

Imferon Agar: Improved Medium for Isolation of Pathogenic *Neisseria*

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Imferon, an iron-dextran complex, enhances the growth of *Neisseria gonorrhoeae* and *N. meningitidis*. The use of Imferon as a replacement for ferric nitrate, in a defined supplement for GC agar, significantly increased the average colony sizes of both gonococci and meningococci. In comparison with Thayer-Martin medium, Imferon agar increased the speed and rate of isolation of gonococci from clinical specimens.

The incidence of gonorrhea has currently reached epidemic proportions: in the United States last year, one million new cases were reported, and this is only a fraction of the incidence of infection with *Neisseria gonorrhoeae* (3). Control of the disease is complicated by the large reservoir of asymptomatic carriers (5, 11). Detection of asymptomatic, as well as symptomatic, infections necessitates the use of a suitable culture medium. The most widely used medium, Thayer-Martin medium (12), is less than 100% efficient in isolation of *N. gonorrhoeae*. Various reports suggest that isolation rates range from 40% (6) to approximately 90% (2), i.e., that 10 to 60% of infected individuals would remain unrecognized after a single culture. Improvements in the efficacy of diagnostic media could greatly assist efforts to interrupt the spread of gonorrhea. Speed and accuracy are even more important in the diagnosis of infections with *N. meningitidis*, a potentially life-threatening pathogen for which Thayer-Martin medium is also used.

Alterations in the availability of the essential element iron have previously been shown to affect both the growth (7) and the virulence (10) of the gonococcus. During our studies on the effects of iron on virulence of gonococcal colony types (10), it was observed that colonies of gonococci, plated by a modification of the drop technique of Miles and Misra (9), were larger when the inocula contained added iron in the form of an iron-dextran complex (Imferon). Accordingly, we evaluated the effects of Imferon on growth of the pathogenic *Neisseria* in vitro and extended the observations by comparisons of the efficacies of various isolation media in detecting *N. gonorrhoeae*.

MATERIALS AND METHODS

Bacterial strains. *N. gonorrhoeae* strain F62 was

provided by D. S. Kellogg, Jr., Center for Disease Control, Atlanta, Ga. Colony types T1 (virulent) and T3 (avirulent) were selected and maintained as previously described (1). Other *N. gonorrhoeae* strains used were local isolates. *N. meningitidis* strain B-11 was obtained from H. Schneider, Walter Reed Army Institute for Research, Washington, D.C. Additional local isolates were also used.

Media. Imferon agar consisted of GC medium base (Difco Laboratories, Detroit, Mich.) plus 0.04% Imferon (Lakeside Laboratories, Inc., Milwaukee, Wis.) to give a final concentration of 20 µg of iron per ml; 1% Kellogg supplement (4), omitting the ferric nitrate; and the selective antibiotics vancomycin, colistin, and nystatin (V-C-N; Baltimore Biological Laboratory [BBL], Cockeysville, Md.) Other supplements tested included IsoVitaleX (BBL), 1% Kellogg supplement with ferric nitrate in varying concentration, 1% hemoglobin (BBL), and dextran, T10, T70, and T500 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). All supplements, including Imferon, were added aseptically to GC base that had been sterilized by autoclaving. The Imferon used in this study was generously provided by H. M. Leyland, Merrell-National Laboratories, Cincinnati, Ohio. Thayer-Martin medium consisted of GC medium base, 1% hemoglobin, 1% IsoVitaleX, and V-C-N. All media used in this study contained V-C-N.

Laboratory evaluation of media. Equal-volume droplets of dilutions of suspensions of gonococci or meningococci were plated on the media being compared. Plates were incubated at 37°C in 10% CO₂. After 20 h of incubation, the numbers of colonies and their average size were determined by using a dissecting microscope fitted with an optical reticle.

Clinical evaluation of media. Imferon agar and Thayer-Martin medium were compared for their ability to isolate gonococci from both males and females attending the Dallas Public Health Department Venereal Disease Clinic. A single swab was used to inoculate both types of medium, alternating the order of inoculation. Plates were incubated at 37°C in 10% CO₂. Imferon plates were read after overnight incubation (16 to 24 h), whereas Thayer-Martin plates were only examined after 40 to 48 h. The two types of plate were read independently, and

a presumptive diagnosis of gonorrhoea was based on the finding of oxidase-positive, gram-negative diplococci. Sugar fermentations were used to confirm cultures when a positive result was obtained on only one type of medium.

