Comparison Of Different Liquid Cell Culture Media For Culturing *Neisseria meningitidis*

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Objective: To compare and evaluate different liquid media for culturing *Neisseria meningitidis* (meningococci).

Design: Strains of *N. meningitidis* were grown in different cell culture media under different conditions (e.g. with or without iron source; with or without serum source). As serum source fetal calf serum (FCS) was used to avoid the presence of anti meningococcal antibodies. Growth rate was determined in 30 min intervals for the first six hours and than in longer intervals until 48 h of culture were completed. Optical density (OD) of the culture media were measured (λ=600 nm). Following 24 h of incubation 10^4 viable meningococci were used in a whole blood model of infection. The bacterial survival and cytokine release from leukocytes were compared to results obtained by using meningococci grown on GC agar.

Results: Growth rate was different in various cell culture media. The OD_{600} for 10^6 meningococci/mL was higher for those meningococci grown in liquid media than for those grown on solid media. Most interestingly, when grown in standard cell culture medium (RPMI 1640; Gibco BRL, Paisley, UK) without iron in the medium but supplemented with 10% FCS meningococci grew to a logarithmic growth phase within the first six to eight hours. Between 8 and 24 h of culture we observed an equilibrium phase or a decrease in *cfu*. The behavior of those meningococci was significantly different in a whole blood model when compared to meningococci grown on GC agar. If, for instance, serogroup B meningococci were grown under iron starvation, they were rapidly killed in human whole blood whereas those grown on GC showed logarithmic growth.

Conclusions: There are considerable differences concerning the growth rate of meningococci and subsequent behavior in a whole blood model of infection depending on the culture medium and the culture conditions used. This should be taken into account when meningococci are used for the study of pathogenicity or host—pathogen interactions.

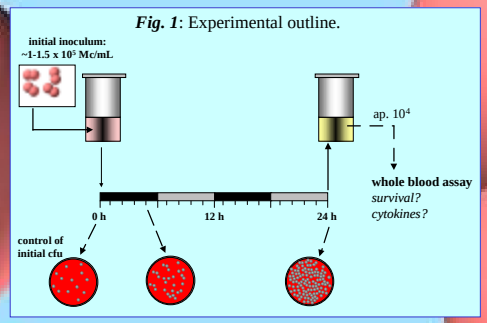


Fig. 1: Experimental outline.

Tab. 1: Commercially available media used in this work:

	Medium	iron-source	glucose (mg/L)
(1)	MEM (*Modified Eagle Medium*)	$Fe(NO_3)_3$ x 9 H_2O	9.000
(2)	Ham`s F-12	$FeSO_4$	1.802
(3)	RPMI 1603	$FeSO_4$	2.500
(4)	RPMI 1640	no iron in media	2.000
(5)	Medium 199	$Fe(NO_3)_3$ x 9 H_2O	1.000
(6)	serum-free medium (Neumann & Tytell)	no iron in media	3.000

All media were supplied by (GibcoBRL) and were supplemented with 10% fetal calf serum (FCS; South American origin, GibcoBRL). All media with the exception of MEM contained phenol red.

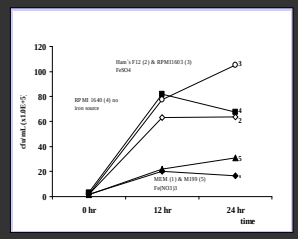


Fig. 2: Comparison of different cell culture media for liquid culture of meningococci. Samples were taken at 0, 12 and 24 hrs of culture.

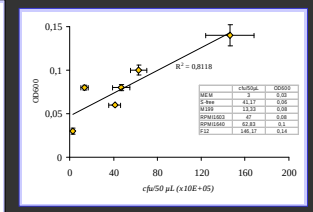
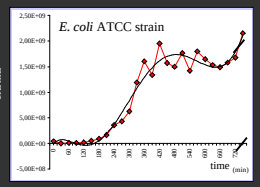


Fig. 3: Correlation between growth (cfu/50 µL +/- SE [X-axis]) and respective $OD_{(600)}$ (OD +/- SE [Y-axis]) in liquid culture media.



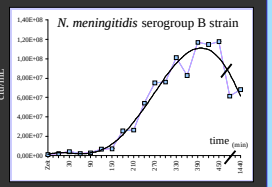


Fig. 4: Growth of *E. coli* (***a.***) and *N. meningitidis* (***b.***) within 24 hrs in RPMI1640 cell culture. Medium supplemented with 10% FCS.

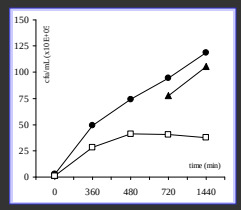


Fig. 5: Growth of *N. meningitidis* in serum-free medium (N°. (6); open squares) & RPMI 1603 (N°. 3, duplicate, closed symbols) over 24 hrs. Both media were supplemented with 10% FCS.

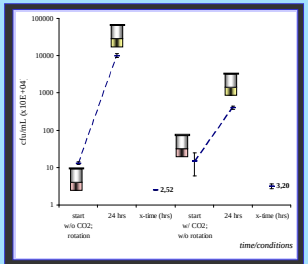


Fig. 6: Doubling time (x-time) of meningococci in liquid media depending on the growth conditions (availability of CO_2, rotation)

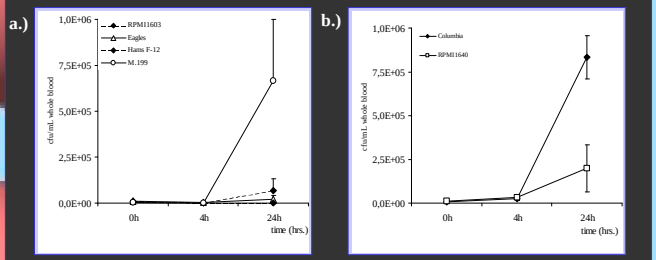
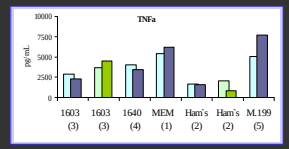


Fig. 7: Survival kinetics of differently cultured *N. meningitidis* strain B6256 (B:4:P1.7,16) in human whole blood. **a.)** initial cfu 7200 +/- 1600 cfu; meningococci were propagated in media 2, 3, 5, or 6, and **b.)** initial cfu 10070 +/- 2270 cfu; meningo-cocci were cultured in medium 4 or on sheep blood supplemented Columbia agar (Nolte et al in preparation).



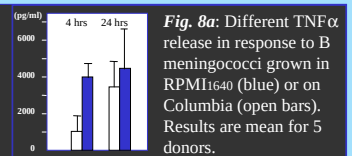
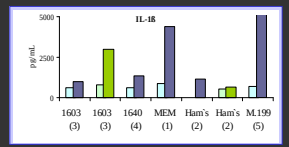


Fig. 8a: Different TNFα release in response to B meningococci grown in RPMI1640 (blue) or on Columbia (open bars). Results are mean for 5 donors.



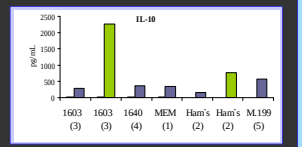


Fig. 8: Cytokine release in human whole blood in response to differently cultured meningococci. One mL heparinized blood samples from two female donors (29 years, blue bars and 52 years of age , green bars) was inoculated with 10^4 viable meningo-cocci. Samples were taken at 4 hrs. (light colored bar) and 24 hrs. (dark colored bar). Cytokines were detected using OptEIA sets (Pharmingen).