

Supplementary data

GroEL Chaperonin-Based Assay for Early Diagnosis of Scrub Typhus

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M31887.1	TEQLGIKLEKVNDTSKLGATANRVIVTKDHTTIVHDKNNSDIEKKVNSRCEQIREAIKDTT
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BAG39717.1	ALFYASRVLD SLKFDNEDQRVGINI IKKVLEAPVRQIVKNAGGKEDVVVNELSKSNDKNR
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M31887.1	GFDARTMQYVDMIKAGIVDPTKVVRTALQDAFSVASLVIATSAMITDHEEDNNTGNRSGG
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EF551295.1	-----
EF551296.1	-----
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BAG39717.1	GVGGGHHGGMGGMDF
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GVGGGHHGGMGGMDF

Consensus sequence of the GroEL chaperonin of *O. tsutsugamushi*

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GFDARTMQYVDMIKAGIVDPTKVVRTALQDAFSVASLVIATSAMITDHEEDNNTGNRSGGGVGGGHHGGMGGMDF

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Figure S1. CLUSTAL Omega (1.2.0) multiple sequence alignment of GroEL chaperonin of *O. tsutsugamushi* and the consensus sequence.

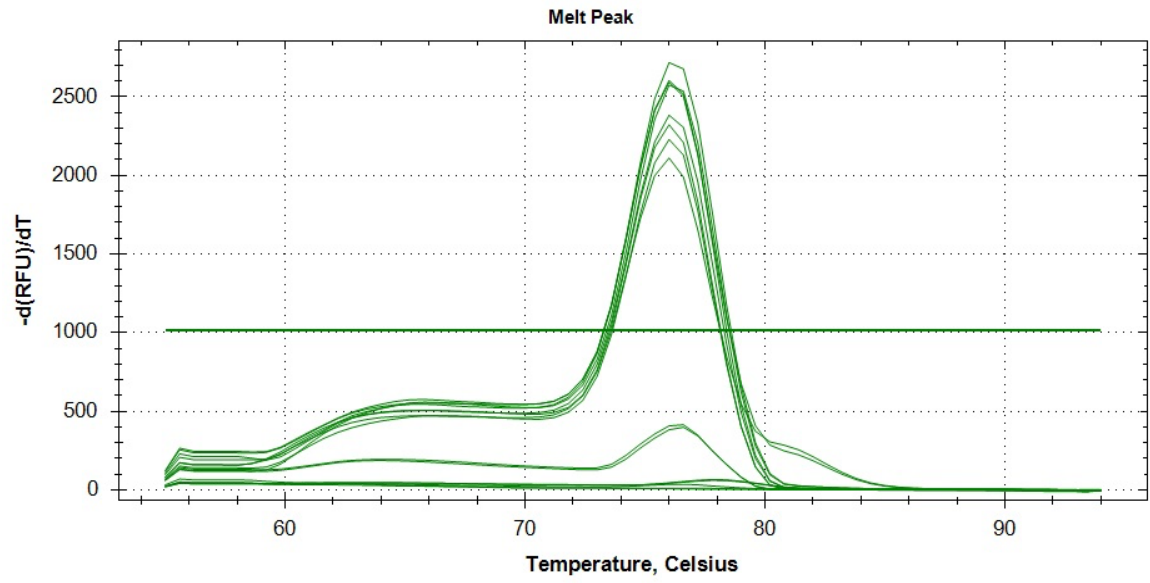


Figure S2. Melting curve of real-time PCR for detecting gene coding for *O. tsutsugamushi* GroEL. Melting curve analysis was performed with increments of 1 °C/step (72-95 °C) to determine the change in peak fluorescence over time (dF/dT).

Table S1. Performance of the ICT AgTK on the 40 frozen serum samples compared with the original diagnoses based on clinical features and laboratory tests including *Leptospira* IFA for leptospirosis and *O. tsutsugamushi* IFA for scrub typhus (Department of Medical Sciences, Ministry of Public Health, Thailand) and Dengue NS1 antigen and Dengue IgG/IgM ELISA for Dengue fever (SD Biosensor, Korea). ST = scrub typhus diagnosis; DF = Dengue fever diagnosis; LT = leptospirosis diagnosis.