RESULTS

The addition of Imferon to GC medium led to enhanced growth of both gonococci and meningococci (Fig. 1). A three- to sixfold increase in colony diameter was observed on Imferon agar (20 μg of iron per ml) when compared to the same medium lacking Imferon or another iron source. Typical meningococcal and gonococcal colonies on agar containing varied amounts of Imferon, Thayer-Martin medium, and medium containing commercial supplement (Iso-VitaleX) are pictured in Fig. 2 and 3. Three additional gonococcal strains and two additional meningococcal strains were tested, and in each case the average colony size was significantly larger ($P < 0.01$, Student's t test) on Imferon agar than on the other media (Thayer-Martin, IsoVitaleX, or Kellogg supplement). Although the colony sizes differed, plating efficiencies were similar with all media and laboratory strains tested.

Imferon and ferric nitrate were compared as iron sources in otherwise identical media. In agreement with Kellogg's observations (7), iron in the form of ferric nitrate produced maximal stimulation at concentrations of $<5 \mu\text{g}/\text{ml}$, and increasing the concentration to 20 μg of iron per ml had no effect on colony size (Fig. 4). Imferon was equally or less effective at lower iron con-

centrations ($\leq 10 \mu\text{g}/\text{ml}$) but was more effective than ferric nitrate at higher iron concentrations (Fig. 4). In experiments not shown, little or no increase in colony size was observed at Imferon concentrations higher than 20 μg of iron per ml.

Imferon is a complex of iron and dextran. Therefore, the possible contribution of the dextran moiety was investigated. We were unable to obtain the precise dextran included in Imferon. Instead, various combinations of iron (ferric nitrate) and dextrans (T10, T70, and T500) were substituted for Imferon. The dextrans alone were found to be somewhat stimulatory for gonococci and meningococci, and in combination with ferric nitrate, colony sizes approached those obtained with Imferon. However, the use of Imferon rather than a combination of iron and dextran was preferable since results were more consistent and predictable with Imferon.

Hemoglobin is a constituent of most media used for the isolation of gonococci and meningococci and has been shown to enhance growth of the gonococcus (4). The addition of hemoglobin to Imferon agar led to a slight increase in the size of gonococcal colonies but had no effect on growth of the meningococcal strain tested (Table 1). Despite its stimulation of gonococcal growth, hemoglobin was not used further since its presence in the medium made growth more difficult to see, especially when the inoculum was small. The clear Imferon agar has an additional advantage over chocolate agar in that gonococci and meningococci can be distinguished from each other and from contaminating colonies on the basis of colonial morphology when illuminated as described by Kellogg (8).

Since Imferon agar was found to produce consistently larger gonococcal colonies than did Thayer-Martin medium, the medium was further tested for its ability to isolate gonococci in a clinical trial. A total of 406 cultures were taken from 389 patients attending a venereal disease clinic. This group consisted of 151 females and 238 males. A total of 182 (44.8%) of the cultures were positive for gonococci on at least one of the two media (Table 2). Of these positive cultures, 182 (100%) were detected within 24 h with Imferon agar, whereas significantly fewer, 164 (90.1%), were detected with Thayer-Martin medium despite an additional 24 h of incubation. No additional positive cultures resulted when the Imferon agar was incubated for an additional 24 h. Of the 18 cultures that were positive only on Imferon agar, 4 were oral or rectal cultures from subjects from whom the corresponding cervical or urethral cultures

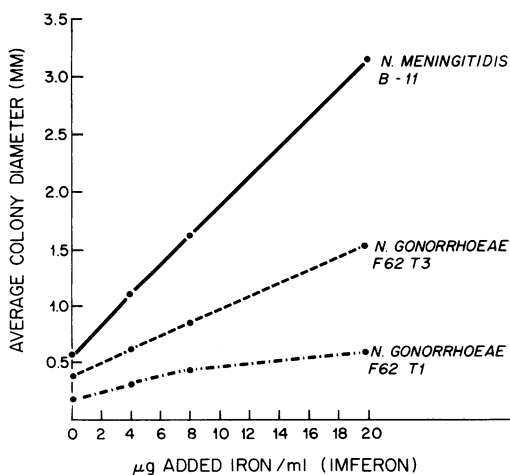


Fig. 1. Stimulatory effect of Imferon on growth of *Neisseria*. Each point represents the mean diameter of 25 colonies after 20 h of incubation at 37°C in 10% CO_2 .

