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Genetic Transformation as a Tool for Detection of *Neisseria gonorrhoeae*

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A rapid method for the detection of *Neisseria gonorrhoeae*, making use of the ability of deoxyribonucleic acid samples from clinically isolated strains of this organism to transform nutritional mutants of a particular strain of *N. gonorrhoeae*, has been described. In addition to using isolated cultures, transforming deoxyribonucleic acid can be obtained directly from the material that adheres to swabs of the cervix or the urethra. The time interval for transfer of swabs to the diagnostic laboratory is not a significant factor. It is not necessary to use pure cultures on primary isolation plates to obtain definitive results. Nongonorrhoeae neisserias, as well as a large variety of commonly encountered unrelated bacteria, do not react or interfere in the transformation assay when using one of the mutant strains under a standardized set of conditions. The entire assay can be completed in less than 24 h. It has also been shown that type T4 cells of the strain of *N. gonorrhoeae* employed in the present study are competent for genetic transformation, although type T4 cells are transformed at a significantly lower frequency than are type T2 cells of the same strain.

Bacterial transformation is a process whereby homologous deoxyribonucleic acid (DNA) is taken up actively by certain so-called competent strains and recombined with the DNA of the recipient cell chromosome (27). Through the use of specific genetic markers, which are determined by particular sequences of nucleotides in chromosomal DNA, the properties of the competent recipient strains can be observed to be altered after the recombinational event. Although all kinds of DNA can be taken up by competent bacteria, only homologous DNA or DNA from closely related strains will recombine with the recipient cell chromosome. Because of the relative complexity of the procedures generally employed, transformation has usually been considered to be unsuitable for the development of routine diagnostic tests. It has been shown recently, however, that the use of a simple technique has made it possible to devise transformation assays for the identification of strains of *Acinetobacter calcoaceticus* (11) and *Moraxella osloensis* (12) which can be employed routinely in clinical diagnostic laboratories. Since species of *Neisseria* are known to be competent for genetic transformation (6, 26), we have investigated the possibility of devising a similar assay procedure for the detection and identification of *Neisseria gonorrhoeae* from clinical materials.

In *Neisseria gonorrhoeae* it has been shown that both virulence and competency for transformation are properties of cells which display a special colonial morphology when plated on certain media (3, 9, 14) and which are piliated (10, 24, 29). The present investigation demonstrates a transformation assay for *N. gonorrhoeae*, making use of the nutritional requirements of recipient indicator strains. This assay can be used as a simple and rapid diagnostic procedure in the clinical laboratory.

(A preliminary report of this work has been presented [E. Juni et al., Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 219, 1974].)

MATERIALS AND METHODS

Organisms and media. All clinical specimens or cultures were obtained from the Elkhart, Ind. VD Clinic or from the diagnostic laboratory of St. Joseph Mercy Hospital, Ann Arbor, Mich. Such material was processed and identified in our laboratories by recognized methods (25). Strains of *N. gonorrhoeae* were distinguished from other neisserias by their ability to produce acid only from glucose. Ability to produce acid from sugars was tested using glucose, maltose, sucrose, fructose, lactose, and mannitol by the method of Kellogg and Turner (15) and also by the use of phenol red agar base (Difco) slants containing 1% sugar and 0.15% corn starch. Other strains of *Neisseria* were either isolated from throats of healthy individuals or obtained from the American Type Culture Collection. Cultures were

incubated at 36° to 37°C in either an air-8% CO_2 incubator or in a candle jar. All strains were stored at −60°C in 3% (vol/vol) Trypticase soy broth (Difco) containing 30% (vol/vol) agamma horse serum and 15% (vol/vol) glycerol. Thawed cultures were inoculated onto medium M1 plates (see below) and incubated overnight.

Cultures were grown on Thayer-Martin plates (30) or on medium M1 plates. Double strength medium M1, lacking defined carbon sources, is prepared by dissolving the following ingredients in approximately 150 ml of distilled water: proteose peptone no. 3 (Difco), 6 g; K_2HPO_4, 1.6 g; KH_2PO_4, 0.4 g; and NaCl, 2 g. The final volume is adjusted to 200 ml with distilled water and sterilized by autoclaving for 20 min. Medium M1 agar plates are prepared by pouring the above medium (200 ml, medium at room temperature) into 200 ml of recently melted (90° to 100°C) sterile 3% agar (Difco) and, after mixing, adding 3.2 ml of 50% glucose (sterilized by Millipore filtration), 2.0 ml of 60% sodium lactate (commercial solution, sterilized by autoclaving), and 40 ml of a sterile 1.5% cornstarch (Argo) sol (19). After the final addition is made, this medium (medium M1) is mixed thoroughly and poured into 20 sterile petri dishes. Medium M1 may also be prepared by appropriate modification of GC medium base (Difco).

