged at 60,000 x g for 30 min at 21°C.

Examination of synovial fluid samples by Amplicor® Chlamydia PCR
For examination by Amplicor® *Chlamydia* PCR (Roche Molecular Diagnostics, Basel, Switzerland) DNA was extracted using either the QIAEX-gel extraction kit (see below) or a "modified Amplicor extraction". The SF pellet was resuspended in 2 ml of urine dilution solution buffer and added to 2 ml of specimen preparation buffer supplied with the Amplicor specimen preparation kit for urine samples. After excessive vortexing, 50 l of the sample was added to 50 l of the Amplicor mastermix and further processed according to the manufacturer's instructions for urine samples using a Perkin-Elmer 9600 thermocycler. The target of Amplicor PCR lies within the cryptic plasmid of *C. trachomatis*; neither the primer sequence nor the precise location of the primers is given by the manufacturer.
Alternatively 20 l of DNA extracted by the QIAEX gel extraction kit was added to 50 l of the Amplicor mastermix and analyzed according to the manufacturer's instructions.

Examination of synovial fluid samples by LCX Chlamydia LCR®
For examination by LCX® Chlamydia (Abbott Diagnostics, Wiesbaden, Germany), DNA was extracted using either the QIAEX-gel extraction kit (see below) or using a "modified LCX extraction". The SF pellet was resuspended in 1 ml of sample buffer supplied by the manufacturer for urine samples, vortexed extensively, and incubated at 97°C for 15 min. After incubation at room temperature for another 15 min, 100 l was transferred to the LCX mastermix and further processed according to the manufacturer's

instructions for urine samples. Alternatively 20 l of DNA extracted by the QIAEX gel extraction kit was added to 80 l of the LCX resuspension buffer and then analyzed according to the manufacturer's instructions. LCX amplifies a portion of the cryptic plasmid of *C. trachomatis*. The precise location of the primers is not given by the manufacturer.

DNA extraction using QIAEX gel extraction
For DNA extraction using QIAEX the SF pellet was incubated in lysis buffer (0.1 M NaCl, 1 mM EDTA, 10 mM Tris HCl, pH 8, 0.5% Tween 20) containing proteinase K (100 g/ml) at 56°C overnight. Then DNA was isolated using the QIAEX gel extraction kit (Qiagen, Hilden, Germany) and resuspended in 60 l of TE-buffer. 20 l of this was subjected to the three different detection methods tested (Amplicor-PCR, LCR and omp1-gene specific PCR).

