This invention relates to a PCR method for the detection of Chlamydia trachomatis.

FIELD OF THE INVENTION:

This invention relates to a PCR method for the detection of Chlamydia trachomatis.

This invention further relates to specific primers pairs for the amplification of PCR products, in a PCR method for the detection of Chlamydia trachomatis.

BACKGROUND OF THE INVENTION:

There is no suitable method available in the art for the detection of Chlamydia trachomatis infection. Accordingly the need exists to provide a method which is efficient and inexpensive and can be easily carried out in laboratories, using easily available means.

OBJECTS OF THE INVENTION:

It is an object of this invention to propose a PCR method for the detection of Chlamydia trachomatis, which is simple.

It is a further object of this invention to propose a PCR method for the detection of Chlamydia trachomatis, which is fast and efficient.

Another object of this invention is to propose a PCR method for the detection of Chlamydia trachomatis, which is cost effective.

These and other objects of the invention will be apparent from the ensuing description.

BRIEF DESCRIPTION OF THE INVENTION:

According to this invention is provided a PCR method for the detection of Chlamydia trachomatis.

This invention further relates to novel primer pairs for the amplification of PCR products.

In accordance with this invention, cervical swabs samples are used for the detection of Chlamydia trachomatis infection.

Steps involved are DNA extraction, PCR of Major Outer Membrane Protein (MOMP) gene of Chlamydia trachomatis using specific primers. Specimen should have adequate cells for processing. The detail of the procedure is as follows;

i. DNA extraction: The DNA is extracted from the cervical sample using any commercially available DNA extraction kit.
ii. PCR for amplification of the extracted DNA for detection of Chlamydia trachomatis:

The Instrument required for PCR is a Thermal Cycler (any version)

The reagents and DNA specimen are added in the following order as given below and then PCR is carried out.

Table 1: Concentration/Volumes of reaction buffer and Taq. Polymerase for PCR analysis.

Component	**Volume**	**Final Concentration**
Reaction buffer	19.75µl	1.0X
Taq. DNA Polymerase	0.25 µl	1.25 units
Template DNA/Control DNA*	5.00 µl	Approx. 250 ng/1ngm*
Final Volume	25.00 µl	

*Control DNA=1 ngm/5 µl

PCR protocol for C. Trachomatis:

Step	Temp	Time	No. of cycles
Initial Denaturation	94°C	2 min	1 cycle
Denaturation	94°C	10 sec	
Annealing	52°C	20 min	35 cycle
Extension	72°C	5 min	
Final Extension	72°C	5 min	1 cycle
	4°C	Indefinite	

Stability of reagents at different temperatures:

1. At room temperature: Reagents are stable up to 28 weeks, further stability testing is ongoing.

2. At 4°C temperature: Reagents are stable up to 7 months, further stability testing is ongoing.

3. At -20°C temperature: Reagents are stable up to 7 weeks, further stability testing is ongoing.

Validation of the method:

This method has undergone 2nd party (Metropolis Health Care Ltd. Mumbai) and 3rd party validation (Postgraduate Institute of Medical Education and Research, chandigarh) with 100% sensitivity and specificity.

The method is novel and none of the primer pairs used to amplify the PCR products, has been used previously.

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