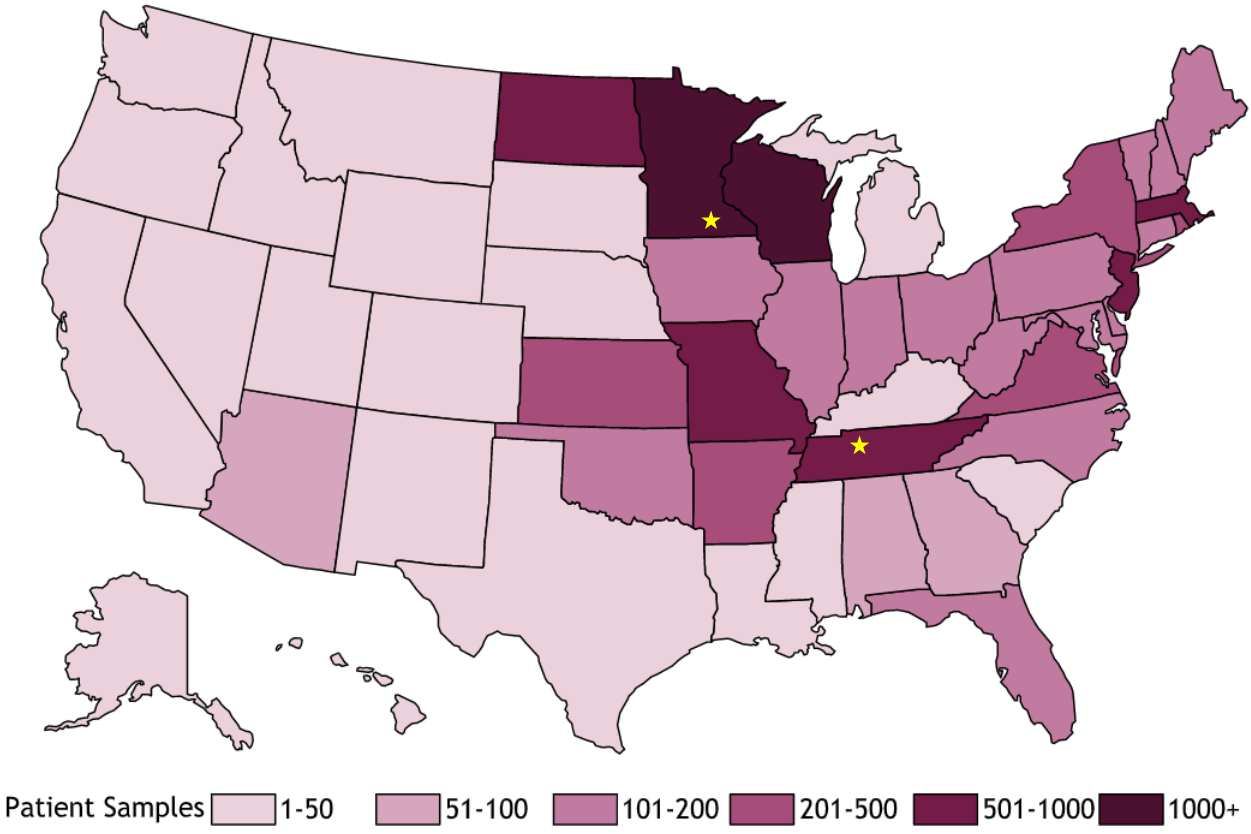
Supplemental Figure 1

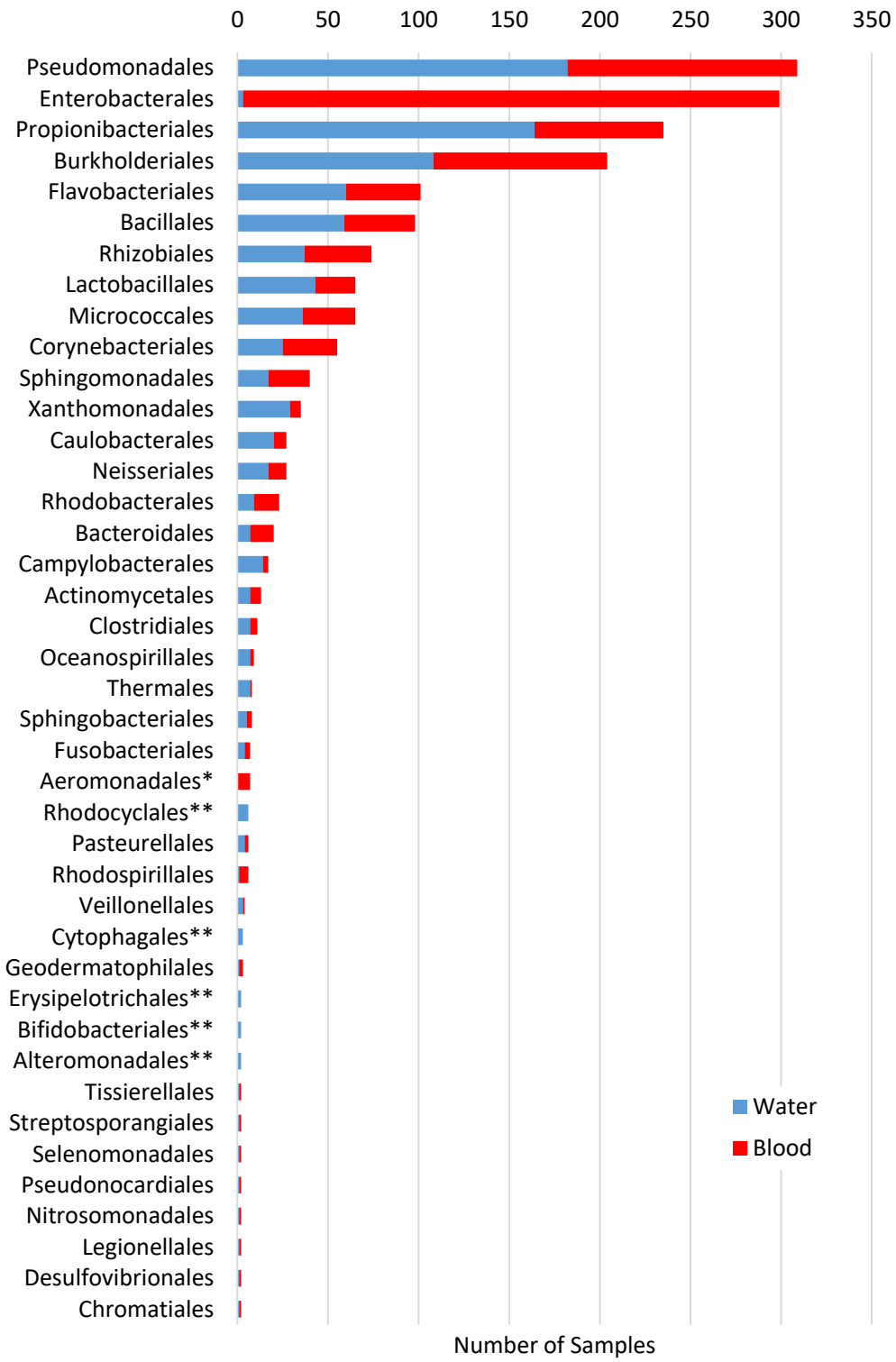


Supplemental Fig 1: Geographic distribution of the ordering provider for the 13,038 residual clinical specimens tested in this study. Differential shading indicates the number of patient specimens submitted from each state. The location of Mayo Clinic (Minnesota) and Vanderbilt University (Tennessee) are indicated by stars. Map generated with Google Charts: https://developers.google.com/chart/

Supplemental Fig 2

Supplemental Fig 2: V1-V2 partial alignment corresponding to 28 tickborne bacterial species and E. coli. Forward and reverse binding sites are boxed. The numbers above the boxes represent the nucleotide positions based on the B. burgdorferi B31 16S rRNA gene sequence (NC_001318.1). The asterisks indicate conserved nucleotides.

