NAATS FOR GONORRHOEA DIAGNOSIS IN WOMEN: EXPERIENCE OF A TERTIARY CARE HOSPITAL IN NORTH INDIA

Methods

Endocervical samples collected from 250 female patients attending the Dermatology and Gynaecology OPD of AIIMS and STD clinic of Dr. R.M. L Hospital, New Delhi, India were tested using 16S ribosomal PCR. Additionally, they were processed by conventional methods. True positives were defined as those which were positive by culture and/or positive by both 16S ribosomal gene and 16S gene based PCR.

Results

Of the 250 female patients, only 1 was positive by conventional methods, i.e., microscopy and culture. Based on PCR results, 16S ribosomal gene based PCR was positive in 17 patients who were also positive by 16S ribosomal PCR. However, 16S ribosomal gene based PCR picked up 8 extra positives. Overall, 17 patients were found to be true positives.

Conclusion

The clinical sensitivity of conventional methods for the detection of Neisseria gonorrhoeae in female patients is low. The gonococcal detection rates increased significantly when molecular method was used giving a prevalence rate of 6.8%. However, it is important to mention that the widespread use of NAATs might result in lack of isolates and ignorance of possible emergence of resistant organisms.

A COST-EFFECTIVE AND SIMPLE ALTERNATIVE TECHNIQUE FOR RESUSCITATION OF FREEZE-DRIED CULTURES OF NEISSERIA GONORRHOEAE

Methods

Freeze drying (lyophilization) of bacteria is a very well established method for the archiving and long-term storage. The recommended medium for resuscitation of freeze-dried cultures of Neisseria gonorrhoeae is 1 ml of a nutrient blood broth or a rich peptone broth supplemented with 10% blood or nutrient broth. Sheep blood or horse blood is not easily available in most of the labs. Human blood is not recommended. Normal saline (0.9% W/V Sodium chloride) is mostly available in all the labs. This study compared the nutrient blood broth and normal saline for resuscitation of freeze-dried cultures of reference and clinical strains of N. gonorrhoeae and evaluated their performance characteristics for the growth of N. gonorrhoeae strains.

Conclusion

A prospective study was undertaken between January 2011 and December 2012. Ninety three N. gonorrhoeae lyophilized strains including 83 clinical isolates, ATCC 49226 and nine WHO reference strains were tested using both the methods. Reconstituted material from both the techniques was subcultured on to chocolate agar and was incubated for 24–48 hrs at 36°C in a moist atmosphere containing 5 to 10% carbon dioxide. The results were recorded in terms of viable gonococci, colony morphology and colony size.

Results

N. gonorrhoeae was successfully isolated from 89 (95.7%) lyophilized strains by both the techniques used for revival. In other 4 cases, it was difficult to state with absolute certainty that the gonococci were nonviable or that unsatisfactory lyophilization practises were responsible for these results as the quality of the lyophilized strains also determined the growth on revival.

Conclusion

Preliminary data suggest that nutrient blood broth and normal saline were equal in performance for revival of lyophilized strains and normal saline could be recommended as convenient and inexpensive alternative for universal use.

DEVELOPMENT OF MOLECULAR BEACON BASED DIAGNOSTIC ASSAY FOR DETECTION OF NEISSERIA GONORRHOEAE AND CHLAMYDIA TRACHOMATIS

Methods

Endocervical swabs were collected from patients visiting gynaecology department of various hospitals in Delhi. We standardised and evaluated in-house uniplex PCR (uPCR) for diagnosis of Neisseria against Roche Amplicor Micro Plate CT/NG kit. Method was modified to co-detect N. gonorrhoeae and C. trachomatis in single test. Further we developed visual assay for detection of Chlamydia and Neisseria using molecular beacon probe.

Conclusion

The in-house dPCR assay is rapid and as sensitive as commercially available tests. Use of molecular beacons provides a highly specific and easy to use detection method, making it a better option for routine diagnosis of genital infection in developing countries.

ANALYTICAL EVALUATION OF THE SPECIFICITY AND NEISSERIA GONORRHOEAE DETECTION OF THE NOVEL GENEPROOF N. GONORRHOEAE DUAL-TARGET PCR ASSAY

Methods

A prospective study was undertaken between January 2011 and December 2012. Ninety three N. gonorrhoeae lyophilized strains including 83 clinical isolates, ATCC 49226 and nine WHO reference strains were tested using both the methods. Reconstituted material from both the techniques was subcultured on to chocolate agar and was incubated for 24–48 hrs at 36°C in a moist atmosphere containing 5 to 10% carbon dioxide. The results were recorded in terms of viable gonococci, colony morphology and colony size.

Results

N. gonorrhoeae was successfully isolated from 89 (95.7%) lyophilized strains by both the techniques used for revival. In other 4 cases, it was difficult to state with absolute certainty that the gonococci were nonviable or that unsatisfactory lyophilization practises were responsible for these results as the quality of the lyophilized strains also determined the growth on revival.

Conclusion

Preliminary data suggest that nutrient blood broth and normal saline were equal in performance for revival of lyophilized strains and normal saline could be recommended as convenient and inexpensive alternative for universal use.