Evaluation of the sensitivity and specificity of a ligase chain reaction test kit for the detection of *Chlamydia trachomatis*

N Miyashita, A Matsumoto, Y Niki, T Matsushima

Abstract

The sensitivity and specificity of a newly developed ligase chain reaction (LCR) test kit were examined by the use of highly purified elementary bodies (EBs) and in situ inclusions containing reticulate bodies only. The performance of the LCR kit was compared with a commercially available polymerase chain reaction (PCR) test kit, AMPLICOR *Chlamydia trachomatis*. The number of EBs and inclusions of *C. trachomatis*, respectively, at the detection limit of both kits were two EBs and one inclusion per assay. Neither kit cross-reacted with *C. psittaci* and *C. pecorum* EBs or reticulate bodies.


Keywords: *Chlamydia trachomatis*, ligase chain reaction.

*Chlamydia trachomatis* is the cause of a large array of sexually transmitted diseases (STDs), as well as ocular infections. Recent epidemiological studies have revealed that *C. trachomatis* is the major cause of STDs such as nongonococcal urethritis, cervicitis and many related diseases, especially in well developed countries. Given its importance, a number of commercially available test kits for detecting *C. trachomatis* in clinical specimens have been developed and are used routinely for the diagnosis of *C. trachomatis* infection. Among these test kits, the polymerase chain reaction (PCR) test kit for detecting *C. trachomatis* specific 7.5 kilobase plasmid DNA has revolutionised diagnostic sensitivity and specificity.

*C. trachomatis*, as well as other members of the genus *Chlamydia*, replicate through a unique developmental cycle that involves serial conversion of two distinct forms, the elementary body (EB) and the reticulate body (RB). After internalisation of the EB into the host cell, this extracellular form transforms into the RB, multiplies by binary fission, then subsequently converts back into the EB form. The EBs are uniform in size (about 0.3 μm in diameter) but the RBs range in size from 0.5 to 1 μm, occasionally reaching more than 2 μm in diameter. This strongly suggests that the target of the test kits is constant in EBs, but not RBs. Based on this supposition, we have examined the sensitivities of many test kits, such as MicroTrak (Syva, Palo Alto, California, USA), IDEIA Chlamydia (Dako, Ely, Cambs, UK), Gen-probe PACE 2 (Gen Probe, San Diego, California, USA), and the PCR test kit, AMPLICOR *C. trachomatis* (Roche Diagnostics, Branchburg, New Jersey, USA). Using the same methodology, we have also examined the sensitivity and specificity of a newly developed ligase chain reaction (LCR) test kit (Abbott, Chicago, Illinois, USA) targeting the 7.5 kilobase plasmid of *C. trachomatis*.

Methods

The following chlamydial strains were used: *C. trachomatis* D/UW-3/Cx, E/UW-5/Cx, 1,2/434/Bu, and 20 clinical isolates; *C. pneumoniae* TW-183, AR-39, AR-388, IOL-207, Kajaani-6, YK-41, KKpn-15, and 14 clinical isolates; *C. psittaci* Prt/GCP-1, Or/B577, Frt/Hu/Cal 10, Bud/Budgerigar-1, Bud/IZAWA-1, Hu/30A, and three clinical isolates; and *C. pecorum* Bo/E58. The species of all clinical isolates of *C. trachomatis* and *C. pneumoniae* was determined by iodine staining and immunofluorescent staining with MicroTrak and RR-402 (Washington Research Foundation, USA) monoclonal antibody specific for *C. pneumoniae*. The propagation of all strains was performed according to previously reported methods.

The purification of EBs was also carried out by a previously reported method, except that the Renografin gradient centrifugation was repeated three times in order to enhance the purity of the EB preparations. Clinical isolates were tested without purification for chlamydial organisms and therefore contained a lot of cell debris. Due to their fragility, it was difficult to purify intact RBs of all strains used. Therefore, we prepared appropriate strain infected cells at less than 1 inclusion forming unit (IFU)/cell, harvesting 15 and 19 hours post-inoculation in order to test reactivity against RBs contained in the inclusions, as described previously. The number of EBs and inclusion bearing cells was counted by previously reported methods. Briefly, the purified EB suspension was appropriately diluted and spun down on a coverslip. After shadowcasting, intact EBs were counted in 30 fields of a scanning electron microscope (×8000). Based on the count, duplicate series of a twofold dilution were prepared. The number of inclusions in 10² cells was counted by flow cytometry (FACStar, Becton Dickinson, USA) after first being prepared by a direct immunofluorescent antibody staining method with a genus specific monoclonal antibody, Cultureset (Ortho Diagnostic Systems), and then being diluted 10-fold in series, in duplicate, as for the EB suspension. To compare the sensitivities of the PCR and LCR kits, each suspension in the dilution series was
mixed with 10 times the volume of transport medium provided in the PCR kit. Similarly, the extraction medium in the LCR kit was added to the other series of EB and inclusion bearing cell suspensions in order to release the target 7.5 kilobase plasmid DNA. Thereafter, all steps in both tests were carried according to the manufacturers’ instructions.

Results
Electron microscopy, as performed in our previous study, demonstrated that the newly prepared EB fractions of the C trachomatis, C psittaci and C pecorum strains consisted of almost 100% intact EBs. However, the EB suspensions of the C pneumoniae strains contained 97–98% intact EBs and also a few EB envelopes.

The results obtained in several preliminary tests of the LCR kit showed that the EB number at the detection limit ranged from two to four EBs per assay; however, the cut off level (detection limit) was changed from 339 to 400 counts/second/second, as this level must be determined every time an assay is run in this test system. Therefore, in the present study, we assayed all samples at the same time to compare the sensitivities of the PCR and LCR kits accurately.

