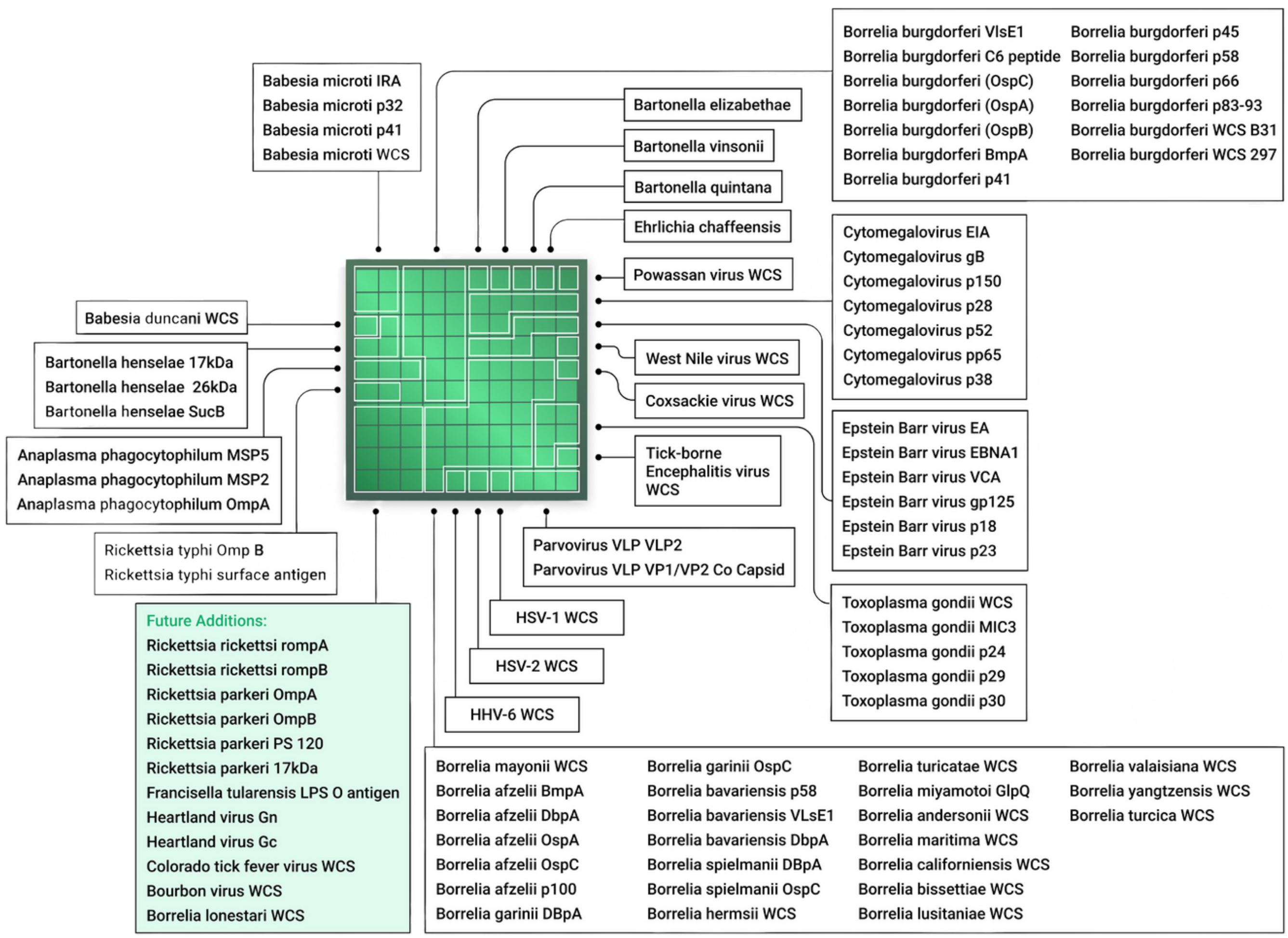
A customizable multiplex protein microarray for antibody testing and its application for tickborne and other infectious diseases

