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Thesis Title: **Development of an Efficacious and Reliable Molecular Assay for Rapid detection of *Neisseria gonorrhoea***

GONORRHEA, is the second most frequently notifiable sexually transmitted infections (STI) with 106.1 million cases occurring annually worldwide. Untreated STI caused by *Neisseria gonorrhoeae* may cause serious complications both in male and female. The principal strategy for curbing gonorrhea requires accurate diagnosis for timely treatment.

Conventional diagnostic approaches like microscopy and culture show significantly decreased sensitivity in case of asymptomatic patients which remains about 30-40% only. Whereas, Nucleic Acid Amplification technology (NAATs) are not possible to perform in resource-limiting settings like mostly prevalent in India, due to inadequate infrastructure. PCR typically is expensive, may not be accessible to many people, and the time in receiving results may be time taking, resulting into loss of follow-up. Thus, the high proportion of asymptomatic patients (50-80% women along with ~10% of men) remains under-diagnosed and under-reported, necessitating the need for feasible laboratory diagnostic procedures for the disease (WHO 2012, Tapsall 2001).

It is in this background that the present study describes the development and standardization of LAMP for detection of *N. gonorrhoeae* and its evaluation in known positive and negative samples based on culture and PCR results. In addition, an in-house PCR assay based on *opa* gene was developed and evaluated against the existing *porA* pseudogene and 16S ribosomal gene based assays in clinical samples.

The LAMP assay conditions, temperature and time were optimized and standardized whereas, determination of sensitivity of LAMP assay was established by using 10- fold serial dilutions

of genomic DNA extracted from pure culture of *N. gonorrhoeae* (ATCC: 49226), the specificity was determined, this was done for both *opa* and *porA* pseudogene LAMP assays separately. To facilitate the field applicability of LAMP assay, LAMP reaction was visualized for color change by SYBR green I and HNB (Hydroxyl napthol blue) separately.

Conventional methods worked well for male patients but had inadequate efficacy in female patients. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the *opa* assay with 95% confidence interval was found to be 100% (91.6-100%), 97.6 (94.5-99%), 89.8% (78.5-95.8%) and 100% (98.0-100%).

Developed *opa* and *porA* pseudogene LAMP assays were able to amplify signature sequence from *N .gonorrhea* and typical ladder like bands appeared within 30 minutes of reaction run time which was four times lesser than the regular PCR (i.e. 2-3 hrs). Analytical sensitivity of each LAMP assay with 10-fold serial dilutions of the genomic DNA from *N. gonorrhoeae* (ATCC: 49226) was found to be in Femto grams (10^{-15}), which is much higher than PCR (Picograms 10^{-12}), signifying its potential of having high sensitivity. Both *opa* gene and *porA* pseudogene specific LAMP assays demonstrated high degree of specificity by only identifying *N. gonorrhea* from the selective panel of bacteria.Visualization of end product with SYBR Green I dye made LAMP more practical in field as compared to PCR which requires Gel electrophoresis to visualize end product.

Statistical analysis of LAMP showed specificity, sensitivity PPV, NPV and accuracy of LAMP was 100%, 99.7%, 100%, 100% and 99.7% respectively. LAMP assay showed 91.3%, concordance and 97.2% agreement with PCR.

Thus, the LAMP assay developed in this study may serve as a promising replacement for PCR assay because not only it is highly sensitive, specific affordable, simple, and robust in performance but also has less turnaround time. Furthermore, this LAMP assay could be employed for detection and confirmation of *N. gonorrhoeae* not only under resource limiting settings but also could be employed as point-of-care test that also has the ability to resolve the problem of gonorrhea diagnosis in asymptomatic females.