Supplementary materials

Accurate detection of methicillin-resistant *Staphylococcus aureus* in mixtures utilizing single bacterial duplex droplet digital PCR

Jun Luo[1,2], Junhua Li[1], Hang Yang[1], Junping Yu[1], Hongping Wei[1#]

1.Key Laboratory of Special Pathogens and Biosafety, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

2. University of Chinese Academy of Sciences, Beijing 100039, China

#To whom correspondence should be addressed.

Email: hpwei@wh.iov.cn; Tel/Fax: +86 27 51861076

Table S1 Bacteria strains used in this study.

Organism group	Strain	SCC*mec* type
MSSA	91118	—
MRSA	N315	II
	ZX3	II
	ZX108	I
	FY16	III
	WH70	V
	YN22	IV
	KQ6	V
	FY17	III
	ZX54	IV
MR-CoNS (*S. haemolyticus*)	WH01	
Control bacteria	*E. coil*	—

–: not applicable (no *mecA* gene)

Table S2 The MRSA Index Ratio (MIR) of MRSA N315 under different concentrations.

Sample N315	Average *mecA*-positive droplets No	Average *Nuc*-positive droplets No	Average duplex positive droplets No	MRSA Index Ratio
$1x10^6$ CFU/mL	7988	7562	6806	90±1.175
$1x10^5$ CFU/mL	629	607	548	90.26±0.13
$1x10^4$CFU/mL	93	84	77	91.67±0.705
$1x10^3$ CFU/mL	4	4	4	100

Table S3 The MRSA Index Ratio (MIR) of mixtures of MSSA and MR-CoNS under different concentrations.

Mixture No	Average *mecA*-positive droplets No	Average *Nuc*- positive droplets No	Average duplex positive droplets No	MRSA Index Ratio
1	1 x 10^6 CFU/mL WH01 + 1 x 10^6 CFU/mL 91118			
	2919	5141	952	29.48±4.07
2	1 x 10^5 CFU/mL WH01+ 1 x 10^5 CFU/mL 91118			
	240	679	16	6.75±0.95
3	1 x 10^4 CFU/mL WH01+ 1 x 10^4 CFU/mL91118			
	45	77	0	0
4	1 x 10^3 CFU/mLWH01+ 1 x 10^3 CFU/mL 91118			
	1	2	0	0
5	1 x 10^6 CFU/mL WH01+ 1 x 10^5 CFU/mL91118			
	1696	253	52	20.74±1.56
6	1 x 10^6 CFU/mL WH01+ 1 x 10^4 CFU/mL 91118			
	2575	76	14	18.24±1.24
7	1 x 10^6 CFU/mL WH01+ 1 x 10^3 CFU/mL91118			
	2511	6	0	0
8	1 x 10^5 CFU/mL WH01+ 1 x 10^4 CFU/mL 91118			
	322	144	3	2.1±0.45
9	1 x 10^5CFU/mL WH01+ 1 x 10^3 CFU/mL 91118			
	349	7	0	0
10	1 x 10^4 CFU/mL WH01+ 1 x 10^3 CFU/mL91118			
	56	2	0	0
11	1 x 10^5 CFU/mL WH01+ 1 x 10^6 CFU/mL 91118			
	661	4140	142	21.48±1.24
12	1 x 10^4 CFU/mL WH01+ 1 x 10^6 CFU/mL 91118			
	85	4160	19	22.29±1.13
13	1 x 10^3 CFU/mL WH01+ 1 x 10^6 CFU/mL 91118			
	3	1920	0	0
14	1 x 10^4 CFU/mL WH01+ 1 x 10^5 CFU/mL 91118			
	68	557	2	2.9±0.6
15	1 x 10^3 CFU/mL WH01+ 1 x 10^5CFU/mL 91118			
	8	1426	0	0
16	1 x 10^3 CFU/mL WH01+ 1 x 10^4CFU/mL 91118			
	4	20	0	0

Table S4 The detection results of the dulpex ddPCR and the duplex qPCR for 104 nasal specimens.

