

**Table S1.** Number of days to first visible colonies for the 264 different conditions/samples of *Leptospira interrogans* strain NR-20157

Type of agar <sup>a</sup>	Concentration of <i>Leptospira</i> inoculum (CFU/mL)	Number of days incubated in carbon dioxide prior to further incubation in air <sup>b</sup>										
		0	1% CO <sub>2</sub>					5% CO <sub>2</sub>				
			1	2	3	4	5	1	2	3	4	5
BA with 3% RS	10 <sup>2</sup>	>28	24	20	20	24	>28	>28	21	22	25	>28
BA with 5% RS	10 <sup>2</sup>	24	18	20	20	24	25	20	21	20	24	>28
BA with 10% RS	10 <sup>2</sup>	20	16	19	15	19	20	20	19	17	20	21
NA with 3% RS	10 <sup>2</sup>	17	13	14	12	13	19	13	14	13	16	19
NA with 5% RS	10 <sup>2</sup>	>28	20	20	20	20	20	20	20	>28	20	24
NA with 10% RS	10 <sup>2</sup>	>28	18	18	20	20	20	20	20	20	20	22
BA with 3% RS	10 <sup>3</sup>	22	15	14	14	13	16	13	25	>28	19	20
BA with 5% RS	10 <sup>3</sup>	21	12	12	12	12	12	12	13	15	14	17
BA with 10% RS	10 <sup>3</sup>	>28	>28	>28	>28	>28	14	22	20	22	>28	20
NA with 3% RS	10 <sup>3</sup>	>28	>28	>28	>28	>28	13	14	18	15	21	20
NA with 5% RS	10 <sup>3</sup>	22	>28	>28	>28	12	12	13	19	12	22	17
NA with 10% RS	10 <sup>3</sup>	22	>28	17	>28	10	10	12	13	12	15	13
BA with 3% RS	10 <sup>4</sup>	>28	>28	20	>28	>28	22	25	20	24	21	25
BA with 5% RS	10 <sup>4</sup>	20	>28	18	>28	>28	22	18	18	20	21	20
BA with 10% RS	10 <sup>4</sup>	19	>28	13	15	>28	19	15	15	20	19	16
NA with 3% RS	10 <sup>4</sup>	17	12	12	12	>28	16	13	13	15	16	13
NA with 5% RS	10 <sup>4</sup>	20	15	14	14	20	21	18	20	18	20	20
NA with 10% RS	10 <sup>4</sup>	20	14	13	14	20	18	15	18	17	20	20
BA with 3% RS	10 <sup>5</sup>	16	13	12	13	13	13	13	14	13	19	17
BA with 5% RS	10 <sup>5</sup>	13	12	10	12	12	10	12	12	12	13	13
BA with 10% RS	10 <sup>5</sup>	18	14	14	14	18	14	13	13	13	13	16
NA with 3% RS	10 <sup>5</sup>	18	13	13	13	16	13	12	12	12	12	16
NA with 5% RS	10 <sup>5</sup>	13	12	12	12	12	12	11	11	11	12	13
NA with 10% RS	10 <sup>5</sup>	12	10	10	11	11	11	10	10	10	10	11

<sup>a</sup> BA = Bacteriological agar base, NA= Noble agar base, RS = rabbit serum

<sup>b</sup> Initial incubation in CO<sub>2</sub> for 1 to 5 days was followed by incubation in air to a total of 28 days

\* Ten conditions yield lowest number of 10 days to first visible colonies (highlighted in yellow).