Examination of clinical SF specimens by an omp1-gene specific nested PCR
The PCR used is specific for the *C. trachomatis* omp1-gene and has been described recently (32). The primers ct05 (bp 67-90 of the chromosomally encoded omp1-gene, gene bank accession number M14738) and ct06 (bp 312-347) were used for outer PCR, the primer ct03 (bp 157-182) and ct04 (bp 285-308) were used for nested PCR. Target DNA extracted using the QIAEX gel extraction kit was amplified for outer PCR through forty cycles (90 sec denaturation at 94°C, 90 sec primer annealing at 55°C, 90 sec primer extension at 72°C) using 50 pmol ct05 and ct06 for the outer PCR in a 100 l volume containing 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM TRIS/HCl (pH 8.4), 0.1% gelatine (w/v), 0.2 mM dNTPs (Pharmacia, Uppsala, Sweden) and 2.5 U Taq-DNA-polymerase (Pharmacia). The reaction product (10 l) was amplified for a second time through 25 cycles using 50 pmol of the nested primers ct03 and ct04 (primer annealing at 60°C) with all other conditions remaining the same. For both the outer as well as the nested PCR two negative
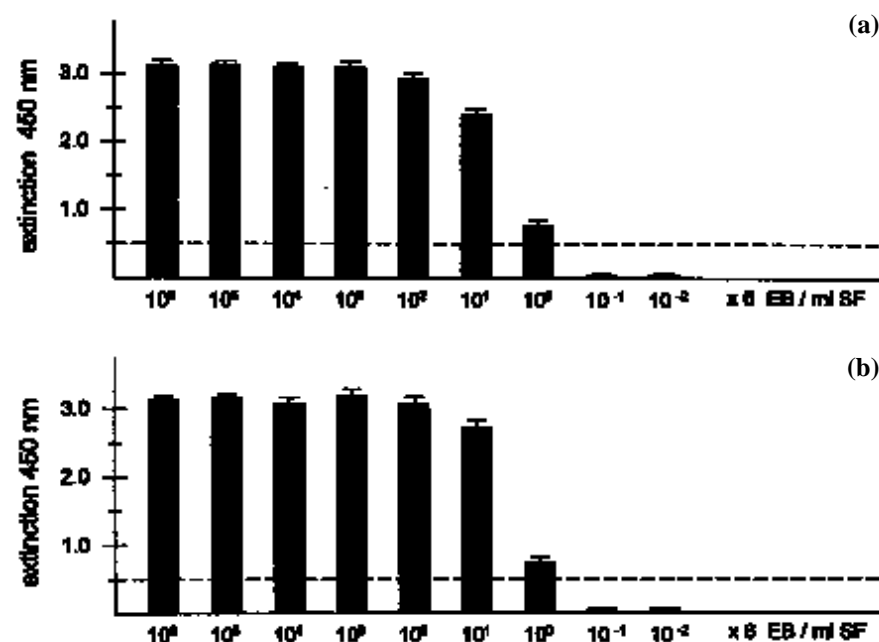
Fig. 1. Results of Amplicor PCR analyses of serial dilutions of purified *C. trachomatis* EB in synovial fluid following DNA extraction by modified Amplicor PCR (**a**) and QIAEX gel extraction (**b**), respectively. On the y-axes, extinction at 450 nm for the Amplicor-PCR-product and relative light units (RLU) for the LCX-product are given. Error bars show standard deviations of the six different dilution series tested. The broken horizontal line indicates the average cutoff-level; results below the cutoff were defined as negative.

controls (sterile distilled water and SF not spiked with CT) were included which were prepared together with the other samples. Amplified product (10 l) was visualized by electrophoresis in a 1.5% agarose gel and stained with ethidiumbromide.

Results
Sensitivity of Amplicor-PCR and LCX for the detection of C. trachomatis *DNA in synovial fluid spiked with defined numbers of purified* C. trachomatis *elementary bodies (EB)*
Five different dilution series of defined numbers of purified EB in synovial fluid were processed by the modified extraction methods for Amplicor-PCR and LCX, respectively as described in Materials and Methods. In addition DNA was extracted from five different dilution series using the QIAEX gel extraction kit and analyzed using Amplicor-PCR and LCX, respectively. Figures 1 and 2 show the results of Amplicor-PCR and LCX for the six different *C. trachomatis* dilution series in the synovial fluid tested. Their sensitivities correspond to the sensitivity of the in-house *C. trachomatis* omp1-gene

specific PCR developed by Bobo *et al*. (33) and modified in our laboratory as has been published recently (32,34,35) (Fig. 3). Both methods allowed the detection of 6 purified elementary bodies per SF sample regardless of the method of DNA extraction used (see Table II).

