

Continuous real-time evaluation of microorganism growth kinetics & interactions with antimicrobial materials by isothermal micro-nano calorimetry (IMNC)

U. von Ah¹, D. Wirz¹, A. U. Daniels¹

¹ [Lab. for Orthopedic Biomechanics](#), Faculty of Medicine, Univ. of Basel, Switzerland.

INTRODUCTION: Cultured cell metabolism and growth processes produce heat measurable by IMNC.¹ The heat production rate ($W=J/s$) at any time t is the aggregate cell metabolic rate, and the amount of heat produced (J) between t_1 and t_2 is proportional to the number of cells produced. IMNC detects changes in heat production rate as low as 22 nJ/s--equivalent to a change of only $\sim 10^4$ in the number of active bacteria present. It is thus a non-invasive method for detecting and quantitating growth kinetics of bacteria in culture. IMNC can determine the effect of culture environment (including antimicrobials) on bacterial growth. After IMNC studies, the undisturbed culture specimens (cells, medium, solids) can be evaluated by any conventional means desired.

METHODS: The IMNC instrument is first equilibrated at a chosen temperature (e.g. 37°C). Bacterial cultures of known types and concentrations (cfu/ml) are prepared conventionally. Studies are done in sterile 4 ml glass ampoules. Typically, 2.97 ml of a growth medium (with or without an antimicrobial) are added, followed by 0.03 ml PBS containing 10^4 cfu of bacteria. The ampoule is then septum sealed. Measurements start ~ 60 minutes later--after the ampoule is lowered first into the equilibration position in one of the instrument's calorimeters and then to the measurement position. The instrument used has 48 independent calorimeters, and can thus rapidly evaluate multiple culture variables and replicate specimens (TAM III-48, Waters/TA Inc., New Castle DE, USA). Heat produced by an ampoule can be monitored as long as desired, typically hours to days.

RESULTS: Our IMNC method rapidly determined whether a sample contained methicillin-resistant or susceptible *Staphylococcus aureus* (MRSA or MSSA).¹ The determination could be made in ~ 4 hours vs. 24 hours by standard means. In addition IMNC provided the MIC (minimum inhibitory concentration) of the antibiotic used.

We expanded this approach to determine MICs of 10 different antibiotics for 5 different surgically important bacteria.² Results correlated exactly with parallel standard assessments and

reference values from the Clinical Laboratory Standards Institute (USA). IMNC was simple and accurate. At subinhibitory concentrations, growth curves (time histories of heat flow rate and aggregate heat) were reproducible for a given bacteria and medium. Differences in the array of growth patterns for a group of media may be a means of bacterial identification.

IMNC also determined the antimicrobial action of Ag^+ ions.³ The MIC (no growth at 24 hours) of $AgNO_3$ was 8 mg/l. For an antimicrobial biomaterial (silica agglomerates containing silver particles $d \sim 5-20$ nm) the MICs were much higher (e.g. 250 mg/l for silica/20% silver) because of the reduced amount of Ag^+ available. More importantly, IMNC showed something new. Subinhibitory concentrations of Ag^+ delay growth, but once it starts, initial concentration has no effect on the rate (Fig 1).

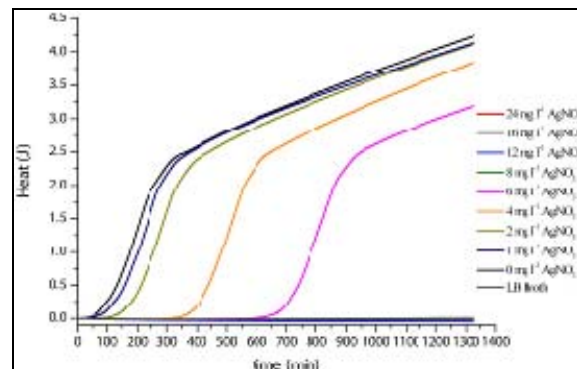


Fig. 1: *E.coli* growth in LB broth at 37°C. Aggregate heat as $f(t)$ & $AgNO_3$ concentration.

DISCUSSION & CONCLUSIONS: Results suggest IMNC can be an important new tool for clinical and research microbiology. The power lies in its ability for continuous, real-time, quantitative monitoring of growth kinetics.

REFERENCES: ¹ von Ah, Wirz, Daniels (2008) *J Clin Microbiol* (on-line 14/4/08). ² von Ah, Wirz, Daniels (2008) *Report 301-MS4*, Velux Foundation, Zürich CH. ³ von Ah, Wirz, Pieles, Daniels (2008) *Abstract O9*, Ann. Mtg. Swiss Soc. for Biomaterials.

ACKNOWLEDGEMENTS: Velux Stiftung (Zürich CH), HeiQ AG (Bad Zurzach CH).