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Working

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'CTCAACTGTAACTGCGTATTT3') 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'-TTCCCCTTGTAATTCGTTGC-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.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kiguen AX, Marramá M, Ruiz S, Estofan P, Venezuela RF, Mosmann JP, Monetti MS, Rivero V, Cuffini CG (2019) Prevalence, risk factors and molecular characterization of *Chlamydia trachomatis* in pregnant women from Córdoba, Argentina: A prospective study. PLoS ONE 14(5): e0217245. doi: 10.1371/journal.pone.0217245

MATERIALS

NAME	CATALOG #	VENDOR
Agarose	A5304	
primers		
MINT Master Mix 2X	M024	Inbio Highway
Agua ultrapura	A0103	Inbio Highway
ECO-Gel 20.000X Highway staining	B0310	Inbio Highway

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