Convenient and Reliable Extraction of Borrelia burgdorferi from Ticks Using Pressure Cycling Technology and the PCT Shredder

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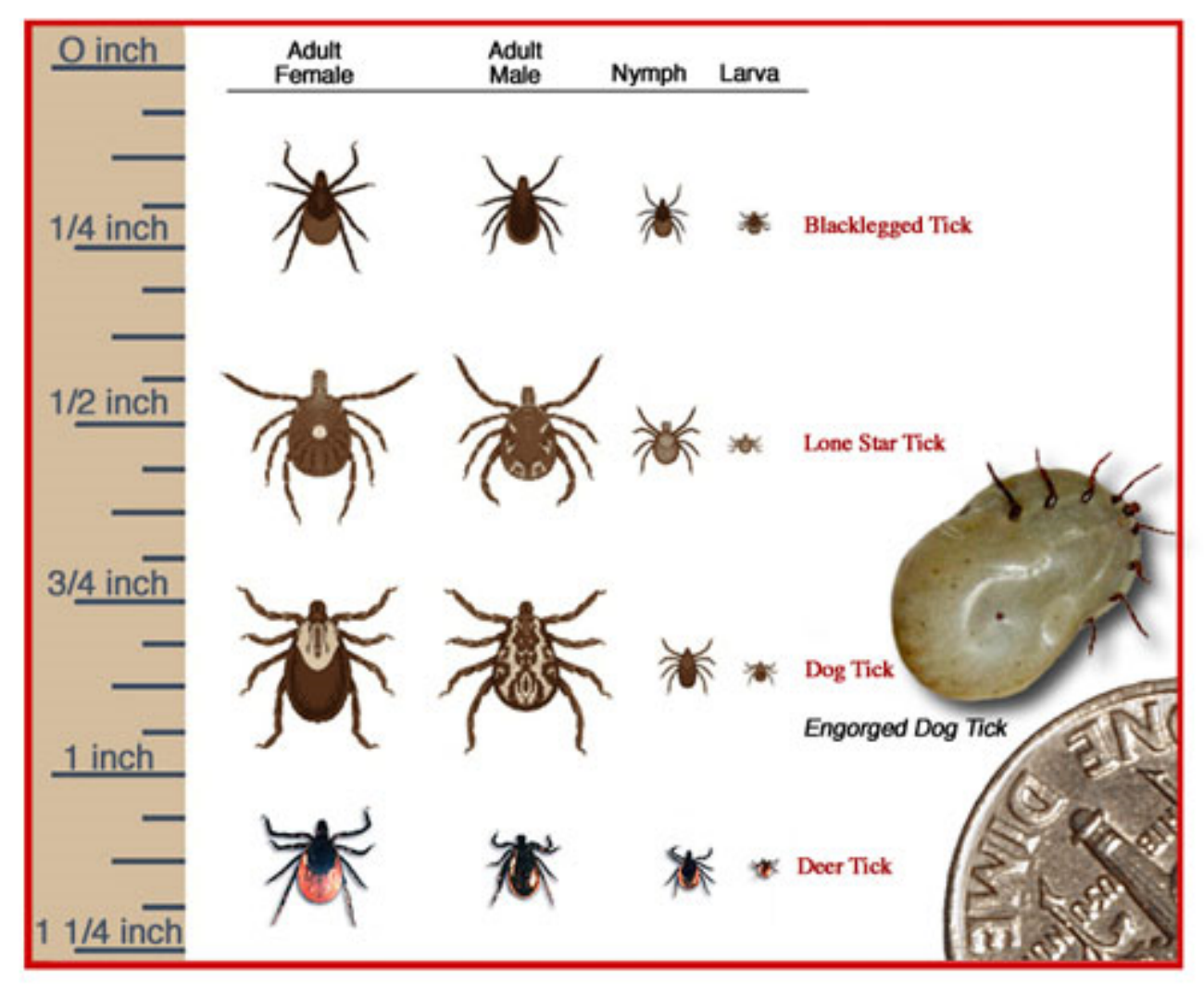
North American Tick Species