Detection of C. trachomatis *DNA in clinical SF specimens*
Using the in-house *C. trachomatis* omp1-gene specific PCR, DNA was detected in 5 out of 21 ReA patients (24%) and in 5 out of 79 UA patients (6%) (Table III). Although patients with polyarticular arthritis were included in the ReA and UA group, all of the PCR positive patients had either mono- or oligoarthritis with a preponderance of involvement of the lower extremities (Table III). In the control group (50 RA patients) no positive MOMP-PCR-result was observed. When the same DNA, extracted by the QIAEX gel extraction kit, was analyzed using Amplicor-PCR and LCX 3/10 (1 ReA, 2 UA) tested positive in both, Amplicor-PCR and LCX. One out of the 10 PCR-positive SF samples (1 UA) was also positive in Amplicor PCR (Table III).
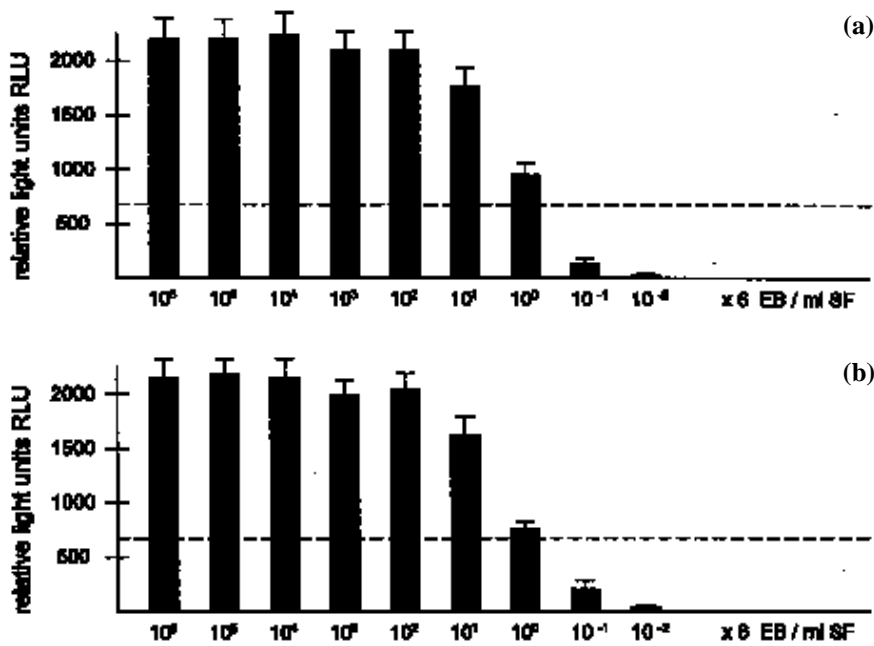
(a)

(b)

Fig. 2. Results of LCX analyses of serial dilutions of purified *C. trachomatis* EB in synovial fluid following DNA extraction by modified LCX extraction (**a**) and QIAEX gel extraction (**b**), respectively. See legend to Fig. 1.

Table II. Comparison of the sensitivity of Amplicor-PCR and LCX for the detection of *C. trachomatis* elementary bodies (EB) in synovial fluid (SF) depending on different DNA extraction methods

A. Sensitivity of Amplicor-PCR for spiked SF.

SF dilution series	DNA extraction by modified Amplicor extraction	DNA extraction by QIAEX gel extraction
#1	6	6
#2	6	6
#3	6	6
#4	6	6
#5	60	6

B. Sensitivity of LCX-PCR for spiked SF.

Dilution series	DNA extraction by modified LCX extraction	DNA extraction by QIAEX gel extraction
#1	6	6
#2	6	6
#3	6	6
#4	6	60
#5	6	60

ine. Only one of the 10 PCR-positive patients was HLA-B27 positive. Most patients showed elevated ESR and CRP values with increased SF leucocytes (Table III).

