

Supplementary Materials

Development of a recombinase-aided amplification assay for rapid detection of the human pathogen *Nocardia farcinca*

Fang Li^{1,2#}, Shuai Xu¹, Ruihuan Wang¹, Lichao Han¹, Min Yuan¹, Xiaotong Qiu¹, Yutong Kang¹, Jirao Shen¹, Xueping Liu³, Shenglin Chen⁴, Jiang Yao³, Zhenjun Li^{1,2*}

¹State Key Laboratory of Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, PR China

²Department of Medicine, Tibet University, Lhasa 850000, Tibet, PR China

³Key Laboratory of Laboratory Medicine, Ministry of Education of China, Wenzhou Medical University, Wenzhou 325035, Zhejiang, PR China

⁴Shanxi Medical University, Taiyuan 030001, Shanxi, PR China

*** Correspondence:** Zhenjun Li.

Email: lizhenjun@icdc.cn.

Table S1. Bacterial strains used in this study.

Sequence	Bacteria	Strain number	Strain source	Number of strains
1	<i>N. farcinica</i>	DSM 43063, DSM 43289, DSM 43665, DSM 43003, DSM 43006, DSM 43244, DSM 43131, DSM 43298, DSM 43599, DSM 43668, DSM 43669, DSM 44562, DSM 46004, DSM 46005, DSM 46028, IFM 10152	15 standard strains of <i>N. farcinica</i> came from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ); one standard strains of <i>N. farcinica</i> came from National Institute of Advanced Industrial Science and Technology	16
2	<i>N. farcinica</i>	CDC25, CDC26, CDC27, CDC28, CDC29, CDC30, CDC31, CDC32, CDC33, CDC34, CDC35, CDC38, CDC39, CDC42, CDC43, CDC44, CDC46, CDC48, CDC49, CDC50, CDC51, CDC52, CDC53, CDC54, CDC55, CDC56, CDC58, CDC59, CDC60, CDC61, CDC62, CDC63, CDC65, CDC68, CDC118, CDC142, CDC143, CDC145, CDC146, CDC147, CDC148, CDC149, CDC150, CDC151, CDC154, CDC158, CDC173, CDC177, CDC178, CDC201, CDC202, CDC239, CDC297, CDC303, CDC335, CDC352, CDC359, CDC367, CDC368, CDC373, CDC392, CDC394, CDC395, CDC396, CDC400, CDC410, CDC414, CDC419, CDC429, CDC434	Clinical strains came from Chinese Center for Disease Control and Prevention (CCDC)	70
3	<i>N. veterana</i>	DSM 40777, DSM 44538	standard strains came from DSMZ	2
4	<i>N. carnea</i>	DSM 44644	standard strain came from DSMZ	1
5	<i>N. asteroides</i>	DSM 44252	standard strain came from DSMZ	1
6	<i>N. amikacinitolerans</i>	DSM 45538	standard strain came from DSMZ	1
7	<i>N. nova</i>	DSM 43256, DSM 44481, CDC370	standard strains came from DSMZ and the strain came from CCDC	3
8	<i>N. brasiliensis</i>	DSM 43758, CDC196	standard strain came from DSMZ	2

9	<i>N. otitidiscaviarum</i>	DSM 43400, DSM 44565, CDC6	and the strain came from CCDC standard strains came from DSMZ and the strain came from CCDC	3
10	<i>N. cyriacigeorgica</i>	DSM 43208, DSM 46058, CDC327, CDC332, CDC342, CDC346, CDC347, CDC376, CDC389, CDC413	standard strains came from DSMZ and the strains came from CCDC	10
11	<i>N. abscessus</i>	CDC184, CDC189	The strains came from CCDC	2
12	<i>N. gipuzkoensis</i>	CDC387, CDC423	The strains came from CCDC	2
13	<i>N. asiatica</i>	CDC416	The strain came from CCDC	1
14	<i>N. aobensis</i>	CDC203	The strain came from CCDC	1
15	<i>Corynebacterium pseudodiphtheriticum</i>	SWAQ9	The strain came from CCDC	1
16	<i>C. propinquum</i>	SWAQ4	The strain came from CCDC	1
17	<i>C. diphtheriae</i>	SWAQ1	The strain came from CCDC	1
18	<i>C. simulans</i>	CDC421	The strain came from CCDC	1
19	<i>Streptomyces flavofungini</i>	CDC127	The strain came from CCDC	1
20	<i>S. cavourensis</i>	CDC185	The strain came from CCDC	1
21	<i>Rhodococcus coprophilus</i>	CDC131	The strain came from CCDC	1
22	<i>Mycobacterium peregrinum</i>	CDC235, CDC258	The strains came from CCDC	2
23	<i>M. syngnathidarum</i>	CDC249	The strain came from CCDC	1
24	<i>M. houstonense</i>	CDC250	The strain came from CCDC	1
25	<i>M. fortuitum</i>	CDC264	The strain came from CCDC	1
26	<i>M. vaccae</i>	CDC303	The strain came from CCDC	1
27	<i>Tsukamurella pulmonis</i>	CDC267	The strain came from CCDC	1
28	<i>Gordonia terrae</i>	CDC300	The strain came from CCDC	1
29	<i>Prauserella coralliicola</i>	CDC337	The strain came from CCDC	1
30	<i>Saccharopolyspora hordei</i>	CDC338	The strain came from CCDC	1