Research Square

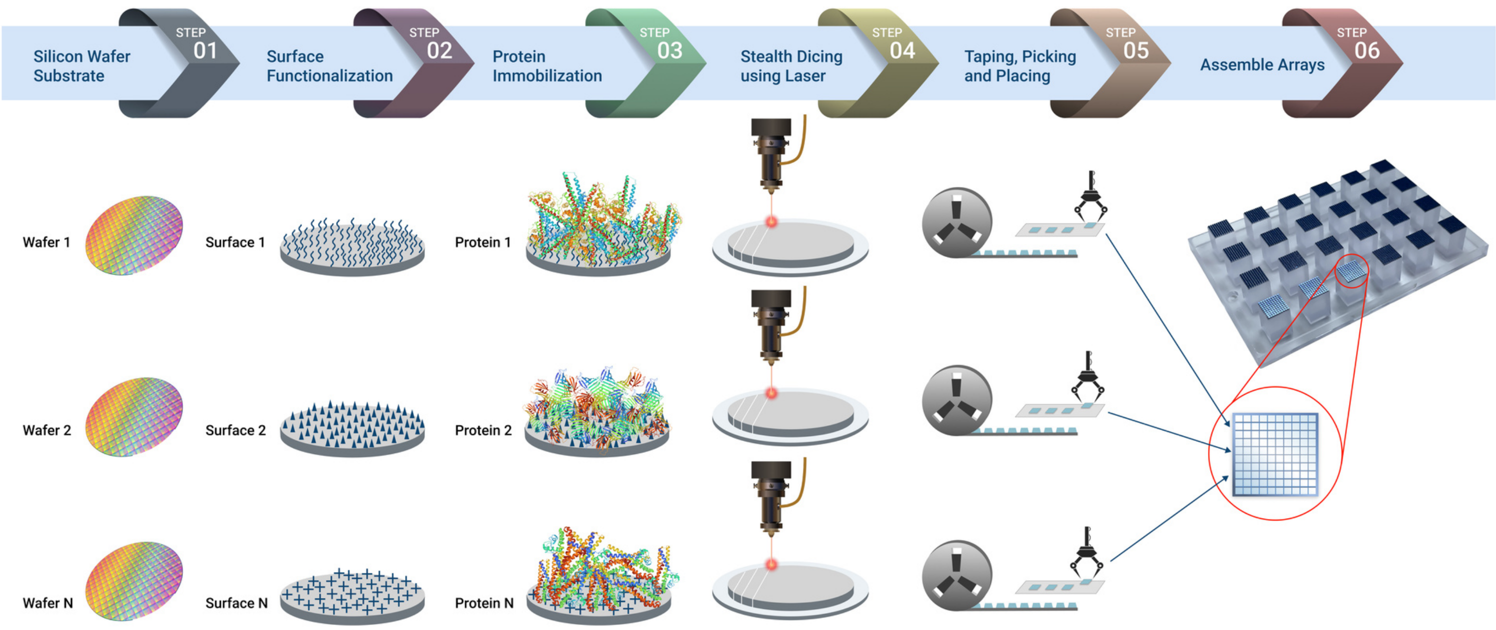
A tick can carry and pass on multiple infectious agents in a single bite. Testing needs to be for a comprehensive broad panel of all possible infections at the outset to avoid delayed diagnosis. Additionally, prolonged exposure to Lyme and its co-infections can compromise the immune system, rendering individuals more susceptible to infections like Epstein Barr virus and opportunistic pathogens which also needs to be tested in cases of chronic infection. The tickborne zoomer is a product to meet this requirement.

In this publication, we present the comprehensive evaluation of the Tickborne Zoomer with various associated infections involving more than 2,900 individuals. The study presents the key performance metrics of the Tickborne immunochip's analytical performance, including precision, sensitivity, reportable range, linearity, along with matrix equivalency studies, paving the way for a paradigm shift in tickborne disease diagnosis. Additionally, the report presents striking clinical sensitivity and specificity findings, particularly in the combined evaluation of IgM and IgG, showcasing a remarkable 100% accuracy for multiple infectious agents(Table on page 2). Image below is of chips on a single pillar containing one chip for one antigen. A patient's serum is tested against all the chips at the same time. Current antigens and future antigens are indicated.



Pathogen Antibodies								
Pathogen	IgM		IgG		IgM+IgG		Gold Standard [34-48]	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Borrelia burgdorferi	60	100	94	100	100	100	***Early:*** 30-40 ***Late:*** 70-100	95-100
Babesia microti	79	98	91	97	99	96	70-80	94-100
Bartonella henselae	81	99	96	99	100	98	100	96.8
Anaplasma phagocytophilum	94	100	100	100	100	100	80-100	95-100
Ehrlichia chaffeensis	84	100	100	99	100	99	83	100
Rickettsia typhi	86	100	83	100	97	100	80-100	91-100
Powassan virus	100	100	100	100	100	100	89	35-84
Tickborne encephalitis	78	99	92	99	93	98	94-100	>95
West Nile virus	25	100	80	100	85	100	80-95	94-100
Coxsackie virus	56	100	100	100	100	100	94-97	100
Cytomegalovirus	100	100	100	100	100	100	89.2	95
Epstein Barr virus VCA	100	100	96	100	97	100	80-95	>95
Epstein Barr virus EBNA1	95	100	96	100	97	100	80-95	>95
Parvovirus B19	90	99	98	98	99	97	>90	>90
Toxoplasma gondii	100	99	100	100	100	99	>90	>95
HSV-1	100	100	98	100	98	100	97-100	98
HSV-2	100	100	98	100	98	100	97-100	98
HHV-6	95	100	95	100	95	100	>95	>95