Sample	qPCR (Ct)			ddPCR (droplet)					Culture result
	mecA	*nuc*	Result	*mecA*	*nuc*	Dual positive	MRSA ratio	Result	
1	-	-	-	9	0	0	0	-	-
2	36.71	-	-	34	1	0	0	-	-
3	**32.91**	**-**	**-**	**1410**	**8**	**6**	**75**	**+**	**+**
4	34.02	-	-	30	4	0	0	-	-
5	31.21	-	-	209	2	0	0	-	-
6	31.29	-	-	127	2	0	0	-	-
7	**33.41**	**33.59**	**+**	**160**	**84**	**82**	**97.6**	**+**	**+**
8	37.16	-	-	14	2	0	0	-	-
9	-	-	-	3	2	0	0	-	-
10	-	-	-	6	4	0	0	-	-
11	**-**	**-**	**-**	**2**	**2**	**2**	**100**	**+**	**+**
12	-	-	-	0	0	0	0	-	-
13	**34.72**	**35.91**	**+**	**13**	**5**	**0**	**0**	**-**	**-**
14	**29.98**	**32.37**	**+**	**3052**	**1542**	**1483**	**96.2**	**+**	**+**
15	**32.01**	**32.51**	**+**	**463**	**450**	**379**	**84.22**	**+**	**+**
16	37.39	-	-	0	0	0	0	-	-
17	-	-	-	2	0	0	0	-	-
18	-	-	-	3	3	0	0	-	-
19	**-**	**-**	**-**	**1**	**1**	**1**	**100**	**+**	**+**
20	37.67	-	-	0	0	0	0	-	-
21	-	-	-	0	0	0	0	-	-
22	-	-	-	1	0	0	0	-	-
23	-	-	-	1	0	0	0	-	-
24	**35.17**	**-**	**-**	**195**	**1**	**1**	**100**	**+**	**+**
25	**35.6**	**37.83**	**+**	**10**	**8**	**0**	**0**	**-**	**-**
26	-	-	-	0	3	0	0	-	-
27	33.77	-	-	87	0	0	-	-	-
28	-	-	-	1	1	0	-	-	-
29	34.13	-	-	69	1	0	-	-	-
30	36.01	-	-	9	0	0	-	-	-
31	-	-	-	0	2	0	-	-	-
32	37.12	-	-	7	3	0	-	-	-
33	**34.35**	**-**	**-**	**117**	**3**	**2**	**66.67**	**+**	**+**
34	-	-	-	21	1	0	-	-	-
35	-	-	-	0	0	0	-	-	-
36	35.74	-	-	14	5	0	-	-	-
37	30.32	-	-	292	1	0	-	-	-
38	-	-	-	0	6	0	-	-	-

39	-	-	-	3	0	0	-	-	-
40	34.01	-	-	15	0	0	-	-	-
41	**30.95**	**37.74**	**+**	**51**	**9**	**8**	**88.89**	**+**	**+**
42	33.94	-	-	21	3	0	-	-	-
43	32.18	-	-	33	0	0	-	-	-
44	-	-	-	3	0	0	-	-	-
45	**31.14**	**-**	**-**	**55**	**2**	**2**	**100**	**+**	**+**
46	-	-	-	0	0	0	-	-	-
47	-	-	-	0	0	0	-	-	-
48	31.62	-	-	26	1	0	-	-	-
49	**30.38**	**-**	**-**	**107**	**3**	**3**	**100**	**+**	**+**
50	36.89	-	-	4	0	0	-	-	-
51	35.14	-	-	8	2	0	-	-	-
52	33.21	-	-	7	0	0	-	-	-
53	-	-	-	2	1	0	-	-	-
54	36.56	-	-	6	2	0	-	-	-
55	-	-	-	3	0	0	-	-	-
56	-	-	-	0	0	0	-	-	-
57	-	-	-	1	0	0	-	-	-
58	-	-	-	4	3	0	-	-	-
59	-	-	-	1	3	0	-	-	-
60	-	-	-	0	0	0	-	-	-
61	-	-	-	10	0	0	-	-	-
62	-	-	-	4	0	0	-	-	-
63	32.96	-	-	33	1	0	-	-	-
64	-	-	-	1	1	0	-	-	-
65	-	-	-	0	1	0	-	-	-
66	31.71	-	-	238	1	0	-	-	-
67	-	_	-	1	1	0	-	-	-
68*	**24.6**	**-**	**-**	**10374**	**10**	**10**	**-***	**-**	**-**
69	37.95	-	-	3	0	0	-	-	-
70	32.12	-	-	143	0	0	-	-	-
71	-	-	-	8	0	0	-	-	-
72	**36.14**	**-**	**-**	**18**	**4**	**1**	**25**	**+**	**+**
73	-	-	-	0	4	0	-	-	-
74	**31.48**	**35.41**	**+**	**231**	**60**	**17**	**28.33**	**+**	**+**
75	-	-	-	1	4	0	-	-	-
76	**34.03**	**37.99**	**+**	**31**	**7**	**1**	**14.28**	**+**	**+**
77	-	-	-	1	2	0	-	-	-
78	29.56	-	-	158	2	0	-	-	-
79	31.12	-	-	59	0	0	-	-	-
80	-	-	-	0	0	0	-	-	-
81	-	-	-	0	0	0	-	-	-
82	**-**	**-**	**-**	**4**	**3**	**3**	**100**	**+**	**+**

