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Chlamydia trachomatis PCR

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ABSTRACT

OmpA gene PCR: PCR DNA extract (5 µl) was used to amplify a 1087 pb fragment of the ompA gene of *C. trachomatis*, using primers NRO (5'CTCAACTGTAAGTGCATTTT3') and NLO (5'ATGAAAAAACTCTTGAAATCG3'). PCR amplification processes commenced with a 4-minute denaturation step at 95°C and continued with 49 amplification cycles. Each cycle consisted of a first denaturation step at 95°C for 1 min, an annealing step at 55°C for 1 min and a final step of chain elongation at 72° C for 1.5 min.

Cryptic Plasmid PCR: The primers used to generate a 201-bp fragment from the cryptic plasmid of *C. trachomatis* were CTP1 (5'-TAGTAACTGCCACITCATCA-3') and CTP2 (5'-TTCCCCTTGTAATTCTGTTGC-3'). The PCR amplification consisted of DNA denaturation at 95°C for 4 min followed by 35 cycles of amplification. Each cycle consisted of 1 min at 95°C, 1 min at 55°C and 1.5 min at 72°C followed by a final elongation at 72°C for 4 min. The ompA gene and cryptic plasmid PCR products were visualized after electrophoresis in a 1% agarose gel by ECO-Gel 20.000X Highway staining. Positive and negative controls were used in all determinations of PCR.

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MATERIALS

MATERIALS

✕ Agarose Contributed by users Catalog #A5304

✕ primers Contributed by users

✕ MINT Master Mix 2X Inbio Highway Catalog #M024

✕ Agua ultrapura Inbio Highway Catalog #A0103

✕ ECO-Gel 20.000X Highway staining Inbio Highway Catalog #B0310

1 Pre Amplification Mix

🧴 12.5 µL Mint Master Mix

🧴 6.5 µL Agua ultrapura

🧴 0.5 µL each primer (NRO/NLO for ompA gene, and CTP1/CTP2 for cryptic Plasmid)

2 Add Extracted DNA

🧴 5 µL DNA

3 In a thermocycler , incubate as follows for ompA gene:

🔥 95 °C ⌚ 00:04:00

49 amplification cycles as follows:

🔥 95 °C ⌚ 00:01:00

🔥 55 °C ⌚ 00:01:00

🔥 72 °C ⌚ 00:01:30

Incubate as follows for Cryptic Plasmid:

🔥 95 °C ⌚ 00:04:00

35 amplification cycles as follows:

95 °C	00:01:00
55 °C	00:01:00
72 °C	00:01:30
followed by a final elongation
72 °C	00:04:00

4 Visualization of PCR products in 1.5% agarose gel

0.75 g	Agarose
50 mL	Water
3 µL Eco	Gel