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In-House Growth-Promoting Transport System for *Neisseria gonorrhoeae*▽

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Eno powder (GlaxoSmithKline), an antacid preparation readily available over the counter, was used instead of a CO_2 generator for the growth of 15 strains of *Neisseria gonorrhoeae* obtained from men with urethritis. Due to its easy accessibility and low cost, Eno powder can be useful in developing countries for transporting clinical specimens from resource-poor peripheral labs to reference laboratories.

Despite a sharp decline in the incidence of gonorrhea in developed countries during the last decade, gonococcal infection remains a significant public health problem in developing countries (9, 10). Timely and accurate laboratory detection of gonorrhea is of major public health importance because untreated gonococcal infection may lead to several complications, especially in women, e.g., ectopic pregnancy, pelvic inflammatory disease, and tubal infertility (4, 5). Moreover, gonorrhea increases the risk of human immunodeficiency virus infection. *Neisseria gonorrhoeae* is a nutritionally fastidious organism that requires an enriched medium in a moist environment of 35 to 37°C with an atmosphere of 3 to 5% CO_2 for growth. It does not grow well in the presence of commensal organisms (6, 7, 8).

Ideally, clinical specimens should be directly inoculated onto a suitable gonococcal-culture selective medium and immediately incubated at 36°C in 5 to 10% CO_2. However, the necessary facilities are not available in most venereal disease clinics. To circumvent this need, specialized transport systems like the Gono-Pak (BBL), the JEMBEC (BBL), and the Bio-Bag Type C (Becton Dickinson) systems have been developed (1). These systems work by producing a CO_2-rich environment. In the Gono-Pak system, a modified Thayer-Martin medium plate is placed in a ziplock plastic bag along with a CO_2-generating tablet. The JEMBEC system is made up of a rectangular plastic tray which contains modified Thayer-Martin medium (1). The tray has a well into which a CO_2-generating tablet is placed. The tray is placed inside a ziplock plastic bag (1). The disadvantage of the Gono-Pak and JEMBEC systems is that the CO_2 generation by the tablet is dependent on the release of moisture from the medium. Thus, the efficacy of these systems is dependent on the freshness of the medium. The Bio-Bag Type C system eliminates dependence on the freshness of the medium. In this system, CO_2 is produced by a CO_2 generator which contains a 130-mg sodium bicarbonate tablet and a 0.5-ml ampoule of 1.56 N hydrochloric acid, packaged in a ziplock plastic bag (1). The ampoule is crushed to generate CO_2. The cost of each of these systems would make them prohibitive in small labs of developing countries like India. Here, we are sharing our experience with an in-house transport system which can be made readily in any microbiology laboratory of a developing country.

Our system consists of a ziplock plastic bag in which a plate of modified New York City medium is placed. For the generation of CO_2 in the bag, Eno powder (GlaxoSmithKline, Asia Pvt. Ltd.), an antacid preparation readily available over the counter, is used. Eno powder, which is available in air-sealed 5-gram sachets, contains sodium bicarbonate and citric acid in solid form, which generate CO_2 after becoming wet (3). Our system also contains a wet filter paper which provides moisture to the Eno powder in a gradual manner, resulting in slow and consistent generation of CO_2. Additionally, the wet filter paper creates a humid environment, which supports the growth of gonococci.

The study group consisted of men who presented with symptoms of acute urethritis at the sexually transmitted disease clinics of Nehru Hospital attached to the Postgraduate Institute of Medical Education and Research, Chandigarh, India, and the STD Polyclinic Sector 22 from March 2006 to September 2006. Patients were enrolled and examined after giving oral consent. Two urethral swab specimens were obtained by gently rotating a cotton swab in the anterior urethra. The swabs were used to inoculate the modified New York City agar plates in duplicate. One plate was placed in the CO_2 incubator at 37°C for up to 72 h. Another plate was placed into a ziplock plastic bag. Before the culture plate was placed in the plastic bag, a small packet of 3 g of Eno powder in a paper towel was created and affixed to the outer side of the lid of the culture plate using cellophane tape. A wet rectangular piece of filter paper was then placed inside the plastic bag. The culture plate was placed over it so that the Eno powder packet was in direct contact with the wet filter paper. The ziplock plastic bag was then sealed and incubated at 37°C in a biological oxygen demand incubator.

The plates were examined daily for up to 72 h for colonies typical of *N. gonorrhoeae*. Isolates which were oxidase-producing gram-negative diplococci were confirmed to be *N. gonorrhoeae* by the rapid carbohydrate utilization test (2). Specimens from a total of 20 patients were tested in this manner, and 15 of these specimens were culture positive for *N. gonorrhoeae*. There was complete agreement between the results with the plates incubated in a CO_2 incubator and those incubated in the

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Eno system. Our study shows that the Eno system works as well as the CO_2 incubator to support the growth of gonococci. Due to the easy accessibility and the low cost ($0.04 per sachet) of Eno powder, it may be useful for transporting gonococcal specimens from resource-poor peripheral labs to reference laboratories. The Eno system also has the advantage of a consistent release of CO_2 and is not dependent upon the freshness (moisture content) of the medium, as are the Gono-Pak and JEMBEC systems.

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