To detect transformed cells, either the chemically defined GGM medium (16) lacking specific amino acids or a modified form of medium A of Gould et al. (8), referred to as medium M9, was used. Double strength medium M9 lacking carbon sources is prepared by dissolving the following ingredients in approximately 150 ml of distilled water: casein hydrolysate (vitamin free, salt free, ICN Pharmaceticals), 3.2 g; Na_2HPO_4, 1.12 g; KH_2PO_4, 0.4 g; $MgSO_4 \cdot 7\,H_2O$, 0.18 g; and NaCl, 2 g. The final volume is adjusted to 200 ml with distilled water and sterilized by autoclaving for 20 min. Medium M9 agar plates are prepared by pouring the above medium (200 ml, medium at room temperature) into 200 ml of recently melted (90° to 100°C) sterile 3% agar (Difco) and, after mixing, adding 3.2 ml of 50% glucose (sterilized by Millipore filtration), 2.0 ml of 60% sodium lactate (commercial solution, sterilized by autoclaving), and 40 ml of a sterile 1.5% cornstarch (Argo) sol (19). This medium (medium M9) is then mixed thoroughly and poured into 20 sterile petri dishes. Both medium M1 and medium M9 can be stored at room temperature in plastic bags, to retard evaporation, until ready for use.

Although *N. gonorrhoeae* can grow with either glucose or sodium lactate as the chief carbon and energy source, we have found that the addition of both glucose and sodium lactate to growth media results in better growth than when either substrate is present alone. For this reason both glucose and sodium lactate are incorporated in all media used in the present study.

The strain of *N. gonorrhoeae* used to obtain nutritionally deficient mutants (strain 55) is one which grows well on medium M9 and also reverts readily during cultivation to the highly competent colony type (type T2) from a culture of type T4 cells. Mutants used in the transformation assay were maintained as predominantly type T2 cells by daily transfer of several type T2 colonies (14) to sectors of a medium M1 plate. Although type T2 colonies give rise to type T4 cells during growth on virtually any medium (13), the above procedure does insure that most of the colonies in the streaked area of the plate are clearly type T2. From comparison with published photographs of the various colony types of *N. gonorrhoeae* (3, 9, 14), it would appear that strain 55 and its mutants rarely form type T1 or type T3 colonies.

Isolation of mutants of *N. gonorrhoeae*. Mutants auxotrophic for specific amino acids were obtained after mutagenesis with the use of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine according to the procedure of Miller (22). One mutant (strain 488) unable to grow on medium M9, but still able to grow on medium M1, was obtained after mutagenesis with methyl methane sulfonate (28). Mutant 488 has been shown to be a uracil and arginine auxotroph. Five independently isolated proline auxotrophs (strain no. 503, 505, 508, 516, and 526) were used in this study. All mutants used were extremely stable and have never been observed to revert spontaneously for ability to grow on the indicator plates.

Preparation of transforming DNA. Crude transforming DNA preparations were made using either cell paste of isolated colonies on Thayer-Martin or any other suitable plates or using the material that adheres to a swab from either cervix or the urethra of patients suspected of harboring *N. gonorrhoeae*. The procedure employed is similar to that used for the preparation of transforming DNA from *A. calcoaceticus* (11) and from *M. osloensis* (12). A small amount of bacterial paste on the edge of a 2-mm loop, just visible to the naked eye, is suspended in 0.5 ml of 0.025% sodium dodecyl sulfate in standard saline citrate solution (0.15 M sodium chloride, 0.015 M Na_3 citrate), and the suspension is heated for 15 to 60 min in a 65° to 68°C water bath. Transforming DNA from cells that adhered to swabs was prepared by swirling the swab in 0.5 ml of the lysing solution and then heating the solution in the usual way. When it was not convenient to heat the swab samples immediately, the swab was swirled in the lysing solution and the solution was heated in the laboratory at a later time. The time of transport required to bring samples to the laboratory before heating could take place was found not to be critical. During the heating step, lysis of cells is completed with the release of intracellular DNA. Crude DNA solutions, prepared in the manner described, are sterile and can be stored for many months in the refrigerator, if evaporation of the lysing solution is prevented. For quantitative transformation studies, DNA was prepared by the method of Marmur (21).

Transformation assay. A small amount of cell paste of a nutritionally deficient mutant of *N. gonorrhoeae*, just visible to the naked eye, taken from 16- to 20-h type T2 colonies on a medium M1 plate, is placed on a marked area of a second medium M1 plate. Using a 2-mm loop, a loopful of crude DNA from a culture or a swab is used to suspend and spread the mutant cells over a circular area approximately 5 to 8 mm in diameter. Sterility of the DNA preparation is checked by spreading a loopful of this material on another marked area of the same me-

dium M1 plate. After overnight incubation, no growth should be observed on this control smear area. Cell paste of the mutant is also spread on a third marked area of the plate to serve as a non-DNA-treated control. Several DNA samples may be assayed on a single plate. It has been observed that cells in heavily streaked areas on a medium M1 plate, particularly those of mutant 488, tend to die more rapidly than do cells in well-isolated colonies, possibly because of cell lysis (23). For this reason cell paste used in the preparation of cell-DNA mixtures is taken from isolated colonies or, if these are not readily available, from the outer edges of the growth area on the plate.

After incubation of the plate for 2 to 4 h, a generous portion of the cell-DNA mixture is streaked on a sector of an indicator medium plate upon which transformed cells are capable of growing. For amino acid auxotrophs, the indicator medium used is GGM (16), lacking the specific amino acid required by the mutant. For mutant strain 488, the indicator medium is medium M9. A sector of each indicator plate is also streaked with cell paste from the area of the medium M1 plate containing the non-DNA-treated mutant. The plates are observed after 18 to 24 h of incubation using a low-power dissecting microscope or by the naked eye after 2 days of incubation. The transformation assay procedure is summarized in Fig. 1.