Discussion

By PCR numerous laboratories including our own have been able to detect chlamydial nucleic acids in synovial fluid in a variable percentage in patients with clinically defined reactive arthritis and undifferentiated arthritis (8-12, 29). However, these studies used locally developed DNA extraction methods and PCR systems. Thus, direct comparison of these data and standardization with the aim to carry over this powerful research tool into routine diagnosis is not yet within reach. It was the aim of this study to analyze whether commercially available detection methods might circumvent these problems.
Using SF samples spiked with defined numbers of purified *C. trachomatis* elementary bodies, these commercially available test systems performed well compared to the published sensitivity of the PCR used in our laboratory (34, 35). For clinical samples from patients with reactive arthritis (ReA) and undifferentiated arthritis (UA) Amplicor-
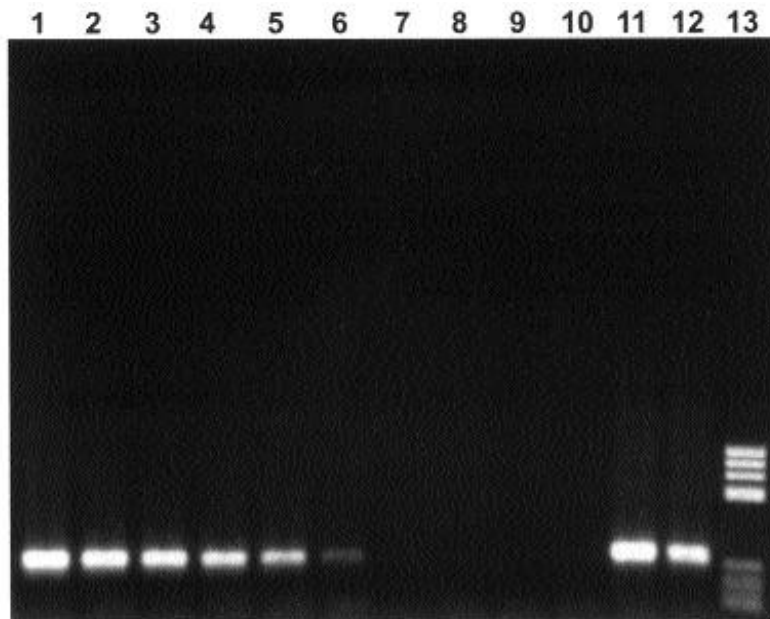

Fig. 3. Electrophoresis of amplified DNA by nested PCR for serial 10-fold dilutions of purified *C. tra - chomatis* EB in synovial fluid. Lane 13, molecular weight marker V (Boehringer, Ingelheim, Germany), lanes 11 and 12 positive controls of purified *C. trachomatis* DNA, lanes 9 and 10 negative controls prepared together with the other samples, lanes 1 to 8, 10-fold serial dilutions of purified EB form 6 x 10⁵ to 6 x 10⁻² EB per ml of synovial fluid. The length of the PCR product is 152 bp.

No PCR-negative SF specimen, including the 50 RA SF samples, tested positive in the two commercial assays. Seven of the 10 PCR-positive patients were tested for chlamydial LPS-specific antibodies (IgA and IgG) using ELISA technology (Medac Co., Ham-

burg, Germany). No confirmatory serological test was performed. Three of these 7 patients tested had *Chamydia* specific antibodies (Table III). Three of the PCR-positive patients had a history of urogenital tract infection, one of these 3 with a positive PCR in the ur-

Table III. Characteristics of the patients with *C. trachomatis*-PCR-positive SF.

Patient number	1	2	3	4	5	6	7	8	9	10
Age [years]	25	67	48	28	44	56	30	46	38	36
Sex	m	f	f	m	m	m	m	m	m	m
Disease duration [months]	48	4	1	6	9	72	5	19	<1	24
Diagnosis	ReA	ReA	UA	ReA	UA	ReA	ReA	UA	UA	UA
Arthritis	Mono	Oligo	Mono	Mono	Oligo	Oligo	Mono	Oligo	Oligo	Oligo
Symmetry		-			+	+		+	+	-
Axial involvement[1]	-	-	-	-	-	-	+	-	-	-
Lower extremity[2]	+	+	+	+	+	+	+	+	+	-
UGT[3]	+	-	-	+[7]	-	+	-	-	-	-
GI[3]	-	+	-	-	-	-	-	-	-	-
Eye[4]	+	-	-	-	-	-	+	-	-	-
ESR [mm in 1 h]	48	59	4	23	25	10	39	21	40	24
CRP [mg/l]	132	15	6	12	2	12	3	n.d.	9	3
WBC	n.d.	7800	5000	7700	n.d.	n.d.	n.d.	6900	4500	11000
RF [>20 U/ml = +]	-	-	-	-	-	-	-	-	-	70
ANA [>1:40 = +]	-	-	-	-	1:80	-	-	-	-	-
HLA-B27	-	-	-	-	-	-	+	-	-	-
IgA-chlamydia[5]	-	-	nd	+	-	-	-	nd	nd	-
IgG-chlamydia[5]	+	-	nd	+	+	-	-	nd	nd	-
SF leucocytes [/ l]	5500	450	1150	8130	300	100	4800	2000	2800	4750
SF-PMN [%]	7	n.d.	29	53	n.d.	n.d.	23	64	61	23
MOMP-PCR in SF[6]	+	+	+	+	+	+	+	+	+	+
Amplicor-PCR in SF[6]	+	+	+	-	-	-	-	-	-	+
LCX in SF[6]	+	+	+	-	-	-	-	-	-	-