No.	Code	Original diagnosis	<i>Leptospira</i> IFA		Dengue NS1 antigen	Dengue IgG/IgM ELISA		<i>O. tsutsugamushi</i> IFA		ICT AgTK
			IgM	IgG	NS1	IgM	IgG	IgM	IgG	GroEL
1	11-1-003	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:3,200	1:100	Negative
2	11-1-004	ST	<1.50	<1.50	Negative	<1.50	<1.50	≤1:6400	≤1:6400	Positive
3	11-1-014	ST	<1.50	<1.50	Negative	<1.50	<1.50	≤1:6400	1:1600	Positive
4	11-1-035	ST	<1.50	<1.50	Negative	<1.50	<1.50	≤1:6400	1:800	Positive
5	11-1-118	ST	<1.50	<1.50	Negative	<1.50	<1.50	≤1:6400	≤1:6400	Positive
6	11-1-124	ST	<1.50	<1.50	Negative	<1.50	<1.50	≤1:6400	≤1:6400	Positive
7	11-1-154	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:1600	≤1:6400	Positive
8	11-1-171	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:800	1:3200	Positive
9	11-1-174	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:800	1:1600	Positive
10	11-1-179	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:3200	1:3200	Positive
11	11-1-192	ST	<1.50	<1.50	Negative	<1.50	<1.50	≤1:6400	1:800	Negative
12	11-1-227	ST	<1.50	<1.50	Negative	<1.50	<1.50	≤1:6400	1:3200	Positive
13	11-1-247	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:200	1:3200	positive
14	11-1-248	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:400	≤1:6400	Positive
15	11-1-259	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:800	1:800	Positive
16	11-1-286	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:400	1:3200	Positive
17	11-1-295	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:100	1:800	Positive
18	11-1-334	ST	<1.50	<1.50	Negative	<1.50	<1.50	<1.50	1:1600	Negative
19	11-1-353	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:1600	1:1600	Positive
20	11-1-379	ST	<1.50	<1.50	Negative	<1.50	<1.50	1:400	1:1600	Positive
21	4-0079.2	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
22	4-0623	DF	<1.50	<1.50	positive	<1.50	<1.50	<1.50	<1.50	Negative
23	4-0640	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
24	4-0651	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
25	4-0660	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
26	4-0661	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
27	4-0682	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
28	4-0700	DF	<1.50	<1.50	positive	<1.50	<1.50	<1.50	<1.50	Negative
29	4-0706	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
30	4-0718	DF	<1.50	<1.50	Positive	<1.50	<1.50	<1.50	<1.50	Negative
31	11-1-116	LT	1:800	1:1600	Negative	<1.50	<1.50	<1.50	<1.50	Negative
32	11-1-144	LT	≤1:6400	1:100	Negative	<1.50	<1.50	<1.50	<1.50	Negative
33	11-1-183	LT	1:200	1:200	Negative	<1.50	<1.50	<1.50	<1.50	Negative
34	11-1-187	LT	1:400	<1.50	Negative	<1.50	<1.50	<1.50	<1.50	Negative

No.	Code	Original diagnosis	<i>Leptospira</i> IFA		Dengue NS1 antigen	Dengue IgM/IgG ELISA		<i>O. tsutsugamushi</i> IFA		ICT AgTK
			IgM	IgG	NS1	IgM	IgG	IgM	IgG	GroEL
35	11-1-253	LT	1:400	1:50	Negative	<1:50	<1:50	<1:50	<1:50	Negative
36	11-1-272	LT	1:400	1:100	Negative	<1:50	<1:50	<1:50	<1:50	Negative
37	3607	LT	1:100	1:400	Negative	<1:50	<1:50	<1:50	<1:50	Negative
38	3261	LT	1:800	1:3200	Negative	<1:50	<1:50	<1:50	<1:50	Negative
39	2692	LT	1:100	1:400	Negative	<1:50	<1:50	<1:50	<1:50	Negative
40	2454	LT	1:400	1:100	Negative	<1:50	<1:50	<1:50	<1:50	Negative

Table S2. Results of the GRoEL detection by using the ICT AgTK and real-time PCR performed blindly in our laboratory in comparison to the laboratory diagnoses performed at laboratory of Nan Hospital using test kits from SD Biosensor, Korea.



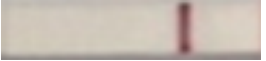

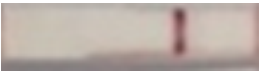
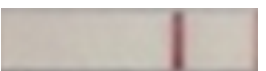




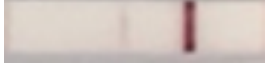




Code no.	Age (years)	Sex	Clinical and lab diagnosis at Nan hospital	Scrub typhus real-time PCR using either plasma or buffy coat samples	Scrub typhus ICT AgTK using either plasma or buffy coat samples	Results of ICT AgTK using plasma	
						T	C
1	47	F	Scrub typhus	+	+		
2	35	F	Dengue fever	-	-		
3	35	M	Leptospirosis	-	-		
4	52	M	Scrub typhus	+	+		
5	72	M	Leptospirosis	-	-		
6	18	M	Dengue fever	-	-		
7	59	F	Dengue fever	-	-		
8	52	F	Scrub typhus	+	+		
9	39	M	Scrub typhus	+	+		
10	28	M	Scrub typhus	+	+		
11	42	F	Scrub typhus	+	+		
12	55	F	Scrub typhus	+	+		
13	21	F	Scrub typhus	+	+		
14	38	M	Scrub typhus	+	+		
15	50	F	Scrub typhus	+	+		

Table S3. Performance of the ICT AgTK evaluated independently at Prasat Hospital, Surin Province, by the hospital's medical technologist in comparison with the results of commercialized test kit (SD Biosensor, Korea) for detection of antibody (IgM/IgG) to *O. tsutsugamushi*.

No.	IgM/IgG to <i>tsutsugamushi</i>	O.	ICT AgTK	ICT AgTK strip	
				T	C
1	IgM Positive IgG Positive (weakly)		Positive		
2	IgM Negative IgG Negative		Negative		
3	IgM Negative IgG Negative		Negative		
4	IgM Negative IgG Negative		Negative		
5	IgM Positive IgG Negative		Positive		
6	IgM Positive IgG Negative		Weakly positive		
7	IgM Negative IgG Positive		Positive		
8	IgM Positive IgG Negative		Positive		
9	IgM Negative IgG Negative		Negative		
10	IgM Positive (weakly) IgG Positive (weakly)		Positive		
11	IgM Negative IgG Negative		Negative		
12	IgM Negative IgG Negative		Negative		
13	IgM Negative IgG Negative		Negative		
14	IgM Negative IgG Negative		Negative		
15	IgM Negative IgG Negative		Negative		
16	IgM Negative IgG Negative		Negative		
17	IgM Negative IgG Negative		Negative		
18	IgM Negative IgG Negative		Negative		
19	IgM Negative IgG Negative		Negative		
20	IgM Negative IgG Negative		Negative		