83	**36.21**	**36.65**	**+**	**11**	**10**	**7**	**70**	**+**	**+**
84	-	-	-	2	1	0	-	-	-
85	35.1	-	-	29	0	0	-	-	-
86	-	-	-	6	0	0	-	-	-
87	35.62	-	-	11	0	0	-	-	-
88	-	-	-	3	0	0	-	-	-
89	-	-	-	1	4	0	-	-	-
90	37.19	-	-	8	0	0	-	-	-
91	31.91	-	-	157	0	0	-	-	-
92	-	-	-	5	0	0	-	-	-
93	-	-	-	**5**	**7**	**3**	**60**	**+**	**+**
94	31.56	-	-	230	0	0	-	-	-
95	32.79	-	-	151	0	0	-	-	-
96	**32.61**	-	-	**145**	**1**	**1**	**100**	**+**	**+**
97	-	-	-	1	1	0	-	-	-
98	-	-	-	1	0	0	-	-	-
99	-	-	-	1	1	0	-	-	-
100	37.23	-	-	11	1	0	-	-	-
101	-	-	-	1	1	0	-	-	-
102	31.98	-	-	125	5	0	-	-	-
103	-	-	-	2	0	0	-	-	-
104	33.1	-	-	101	1	0	-	-	-

Note: "-" means no signal (for qPCR) or MRSA negative result. "+" means MRSA positive result.