Table S2. Partial of the alignment result.

sequence	sseqid	pident	length	qcovs	qstart	qend	evalue	bitscore	taxonomy_ID	tax
1	gi 1444379873 gb CP031418.1	100	1191	100	1	1191	0	2149	37329	<i>Nocardia farcinica</i>
2	gi 873551602 emb LN868939.1	100	1191	100	1	1191	0	2149	37329	<i>Nocardia farcinica</i>
3	gi 1417721500 gb CP030158.1	100	26	2	282	307	1.5	48.2	2026787	<i>Rhodothermaceae</i> <i>bacterium</i>
4	gi 1167574847 gb CP020382.1	100	26	2	282	307	1.5	48.2	1779382	<i>Rhodothermaceae</i> <i>bacterium RA</i>
5	gi 1747867215 gb CP043959.1	100	25	2	734	758	5.2	46.4	1932	<i>Streptomyces tendae</i>
6	gi 1560905426 gb CP032301.1	100	25	2	463	487	5.2	46.4	30069	<i>Anopheles stephensi</i>
7	gi 54013472 dbj AP006618.1	98.825	1191	100	1	1191	0	2085	247156	<i>Nocardia farcinica</i> IFM 10152
8	gi 1860152443 gb CP054916.1	96.667	30	2	872	900	5.2	47.3	2742137	<i>Streptomyces sp.</i> NA02950
9	gi 1815626848 gb CP049037.1	96.429	28	2	192	219	5.2	47.3	1591409	<i>Pseudohalocynthiibacter</i> <i>aestuariivivens</i>
10	gi 1806479221 dbj AP022619.1	96.429	28	2	442	469	5.2	47.3	590652	<i>Mycobacterium</i> <i>paraseoulense</i>
11	gi 1806309652 dbj AP022582.1	96.429	28	2	442	469	5.2	47.3	386911	<i>Mycobacterium</i> <i>seoulense</i>
12	gi 1519110434 gb CP033854.1	96.429	28	2	103	130	5.2	47.3	87883	<i>Burkholderia</i> <i>multivorans</i>
13	gi 1061771052 gb CP013464.1	96.429	28	2	103	130	5.2	47.3	101571	<i>Burkholderia ubonensis</i>
14	gi 1061735496 gb CP013447.1	96.429	28	2	103	130	5.2	47.3	101571	<i>Burkholderia ubonensis</i>
15	gi 1061657888 gb CP013416.1	96.429	28	2	103	130	5.2	47.3	101571	<i>Burkholderia ubonensis</i>
16	gi 1061548849 gb CP013371.1	96.429	28	2	103	130	5.2	47.3	101571	<i>Burkholderia ubonensis</i>