The concentration of sodium dodecyl sulfate in the lysing solution (0.025%) is the highest that can be used during heating without possible lysis of mutant cells on the surface of the plate when the DNA solutin is applied. If any evaporation of this solution has taken place the detergent concentration may rise to dangerous levels, as evidenced by the appearance of lysis after 18 to 24 h of incubation of the mutant-DNA mixtures; these should normally show confluent growth (Fig. 2). If cell lysis is observed, dilution of the original DNA solution with 0.5 ml of sterile water will eliminate this problem.

RESULTS

Interstrain transformation of mutants of *N. gonorrhoeae*. Crude DNA solutions from 128 clinically isolated strains of *N. gonorrhoeae* were tested for the ability to transform the available nutritionally deficient mutants. Figure 2 shows the growth of the recipient cell-DNA mixtures as well as the growth of the non-DNA-treated mutant control and the DNA sterility controls for several of these DNA samples. Positive transformation is seen as prototrophic colonies in the areas of the indicator plate where the DNA-mutant cell mixtures were streaked (Fig. 3). One sector streaked with cells of the non-DNA-treated mutant is always included as a control on each plate to verify that the mutant strain used has not given rise to spontaneous revertant prototrophic cells capable of forming colonies on the

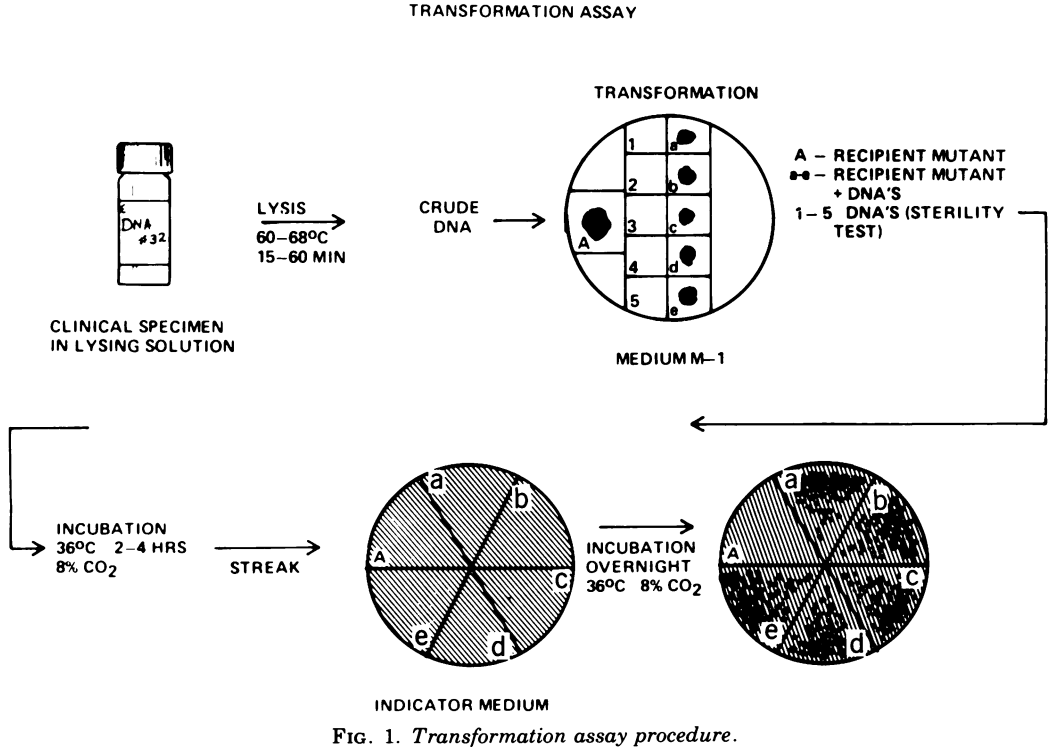

FIG. 1. *Transformation assay procedure.*

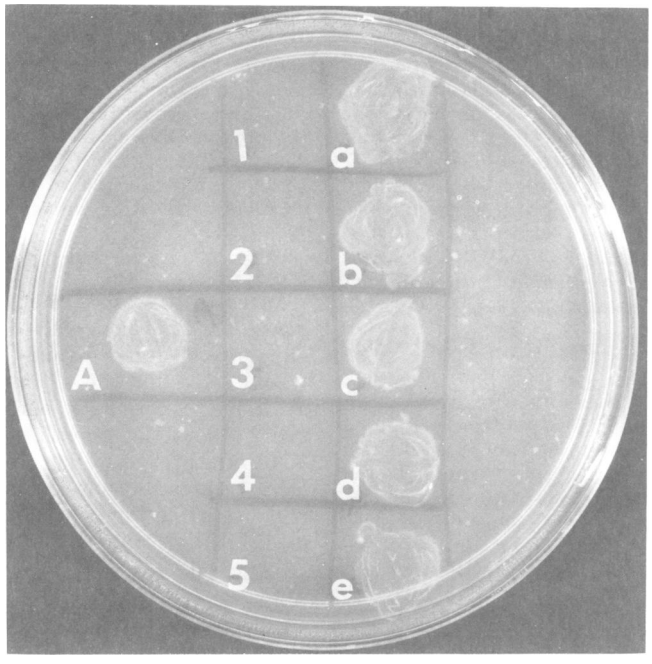

FIG. 2. *Growth of the recipient cell-DNA mixtures on medium M1. The growth areas contained: (A) mutant 488 without DNA addition; (a–e) mutant 488 to which crude DNA samples from pure cultures have been added; (1–5) DNA samples to certify that these preparations were sterile. The recipient cell-DNA mixtures were streaked on indicator medium (Fig. 3) after 3 h of incubation, and the plate shown above was incubated for 24 h.*