***: Specimen 68 was diluted 10 times for determining the MIR in the ddPCR assay.**

Fig.S1

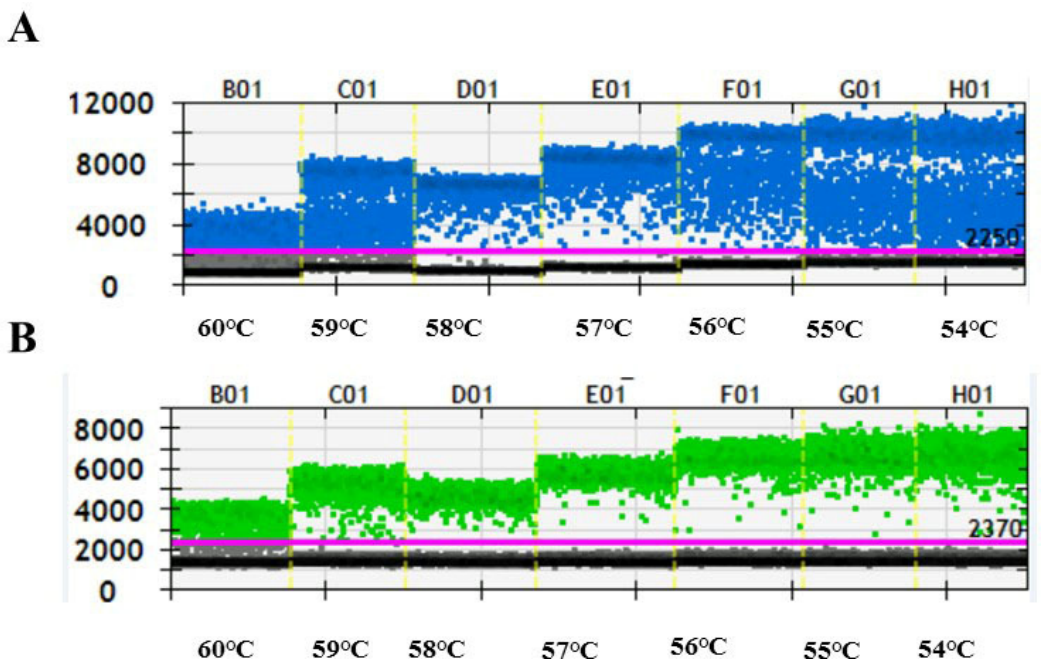

Fig.S1 One-dimensional (1D) plots of the duplex ddPCR assay under different annealing temperatures (54–60°C). MRSA strain N315 (1 *10^7 CFU/mL) is used as the template (A. *mecA* gene and B. *nuc* gene). Each point represents a droplet with a given fluorescence level and the droplet colors indicate which target was amplified. The x-axis shows various temperature, and the y-axis represents the fluorescence amplitude.

Fig.S2

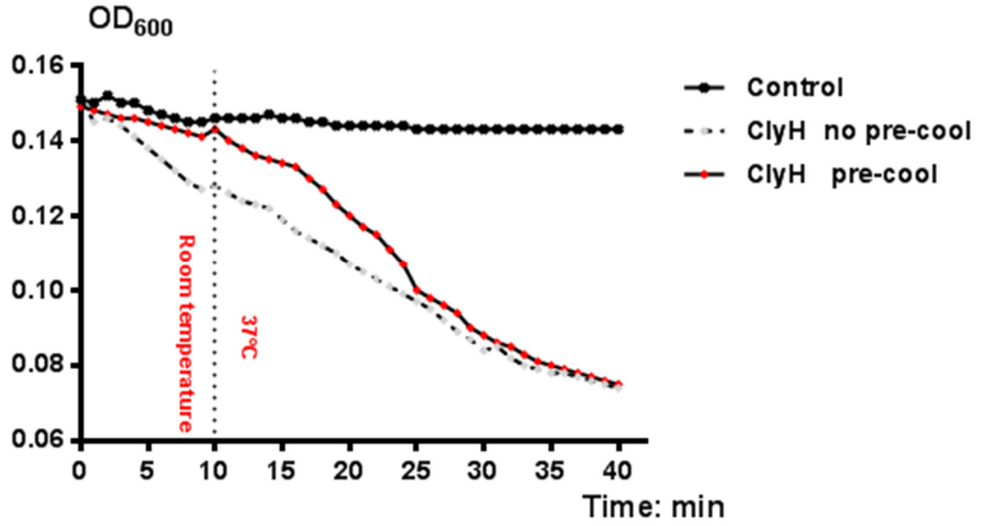

Fig. S2 The activity changes of ClyH after cooling on ice. The concentration of ClyH: 10 μg/mL.

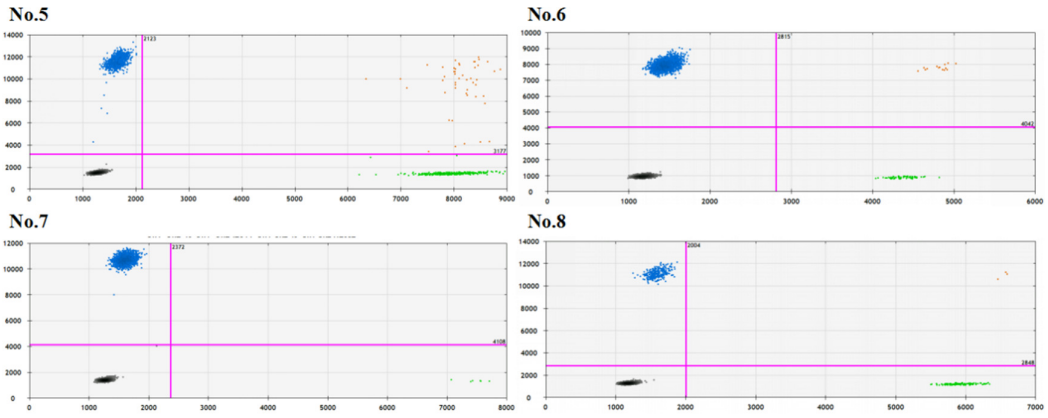

Fig. S3 Two-dimensional (2D) plots of the duplex ddPCR for detection of the mixture of MSSA(91118) and MR-CoNS under different concentrations (Sample No 5 - No 8 are as shown in Table S3). The x-axis shows the fluorescence amplitude corresponding to the HEX fluorophore (*nuc*), and the y-axis represents the fluorescence amplitude corresponding to the FAM fluorophore (*mecA*). Each point represents a droplet with a given fluorescence level and the droplet colors indicate which target was amplified (blue: *mecA* positive; green: *nuc* positive; black: negative, Orange: both *nuc* and *mecA* positive).