17	gi 941153505 gb CP007213.1	96.429	28	2	882	909	5.2	47.3	41899	<i>Burkholderia plantarii</i>
18	gi 772920427 gb CP009486.1	96.429	28	2	103	130	5.2	47.3	1249668	<i>Burkholderia ubonensis</i> MSMB22
19	gi 1904664466 gb CP061281.1	96.429	28	2	453	480	5.2	47.3	2768069	<i>Streptomyces</i> sp. CRXT- Y-14
20	gi 1987709169 ref XM_039556082.1	93.75	32	3	774	805	5.2	46.4	932674	<i>Corvus cornix cornix</i>
21	gi 1215222647 gb CP022316.1	93.548	31	3	983	1013	1.5	48.2	2017485	<i>Brachybacterium avium</i>
22	gi 1041165931 emb LN890655.2	93.548	31	3	448	478	1.5	48.2	1806508	<i>Candidatus</i> <i>Promineofilum breve</i>
23	gi 1861362334 gb CP054587.1	93.333	30	3	662	691	5.2	46.4	1853690	<i>Halolamina</i> sp. CBA1230
24	gi 1896266485 gb CP059894.1	93.333	30	3	997	1026	5.2	46.4	258505	<i>Mycolicibacterium</i> <i>fluoranthenvivorans</i>
25	gi 703597103 gb CP009755.1	91.667	36	3	456	491	0.43	50	1561023	<i>Curtobacterium</i> sp. MR_MD2014
26	gi 1733454846 gb CP042331.1	91.429	35	3	821	855	0.43	50.9	2599640	<i>Bosea</i> sp. F3-2
27	gi 1607200680 gb CP037895.1	91.429	35	3	775	809	0.43	50.9	2546351	<i>Thermaerobacter</i> sp. FW80
28	gi 1124777708 emb LK995510.1	91.429	35	3	374	407	5.2	47.3	1522002	<i>Actinomyces</i> <i>succiniciruminis</i>
29	gi 1885266710 emb LR862143.1	91.429	35	3	504	537	5.2	47.3	296719	<i>Ananas comosus</i> var. <i>bracteatus</i>
30	gi 1789835889 gb CP047140.1	91.176	34	3	858	891	1.5	49.1	2676871	<i>Streptomyces</i> sp. Tu 2975
31	gi 944389169 gb CP012184.1	91.176	34	3	193	226	1.5	49.1	1096856	<i>Pseudonocardia</i> sp. EC080619-01

32	gi 944383274 gb CP012181.1	91.176	34	3	193	226	1.5	49.1	1688404	<i>Pseudonocardia sp.</i> EC080610-09
33	gi 928461215 gb CP010989.1	91.176	34	3	193	226	1.5	49.1	1096868	<i>Pseudonocardia sp.</i> EC080625-04
34	gi 924532124 gb CP011340.1	91.176	34	3	858	891	1.5	49.1	38300	<i>Streptomyces</i> <i>pristinaespiralis</i>
35	gi 723622094 gb CP009896.1	91.176	34	3	447	480	1.5	49.1	2045	<i>Pimelobacter simplex</i>
36	gi 1996341204 gb CP039124.1	91.176	34	3	65	98	1.5	49.1	2006	<i>Thermobispora bispora</i>
37	gi 1885753887 gb CP059696.1	91.176	34	3	858	891	1.5	49.1	38300	<i>Streptomyces</i> <i>pristinaespiralis</i>
38	gi 296090785 gb CP001874.1	91.176	34	3	65	98	1.5	49.1	469371	<i>Thermobispora bispora</i> DSM 43833
39	gi 1750980319 gb CP044067.1	90.909	33	3	448	480	5.2	47.3	82633	<i>Cupriavidus pauculus</i>
40	gi 1578802915 gb CP036164.1	90.909	33	3	270	302	5.2	47.3	53458	<i>Janibacter limosus</i>
41	gi 1539310850 emb LR134136.1	90.909	33	3	30	62	5.2	47.3	565	<i>Atlantibacter hermannii</i>
42	gi 1418370697 gb CP030286.1	90.909	33	3	1145	1177	5.2	47.3	2099584	<i>Streptomyces sp.</i> ICC4
43	gi 1418363025 gb CP030287.1	90.909	33	3	1145	1177	5.2	47.3	2099583	<i>Streptomyces sp.</i> ICC1
44	gi 1248666634 gb CP023564.1	90.909	33	3	424	456	5.2	47.3	1331682	<i>Brachybacterium</i> <i>ginsengisoli</i>
45	gi 1025712797 gb CP015511.1	90.909	33	3	135	167	5.2	47.3	1827469	<i>Brevundimonas sp.</i> GW460-12-10-14-LB2
46	gi 1943261987 gb CP065700.1	90.909	33	3	30	62	5.2	47.3	565	<i>Atlantibacter hermannii</i>
47	gi 1891431189 gb CP043652.1	90.909	33	3	656	688	5.2	47.3	2596920	<i>Geodermatophilaceae</i> <i>bacterium</i> NBWT11
48	gi 119534933 gb CP000509.1	90.909	33	3	270	302	5.2	47.3	196162	<i>Nocardioides sp.</i> JS614
49	gi 1317055927 gb CP025407.1	90	40	3	274	311	0.12	52.7	2059884	<i>Streptomyces sp.</i> CMB-