Supplemental Fig 3



Supplemental Fig 3: Full list of identified tax in control water and blood specimens. Blood specimens are shown in red, water specimens are shown in blue.

Supplemental Table 1: Sequencing information and statistics

Run Number	Concentration (pM)	PhiX Spike (%)	>Q30	Clusters Passing Filter	Cluster Density (K/mm2)	PhiX Aligned (%)	Total Reads	Reads Passing Filter	*E. coli* DNA Controls: Average % of total	*E. coli* Spiked in Blood Controls: Average % of Total	Low *Borrelia* Controls: Average % of Total
1	13	10	81.1	77.49 +/- 1.50	766 +/- 47	20.15 +/- 1.09	17800264	13784918	96.86	89.25	No Low Borrelia controls
2	13	10	80.4	82.26 +/- 1.45	835 +/- 71	12.24 +/- 1.13	18924080	15547541	97.51	86.01	76.27
3	13	10	83.0	44.60 +/- 42.3	847 +/- 58	7.50 +/- 7.12	19271578	7915899	98.52	98.33	76.02
4	13	10	83.1	44.11 +/- 41.91	798 +/- 167	6.97 +/- 6.61	9439254	7766794	94.54	92.29	75.65
5	13	10	70.8	43.73 +/- 41.71	801 +/- 175	7.08 +/- 6.73	9363256	7602913	97.32	93.09	77.12
6	12.5	10	79.3	79.94 +/- 2.03	983 +/- 87	8.14 +/- 0.38	21977116	17542822	96.50	98.24	76.26
7	12.5	10	79.3	82.05 +/- 2.4	1039 +/- 76	7.84 +/- 0.63	22548534	18467356	93.92	69.49	70.04
8	12.5	10	78.0	70.5 +/- 25.28	1041 +/- 94	6.68 +/- 2.39	20415080	16099766	94.72	91.17	77.03
9	12.5	10	72.7	82.95 +/- 1.03	1105 +/- 67	5.81 +/- 0.38	23313693	19327582	95.60	95.03	76.90
10	12.5	10	84.6	85.39 +/- 2.80	741 +/- 52	13.20 +/- 1.01	16953914	14453309	94.07	98.07	75.97
11	12.5	10	78.8	64.34 +/- 34.37	854 +/- 79	9.11 +/- 4.90	19749215	12143071	97.34	94.77	76.15
12	12.5	10	82.2	84.77 +/- 2.88	831 +/- 102	11.97 +/- 1.29	18312236	15432047	91.07	86.67	76.63
13	12.5	10	84.1	83.92 +/- 4.44	712 +/- 69	13.85 +/- 1.21	16371565	13668279	94.97	93.87	76.23
14	12.5	10	81.8	79.81 +/- 1.35	739 +/- 23	18.51 +/- 0.59	17082129	13634026	94.10	93.11	76.61
15	12.5	10	84.2	80.03 +/- 1.63	709 +/- 23	18.23 +/- 0.53	16389256	13121993	93.19	90.95	75.87
16	12.5	10	73.2	75.12 +/- 1.00	1186 +/- 48	2.28 +/- 0.14	24402085	18325995	95.26	93.69	77.65
17	12.5	10	79.0	83.37 +/- 1.27	1088 +/- 66	6.92 +/- 0.29	23202496	19333202	95.84	91.16	77.48
18	12.5	10	76.2	80.54 +/- 1.24	1092 +/- 63	7.03 +/- 0.21	23350132	18798341	95.93	97.83	76.44
19	12.5	10	80.1	79.98 +/- 3.23	811 +/- 72	11.52 +/- 0.76	18735551	14948127	97.11	97.49	76.64
20	12.5	10	81.5	82.99+/- 0.82	776 +/- 49	12.82 +/- 0.36	18112928	15031053	95.45	95.17	76.68