**Table S2. MIC of for 109 pathogenic *Leptospira* isolates as determined by the Etest**

<i>Species</i>	<i>Serovar</i>	<i>Country</i>	<i>Source</i>	<i>ST</i> <sup>a</sup>	<i>Strain</i> <sup>b</sup>	<i>PG</i> <sup>c</sup>	<i>Do</i> <sup>c</sup>	<i>CT</i> <sup>c</sup>	<i>TX</i> <sup>c</sup>	<i>C</i> <sup>c</sup>
<i>L. interrogans</i>	Autumnalis	Japan	Human	27	Akiyami A	0.002	0.047	0.002	0.047	0.75
	Autumnalis	Lao PDR	Human	34	UI13016	0.032	0.064	0.012	0.094	0.5
	Autumnalis	Lao PDR	Human	34	UI12268	0.047	0.047	0.023	0.094	1.5
	Autumnalis	Lao PDR	Human	34	UI15218	0.032	0.125	0.023	0.125	1.5
	Autumnalis	Lao PDR	Human	34	U 018597	0.012	0.032	0.004	0.064	0.125
	Autumnalis	Lao PDR	Human	34	UI12830	0.016	0.064	0.023	0.125	1.5
	Autumnalis	Thailand	Human	22	NR-20161*	0.023	0.064	0.008	0.125	1
	Autumnalis	Thailand	Human	34	UT285	0.016	0.094	0.012	0.125	1
	Autumnalis	Thailand	Human	34	L0910	0.016	0.064	0.023	0.25	0.5
	Autumnalis	Thailand	Human	34	L0474	0.016	0.19	0.016	0.19	1.5
	Autumnalis	Thailand	Human	34	L0468	0.047	0.064	0.016	0.38	1
	Autumnalis	Thailand	Human	34	L1000	0.032	0.064	0.012	0.19	1.5
	Autumnalis	Thailand	Human	34	L1118	0.016	0.094	0.016	0.125	0.38
	Autumnalis	Thailand	Human	34	L1160	0.032	0.047	0.002	0.064	1
	Autumnalis	Thailand	Human	34	RY21	0.016	0.125	0.016	0.25	2
	Autumnalis	Thailand	Human	34	L0020	0.016	0.032	0.008	0.064	0.5
	Autumnalis	Thailand	Human	34	L0036	0.023	0.047	0.012	0.19	0.75
	Autumnalis	Thailand	Human	34	L0521	0.032	0.064	0.032	0.19	1
	Autumnalis	Thailand	Human	34	L0528	0.032	0.064	0.012	0.19	0.75
	Autumnalis	Thailand	Human	34	L0562	0.012	0.047	0.012	0.094	0.5
	Autumnalis	Thailand	Human	34	UT667	0.012	0.064	0.006	0.094	0.5
	Autumnalis	Thailand	Human	34	UT571	0.012	0.032	0.006	0.064	0.38
	Autumnalis	Thailand	Human	34	UT226	0.008	0.047	0.016	0.064	1.5
	Autumnalis	Thailand	Human	34	L1104	0.008	0.032	0.008	0.125	0.5
	Autumnalis	Thailand	Human	34	L0752	0.047	0.125	0.064	0.5	1.5
	Autumnalis	Thailand	Human	34	UT294	0.012	0.064	0.023	0.125	0.75
	Autumnalis	Thailand	Human	34	UT105	0.023	0.064	0.016	0.125	1
	Autumnalis	Thailand	Human	34	UT227	0.023	0.023	0.002	0.047	0.25
	Autumnalis	Thailand	Human	34	UT560	0.023	0.125	0.016	0.094	1
	Autumnalis	Thailand	Human	34	UT567	0.023	0.023	0.006	0.006	0.75
	Autumnalis	Thailand	Human	34	L0088	0.008	0.047	0.003	0.016	0.75
	Autumnalis	Thailand	Human	34	L0382	0.023	0.064	0.006	0.032	0.5
	Autumnalis	Thailand	Human	34	L0388	0.002	0.023	0.016	0.094	0.125
	Autumnalis	Thailand	Human	34	L0615	0.75	0.064	0.047	0.5	1.5
	Autumnalis	Thailand	Human	34	L0810	0.002	0.032	0.002	0.094	0.38
	Autumnalis	Thailand	Human	34	L0984	0.032	0.094	0.006	0.094	1.5
	Autumnalis	Thailand	Human	34	L1229	0.047	0.094	0.002	0.064	1
	Autumnalis	Thailand	Human	34	L1059	0.023	0.047	0.012	0.19	0.75
	Autumnalis	Thailand	Human	34	L1064	0.008	0.064	0.016	0.125	1
	Autumnalis	Thailand	Human	34	L1254	0.016	0.064	0.016	0.19	0.75

