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Detection of *Neisseria gonorrhoeae* by PCR using *orf*1 gene as target

Nucleic acid amplification tests have the ability to specifically amplify small quantities of DNA and hence have been used successfully in the diagnosis of STDs.[1 2] An in-house polymerase chain reaction (PCR) method was developed and evaluated for the detection of *Neisseria gonorrhoeae* DNA in the urogenital specimens collected (with consent) from patients visiting an STD clinic in India.

The primers (forward primer 5′-CAACTATTCCCGATTGCGA-3′ and reverse primer 5′-GTTATACAGCTTCGCCTGAA-3′) amplify the 221–480 bp region of *orf*1 gene. Clinical isolates (n = 40) of *N gonorrhoeae* were recovered from urethral or cervical swabs by inoculation onto modified Thayer-Martin medium and identified by Gram stain, colony morphology, positive oxidase, and rapid carbohydrate utilisation test. For PCR the clinical samples (n = 489) were centrifuged (30 minutes, 14 000 *g*) and the cell pellet was lysed with 50 mM TRIS-HCl (pH 7.5) 1% Triton X-100, 1 mM EDTA, 250 µg of proteinase K per ml at 37° for 1 hour, boiled for 10 minutes, and centrifuged. Eight µl of lysate was used for amplification (40 cycles) under standard conditions. Each cycle consisted of 30 seconds at 94°C, 30 seconds at 52°C, and 1 minute at 72°C. The amplified PCR product (10 µl) was analysed by electrophoresis in a 2% agarose gel and characterised by sequencing.

An amplified product of 260 base pairs (bp) of *orf*1 gene was observed with all *N gonorrhoeae* isolates but not when DNA from the other non-gonococcal strains (17 closely related *Neisseria* species, *Corynebacterium*, *Chlamydia trachomatis*, *Candida*, syphilis, and members of Enterobacteriaceae) was used as template. For the 427 clinical swabs collected from men, 379 were positive and 46 were negative by both culture method and *orf*1-PCR assay. Urethral specimens from two men were culture negative but PCR positive for *orf*1 gene. Since these two samples tested PCR positive for *cpp*B gene of *N gonorrhoeae*[3] they were considered true positives. Thus, a total of 381 men (89%) were classified as true positives based on the PCR assay (table 1). Of the 62 women tested, 52 were true positives, and five were true negative as they gave concordant results irrespective of the site of collection and the diagnostic method used (table 1). Four culture negative specimens tested positive by the PCR assays using primers specific to *orf*1 as well as *cpp*B gene and were, therefore, considered positive. One culture negative specimen was positive by the *orf*1-PCR assay for its endocervical specimen but negative for urethral specimens. For the *cpp*B gene amplification, the specimen yielded a negative result for both the sites. This was therefore classified as true negative. The sensitivity, specificity, positive predictive value, negative predictive value for the PCR method described here would be 100%, 98%, 99.7%, and 100% respectively. The gold standard has been reported as having a sensitivity of 85–95%.[4 5]

The high specificity and sensitivity (25 fg DNA per assay, equivalent to 10 cells) coupled with low cost and rapidity of the in-house PCR assay described here can serve as a promising diagnostic method for the detection of gonococcus directly from clinical swab samples.

Table 1 Comparison of culture and PCR method for detection of *Neisseria gonorrhoeae* in urogenital specimens from men and women

No of specimens from men	Urethra				Patient status
	Culture	Gram stain	PCR (*orf*1/*cpp*B gene)		
46	–ve	–ve	–ve		Not infected
367	+ve	+ve	+ve		Infected
12	+ve	–ve	+ve		Infected
2	–ve	–ve	+ve/+ve		Infected
No of specimens from women	**Urethra**		**Endocervix**		
	Culture	**PCR**	**Culture**	**PCR**	**Patient status**
5	–ve	–ve	–ve	–ve	Not infected
52	+ve	+ve	+ve	+ve	Infected
1	–ve	–ve	–ve	+ve	Not infected*
4	–ve	+ve	–ve	+ve	Infected

*The individual was categorised as not infected after confirming with the *cpp*B gene PCR.

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References

1 **Farrell DJ**. Evaluation of Amplicor Neisseria gonorrhoeae PCR using cppB nested PCR and 16S rRNA PCR. *J Clin Microbiol* 1999;**37**:386–90.
2 **Kehl SC**, Georgakas K, Swain GR, *et al*. Evaluation of the Abbott LCx assay for detection of Neisseria gonorrhoeae in endocervical swab specimens from females. *J Clin Microbiol* 1998;**36**:3549–51.
3 **Ho BS**, Feng WG, Wong BK, *et al*. Polymerase chain reaction for the detection of Neisseria gonorrhoeae in clinical samples. *J Clin Pathol* 1992;**45**:439–42.
4 **Hook EW III**, Handsfield HH. Gonococcal infections in the adult. In: Holmes K, Straling F, Wiesner P, Cates W, Lemon S, Stamm W, ed. *Sexually transmitted diseases*. New York: McGraw Hill, 1990:149–65.
5 **Ching S**, Lee H, Hook E, *et al*. Ligase chain reaction for detection of Neisseria gonorrhoeae in urogenital swabs. *J Clin Microbiol* 1995;**33**:3111–14.

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Nevirapine + efavirenz based salvage therapy in heavily pretreated HIV infected patients

The emergence of protease inhibitors (PIs) and multiple drug therapy for HIV infection has greatly decreased mortality in countries where these medications are available. Unfortunately, many patients eventually develop viral resistance to treatment because of HIV virus mutations. As clinicians await development of new drugs to combat resistant virus, innovative strategies with existing drugs may be particularly valuable. Patients having failed regimens containing nucleoside reverse transcriptase inhibitors (NRTIs) and PIs face limited options for future therapy. A regimen containing the two potent non-nucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine (NVP) and efavirenz (EFV), could provide an effective alternative, since both can be conveniently dosed once daily[1 2] and have demonstrated efficacy in patients with high viral loads.[2–4]

A retrospective chart review at an urban HIV hospital clinic identified 13 patients who had initiated an NVP + EFV based salvage