21	12.5	10	82.6	85.42 +/- 1.48	885 +/- 63	9.15 +/- 0.43	19276560	16447463	94.28	91.64	77.16
22	12.5	10	77.0	74.06 +/- 3.92	1008 +/- 75	8.10 +/- 0.37	22523040	16664736	96.71	96.09	66.84
23	12.5	10	82.3	76.06 +/- 15.98	741 +/- 59	15.93 +/- 3.53	16707118	13099067	95.54	94.43	75.52
24	12.5	10	85.7	82.64 +/- 3.26	756 +/- 28	25.42 +/- 1.8	16665204	13727928	95.62	96.81	76.99
25	12.5	10	84.4	79.38 +/- 3.43	696 +/- 18	14.7 +/- 1.13	15847596	12558303	98.02	93.55	75.12
26	12.5	10	81.2	80.38 +/- 3.60	787 +/- 59	14.18 +/- 1.19	18586619	14899088	97.06	95.25	75.60
27	12.5	10	85.9	86.09 +/- 1.34	755 +/- 58	13.66 +/- 0.66	17377864	14946444	97.65	94.75	76.99
28	12.5	10	81.1	82.61 +/- 3.85	889 +/-86	11.66 +/- 0.92	20277941	16678700	98.72	97.52	44.98
29	12.5	10	79.0	83.37 +/- 1.27	1088 +/- 66	6.92 +/- 0.29	23202496	19333202	88.05	87.42	77.83
30	12.5	10	82.3	76.06 +/- 15.98	741 +/- 59	15.93 +/- 3.53	16707118	13099067	96.74	94.59	75.76
31	12.5	10	84.0	83.95 +/- 2.34	738 +/- 57	13.35 +/- 1.21	16829236	14087992	95.84	92.70	75.57
32	12.5	10	84.6	84.69 +/- 1.13	723 +/- 44	14.05 +/- 1.02	16318234	13806920	97.17	94.45	74.99
33	12.5	10	85.3	84.3 +/- 1.17	673 +/- 44	14.08 +/- 0.81	15688812	13213318	98.37	96.90	75.92
34	12.5	10	83.2	83.46 +/- 2.20	755 +/-47	15.78 +/- 1.37	17303490	14405907	97.93	94.45	76.20
35	12.5	10	84.9	83.46 +/- 1.97	707 +/- 43	16.42 +/- 0.09	16293986	13567605	98.15	97.15	75.34
36	12.5	10	83.6	80.98 +/- 1.04	670 +/- 22	17.29 +/- 0.62	15573848	12614597	98.03	97.76	72.24
37	12.5	10	83.2	81.46 +/- 0.95	696 +-/ 31	16.89 +/- 0.55	16303711	13278946	98.08	97.09	75.72
38	12.5	10	87.3	86.72 +/- 1.18	589 +/- 47	15.85 +/- 0.73	13675639	11845317	98.34	96.78	69.59
39	12.5	10	85.3	84.24 +/- 1.62	677 +/- 40	16.05 +/- 0.91	15712387	13214268	98.52	96.08	60.56
40	12.5	10	82.0	78.14 +/- 15.43	731 +/- 59	14.84 +/- 3.05	16422703	13276807	96.72	95.81	75.35
41	12.5	10	79.4	77.55 +/- 1.97	763 +/- 42	17.05 +/- 0.50	17932906	13912961	98.29	97.42	77.02
42	12.5	10	86.8	82.06 +/- 1.73	609 +/- 18	19.48 +/- 0.53	14112136	11584263	97.10	97.26	75.57
43	12.5	10	84.5	82.89 +/- 0.80	667 +/- 32	16.05 +/- 0.33	15778874	13078902	96.27	96.29	75.18
44	12.5	10	83.7	83.04 +/- 0.92	693 +/- 45	16.14 +/- 0.75	16277052	13506896	98.14	95.75	75.30
45	12.5	10	82.5	83.07 +/- 1.94	780 +/- 63	14.21 +/- 0.89	18004388	14925086	96.53	93.65	77.15
46	12.5	10	83.8	84.00 +/- 0.92	757 +/- 33	11.79 +/- 0.73	16337282	13716049	96.76	93.55	45.87