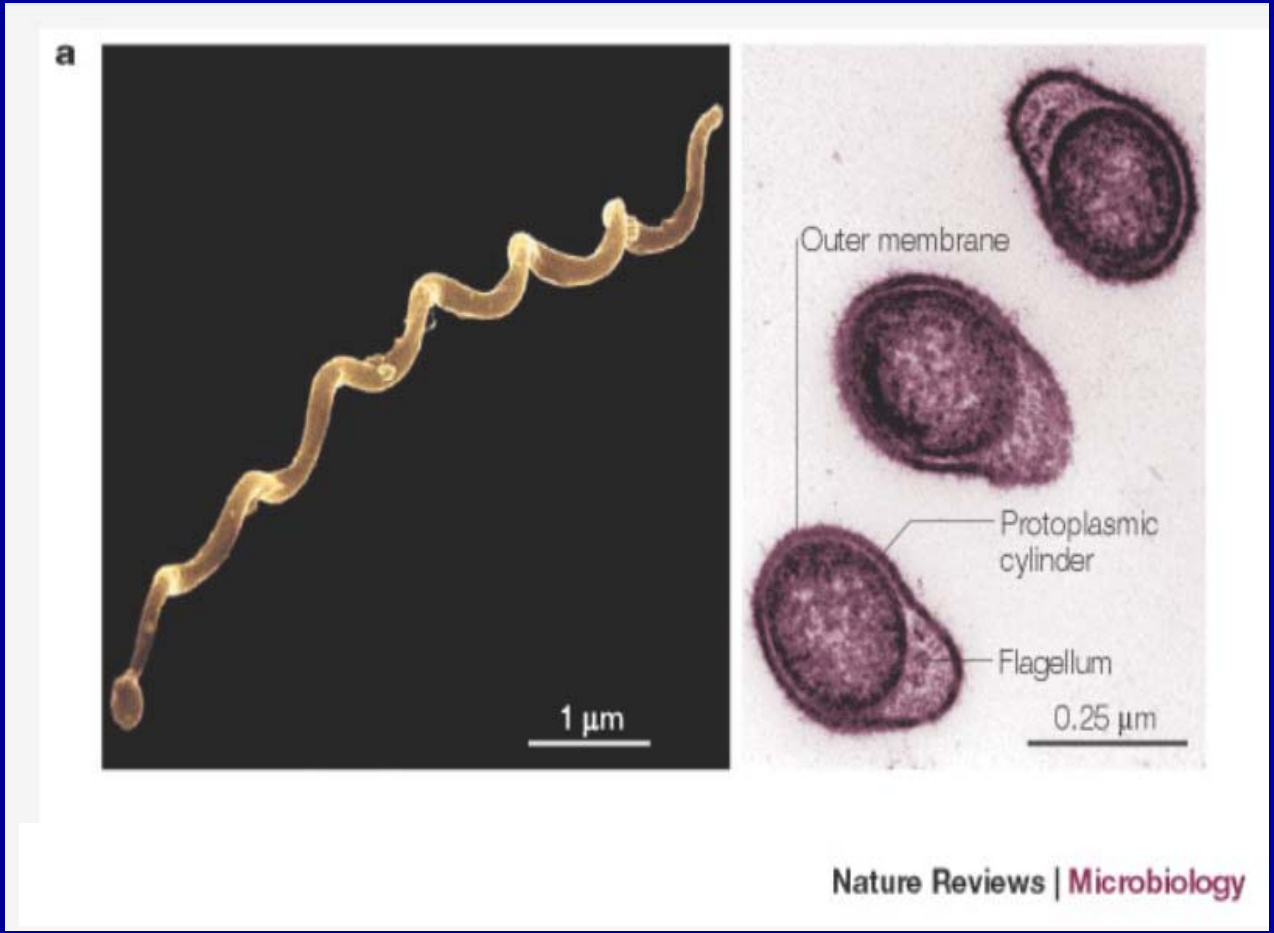
Ixodes scapularis

Amblyomma americanum

Dermacentor variabilis

Ixodes scapularis

Borrelia burgdorferi – pathogenic *spirochaeta*



The burgeoning molecular genetics of the Lyme disease spirochaete
P. A. Rosa, *et al*., Nature Reviews Microbiology 3, 129-143 (2005)

Clinical manifestations of Lyme borreliosis in children and adolescents.