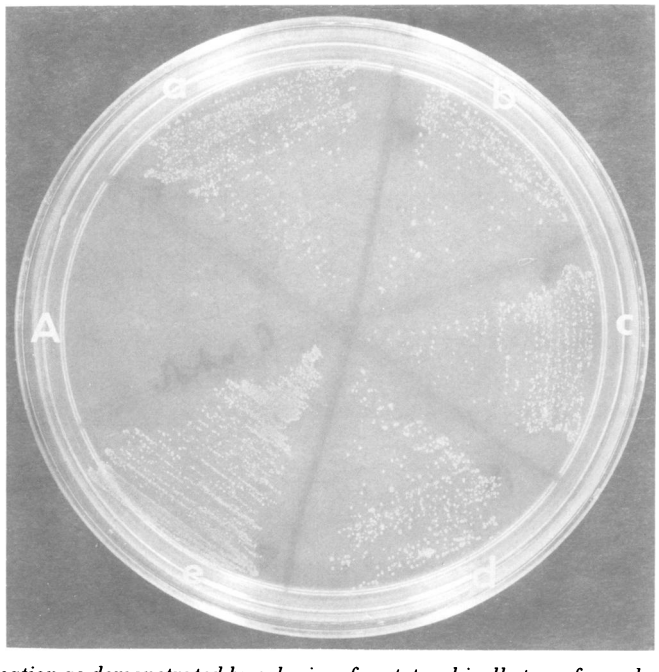

FIG. 3. *Transformation as demonstrated by colonies of prototrophically transformed cells of mutant 488 by DNA samples from pure cultures, which are now capable of growing on medium M9. The letters in this figure correspond to the similarly lettered growth areas of Fig. 2. The plate was incubated for 2 days before the photograph was taken.*

indicator agar. To eliminate artifacts due to possible airborne contaminants, prototrophic colonies which appeared on indicator plates were, on occasion, covered with the oxidase reagent to confirm the oxidase-positive reaction of the transformants. Experience with air contaminants on plates in our laboratories has shown that a great majority of these has proven to be oxidase negative. DNA samples from all 128 isolated cultures of *N. gonorrhoeae* were shown to transform each of the mutants tested for ability to grow on the indicator agar.

Specificity of the transformation assay. DNA samples from different species of *Neisseria*, from *Moraxella*, and from a large variety of bacteria and a few yeasts isolated from urinary and throat infections and from stool specimens were examined for the ability to transform a selected group of stable mutants of *N. gonorrhoeae* (Table 1). With the exception of the DNA samples from a few nongonorrhoeae *Neisseria* cultures, all other DNA samples failed to transform any of the *N. gonorrhoeae* mutants.

Examination of strains of several species of nongonorrhoeae neisserias revealed that DNA samples from a few strains of *N. meningitidis*, *Neisseria lactamica*, and *Neisseria sicca* were able to transform some of the proline auxotrophs of *N. gonorrhoeae* (Table 1). Transformation of these proline auxotrophs with DNA samples from *N. meningitidis* serological types A and B was highly reproducible, whereas transformation of these auxotrophs with DNA samples from strains of *N. meningitidis* serological type C, *N. lactamica*, and *N. sicca* gave positive results only at an irregular frequency.

By contrast, using *N. gonorrhoeae* mutant 488, however, only three DNA samples of *N. meningitidis* showed any transformation whatsoever, with DNA samples from all other nongonorrhoeae neisserias being completely unable to transform mutant 488. For the three DNA samples from strains of *N. meningitidis* which did transform mutant 488 for ability to grow on medium M9, it was necessary to incubate the DNA-mutant cell mixtures for at least 12 h before streaking on the indicator medium to observe any transformant colonies and, under these conditions, only from one to approximately 20 transformant colonies were observed. When the incubation time of the DNA-mutant cell mixtures was reduced to 2 to 4 h, DNA from none of the nongonorrhoeae neisserias was able to transform mutant 488, whereas DNA samples from all of the isolated strains of *N. gonorrhoeae* were able to do so under the same conditions. It is clear, therefore, that mutant 488 is almost completely specific for the DNA samples from strains of *N. gonorrhoeae*.

TABLE 1. *Test for the nonspecific transformation of N. gonorrhoeae mutants with crude DNA samples from various bacteria*

No. of cultures	DNA donor strain	Recipient mutant of *N. gonorrhoeae*[a]	No. of cultures with transforming DNA[b]
23	*Neisseria meningitidis*	505, 508, 516	17
		488	0
2	*Neisseria lactamica*	526	1
		488, 503, 505, 516	0
4	*Neisseria flava*	488, 505	0
2	*Neisseria perflava*	488, 505, 516	0
22	*Neisseria sicca*	526	1
		488, 505, 516	0
1	*Neisseria flavescens*	488	0
52	Throat neisserias[c]	488	0
8	*Branhamella catarrhalis*	488, 505, 516	0
5	*Moraxella osloensis*	488, 505, 508, 516	0
3	*Moraxella nonliquefaciens*	488	0
3	*Moraxella phenylpyruvica*	488	0
6	*Moraxella urethralis*	488	0
1	*Flavobacterium meningosepticum*	488	0
2	*Escherichia coli*	488, 505, 516	0
1	*Proteus mirabilis*	488, 505, 516	0
43	*Candida albicans*	488, 505, 516	0
318	Clinically isolated gram-positive and gram-negative bacteria[d]	488, 505, 516	0

[a] Mutant strain number.