Supplemental Table 2: Full list of identified taxa in clinical specimens after background subtraction.

Order	Relative Percentage After Subtraction
Rickettsiales	54.63%
Spirochaetales	9.99%
Deinococcales	6.15%
Chitinophagales	3.33%
Thiotrichales	2.89%
Synechococcales	1.95%
Verrucomicrobiales	1.88%
Sphaerobacterales	1.59%
Leptospirales	1.37%
Acholeplasmatales	1.16%
Glycomycetales	1.16%
Cellvibrionales	1.09%
Syntrophobacterales	1.09%
Micromonosporales	1.01%
Bryobacterales	0.80%
Solirubrobacterales	0.80%
Nakamurellales	0.72%
Pelagibacterales	0.65%
Myxococcales	0.58%
Desulfuromonadales	0.51%
Deferribacterales	0.43%
Fimbriimonadales	0.43%
Mycoplasmatales	0.36%
Nitrospirales	0.36%
Planctomycetales	0.36%
Vibrionales	0.36%
Gemmatimonadales	0.29%
Thermotogales	0.29%
Acidiferrobacterales	0.22%
Bdellovibrionales	0.22%
Chroococcidiopsidales	0.22%
Coriobacteriales	0.22%
Eggerthellales	0.22%
Holosporales	0.22%
Nevskiales	0.22%
Synergistales	0.22%
Acidimicrobiales	0.14%
Anaerolineales	0.14%
Aquificales	0.14%
Chroococcales	0.14%
Dehalococcoidales	0.14%
Frankiales	0.14%
Kineosporiales	0.14%
Nostocales	0.14%
Saprospirales	0.14%

Supplemental Table 3: Assays used for secondary verification of taxonomic prediction/identifications

Assay	Primer/Probe	Primer/Probe Sequence (5'-> 3')	Final Concentration (µM)	Annealing Temp (°C)	Reference
pan-*Borrelia* Taqman PCR	Forward Primer	AGC YTT TAA AGC TTC GCT TGT AG	0.6	60	Kingry *et al*. 2018
	Reverse Primer	GCC TCC CGT AGG AGT CTG G	0.6		
	Probe	/6FAM/-CCG GCC TGA /ZEN/ GAG GGT GAW CGG-/3IABkFQ/	0.2		
***Anaplasma/ Ehrlichia* real-time PCR**	Forward Primer (Esp-F)	TAC TCA GAG TGC TTC TCA ATG T	0.5	55	Johnson *et al*. 2015
	Reverse Primer (Esp-R)	GCA TAC CAT CAG TTT TTT CAA C	0.5		
	Ehrlichia Probe (948-EHR)	ATT TCA GCT AAT GGA GAT AAG AAT ATA-/3FlourT/	0.2		
	Anaplasma Probe (949-AP)	CAT TGT CTG CGA ATG GAG ACA AGA ACA TAG GA-/3FlourT/	0.2		
	LC640 Probe	/LC640/-GTA AGA TTG CAC AGT GTG TTC AAG AAG TCG GTA-/3Phos/	0.4		
16S V1-V4 Sequencing	Forward Primer 1*	TAA /i5NitInd/AC ATG CAA GTC RAR CG	0.2-1.6	55	Modified based on Marchesi *et al*. 1998
	Forward Primer 2#	TAA CAC ATG CAA GTC GAA CG	0.4		
	Reverse Primer (926R)	CCG TCA ATT CCT TTG AGT TT	0.2-0.4		Schwieger *et al*. 1998
pan-*Rickettsia* Taqman PCR	Forward Primer (PANR8_F)	AGC TTG CTT TTG GAT CAT TTG G	1	60	Kato *et al*. 2013
	Reverse Primer (PANR8_R)	TTC CTT GCC TTT TCA TAC ATC TAG T	1		
	Probe (PANR8_P)	/5FluorT/-CCT GCT TCT ATT TGT CTT GCA GTA ACA CGC CA-/3BHQ1/	0.04		
***Francisella* Taqman PCR**	Forward Primer	GAG ACA TCA ATT AAA AGA AGC AAT ACC TT	0.75	65	Kugeler *et al*. 2006
	Reverse Primer	CCA AGA GTA CTA TTT CCG GTT GGT	0.75		
	Probe	/6FAM/-AAA ATT CTG C/BHQ1/C AGC AGG ATT TTG ATT TGG T	0.2		

*Forward Primer 1 was used for non-Rickettsiales

Forward Primer 2 was used for Rickettsiales

Supplemental Table 4: Genetic pairwise distance table of an 837 bp fragment of 16S rDNA.