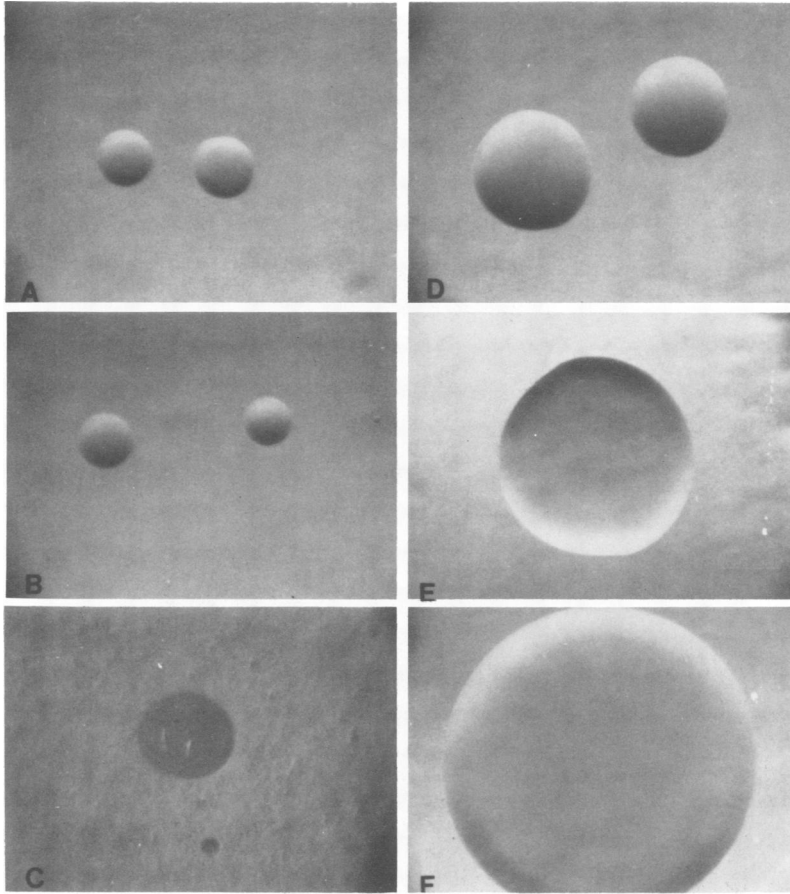


FIG. 2. Comparison of growth of *N. meningitidis* B-11 on: (A) GC base + Kellogg supplement without iron + V-C-N; (B) GC base + IsoVitaleX + V-C-N; (C) Thayer-Martin medium; (D) Same as (A), supplemented with Imferon (4 μg of iron per ml); (E) Same as (A), supplemented with Imferon (8 μg of iron per ml); (F) Same as (A), supplemented with Imferon (20 μg of iron per ml). All media were inoculated simultaneously and incubated at 37°C for 20 h in 10% CO₂. $\times 13.5$.

were positive on both types of medium. An additional five cultures that did not grow on Thayer-Martin produced only a small number of colonies on Imferon agar, indicating a relatively small inoculum. No difference was noted in the number of contaminating colonies on the two types of medium. In no case was the level of contamination great enough to interfere with the identification of gonococcal growth. No difference in efficiency of isolation was associated with the order of inoculation: nine additional isolates were recovered on Imferon agar plates inoculated before Thayer-Martin medium, and nine were isolated on Imferon plates streaked after Thayer-Martin medium.

DISCUSSION

Imferon, an iron-dextran complex, has been

found to stimulate growth of both gonococci and meningococci when used as a replacement for ferric nitrate in the supplement described by Kellogg (4, 7, 8). Using increased colony diameter as a measure of stimulation, both laboratory-adapted strains and recent clinical isolates exhibited enhanced growth on Imferon agar compared with that obtained with Thayer-Martin medium, GC base supplemented with Iso-VitaleX, or Kellogg supplement containing ferric nitrate.

The stimulatory effect of Imferon appears to be attributable to both the iron and the dextran components. Iron is an essential element for growth, but the function of the dextran in this medium is not known. The dextran may serve a purpose similar to that of the starch present in most gonococcal media, i.e., the binding of fatty acids or other toxic materials in the medium.