Figure 1 shows the relation between the number of EBs and the intensity of the signal produced by the PCR (fig 1A) and LCR (fig 1B) kits. The cut off level clearly occurs between one and two EBs per assay (broken lines) in the PCR kit, in agreement with our previous study. An identical result was obtained with the LCR kit for three different series of diluted samples. We therefore conclude that the sensitivities of the PCR and LCR kits are equivalent and that the newly developed LCR test kit can detect two or more EBs per assay. We emphasise that, based on identical PCR sensitivities in the present and previous studies, the LCR sensitivity obtained from the same dilution series is reliable. No cross-reaction with the EBs of C pneumoniae, C psittaci and C pecorum strains was observed for any of the EB concentrations used (data not shown), indicating that the LCR and PCR kits are highly specific for C trachomatis.

Table 1 shows the number of inclusions detected by the PCR and LCR test kits. One inclusion of C trachomatis was detected by both assays irrespective of the harvest time after inoculation, indicating that RBs could also be detected by both test kits, although the number of RBs at the detection limit was not determined. As for the EBs of C pneumoniae, C psittaci and C pecorum, the RB inclusions of these species were not detected by either kit.

All clinical C trachomatis isolates containing cell debris were also detected with the LCR and PCR kits. This indicates that these kits are applicable to specimens containing cell debris, such as clinical swabs. None of the clinical isolates of other species was detected with either test kit (data not shown).

Discussion
The target of the LCR test kit and the PCR test kit, AMPLICOR C trachomatis, is the C trachomatis specific 7.5 kilobase plasmid. We previously reported that the number of EBs at the detection limit (or cut off level) determined by the PCR kit was two to four per assay. In the present study, an identical result was obtained, confirming the accuracy of our particle counting method applied to purified EBs. The samples tested with the LCR kit were the same as those tested with the PCR kit, and the EB particle number at the detection limit of the LCR test was also identical with that of the PCR test. Therefore, we conclude that the
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sensitivity of the LCR kit is identical with that of the PCR kit and that the LCR kit can detect two or more EBs per assay. The average number of plasmids in one EB was estimated to be about 10. These results, therefore, indicate that 20 copies or more of the plasmid can be amplified and detected by the LCR kit under the conditions used. Dille et al. reported that the sensitivity of the LCR kit which targets the major outer membrane protein gene and the plasmid appeared to be three EBs per assay using purified EBs which were counted by optical microscopy with Giemsa staining. Their results lead us to wonder whether the accuracy of the particle counting, together with the purity of the EB fraction used in their tests, were sufficient for this highly sensitive assay.

C. psittaci has also been reported to contain plasmids, but they were not amplified in either of the assays used in the present study, indicating the sequence differences between C. trachomatis and C. psittaci. No evidence was obtained for the presence of plasmids in C. pneumoniae organisms isolated from humans.

In conclusion, the newly developed LCR test kit, which can be used as a non-radioactive method, is extremely sensitive and specific for the detection of C. trachomatis organisms. The manipulation procedures for this assay are simple and designed to minimise carry-over contamination which can cause false positive results. Therefore, we recommend the LCR and PCR kits for the routine diagnosis of C. trachomatis infection.

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Caseous necrosis in cutaneous leishmaniasis

E Peltier, P Wolkenstein, M Deniau, E-S Zafrani, J Wechsler

Service d’Anatomie et de Cytologie Pathologiques, Hôpital Antoine Béclère, 157 rue de la Porte de Trivaux, 92141 Clamart, France E Peltier

Service de Dermatologie, Hôpital Henri Mondor, 51 Avenue du Maréchal de Lattre de Tassigny, 94010 Créteil Cédex, France P Wolkenstein

Service de Parasitologie M Deniau

Service d’Anatomie et de Cytologie Pathologiques E-S Zafrani J Wechsler

Correspondence to: Janine Wechsler, M.D.

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Abstract

A case of late stage cutaneous leishmaniasis with focal caseous necrosis is reported. The patient, a 30 year old Tunisian man, presented with idiopathic bone marrow aplasia. Microscopically, minimal changes were observed in the epidermis: slight hyperkeratosis and moderate acanthosis. Lesions predominated in the dermis. Epitheloid granulomas were found in the lower dermis. Some of these lesions were clearly surrounded by a ring of lymphocytes and were rarely confluent. A peculiar histological feature was the presence of focal acidophilic and slightly granular necrosis at the centre of some the tuberculoid lesions. Focal fibrinoid necrosis was found in the upper dermis, outside granulomas. A mild to moderate infiltrate of histiocytes, lymphocytes and plasma cells, with scanty neutrophils, was observed mainly in the upper dermis. No intracellular or extracellular Leishman–Donovan bodies were observed. Acid fast mycobacteria, however, were not detected.

Leishmaniasis was diagnosed on culture of skin biopsy specimens. The presence of caseous necrosis could lead to diagnostic confusion and result in an erroneous diagnosis of, for example, tuberculosis, syphilis, acne agminata, and sarcoidosis with fibrinoid necrosis. This is especially the case when parasites are scanty or absent.


Keywords: cutaneous leishmaniasis, caseous necrosis, pathology.

Cutaneous leishmaniasis, caused by infection with a flagellate protozoon, is endemic in the Mediterranean region. The disease can occur in one of four clinical forms: acute leishmaniasis, chronic leishmaniasis, leishmaniasis recidivans, and diffuse cutaneous leishmaniasis. Ninety per cent of cases of acute cutaneous leishmaniasis resolve without treatment, healing with scar formation. The remaining cases generally evolve into chronic disease.

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