Organism	**GenBank Number**		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	9
Blood sample- OK (15-3642)	MG429812	**1**									
Blood sample- MN (15-6842)		**2**	0.000								
Uncultured *Anaplasma* sp.	JN862824.1	**3**	0.013	0.013							
A. bovis S_T	U03775.1	**4**	0.017	0.017	0.013						
A. platys	AF156784.1	**5**	0.033	0.033	0.032	0.026					
A. phagocytophilum	NR_044762.1	**6**	0.041	0.041	0.035	0.033	0.019				
A. ovis	AF309865.1	**7**	0.055	0.055	0.055	0.046	0.037	0.040			
A. marginale	NC_012026.1	**8**	0.055	0.055	0.055	0.046	0.037	0.040	0.000		
A. centrale	NC_013532.1	**9**	0.054	0.054	0.051	0.045	0.040	0.038	0.005	0.005	
R. rickettsii	L36217.1	**10**	0.191	0.191	0.201	0.201	0.193	0.185	0.186	0.186	0.188

Supplemental Table 5: Breakdown of number of blood specimens by Lot Number with a given taxonomic prediction.

Lot #	1	2	3	4	5	6	Total
n	93	90	97	45	53	54	432
Enterobacterales	64	65	68	29	33	37	296
Pseudomonadales	31	20	26	6	22	22	127
Burkholderiales	20	24	27	9	8	8	96
Propionibacteriales	12	10	15	0	20	14	71
Flavobacteriales	9	13	13	0	4	2	41
Bacillales	7	8	10	5	3	6	39
Rhizobiales	10	12	8	0	3	4	37
Corynebacteriales	7	8	8	0	4	3	30
Micrococcales	6	8	10	2	2	1	29
Sphingomonadales	5	6	5	1	5	1	23
Lactobacillales	7	0	4	2	6	3	22
Rhodobacterales	1	3	6	0	1	3	14
Bacteroidales	0	6	2	2	2	1	13
Neisseriales	2	0	3	1	2	2	10
Aeromonadales	2	3	2	0	0	0	7
Caulobacterales	0	2	3	1	0	1	7
Actinomycetales	0	1	2	0	1	2	6
Xanthomonadales	0	1	3	2	0	0	6
Rhodospirillales	2	1	1	0	1	0	5
Clostridiales	0	2	2	0	0	0	4
Campylobacterales	0	0	0	0	3	0	3
Fusobacteriales	0	1	1	0	1	0	3
Sphingobacteriales	0	1	1	1	0	0	3
Geodermatophilales	0	0	0	0	1	1	2
Oceanospirillales	0	0	1	0	0	1	2
Pasteurellales	0	0	1	0	0	1	2
Acidobacteriales	0	0	0	0	1	0	1
Bdellovibrionales	0	0	0	0	1	0	1
Chromatiales	0	0	1	0	0	0	1
Deferribacterales	0	0	1	0	0	0	1
Desulfovibrionales	0	0	1	0	0	0	1
Legionellales	0	0	1	0	0	0	1
Nitrosomonadales	0	1	0	0	0	0	1
Nostocales	0	0	0	0	1	0	1
Oscillatoriales	0	0	0	0	0	1	1
Pseudonocardiales	0	0	1	0	0	0	1
Rubrobacterales	0	0	1	0	0	0	1
Selenomonadales	0	0	0	0	1	0	1
Streptomycetales	0	0	1	0	0	0	1
Streptosporangiales	0	1	0	0	0	0	1
Thermales	0	0	1	0	0	0	1
Thermotogales	0	0	1	0	0	0	1
Thiotrichales	0	0	1	0	0	0	1
Tissierellales	0	1	0	0	0	0	1
Veillonellales	0	0	0	0	1	0	1

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