										StM0423
50	gi 822591927 gb CP011492.1	90	40	3	274	311	0.12	52.7	444103	<i>Streptomyces</i> sp. CNQ-509

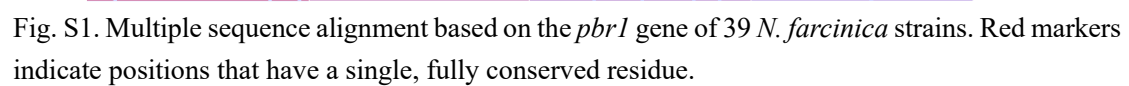


Fig. S1. Multiple sequence alignment based on the *pbrl* gene of 39 *N. farcinica* strains. Red markers indicate positions that have a single, fully conserved residue.

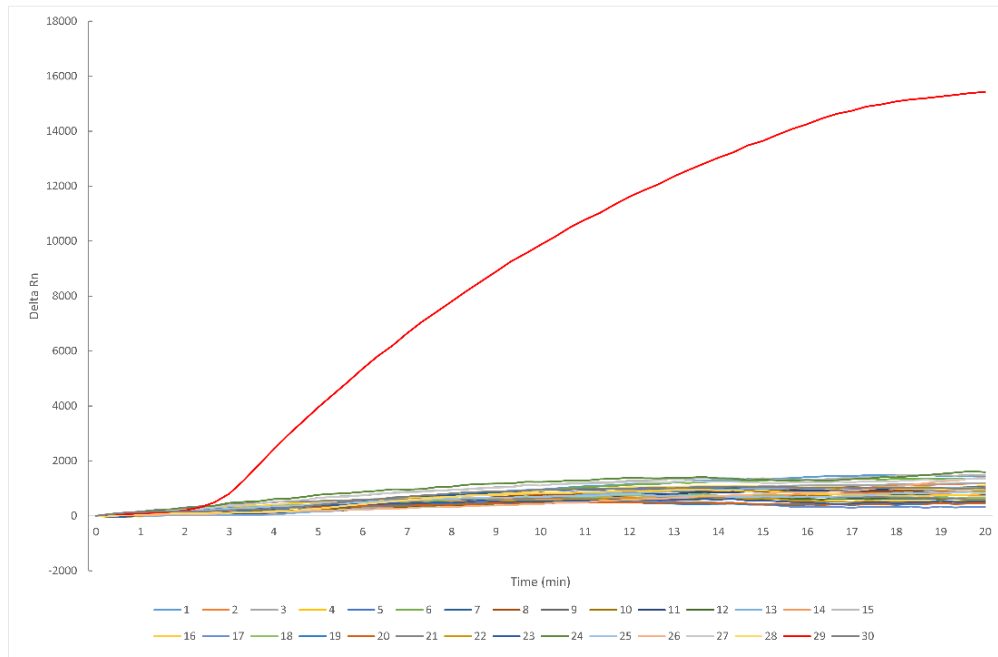


Fig. S2. Specificity of the *N. farcinica* RT-RAA assay at 37°C for 20 min. 1–28, *N. farcinica* closely related strains; 29, positive control; 30, negative control.

