Preservation of Neisseria gonorrhoeae at -80C
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Objectives: In most laboratories, bacterial strains need to be maintained for quality control, teaching, and research. To explore the feasibility of preserving N. gonorrhoeae at -80C, we studied its viability in three different medias. Materials And Methods: 84 N. gonorrhoeae isolates preserved in our laboratory, 77 were viables: 4/5 in 2008, 18/20 in 2009, 30/34 in 2010 and 25/25 in 2011. Skim Milk (SM), Commercial Media (CM. AES laboratoire) and Trypticase Soy Broth (Oxoid) with 20% glycerol (Prolabo) (TSBG) were used to preservation. All isolates were cultured on supplemented GC agar (GCagar with Vitox supplement, Oxoid). The cultures were incubated during 72h at 35 C in a 5% CO2, humid atmosphere. The nonviable isolates were subcultured on chocolate (Oxoid). The viability was determined quantitatively by plate counts on agar (Growth0: 0CFU, Growth1: 1-20CFU, Growth2: 20-100CFU and Growth3: >100CFU). The oxidase test and Maldi Biotyper 3 (Bruker) were used to confirm the presence of N. gonorrhoeae. Results: 75(97.4%) out of the 77 isolates remained viable in the TSBG, 63(81.82%) in the CM and 53(68.84%) in the SM. The 90,90% of isolates preserved in TSBG, the 36,38% in CM and the 28,55% in SM had a growth >100 CFU. 37(48.05%) isolates grew with more CFU in the TSBG, 5(6.49%) in the CM and one in the SM. 11(14.6%) out of the 77 isolates didn’t grow on GC medium, but did it on chocolate. In 1979, Yamai et al.(1) used to preserve gelatine-disc method for over three years; Cody(2) CAS under oil at 30 C for 12-18 months. Our work shows that the most effective method for preserving N. gonorrhoeae over four years now in common use is TSBG. In 1996, Harbec et al.(3) explored the feasibility in TSBG(10%) at -20 C for 4 months and they noted a sharp drop in bacterial counts. We studied the preservation at -80 C for more time and we used TSBG(20%), for this reason, the results can be different. Knapp and Rice(4) also indicated that some isolates preserved at -20 C in TSBG(20%) may survive storage for up to 2 weeks. Conclusions: This work shows that it is possible to maintain viable cultures of N. gonorrhoeae with an efficient and inexpensive method (TSBG). References: 1. Yamai et al. British Journal of Venereal Diseases 1979; 55: 90-93 2. Cody R.M. Health Laboratory Science 1974; 15: 206-209. 3. Harbec and Turcotte. J Clin Microbiol 1996; 34: 1143-1146. 4. Knapp et al. En Murray et al., Manual of clinical microbiology, 6th ed. 1995 pp324-340