Individual wafers were cut into 0.70 x 0.70mm2 microchips for each antigen. These microchips were placed on carrier tapes using a die-sorting system and then loaded onto a high-throughput surface mount technology (SMT) component placement system. Ultimately, 24 pillar plates were used to mount these microchips, with each pillar containing 87 microchips designated for different antigens, including recombinant protein, peptide, or whole cell sonicates.



This research involved collaboration from over 25 renowned global researchers, including the CDC, NY Department of Health -Parasitology Laboratory/Wadsworth Center (NYSDOH), universities, and private clinics.

Sample Cohort

Pathogen	No.	Source (scientist/company)	Basis of Diagnosis
Borrelia burgdorferi	298	CDC, Private Clinics	RT-PCR, Physician
Babesia microti	70	Parasitology Laboratory, Wadsworth Center (NYSDOH)	RT-PCR, Blood Smear
Babesia microti	118	Seracare, Boca Bio, Private Clinics	Serology, RT-PCR
Babesia microti	26	Renata Welc-Falęciak (University of Warsaw), Agnieszka Pawełczyk (Warsaw Medical University)	Physician
Bartonella henselae	119	Private clinics	RT-PCR, Physician
Bartonella henselae	26	Renata Welc-Falęciak (University of Warsaw), Agnieszka Pawełczyk (Warsaw Medical University)	Physician
Bartonella henselae	10	Dimosthenis Chochlakis (University of Crete)	Physician
Anaplasma phagocytophilum	118	Private clinics, Boca Bio	RT-PCR, Serology
Anaplasma phagocytophilum	26	Renata Welc-Falęciak (University of Warsaw), Agnieszka Pawełczyk (Warsaw Medical University)	Physician
Ehrlichia chaffeensis	120	Private clinics, Boca Bio	RT-PCR, Serology
Ehrlichia chaffeensis	26	Renata Welc-Falęciak (University of Warsaw), Agnieszka Pawełczyk (Warsaw Medical University)	Physician
Rickettsia typhi	70	Lucas Blanton (University of Texas Medical Branch)	Physician
Rickettsia typhi	124	Private clinics	RT-PCR, Physician
Powassan virus	127	Private clinics	RT-PCR, Physician
Tick-borne encephalitis	111	Daniel Ružek (Czech Academy of Sciences)	Physician
Tick-borne encephalitis	124	Private clinics	RT-PCR, Physician
West Nile virus	20	Gheyath K. Nasrallah (Weill Cornell Medicine-Qatar)	Physician
West Nile virus	124	Private clinics	RT-PCR, Physician
Coxsackie virus	45	iSpecimen	Serology
Coxsackie virus	124	Private clinics	RT-PCR, Physician
Cytomegalovirus	43	DLS	Serology
Cytomegalovirus	138	Daniele Lilleri (Fondazione IRCCS Policlinico San Matteo)	Physician
Cytomegalovirus	96	Private Clinics	RT-PCR, Physician
Cytomegalovirus	37	Seracare	Serology
Epstein Barr virus	20	Gheyath K. Nasrallah (Weill Cornell Medicine-Qatar)	Physician
Epstein Barr virus	43	Seracare	Serology
Epstein Barr virus	14	iSpecimen	Serology
Epstein Barr virus	96	Private clinics	RT-PCR, Physician
Parvovirus B19	124	Private clinics	RT-PCR, Physician
Toxoplasma gondii	24	Seracare	Serology
Toxoplasma gondii	124	Private clinics	RT-PCR, Physician
HSV-1	20	Gheyath K. Nasrallah (Weill Cornell Medicine-Qatar)	Physician
HSV-1	31	Seracare	Serology
HSV-1	96	Private clinics	RT-PCR, Physician
HSV-2	19	Gheyath K. Nasrallah (Weill Cornell Medicine-Qatar)	Physician
HSV-2	27	Seracare	Serology
HSV-2	96	Private clinics	RT-PCR, Physician
HHV-6	20	Gheyath K. Nasrallah (Weill Cornell Medicine-Qatar)	Physician
HHV-6	96	Private clinics	RT-PCR, Physician

