

Introduction: *Neisseria gonorrhoeae* is a common bacteria that causes a sexually transmitted disease. This disease can cause a great amount of pain for both men and women. Treatment of the bacterium has been a simple matter of diagnosis and antibiotic treatment in the past, but now the bacteria is becoming antibiotic resistant because of the increased antibiotic use. In search for novel ways to treat *N. gonorrhoeae* infection, we are focusing on finding a bacteriophage that targets *N. gonorrhoeae*. The most effective way to look for new types of bacteriophage are in a liquid culture media, but the current standard media for growing *N. gonorrhoeae* does not grow *N. gonorrhoeae* in an efficacious manner. If we cannot grow *N. gonorrhoeae* in the laboratory, further research into novel treatment methods become almost impossible. The difficulties with growing *N. gonorrhoeae* samples in Fastidious Broth led us to search for other media options that would result in a more timely and effective bacterial growth rate. This experiment characterized different growth media supplementation options with the aim of developing a media that allows for completion of a bacterial growth curve over the course of 8-12 hours, which would be best suited bacteriophage isolation and amplification protocols. Our findings indicate that the supplement media we developed is the most consistent and efficacious in which to grow *N. gonorrhoeae* for laboratory experimentation.

Methods: Growing the initial bacteria colonies:

- Bacteria was cultured from frozen stocks and incubated on chocolate agar plates for two days to ensure growth.

Setting up overnight culture:

- Bacteria colonies were cultured overnight in Medium C.
- Gram stain was performed to ensure no contamination.
- An OD measurement was taken of the overnight cultures. 2mL aliquots were used to inoculate each experimental culture tube.
- The media used for this experiment included Fastidious Broth (FB), Brain Heart Infusion (BHI), and three different supplemented BHI media we called A, B and C.

Data collection:

- Using the overnight culture bacteria, we inoculated three tubes containing 20mL for a total of 15 tubes per experiment using each of the five media we were testing.
- We incubated these samples throughout the day, taking OD readings every hour until lag phase to evaluate bacterial growth. The growth curve represents the overall bacterial culture growth measured in Optical Density (OD). The lag phase is when the bacteria is just beginning to grow, log phase is when the bacteria is growing exponentially, and the stationary phase is when the bacteria has used up the available resources and the growth rate plateaus (Fig 1).
- OD data were used to generate growth curves using Growthrates software.

Figure 1

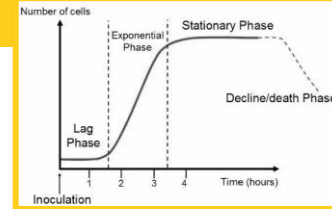
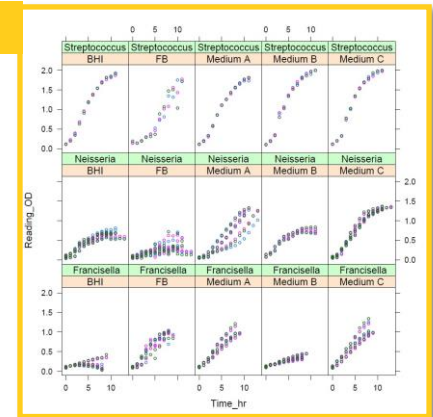


Figure 2 legend: Each separate experiment was run in triplicate at the time, and then the entire process was repeated three times. Each growth curve represented in Figure 2 shows the average OD of the triplicates for each of the separate experiments run with each bacterium species in each media.

Results: In order to study alternative ways to treat *N. gonorrhoeae* infections, scientists need to be able to grow the bacteria easily and reliably in the laboratory setting. The most efficacious way to do this is with a liquid media. The gold standard, Fastidious Broth, is less efficacious than our Medium C. The *S. pneumoniae* cultures grew well in all medias, though it grew more consistently in the BHI media. The *F. novacida* showed consistent growth curves in the FB, Media A and Media C supplementations.

Figure 2



Conclusion: We found that the Medium C was a better medium for growing *N. gonorrhoeae* than the gold standard Fastidious Broth in terms of more consistent rapid growth. The other fastidious bacteria evaluated, *S. pneumoniae* and *F. novacida*, showed different growth curves, but *N. gonorrhoeae* grows better in Medium C than the other media evaluated.

Resources:

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