[b] Transformation was performed using the standard transformation assay.

[c] These were all shown to be gram-negative, oxidase-positive diplococci that form aid from glucose and maltose, but were not characterized further.

[d] These were all oxidase-negative cultures isolated from patients with urinary tract and throat infections and also from stool specimens.

Transformation using crude DNA samples from swab specimens. Crude DNA samples were prepared from a total of 462 swab specimens. These specimens were obtained from symptomatic patients or their contacts, as well as from patients who had undergone antibiotic therapy. Each swab was inserted directly into the lysing solution and treated as described in Materials and Methods. At the time each swab

specimen was taken, a second swab of the same material was used to inoculate a Thayer-Martin plate. The results obtained for these swab studies are summarized in Table 2. For all the 90 swabs where some of the colonies on the Thayer-Martin medium could be identified as *N. gonorrhoeae*, crude DNA samples prepared directly from the material adhering to the swabs gave positive results in the transformation assay. No growth on the Thayer-Martin medium was observed with 301 swab specimens. Nevertheless, crude DNA samples from 39 of these 301 specimens were able to transform the *N. gonorrhoeae* auxotroph to prototrophy. The positive results obtained with these 39 specimens were shown to correlate with the clinical findings, symptoms, and histories of the patients from which the specimens were derived. The 262 swab specimens which failed to yield *N. gonorrhoeae* on culture (Table 2) and which also failed to yield transforming DNA were obtained from patients who had previously been under a regime of antibiotic therapy. The 58 swab specimens for which only oxidase-negative organisms were observed to grow on the Thayer-Martin medium (Table 2) also failed to yield transforming DNA. Oxidase-positive organisms which produced acid from both glucose and maltose could be isolated from 10 of the swab specimens tested (Table 2). In these cases, DNA samples from neither the swab specimens nor the isolated cultures were able to transform the *N. gonorrhoeae* mutants. Similar results were obtained for the three cases where the oxidase-positive cultures isolated failed to form acid from either glucose or maltose and were presumably cultures of *Moraxella*. In no case were cultures of *N. gonorrhoeae* isolated on the Thayer-Martin medium when the DNA sample from the swab specimen failed to transform the *N. gonorrhoeae* mutants.

TABLE 2. *Transformation with crude DNA solutions from swab specimens*[a]

No. of swabs tested[b]	Growth on TM medium[c]	Oxidase reaction	Acid production from:		Transformation[d] using DNA from:	
			Glucose	Maltose	Swabs	Cultures
90	+	+	+	−	+[a]	+
39	NG[e]	NT[f]	NT	NT	+	NT
262	NG	NT	NT	NT	−	NT
58	+	−	NT	NT	−	NT
10	+	+	+	+	−	−
3	+	+	−	−	−	−

[a] +, Transformation occurred; −, transformation did not occur.

[b] Specimens on swabs from the cervix or from the urethra were transferred directly into lysing solution. At the same time, a second swab from the same source was used to inoculate a plate of Thayer-Martin medium.

[c] TM medium, Thayer-Martin medium.

[d] Both mutant strains 488 and 508 were used in these studies.

[e] NG, No growth.

[f] NT, Not tested.

Effect of time of incubation of DNA-mutant cell mixtures. Previous studies of transformation of *N. gonorrhoeae* have shown that maximum competence occurs in the lag and in the early exponential growth phases, although lower levels of competency are evident throughout the remaining phases of growth (26). Because the method used for transformation in the present study is somewhat different from the methods usually employed (7, 26), it was necessary to determine the effect of time of contact of transforming DNA with a competent mutant of *N. gonorrhoeae* on medium M1 prior to streaking the DNA-mutant cell mixture on indicator medium. The results in Fig. 4 show the transformations obtained when DNA-mutant cell mixtures were incubated for periods from 0 to 8 h before streaking these mixtures on the indicator medium. Although a significant number of transformant colonies can be seen for the DNA-mutant cell mixture that was streaked on indicator medium immediately after mixing on medium M1 (Fig. 4), it is evident that longer periods of incubation of DNA-mutant cell mixtures on medium M1 do result in increased numbers of transformant colonies on the indicator medium.

Since the DNA used to transform mutant strain 488 in the experiment depicted in Fig. 4 was derived from a streptomycin-resistant mutant of *N. gonorrhoeae*, it was also possible to check for the transformation of mutant strain 488 to streptomycin resistance. It is well known that streptomycin-sensitive cells transformed to streptomycin-resistant cells require a period of growth in streptomycin-free medium, to overcome a phenotypic lag, before they can survive on streptomycin-containing medium (17). The fact that streptomycin-resistant mutants can be seen on the sections of the streptomycin-containing plate which were streaked after incubation for 2 to 4 h on medium M1 (Fig. 4) indicates that at least some transformation to streptomycin resistance must have taken place very shortly after the mixing of DNA with mutant strain 488 on the medium M1 plate.