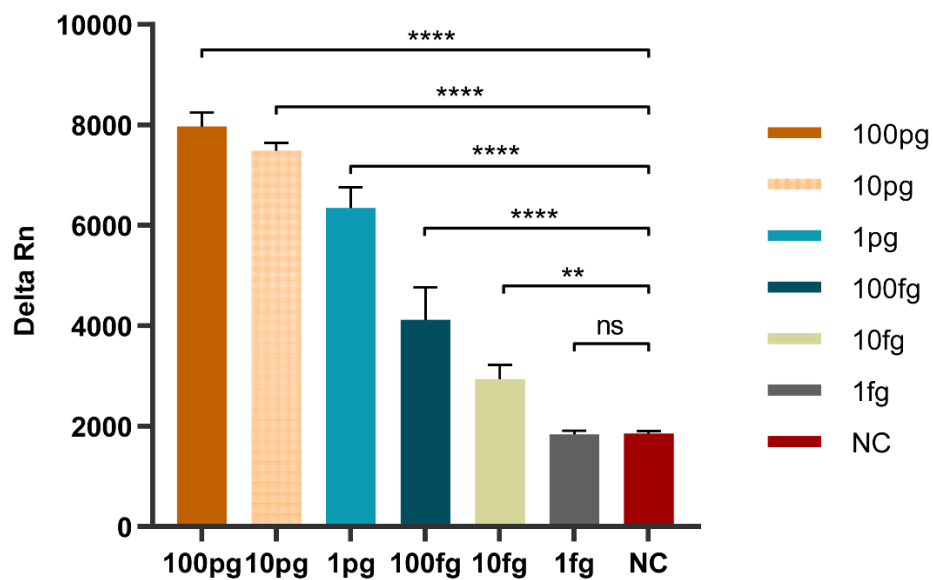


Fig. S3. Sensitivity of *N. farcinica* RT-RAA assay in pure cultures. n = 3 technical replicates, two-tailed Student t-test; ns, no significance; ***p < 0.001; ****p < 0.0001; bars represent mean $\pm$ s.e.m.

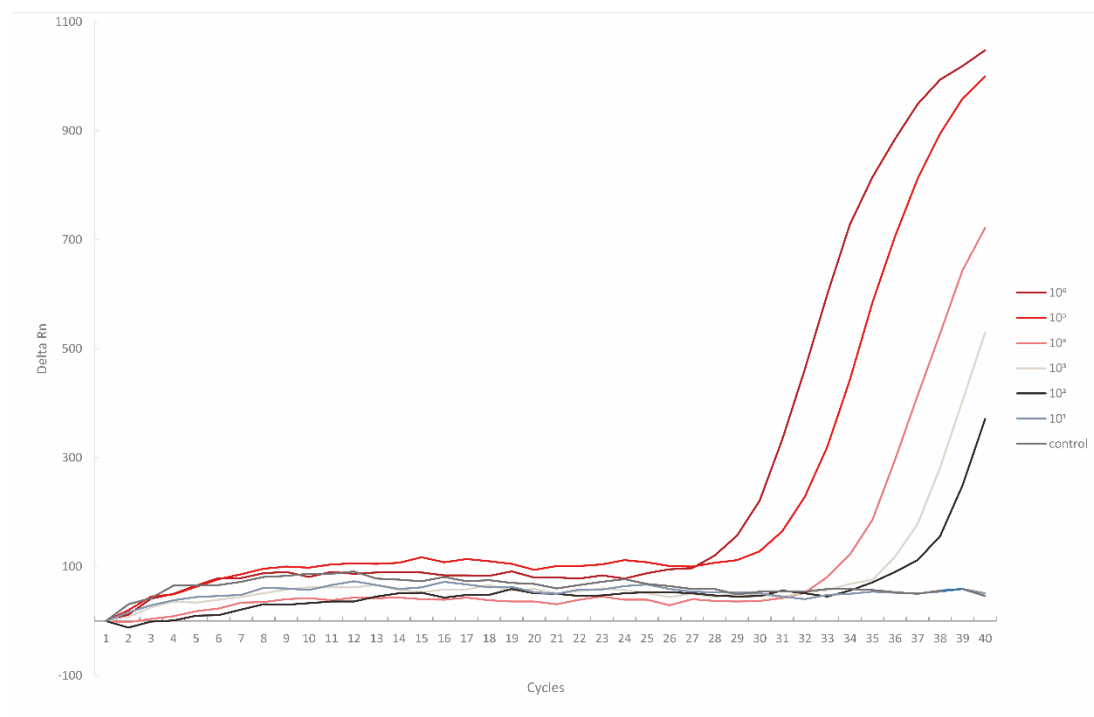


Fig. S4. The sensitivity of the qPCR detection using 10-fold serially diluted recombinant plasmids.

