Identification of *Neisseria gonorrhoeae* by Genetic Transformation: a Clinical Laboratory Evaluation

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Transformation of a *Neisseria gonorrhoeae* auxotroph (uracil and arginine deficient) to prototrophy was attempted with wild-type deoxyribonucleic acid from 71 random clinical *N. gonorrhoeae* cultures. Of these 71 cultures, 97.1% transformed the nutritionally deficient mutant to prototrophy. The procedure was reliable and economical and offered several distinct advantages over other methods used for the confirmation of *N. gonorrhoeae*.

Laboratory identification of *Neisseria gonorrhoeae* by sugar oxidation often has been complicated by non-acid-producing strains, nonviable cultures, and mixed cultures. Several nutritionally deficient, genetically stable mutants were recently isolated and characterized by Janick et al. (1). The use of these mutants for the simplified transformation procedure essentially eliminates the possibility of erroneous identification of *N. gonorrhoeae* as to species in a clinical laboratory by offering several advantages over sugar oxidation methods and fluorescent-antibody techniques used for the identification of *N. gonorrhoeae*. These advantages are: (i) there is no cross-reaction with other *Neisseria* spp.; (ii) the need for a pure culture is eliminated; (iii) nonviable cultures may be tested; (iv) *N. gonorrhoeae* may be identified from deoxyribonucleic acid (DNA) prepared from the purulent discharge; and (v) the transformation reaction is not influenced by the apparent inability of a given culture to oxidize dextrose.

The procedure may be performed on any organisms that are oxidase-positive, gram-negative diplococci. The transformation procedure involves the preparation of wild-type DNA by the suspension of wild-type gonococcus colonies in a lysing solution containing 0.025 M sodium dodecyl sulfate, 0.15 M sodium chloride, and 0.015 M sodium citrate followed by heating at 60 to 70°C for 30 min.

Because they are more competent for transformation than are T4 colonies, type T2 colonies of one of the nutritionally deficient mutants (strain 488) were used and transferred daily on an enriched medium plate as previously described (1). Wild-type DNA and the viable recipient mutant strain were mixed on an enrichment medium plate along with separate sterility

controls of the DNA lysate. In addition, DNA prepared from a known wild-type gonococcus culture was used as a positive control. This plate was incubated at 36°C in a 5% CO₂ incubator. At the end of 6 h or more, the viable growth containing DNA from the unknown preparation, along with the appropriate positive and negative controls, was transferred to a plate containing specific synthetic medium previously described (1) if there was not growth in the sterility controls. The plate was incubated for an additional 24 h at 36°C in a 5% CO₂ incubator and examined for growth under a dissecting microscope. The presence of pinpoint colonies in the positive control and in the wild-type DNA mutant mixture indicated transformation of the nutritionally deficient mutant to prototrophy. A Gram stain of the pinpoint colonies confirmed that the colonies were gram-negative diplococci and not contaminants acquired during the transfer of cells from the enriched medium to the synthetic-deficient medium. This, therefore, confirmed the identity of the unknown wild-type colonies as *N. gonorrhoeae*.

Random cultures of *Neisseria* species were obtained from various body sites of patients seen at the Washtenaw County Venereal Disease Clinic (Ypsilanti, Mich.) and from cultures received at our microbiology laboratory. All cultures were identified as to species by conventional sugar oxidation methods before the transformation procedure was performed. The results of this clinical evaluation are given in Table 1. These data show that, of the 71 gonococcal cultures confirmed by sugar oxidation, 69 were transformation positive. The two remaining *N. gonorrhoeae* cultures, not transformation positive, were found to be nutritional mutants, having chromosomal lesions similar

TABLE 1. Transformation trial of *Neisseria*^a and related organisms

<i>Neisseria</i> species	No. of specimens	Confirmation by sugar reactions	Transformation positive	% Transformation positive
<i>N. meningitidis</i>	71	71	69	97.1
<i>N. (branhamella) catarrhalis</i>	9	9	0	0
<i>N. lactamica</i>	3	3	0	0
<i>N. sicca</i>	2	2	0	0
<i>N. sicca</i>	17	17	0	0
<i>Moraxella osloensis</i>	1	1	0	0

^a All *Neisseria* species used in this trial were clinical isolates.

to that of the mutant used as the transformation indicator strain. Although we have only used one mutant indicator strain (strain 488) in the present study, other mutant strains having different genetic lesions are also available (1) and could be used to assay those rare strains normally having lesions identical to that in strain 488. There were no false-positive reactions from any of the other *Neisseria* species tested.

In summary, when DNA samples from suspect gonococcal cultures were prepared early in the day, it was possible to have confirmation of *N. gonorrhoeae* by late the next day. In those rare instances in which the suspect gonococcal

culture has the same chromosomal lesion as the indicator strain, one may resort to another mutant having a different chromosomal lesion or conventional sugar oxidation methods if the source of specimen warrants such extraordinary procedures.

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LITERATURE CITED

1. Janick, A., E. Juni, and G. A. Heym. 1976. Genetic transformation as a tool for detection of *Neisseria gonorrhoeae*. J. Clin. Microbiol. 4:71-81.