n.d. = not done, [1]defined by inflammatory back pain (41); [2]arthritis predominant in lower limbs; 3signs and symptoms of UGT (urogenital tract infection) and GI (= gastro-intestinal tract infection), respectively, within one month prior to onset of the arthritis; [4]signs and symptoms of conjuncitivitis; [5]*Chlamydia* serology by r-ELISA (Medac, Hamburg, Germany); [6]DNA extracted by QIAEX gel extraction kit from 1 ml of SF as described in Materials and Methods; [7]omp1-gene-PCR in urine positive.

PCR and LCX allowed the detection of CT in synovial fluid, although with a lower sensitivity than the in-house PCR.

One explanation may be of course that the in-house PCR overestimated the frequency of chlamydial DNA in SF specimens. However, none of the RA SF specimens were positive using this PCR. Furthermore, we have recently published that even for *C. trachomatis*-induced arthritis (defined by the AMOR criteria for reactive arthritis) (36) the sensitivity of our PCR for the detection of chlamydial DNA in SF is about 50% (29). Therefore highly un-specific amplification by this PCR with a sensitivity being considerably below 100% even for defined *C. trachomatis* reactive arthritis is very unlikely. In addition, one PCR-positive SF speci-men from a UA patient (patient #4), which was negative on Amplicor PCR and LCX analysis, tested positive when this sample was subjected to the Ampli-fied Gen-Probe *Chlamydia* transcrip-tion mediated amplification assay (Gen-Probe, San Diego, CA).

Another more likely explanation for the differences in sensitivity between PCR and the commercial assays regards the details of sample extraction prior to amplification. We have recently de-monstrated that sample preparation prior to PCR greatly influences the sen-sitivity of PCR (34, 35). The method of DNA extraction not only significantly alters the sensitivity of the amplifica-tion system used, but importantly the extraction method has to be optimized in relation to the amplification method. That is, although the DNA extraction method (QIAEX gel extraction) works well for the in-house PCR, it obviously does not allow CT detection in clinical samples with comparable sensitivity by Amplicor-PCR and LCX, respectively. Interestingly the sensitivity of Ampli-cor-PCR and LCX seemed to be suffi-cient using SF samples spiked with defined numbers of purified elementary bodies EB). This phenomenon can be understood by considering the unique life cycle of *C. trachomatis*. This bac-terium is an obligate intracellular path-ogen. The extracellular form, i.e. EB, adheres to the cell membrane and is then incorporated into the phagosomal compartment of the cell where the bac-teria are transformed into reticular bod-ies (RB), undergo replication by binary fission and finally reorganize to EB prior to release by the infected host cell. To spike the synovial fluid, puri-fied extracellular EB were used be-cause these can be quantified precisely using immunofluorescence. However, in the clinical samples *C. trachomatis* persists intracellularly. Furthermore, these intracellularly persisting *C. tra-chomatis* show an aberrant gene ex-pression profile which also may influ-ence the ease with which DNA extrac-tion methods can release chlamydial DNA (37). These two differences, i.e. the intracellular localization and atypi-cal morphology of the intraarticularly persisting *C. trachomatis,* to our minds

are the most likely explanation for the differences in sensitivity for clinical samples compared to spiked SF specimens.