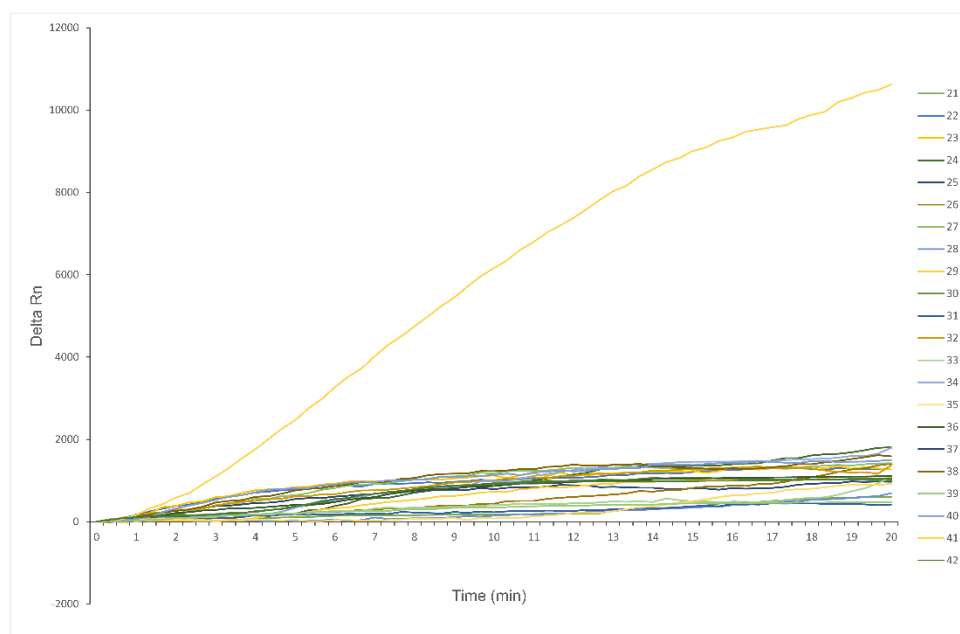


Fig. S5 Feasibility of *N. farcinica* RT-RAA assay using clinical samples. 21-40, 20 sputum samples from healthy people; 41, positive control; 42, negative control.

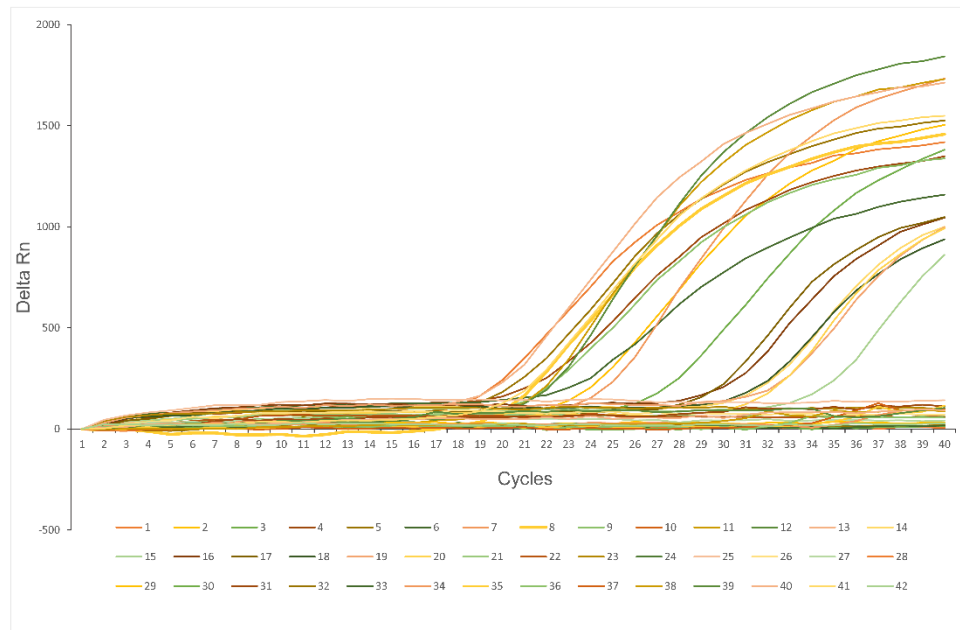


Fig. S6 Feasibility of *N. farcinica* qPCR assay using clinical samples. 1-20, simulated *N. farcinica* positive samples; 21-40, 20 sputum samples from healthy people; 41, positive control; 42, negative control.