Early (days, weeks)	Late (months, years)
Erythema migrans	Acrodermatitis chronica atrophicans
Lymphocytoma	Radiculoneuritis
Lymphocytic meningitis	Encephalomyelitis
Facial palsy	Cardiomyopathy
Myopericarditis	Uveitis, Keratitis
Conjunctivitis	Episodic arthritis
Summer “ flue”	Chronic arthritis
Arthralgias	

Lyme borreliosis

Pediatric Rheumatology Online, June 30th, 2006

Gentle mechanical homogenization prior to pressure-mediated extraction

Serrated Ram

The PCT Shredder

Barocycler NEP 2320

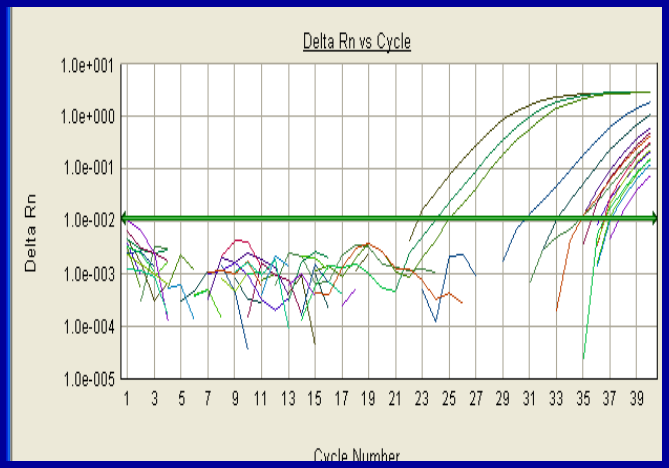
Hydrostatic pressure:
5,000 – 35,000 psi

Temperature control:
Up to 60°C

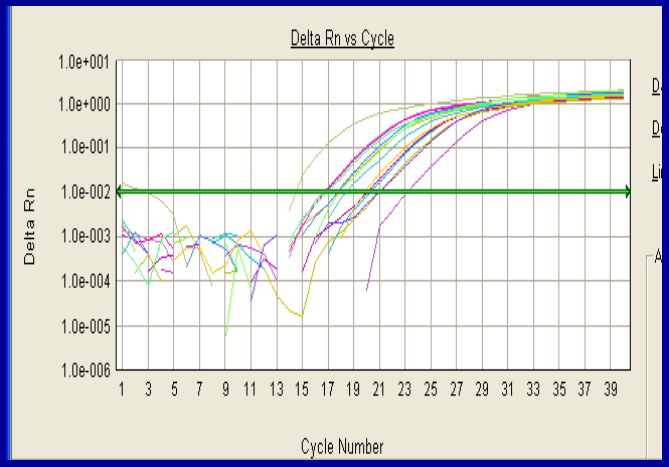
PCT program:
20s at 35,000 psi
20s at atm. pressure
45 cycles

PCT lysis combined with
Proteinase K digestion

Real-time PCR Detection of Borrelia burgdorferi and total bacteria load in tick DNA isolated using the PCT-shredder



Real-time PCR detection of *Borrelia burgdorferi* 23 S rRNA gene, generating a 75-bp fragment with a Taqman probe. Three extractions out of five on *Ixodes scapularis* were positive (about 10^8 to 10^9 copies). Two are less-strongly amplified. One out of nine *Amblyomma americanum* and *Dermacentor variabilis* are less-strongly amplified.



Real-time PCR detection of total bacteria load by amplification of bacteria 16S rDNA genes with a Taqman probe. All 14 DNA samples isolated by PCT-shredder are positive for total bacteria load.

Detection of *B. burgdorferi* in New England ticks

Species	No. of tick	*B. burgdoferi* detection		total bacterial load	
		Positive	Ct value	Positive	Ct value
Ixodes scapularis	5	3	20.28 23.69 22.15	5	15.94 14.11 17.68 17.07 22.41
Amblyomma americanum	6	1	29.67	6	17.87 20.35 20.15 21.04 16.83 19.91
Dermacentor variabilis	3	0	n/a	3	17.75 21.03 17.86
Unidentified ticks	7	1	23.20	7	19.24 17.08 20.11 17.22 16.08 23.20 24.25

Acknowledgements

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