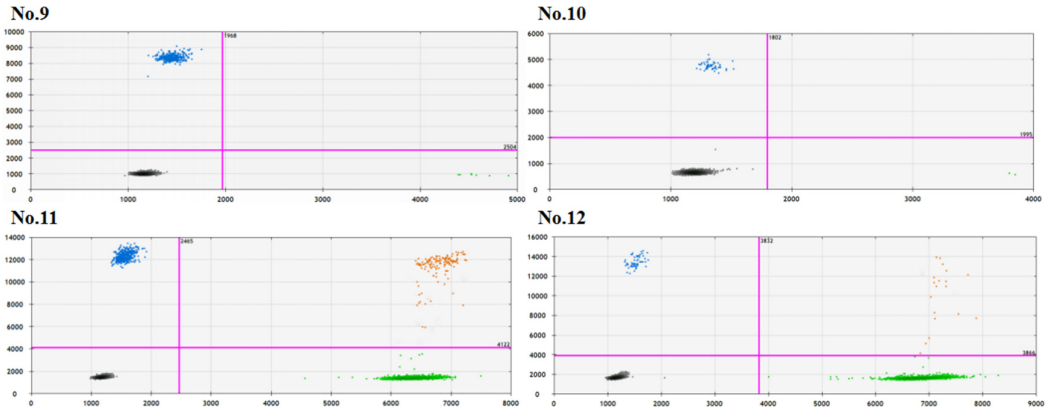

Fig. S4 Two-dimensional (2D) plots of the duplex ddPCR for detection of the mixture of MSSA(91118) and MR-CoNS under different concentrations (Sample No 9 - No 12 are as shown in Table S3). The x-axis shows the fluorescence amplitude corresponding to the HEX fluorophore (*nuc*), and the y-axis represents the fluorescence amplitude corresponding to the FAM fluorophore (*mecA*). Each point represents a droplet with a given fluorescence level and the droplet colors indicate which target was amplified (blue: *mecA* positive; green: *nuc* positive; black: negative, Orange: both *nuc* and *mecA* positive).

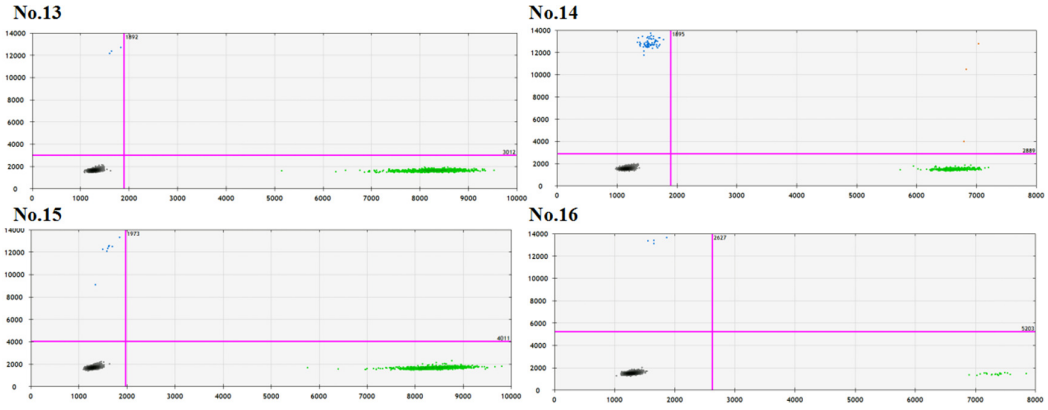

Fig. S5 Two-dimensional (2D) plots of the duplex ddPCR for detection of the mixture of MSSA(91118) and MR-CoNS under different concentrations (Sample No 13 - No 16 are as shown in Table S3). The x-axis shows the fluorescence amplitude corresponding to the HEX fluorophore (*nuc*), and the y-axis represents the fluorescence amplitude corresponding to the FAM fluorophore (*mecA*). Each point represents a droplet with a given fluorescence level and the droplet colors indicate which target was amplified (blue: *mecA* positive; green: *nuc* positive; black: negative, Orange: both *nuc* and *mecA* positive).