

# Growth Characteristics and Antimicrobial Susceptibility of *Borrelia burgdorferi* by Calorimetry

D-736

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## Background

- Borrelia burgdorferi* is difficult to cultivate due to its slow replication and special culture requirements. Antimicrobial susceptibility tests are not standardized and require dark-field microscopy for counting borrelia and evaluate their vitality.
- Measuring heat production of replicating bacteria (calorimetry) may be a more accurate, rapid and real time tool for evaluation of growth characteristics and antimicrobial susceptibility of borrelia.
- Aim of the study was to evaluate: (1) the growth characteristics of borrelia by calorimetry and (2) the potential of calorimetry to determine their antimicrobial susceptibility.

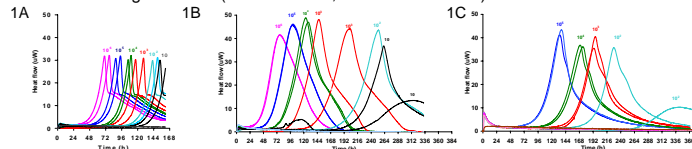
## Methods

- Test isolates of *Borrelia burgdorferi sensu lato* were used from skin biopsies of patients with erythema migrans: *B. burgdorferi sensu stricto* (1408/94), *B. afzelii* (1978/94), *B. garinii* (1375/05).
- The inoculum of *B. burgdorferi* was 1-5 x 10<sup>6</sup> viable borrelia/ml, determined by dark-field microscopy (motile cells). For culture, Barbour-Stoenner-Kelly (BSK-H) medium (Sigma, B8291) with 6% rabbit serum was used, completed immediately before experiment. We used low-passage isolates (<10 growth passages).
- Heat production (in µW) of borrelia was measured at 37°C for up to 21 days using a microcalorimeter (TA Instruments, Newcastle, USA) with a detection limit of 0.2 µW (Figure 1). Peak of heat flow and time to peak were determined. Minimal heat inhibition concentration (MHIC) was defined as the lowest concentration of antibiotics in which after 21 days of incubation at 37°C no heat flow was measured.
- Evaluation of growth characteristics and detection limit**
  - 0.1 ml of 1:10 serial dilutions (containing 10<sup>6</sup> to 1 borrelia/ml) added to 3.9 ml BSK-H medium in duplicate; BSK-H medium alone was used as control.
- Antimicrobial susceptibility testing of *B. burgdorferi sensu stricto***
  - 5 different concentrations **amoxicillin** (0.0625, 0.125, 0.25, 0.5, 1 µg/ml) and **doxycycline** (0.125/0.250/0.5/1.0 und 2.0 µg/ml) were tested in duplicate.
  - Antibiotic stock dilutions were prepared from powder in distilled water and sterile filtered. Dilutions were prepared in sterile water.

## Results

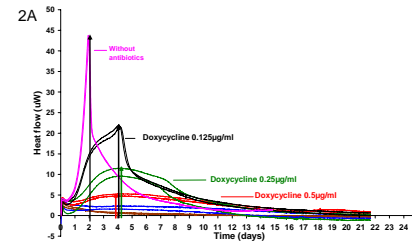
### Growth characteristics and detection limit of *B. burgdorferi sensu lato* (Figure 1)

- All tested isolates of borrelia generated heat with a peak of 30-60 µW. Figure 1A shows *B. burgdorferi sensu stricto* (inoculum 10<sup>6</sup> borrelia/ml) with time to peak at 75 hours and *B. afzelii* (2B) at 77 hours, respectively. *B. garinii* (1C) with initial inoculum of 10<sup>5</sup> borrelia/ml shows time to peak and 135 hours. Time to peak was proportionally delayed with decreasing inoculum (detection limit, 10-100 borrelia/ml).

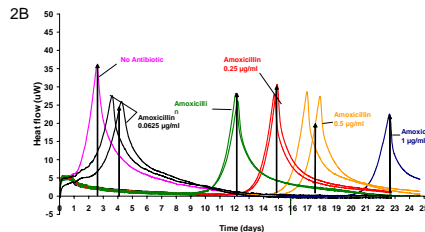


### Antimicrobial susceptibility testing of *B. burgdorferi sensu stricto* (Figure 2)

- Fig. 2A: **Doxycycline** at 2 µg/ml and 1 µg/ml inhibited heat production completely, while lower concentrations (0.5, 0.25 and 0.125 µg/ml) reduced the peak of heat flow curve and increased the time to peak from 2.0 days (no antibiotics) to ~4 days (vertical arrows).



- Fig. 2B: **Amoxicillin** did not reduce the peak of heat flow, but delayed the time to peak from 2.7 days (no antibiotics) to 4.0 days (0.0625 µg/ml), 12.1 days (0.125 µg/ml), 14.9 days (0.25 µg/ml), 17.5 days (0.5 µg/ml) and 22.5 days (1 µg/ml).



## Results – cont.

Antibiotics	Minimal heat inhibition concentration (MHIC)	MIC <sub>90</sub> (colorimetric method)*	MIC range (colorimetric method)*
		(µg/ml)	
Amoxicillin	>1	1	0.03-2
Doxycyclin	0.5-1	0.25	0.06-2

\*Determined by Hunfeld et al. as described in references 1 and 2 (below).

## Interpretation & Conclusions

- Growth characteristics:** Calorimetry is a useful tool for evaluation of borrelia, allowing an accurate, rapid and real-time assessment of their growth characteristics and antimicrobial susceptibility testing.
- Both antibiotics increased the time to peak, but only **doxycyclin** reduced the peak of heat flow in a dose-dependent manner, reflecting a potential bactericidal action on borrelia. In contrast, **amoxicillin** shifted the heat-flow curves with increasing dose, indicating a potential inhibitory action or efflux mechanism.
- The measured MHIC of doxycyclin and amoxicillin correlates with the MIC values determined by microdilution colorimetric assay (Hunfeld et al.).
- In future with calorimetry, the activity of antimicrobial combinations and specific activity against persistent forms (cysts) may be determined.

### Acknowledgments

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## References

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