<i>Species</i>	<i>Serovar</i>	<i>Country</i>	<i>Source</i>	<i>ST</i>	<i>Strain</i>	<i>PG</i>	<i>Do</i>	<i>CT</i>	<i>TX</i>	<i>C</i>
<i>L. interrogans</i>	Autumnalis	Thailand	Human	34	LP085	0.047	0.064	0.016	0.094	2
	Autumnalis	Thailand	Human	34	YT12	0.094	0.19	0.012	0.064	1.5
	Autumnalis	Thailand	Human	34	UT108	0.032	0.19	0.094	0.38	3
	Autumnalis	Thailand	Human	34	UT670	0.016	0.125	0.023	0.19	2
	Autumnalis	Thailand	Human	34	L0013	0.023	0.064	0.012	0.125	1.5
	Autumnalis	Thailand	Human	34	L0607	0.032	0.064	0.023	0.125	1
	Autumnalis	Thailand	Human	34	L1190	0.002	0.125	0.002	0.006	1
	Autumnalis	Thailand	Human	34	L0015	0.004	0.047	0.002	0.032	0.094
	Bataviae	Lao PDR	Human	79	NR-20168*	0.047	0.125	0.032	0.75	1
	Bataviae	Thailand	Human	42	NR-20159*	0.047	0.094	0.032	0.38	1
	Bataviae	Thailand	Human	59	UT075	0.094	0.125	0.023	0.5	2
	Bataviae	Thailand	Human	46	NR-20325*	0.047	0.094	0.016	0.094	0.5
	Bataviae	Thailand	Human	46	UT229	0.064	0.125	0.012	0.25	1.5
	Canicola	Lao PDR	Human	37	NR-20170*	0.047	0.25	0.047	0.38	0.75
	Canicola	Lao PDR	Human	37	UI9661	0.047	0.125	0.032	0.25	1
	Canicola	Lao PDR	Human	37	UI12823	0.125	0.19	0.064	0.5	1.5
	Grippotyphosa	Lao PDR	Human	82	NR-20166	0.047	0.25	0.032	0.38	0.75
	Grippotyphosa	Lao PDR	Human	85	NR-20174*	0.002	0.25	0.032	2	2
	Grippotyphosa	Lao PDR	Human	77	NR-20165*	0.047	0.125	0.023	0.25	2
	Grippotyphosa	Lao PDR	Human	86	NR-20175*	0.047	0.19	0.064	0.25	0.75
	Hardjo	Indonesia	Human	20	Hardjoprajitno	0.003	0.094	0.016	0.75	2
	Hebdomadis	Sri Lanka	Human	80	NR-20164*	0.023	0.047	0.023	0.25	0.5
	Icterohaemorrhagiae	Belgium	Human	NA	RGA	0.012	0.047	0.004	0.064	0.25
	Lai	China	Human	1	Lai	0.064	0.064	0.125	1.5	1.5
	Medanensis	Thailand	Human	46	NR-20178*	0.032	0.125	0.032	0.25	0.75
	Medanensis	Thailand	Human	46	NR-20184*	0.047	0.19	0.094	0.5	2
	Medanensis	Thailand	Human	46	NR-20326*	0.023	0.125	0.012	0.25	1
	Medanensis	Thailand	Human	46	L0941	0.006	0.094	0.003	0.012	0.25
	Pomona	Thailand	Human	38	NR-20180*	0.008	0.064	0.016	0.064	0.75
	Pyrogenes	Lao PDR	Human	37	UI8414	0.094	0.094	0.032	0.094	0.38
	Pyrogenes	Lao PDR	Human	37	UI12344	0.094	0.19	0.047	0.38	1
	Pyrogenes	Sri Lanka	Human	49	NR-20163*	0.032	0.125	0.003	0.19	1.5
	Pyrogenes	Sri Lanka	Human	74	R163	1	0.38	0.023	0.012	0.094
	Pyrogenes	Sri Lanka	Human	49	R480	0.047	0.064	0.012	0.125	0.75
	Pyrogenes	Sri Lanka	Human	49	R358	0.016	0.094	0.003	0.094	1
	Pyrogenes	Sri Lanka	Human	75	R493	0.047	0.094	0.016	0.125	0.75
	Pyrogenes	Sri Lanka	Human	75	R601	0.006	0.047	0.023	0.094	1
	Pyrogenes	Thailand	Human	49	NR-20157*	0.064	0.25	0.094	0.75	1.5
	Pyrogenes	Thailand	Human	49	L0784	0.047	0.5	0.032	1	2
	Pyrogenes	Thailand	Human	37	UD009	0.094	0.25	0.012	0.125	1
	Unknown	Lao PDR	Human	84	NR-20173*	0.023	0.064	0.023	0.38	0.75
Unknown	Lao PDR	Human	87	NR-20177*	0.002	0.094	0.008	0.38	0.75	
Unknown	Lao PDR	Human	46	NR-20167*	0.023	0.125	0.016	0.094	1.5	