Pathogens & Antigens	
Pathogen (Tick, if any)	**Antigen**
B. burgdorferi (I. scapularis)	VlsE1, C6 peptide, DbpB, OspC, p28, p30, OspA, OspB, BmpA, p41, p45, p58, p66, p83-93, WCS B31, WCS 297.
B. mayonii (I. scapularis)	Whole cell sonicate
B. afzelii (I. ricinus, I. persulatus)	BmpA, DbpA, OspA, OspC, p100
B. garinii (I. ricinus, I. persulatus)	DBpA,OspC
B. bavariensis (I. uriae, I. persulcatus)	p58,VLsE1, DbpA
B. spielmanii (I. ricinus)	DBpA,OspC
B. hermsii (O. hermsi)	Whole cell sonicate
B. turicatae (O. turicatae)	Whole cell sonicate
B. miyamotoi (I. dentatus, I. ricinus, I. scapularis, I. pacificus)	GlpQ
B. andersonii (I. dentatus)	Whole cell sonicate
B. maritima (I. spinipalpis)	Whole cell sonicate
B. californiensis (I. jellisonii, I. spinipalpis, I. pacificus)	Whole cell sonicate
B. bissettiae (I.scapularis, I. persulatus, I. spinipalpis, I. pacificus)	Whole cell sonicate
B. lusitaniae (I. ricinus)	Whole cell sonicate
B. valaisiana (I. ricinus, I. nippopensis, I. columnae)	Whole cell sonicate
B. yangtzensis (I. granulatus, I. nipponensis)	Whole cell sonicate
B. turcica (H. aegypticum)	Whole cell sonicate
Babesia microti (I. ricinus, I. scapularis, blood transfusions, perinatal)	IRA, p32, p41, WCS

Pathogen (Tick, if any)	Antigen
Babesia duncani (I. ricinus, I. scapularis, blood transfusions, perinatal)	Whole cell sonicate
Bartonella henselae	17kDa, 26kDa, SucB
Bartonella elizabethae	Whole cell sonicate
Bartonella vinsonii	Whole cell sonicate
Bartonella quintana	Whole cell sonicate
Anaplasma phagocytophilum (I. scapularis, I. ricinus)	MSP5,MSP2,OmpA
Ehrlichia chaffeensis (Amblyomma americanum)	Whole cell sonicate
Rickettsia typhi (Flea Xenopsylla cheopis, Ctenocephalides felis))	Omp B, surface antigen
Powassan virus (Hemaphysalis longicornis, I. scapularis, I.cookei)	Whole cell sonicate
Tick-borne encephalitis virus (I. ricinus, I. persulcatus)	Whole cell sonicate
West Nile virus (I. ricinus, O. moubata)	Whole cell sonicate
Coxsackie virus (Amblyomma americanum)	Whole cell sonicate
Cytomegalovirus	EIA, gB, p150, p28, p52, pp65, p38
Epstein Barr virus	EA, EBNA1, VCA gp125, p18, p23
Parvovirus B19	VLP VLP2,VLP VP1/VP2 Co Capsid
Toxoplasma gondii (Multiple Ticks)	WCS, MIC3, p24, p29, p30
HSV-1	Whole cell sonicate
HSV-2	Whole cell sonicate
HHV-6	Whole cell sonicate

The Immunochip assay for antibody detection is a highly efficient and automated process. Serum samples are diluted at a 1:20 ratio and incubated on a pillar plate at room temperature for 1 hour. Subsequent steps involve washing and incubation with secondary antibodies (Goat Anti-Human IgG HRP and Goat Anti-Human IgM HRP) followed by chemiluminescent substrate addition and imaging. To enhance IgM sensitivity, proprietary assay components are used to strip IgG before IgM testing.

This multiplex antibody detection relies on chemiluminescent immunoassay and requires less than 200 μL of serum. Liquid handlers are programmed to handle sample dilution, multi-step incubation, and multi-solution washing, allowing for the analysis of 192 individual specimens in just 2 hours. Chemiluminescent signals from each probe are converted into intensity plots using specialized software. This automated antigen detection method significantly reduces turnaround time, labor costs, and the need for manual handling and subjective interpretation of results when compared to traditional two-tiered testing methods recommended by the CDC. Additionally, all antibodies are detected in a single run, streamlining the process further.