Use of Real-Time Quantitative PCR To Detect 
Chlamydophila felis Infection

CHRIS HELPS,* NICKY REEVES, SÉVERINE TASKER, AND DAVE HARBOUR
Division of Molecular and Cellular Biology, Department of Clinical Veterinary Science, University of Bristol,
Langford, Bristol, BS40 5DU, United Kingdom

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A real-time PCR assay was developed to detect and quantify Chlamydophila felis infection of cats. The assay uses a molecular beacon to specifically identify the major outer membrane protein gene, is highly reproducible, and is able to detect fewer than 10 genomic copies.

The family Chlamydiaceae has recently been revised on the basis of recent phylogenetic analyses of the 16S and 23S rRNA genes and genetic and phenotypic data (1). In this revision, the feline strain of Chlamydia psittaci is given specific status in a new genus, Chlamydophila. Chlamydophila felis infection is common in cats and is a major cause of conjunctivitis (2, 8, 9). It can also cause fever, lethargy, lameness, reduction in weight gain, and upper respiratory symptoms (5, 7). It can be difficult to diagnose from conjunctival swabs by isolation due to the low number of organisms present and the presence of tear antibodies in chronic infection. It has been shown that conventional PCR is more sensitive than isolation in detecting this infection (3, 6). Some major problems with conventional PCR are amplicon contamination, resulting in false positives, and the inability to accurately quantify the amount of starting template in the reaction mixture. We have developed a real-time quantitative PCR assay using a molecular beacon for C. felis that can detect fewer than 10 genomic equivalents and has a linear dilution curve of 9 log10.

A conjunctival swab was used to obtain cells from a cat known to be infected with C. felis. Genomic DNA was extracted from the swab using a DNeasy tissue kit (Qiagen, Crawley, United Kingdom) according to the manufacturer’s instructions and was used as a template to generate an amplicon using primers Chl for and Chl rev designed to target the major outer membrane protein gene (Table 1) (4). The PCR mixture consisted of 25 μl of Qiagen 2X master mix (Qiagen), 0.2 μM Chl for, 0.2 μM Chl rev, 5 μl of template DNA, 120 nM C. felis molecular beacon (Cruachem Ltd., Glasgow, Scotland) (Table 1), and water to 20 μl. After an initial incubation at 50°C for 3 min to allow uracil DNA glycosylase (UDG) to digest any amplicon carryover and at 94°C for 2 min to inactivate the UDG, 45 cycles of 94°C for 10 s and 50°C for 30 s were carried out. Fluorescence was detected at 525 nm at each annealing step (50°C). All reactions were run in triplicate.

Figure 1 shows a plot of threshold cycle versus log10 copy number for the PCR standard in triplicate. It can be seen that the dilution is linear over a 9-log10 range with a correlation coefficient of 0.999. The estimated starting copy number was 7 × 1010 molecules per PCR, and it was possible to detect as few as 7 molecules of template in two of the three reactions performed at this dilution. None of the reactions at 0.7 molecule per reaction gave a positive result.

Figure 2 shows a dilution curve for a sample isolated from a cat known to have a C. felis infection. Genomic DNA was diluted 10-fold and used as a template in the PCR. It can be seen that, by using a clinical sample of genomic DNA, a dilution curve that is linear over a range of 5 log10 can be produced. Using the standard curve, it is possible to accurately quantify the number of genomic copies present in the starting material. This equates to fewer than 10 genomic equivalents in the 105 dilution. At a 106 dilution no signal was seen for any of the three reactions performed at this dilution. None of the reactions at 0.7 molecule per reaction gave a positive result.

These results demonstrate for the first time the use of real-time PCR and molecular beacons to detect C. felis DNA isolated from a conjunctival swab. The assay is very sensitive, highly reproducible, and can be accomplished in less than 2 h. This assay is about 10 to 100 times more sensitive than our current nested PCR assay for C. felis and has equal specificity.

* Corresponding author. Mailing address: Division of Molecular and Cellular Biology, Department of Clinical Veterinary Science, University of Bristol, Langford House, Langford, Bristol, BS40 5DU, United Kingdom. Phone: 44 117 928 9242. Fax: 44 117 928 9505. E-mail: c.r.helps @bristol.ac.uk.
C. felis assay. A quicker turnaround time and saving resources. From a diagnostic point of view, the main advantages of real-time PCR are that the closed tube system results in a decrease in false positives and that the assay is very easy to use. The inclusion of UDG and dUTP further reduces the chance of false positives occurring. The use of a molecular beacon ensures that only the desired target is detected and gives very low background fluorescence. The accurate quantitative nature of the assay lends itself to the determination of the number of organisms in the swab sample. This can be very useful in determining whether antibiotic treatment has been effective. The use of molecular beacons can allow several infectious organisms to be identified in the same sample at the same time, yielding a quicker turnaround time and saving resources. The use of real-time PCR to diagnose infection and disease will allow results to be obtained more quickly than is currently possible with conventional PCR or isolation. Real-time PCR is also more sensitive than conventional PCR and allows accurate quantification if desired. The use of multiplex real-time PCR with molecular beacons can allow several infectious organisms to be identified in the same sample at the same time, yielding a quicker turnaround time and saving resources.

### REFERENCES