<i>Species</i>	<i>Serovar</i>	<i>Country</i>	<i>Source</i>	<i>ST</i>	<i>Strain</i>	<i>PG</i>	<i>Do</i>	<i>CT</i>	<i>TX</i>	<i>C</i>
<i>L. interrogans</i>	Unknown	Lao PDR	Human	83	NR-20172*	0.023	0.047	0.003	0.032	1
	Unknown	Lao PDR	Human	38	UI15191	0.023	0.047	0.016	0.125	1.5
	Unknown	Lao PDR	Human	NA	UI15253	0.032	0.094	0.032	0.125	1
	Unknown	Sri Lanka	Human	76	R457	0.047	0.19	0.032	0.38	1.5
	Unknown	Sri Lanka	Human	75	R444	0.006	0.094	0.012	0.064	0.25
	Unknown	Thailand	Human	40	NR-20179*	0.016	0.064	0.023	0.25	1
	Unknown	Thailand	Human	46	NR-20158*	0.047	0.19	0.047	0.38	2
	Unknown	Thailand	Human	71	NR-20156*	0.016	0.023	0.016	0.19	0.19
	Unknown	Thailand	Human	35	NR-20153*	0.012	0.032	0.016	0.094	0.75
	Wolffi	Lao PDR	Human	NA	LNT1691	0.002	0.064	0.002	0.032	1.5
Wolffi	Thailand	Human	47	NR-20154*	0.023	0.19	0.008	0.047	1.5	
<i>L. borgpetersenii</i>	Ballum	Denmark	Animal	NA	Mus 127	0.008	0.19	0.006	0.125	2
	Javanica	Thailand	Human	NA	NR-20151*	0.012	0.19	0.006	0.032	0.75
	Javanica	Thailand	Human	NA	NR-20150*	0.004	0.094	0.006	0.047	0.75
	Javanica	Indonesia	Animal	NA	ATCC 23479	0.012	0.125	0.006	0.064	2
	Unknown	Lao PDR	Human	NA	NR-20169*	0.006	0.064	0.023	0.125	0.5
	Unknown	Lao PDR	Human	NA	NR-20171*	0.002	0.094	0.002	0.064	0.75
<i>L. inadai</i>	Lyme	USA	Human	NA	ATCC 43289	0.064	0.19	0.064	0.38	2
<i>L. kirschneri</i>	Cynopteri	Indonesia	Animal	70	3522C	0.003	0.047	0.006	0.19	0.5
	Grippotyphosa	Thailand	Human	68	NR-20327*	0.023	0.064	0.032	0.125	1
	Grippotyphosa	Thailand	Human	68	NR-20155*	0.047	0.125	0.023	0.064	1
<i>L. weilii</i>	Sarmin	Indonesia	Human	NA	Sarmin	0.003	0.19	0.008	0.75	1.5
	Unknown	Lao PDR	Human	NA	NR-20181*	0.012	0.094	0.002	0.047	0.5
	Unknown	Lao PDR	Human	NA	NR-20176*	0.008	0.125	0.012	0.094	0.38
	Unknown	Lao PDR	Human	NA	NR-20182*	0.016	0.125	0.004	0.008	0.75
	Unknown	Lao PDR	Human	NA	NR-20183*	0.032	0.125	0.016	0.064	1.5

<sup>a</sup> ST = Sequence type determined by multilocus sequence typing (MLST), N/A = Not available

<sup>b</sup> PG = Penicillin G (units/ml), Do = Doxycycline ( $\mu\text{g/ml}$ ), CT = Cefotaxime ( $\mu\text{g/ml}$ ), TX = Ceftriaxone ( $\mu\text{g/ml}$ ), C = Chloramphenicol ( $\mu\text{g/ml}$ )

\* NR represents strains deposited with BioDefense and Emerging Infections Research Resources Repository (n=35)