Although, as indicated above, longer periods of incubation of DNA-mutant cell mixtures do

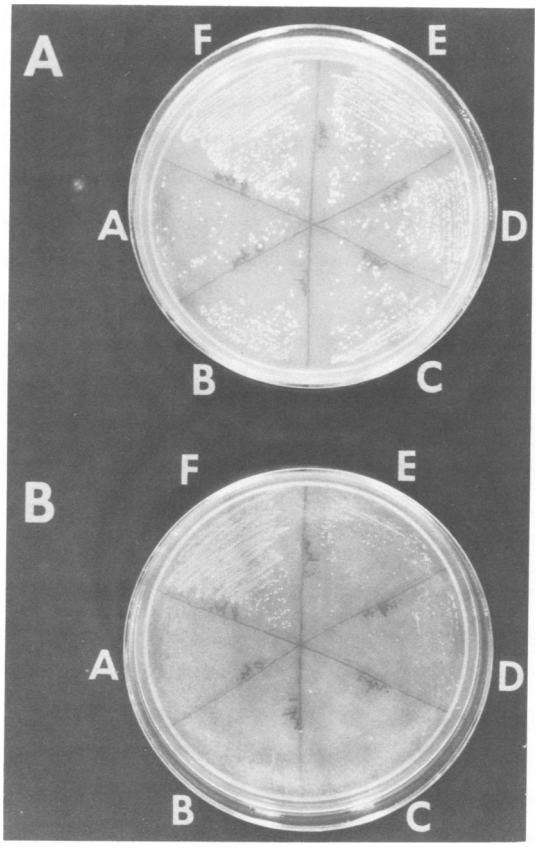

FIG. 4. *Transformation of mutant strain 488 as a function of time of incubation of DNA-mutant cell mixtures. DNA from a streptomycin-resistant mutant of wild-type strain 55 of N. gonorrhoeae was mixed with mutant strain 488 on sections of a medium M1 plate and incubated for 0 h (A), 1 h (B), 2 h (C), 4 (D), 6 h (E), and 8 h (F) before streaking on the indicated sectors of a medium M9 plate (A) and a medium M1 plate containing 100 μg of dihydrostreptomycin sulfate per ml (B). Both plates were incubated for 2 days before the photograph was taken.*

increase the sensitivity of the transformation assay, our experience indicates that incubation times of 2 to 4 h are adequate to detect *N. gonorrhoeae* both in culture and when present in sufficient numbers on swabs from clinical materials.

Minimum number of DNA-yielding cells of *N. gonorrhoeae* required for a positive transformation assay. To determine the smallest amount of DNA that results in transformation under the test conditions used in the present assay, type T2 mutant cells were suspended uniformly in standard saline citrate solution, and the density was adjusted to approximately 10^9 cells/ml. Portions (0.01 ml) of the cell suspension were applied to areas of approximately 1 cm^2 on medium M1 plates and permitted to dry on the agar surface. Using a 2-mm loop, crude DNA solutions (5-μl portions) prepared from suitable dilutions of wild-type cell suspensions were then mixed with the dry mutant cells. After incubation for 1 h the DNA-mutant cell mixtures were spread on sectors of indicator medium plates. After incubation for 2 days, the various sectors streaked were observed for the presence or absence of transformant colonies. The maximum dilution of cells used for the preparation of DNA for which transformant colonies appeared corresponded to a solution containing 50 ± 30 colony-forming units in the 5 μl of solution. Since the DNA content of bacterial cells is approximately 35 mg per g (dry weight) of cells (20) and assuming that the volume per cell is 10^{-12} cm^3, it can be calculated that as little as 3.5×10^{-13} g of DNA can be detected in the transformation assay.

Relationship of colonial morphology to the efficiency of transformation. Strain 55 of *N. gonorrhoeae*, the parent strain from which the mutants used in the present study were derived, was originally isolated as colonies having type T4 morphology. Upon streaking strain 55 for colony isolation on medium M1, an occasional type T2 colony can be observed. A similar situation prevails for mutants of this strain, such as mutant strain 488, for example. Reversion of type T4 colonies to type T2 colonies has been reported (13), although the mechanism for the interconversion of colony types is currently not understood. When spontaneously arising type T2 colonies are picked and transferred regularly to fresh media, they can be maintained indefinitely as type T2 colonies. As observed previously (9, 14), however, type T2 colonies constantly give rise to type T4 cells during normal cultivation, and it has not yet been possible to devise a procedure to maintain a culture of type T2 cells devoid of at least some type T4 cells.