A third explanation for the reduced sensitivity for clinical samples of Amplicor PCR and LCX may be that both assays target the cryptic plasmid of *C. trachomatis*. With regard to the observation that this plasmid is not necessary for chlamydial viability and that plasmid-free *C. trachomatis* have been described in clinical samples, another explanation for their reduced sensitivity may therefore be the intraarticular presence of plasmid-free *C. trachomatis* at least in individual patients (38).

Taking together, in our view differences in sample preparation in concert with intracellular localization and the atypical morphology of the persisting *C. trachomatis* most likely account for the difference in sensitivity observed between the in-house PCR and the two commercial tests. This interpretation is supported by previous work from our group demonstrating clearly the great importance of optimised sample preparation prior to PCR (34, 35).

Amplicor PCR and LCX have recently been analyzed for their sensitivity to detect *C. trachomatis* in synovial fluid (39, 40). Whereas Bas *et al.* failed to detect *C. trachomatis* DNA in *C. trachomatis* PCR-positive SF specimens using commercially available test systems (Amplicor PCR, LCX and Gen-Probe PACE2), Nikkari *et al.* demonstrated chlamydial DNA in SF cells from 4 of 12 patients with *Chlamydia*-induced arthritis using the LCX Chlamydia LCR (38,40). The data of Nikkari are in agreement with our findings (40). We were also able to detect chlamydial DNA by LCX and, in addition, also by Amplicor PCR. The negative results reported by Bas *et al.* could have been due to problems in DNA release from the samples in their analysis; for instance, even for SF samples spiked with defined numbers of extracellular EB the sensitivity was very low for inflammatory synovial fluid samples (39).

Chlamydia serology could not compensate for PCR testing. Seven of the 10 PCR positive patients were tested for anti-chlamydial LPS antibodies (IgA and IgG) using ELISA, with only 3 being seropositive. Insensitivity of the ELISA used, the fact that the antibody target (LPS) differs from the PCR target (MOMP) and the intracellular persistence of the bacteria, thus potentially evading a humoral immune response, may explain this phenomenon.

The standardization of molecular biology-based amplification systems for the detection of reactive arthritis-causing bacteria such as *C. trachomatis* is urgently needed. At this stage commercially available test systems, although performing well for spiked samples, do not reach the necessary sensitivity for clinical samples. It is conceivable, however, that by optimized sample preparation adjusted to the respective amplification systems their performance for clinical SF specimens will improve. In studies published recently we were able to demonstrate that optimized sample preparation is able to increase the sensitivity of subsequent molecular biology detection systems by more than 10 to 100-fold (34,35). Thus, optimized sample extraction will most likely allow detection rates of commercial assays with a sensitivity comparable to optimized DNA extraction and in-house PCR systems.

Chlamydia-induced reactive arthritis is a rare consequence of *C. trachomatis* urogenital tract infection (1,2,4). Thus, detection in the inflamed joint would mean greater diagnostic accuracy than detection at the site of entry, provided that the specificity of the PCR for synovial specimens is high, as shown in this study where no positive PCR results were observed in rheumatoid arthritis patients. Since there is no proven effective antibiotic treatment for *C. trachomatis*-induced reactive arthritis, the PCR based detection of chlamydial DNA does not justify antibiotic treatment, but allows us to assure the patient that he is suffering from a benign disease. Furthermore, in those patients with prolongued *Chlamydia*-induced reactive arthritis a positive PCR for synovial specimens will be important once effective antibiotic treatment becomes available. Right now different PCRs for synovial specimens are used in research laboratories. Standardized detection by PCR is urgently needed to allow valid interlaboratory comparisons of the prevalence of positive chlamydial PCR in undifferentiated and reactive arthritis.

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