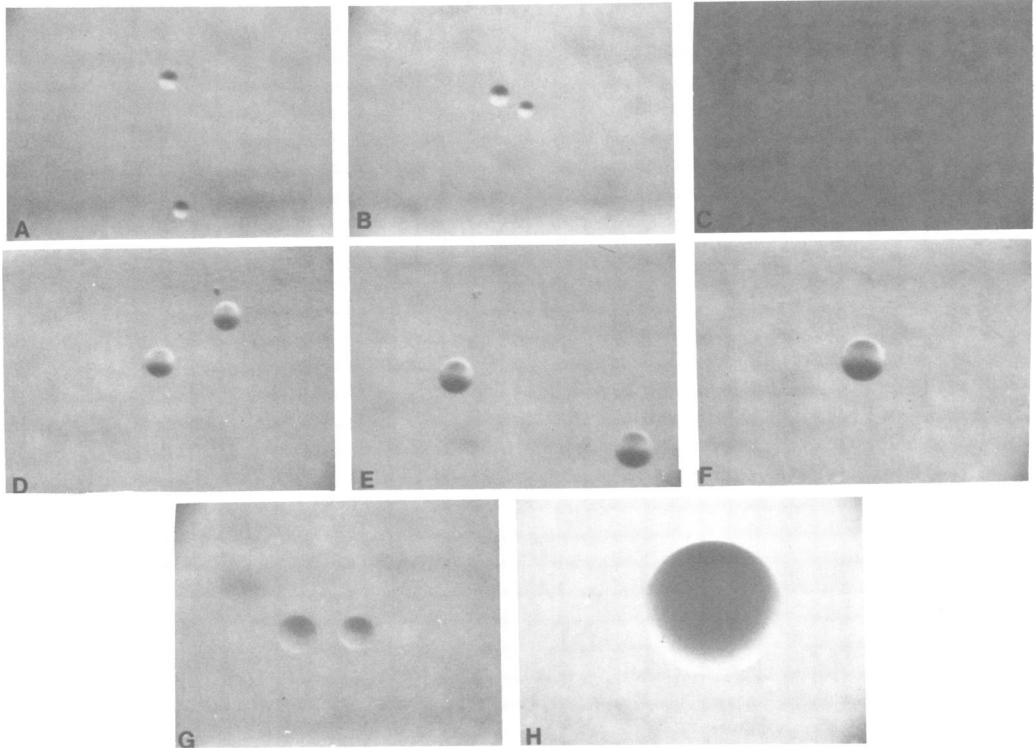


FIG. 3. Comparison of growth of *N. gonorrhoeae* F62 T1 (A-F) and T3 (G, H) on: (A) GC base + Kellogg supplement without iron + VCN; (B) GC base + IsoVitaleX + VCN; (C) Thayer-Martin medium; (D) Same as (A), supplemented with Imferon (4 μg of iron per ml); (E) Same as (A), supplemented with Imferon (8 μg of iron per ml); (F) Same as (A), supplemented with Imferon (20 μg of iron per ml); (G) GC base + IsoVitaleX + VCN; (H) Same as (A), supplemented with Imferon (20 μg of iron per ml). All media were inoculated simultaneously and incubated at 37°C for 20 h in 10% CO₂. $\times 12.5$.

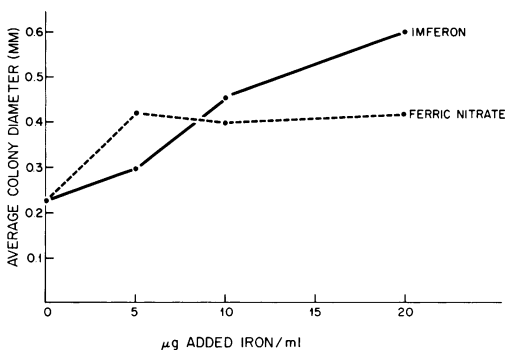


FIG. 4. Comparison of stimulatory effects of Imferon and ferric nitrate on *N. gonorrhoeae* F62 T1. Each point represents the mean diameter of 50 colonies after 20 h of incubation at 37°C in 10% CO₂.

However, attempts to replace the starch with dextran have only been partially successful.

The ability of Imferon to enhance gonococcal growth allows for more rapid identification of infections with *N. gonorrhoeae*. Gonococcal col-

TABLE 1. Effect of hemoglobin on colony size

Strain	Medium	Size (mm)	
		Range	Average ^a
<i>N. gonorrhoeae</i> F62 T1	Imferon agar	0.3-0.9	0.59
	Imferon agar + 1% hemoglobin	0.4-1.2	0.67
<i>N. meningitidis</i> B-11	Imferon agar	2.7-3.3	3.1
	Imferon agar + 1% hemoglobin	2.1-3.3	3.0

^a Mean of 50 colonies.

TABLE 2. Isolation of *N. gonorrhoeae* from clinical specimens: comparison of Imferon and Thayer-Martin media^a

Cultures on Imferon agar	Cultures on Thayer-Martin medium	
	Positive	Negative
Positive	164	18 ^b
Negative	0 ^b	224

^a Total number of cultures, 406.

^b $P < 0.05$, chi-square test.

onies were readily visible after overnight incubation, even when the inocula were apparently small, and further incubation did not result in any increase in the number of positive cultures. Although in our earlier laboratory tests plating efficiency was not significantly higher on Imferon agar, in the clinical trial Imferon agar significantly increased the rate of isolation of gonococci from suspected cases of gonorrhea. This is similar to the experience of Chandler et al. (4), who noted that recovery of gonococci from clinical material was related more to colony size than to plating efficiency. On the basis of these observations, we conclude that Imferon agar represents a significant improvement over previously described and widely used media for the isolation of pathogenic *Neisseria*.

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