In 1966 Sparling (26) demonstrated that colony types T1 and T2 are highly competent for genetic transformation, whereas colony types T3 and T4 are either noncompetent or very weakly competent. Studies with strain 55 revealed that treatment of type T4 cells with transforming DNA invariably gives rise to transformant colonies, although at a considerably reduced rate when compared with the rate of transformation of type T2 cells. The experiment depicted in Fig. 5 illustrates this phenomenon. DNA samples from five different strains of *N. gonorrhoeae* were mixed with both type T2 and type T4 cells of mutant strain 488 and

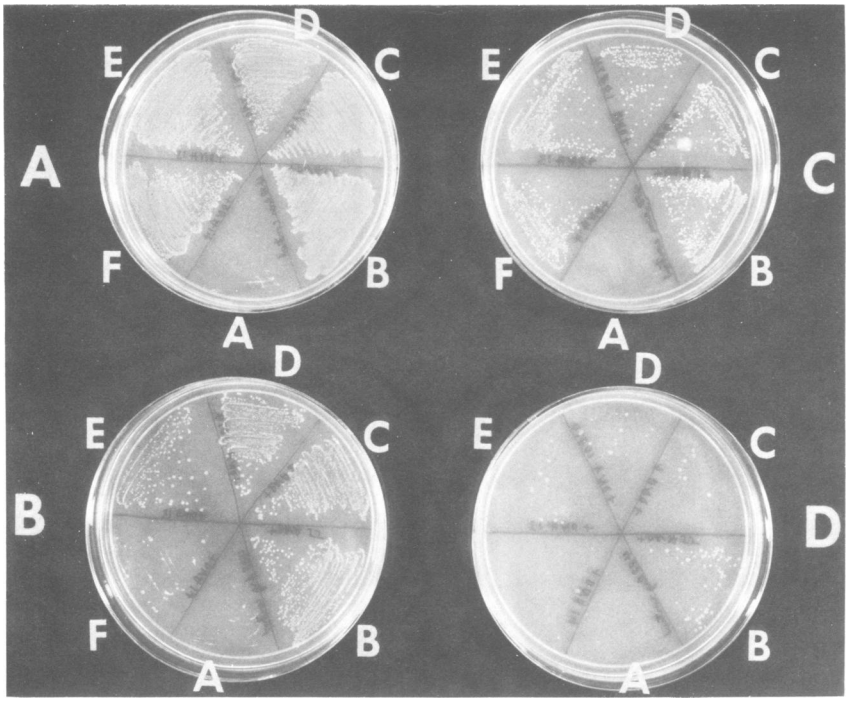

FIG. 5. *Transformation of type T2 and type T4 cells of mutant strain 488 with DNA samples from five different strains of N. gonorrhoeae. DNA-mutant cell mixtures on sections of a medium M1 plate were incubated for 24 h before streaking on sectors of medium M9 plates A and B and for 4.5 h before streaking on sectors of medium M9 plates C and D. Plates A and C were streaked with DNA-type T2 mutant cell mixtures, and plates B and D were streaked with DNA-type T4 mutant cell mixtures. The DNA samples used for the various mixtures were derived from a mutant cell control for which no DNA was used (A); strain 55 (B); strain 2 (C); strain ATCC 10874 (D); strain 13 (E); and strain 14 (F). Strain 55 is the parent strain of mutant strain 488. Strain ATCC 10874 has been reported to be a glucose-negative strain of N. gonorrhoeae. Strains 2, 13, and 14 are typical clinical isolates. All the above plates were incubated for 2 days for photographic purposes.*

incubated for either 4.5 or 24 h before streaking the DNA-mutant cell mixtures on indicator medium M9. Transformation is evident for all five DNA samples when both type T2 and type T4 recipient mutant cells were used (Fig. 5). It is quite clear, however, that type T2 cells are considerably more competent than type T4 cells and, also, that the sensitivity of the assay is significantly greater when the incubation time is increased from 4.5 to 24 h. Nevertheless, transformant colonies can be seen for each of the five DNA samples incubated with type T4 cells for 4.5 h as well as 24 h.

One possible explanation for the low level of transformation of type T4 cells (Fig. 5) involves the assumption that what is observed here is actually the transformation of small numbers of type T2 cells which, as described above, do appear spontaneously at a low rate in cultures of type T4 cells. Although such spontaneously arising type T2 cells will most certainly be transformed with relatively high efficiency, this phenomenon cannot explain entirely the results shown in Fig. 5. When transformant colonies are observed 18 to 24 h after streaking of DNA-type T2 mutant cell mixtures on medium M9, the large majority of these transformant colonies are type T2. By contrast, observation of transformant colonies after streaking DNA-type T4 mutant cell mixtures on medium M9 reveals predominantly type T4 transformant colonies. It seems quite clear, therefore, that type T4 cells do have an intrinsic low level of competency for genetic transformation, at least in *N. gonorrhoeae* strain 55 and its mutants.

When several of the type T4 transformant colonies of mutant strain 488 were picked and subsequently transformed to resistance to streptomycin, it was shown that approximately the same numbers of streptomycin-resistant transformants were obtained as were obtained

using type T4 colonies of mutant strain 488 as the recipient cells. This result indicates that the initially transformed type T4 cells do not represent a particularly competent kind of cell present in the type T4 population which is highly competent and still has type T4 colonial morphology. It was also found that the transformation of type T2 cells to streptomycin resistance resulted in predominantly type T2 streptomycin-resistant transformant colonies, whereas the transformation of type T4 cells gave predominantly type T4 streptomycin-resistant colonies. In this case, for transformation to resistance to streptomycin, as well as in the transformation of type T2 and type T4 cells of mutant strain 488 for ability to grow in medium M9, much smaller numbers of transformants were observed when type T4 recipient cells were used compared with the results obtained when the recipient cells were of type T2.

In quantitative transformation studies, using standard transformation procedures (26), it was found that type T2 cells of mutant strain 516 were transformed at an efficiency of 0.35%, whereas type T4 cells of the mutant strain were transformed at an efficiency of 0.0077%.

DISCUSSION

The data presented above show that it is possible to employ a simple and rapid genetic transformation assay for the detection and identification of *N. gonorrhoeae* using either isolated cultures or swabs from the vaginas, cervixes, or urethras of infected individuals as sources of transforming DNA. The finding that one can detect *N. gonorrhoeae* in the lysates of swab samples, without the isolation of the organisms in culture, greatly aids in the diagnosis of this organism in clinical materials. In addition to eliminating the requirement for Gram stains and sugar tests, the transformation assay for *N. gonorrhoeae* has the advantage of being more rapid and less laborious, of requiring less media, and also of eliminating the necessity of processing samples shortly after they have been taken to avoid death of the organisms.

It should be recognized that any single swab sample may not have adhering to it a sufficiently large number of cells of *N. gonorrhoeae* to yield a DNA preparation containing enough DNA molecules to always give a positive result in the transformation assay. Nevertheless, when a positive transformation assay is obtained using DNA prepared from a swab specimen this result does permit a more rapid diagnosis to be made than is the case using conventional procedures. When using DNA prepared from isolated cultures, however, one need not be concerned about the adequacy of DNA concentration.

Two unique advantages of the assay procedure described in the present paper over transformation methods in common use are the ease with which transforming DNA can be prepared and the speed and simplicity of the remaining steps of the assay. With the use of mutant strain 488, where mutant-DNA mixtures are streaked on medium M9, it is possible to observe transformant colonies in less than 24 h after the start of the transformation assay. Furthermore, medium M9 is simple to prepare when compared with the preparation of GGM (16) or the NEDA medium used by Catlin (5). Another important feature of the present method is the fact that impure cultures of *N. gonorrhoeae*, such as are obtained on original isolation plates, may be used as sources of DNA for the transformation assay, since the presence of DNA from unrelated microorganisms does not affect the qualitative results of the assay. This contrasts with the necessity of using pure cultures when testing for acid formation from sugars, for example. Still another important use of the transformation assay for *N. gonorrhoeae* concerns its application to cultures that are reported not to produce acid from glucose. One such culture (ATCC 10874) was shown to be an authentic strain of *N. gonorrhoeae*, since its DNA was readily able to transform mutant strain 488 to grow on medium M9 (Fig. 5).

It is well known that DNA samples from various species of *Neisseria* are able to transform competent strains of a given species of *Neisseria* (6). These findings, together with the known similarity in DNA composition for all true neisserias (1, 2, 6), serve to emphasize the close relationship of the various types of *Neisseria*. Our findings that the proline auxotrophs, used in the present study, can indeed be transformed to proline independence by a few DNA samples from nongonorrhoeae neisserias are in accord with these results. In spite of these observations, there are several good reasons why a genetic transformation assay is still valid for the detection and identification of *N. gonorrhoeae*. DNA preparations from only a few nongonorrhoeae neisserias are able to transform the proline auxotrophs, and none of such preparations are able to transform mutant 488 under the standard transformation assay conditions. Some strains of *N. meningitidis* of serotypes A and B decrease the specificity of the transformation assay for *N. gonorrhoeae* using proline auxotrophs. In a recent report, Lewis and Alexander (18) studied the occurrence of *N. menin-*

gitidis in the vagina and cervix. They found strains of *N. meningitidis* in the vaginas and/or cervixes of only three young sexually active women out of approximately 700 Transgrow cultures. It would thus appear that the likelihood of false positive results in the transformation assay for *N. gonorrhoeae* due to the presence of *N. meningitidis* in samples from the vagina and the cervix, and probably the urethra as well, is extremely small.

Although all *Neisseria* strains do have some genes in common, it is likely that more detailed studies will reveal that each type of *Neisseria* may have certain genes that are unique for that particular type. For example, there must be some genetic determinants that serve to make the strains of *N. gonorrhoeae* different from other nongonorrhoeae neisserias. Mutations in such *N. gonorrhoeae* genes should then be transformable using DNA samples only from other strains of *N. gonorrhoeae*. Future efforts will be directed toward identification of such genes so that the specificity of the transformation assay for *N. gonorrhoeae* may become as complete as it is in the cases for similar assays for *A. calcoaceticus* (11) and for *M. osloensis* (12).

Carifo and Catlin (4) have shown that 97 of the 251 isolated strains of *N. gonorrhoeae* studied in their laboratory require proline, in addition to other factors, for growth in a chemically defined medium. Since such a large proportion of these strains are proline auxotrophs, it may be questioned whether DNA samples from proline auxotrophs are capable of transforming the proline auxotrophs used in the present study. Although many isolated strains are auxotrophic for proline, it is nevertheless the case that such a phenotype could result from any one of a very large number of different alterations in the DNA structure. Catlin (5) has shown, for example, that DNA from one of her proline auxotrophs readily transformed two other proline auxotrophs in her collection to proline independence. The DNA lesions in the proline auxotrophs used in the present study cannot be identical, since DNA from any one of them could transform any of the others to proline independence. None of the DNA samples from the isolated cultures of *N. gonorrhoeae* examined in the present study failed to transform the indicator proline auxotrophs. It is possible, however, that a naturally occurring proline auxotroph may have a DNA lesion that is the same as, or overlaps, the lesion in the indicator strain. In such rare cases the availability of a second indicator strain would help make the diagnosis of this organism more definitive.

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