Supplemental Online Content

Tay JK, Cross GB, Toh ST, et al. Design and multicenter clinical validation of a 3-dimensionally printed nasopharyngeal swab for COVID-19 testing. *JAMA Otolaryngol*. Published online February 18, 2021. doi:10.1001/jamaoto.2020.5680

This supplemental material has been provided by the authors to give readers additional information about their work.

eMethods

Swab design

Nasopharyngeal (NP) swabs should capture respiratory epithelial cells and mucus (cellular-mucus matrix), retain the cellular-mucus matrix while relaying the swab to a transport container, then release the cellular-mucus matrix into the transport media from which viral RNA can be detected in order to be effective. Intuitively, the greater the surface area of the NP swab, the greater the cellular-mucus matrix volume captured and released, with resultant increased sensitivity of the assay. With that goal in mind and with awareness of the diameter of the nasal passage through which the NP swab must journey, a square shaped, helix design with a reservoir concept was selected. The helix makes 1.5 turns from start to end. This allows for surface manipulation (sculpting, flexing and weaving) while retaining sufficient structural integrity for withstanding pushing and turning impact forces. The gaps between the helix blades and the offset of the blades' inner aspects allow the swab to collect the cellular-mucus matrix via capillary effect and deposit it into the central reservoir. The cellular-mucus matrix contained within the reservoir and adherent to the swab tip is released when the NP swab tip is submerged and agitated in viral transport media.

3D-printing and sterilization

The 3D printed swabs were designed and developed using Rhino 6 for Windows (Rhinoceros®, Robert McNeel and Associates, Seattle, WA). After design development, the CAD files were exported into a STereoLithography (.stl) file and printed by the Formlabs Form3 printer (Formlabs Inc., Somerville, MA, USA), and fabricated in Surgical Guide Resin (Formlabs Inc., Somerville, MA, USA) which is non-cytotoxic, non-sensitizing, non-irritating, and complies with international

standards (ISO 109933-1:2018 Biological evaluation of medical devices). The 3D printed swabs were then steam sterilized according to recommendations from the United States Centers for Disease Control and Prevention (30 minutes at 121 °C / 250 °F in a gravity displacement autoclave) prior to the testing.

Tensile, flexural and torsional strength testing

Mechanical testing was performed by an independent testing facility (TÜV SÜD PSB Singapore). 3D printed swabs samples were tested for tensile and flexural strength in accordance with international standards (ISO 527-1:2012 Plastics – Determination of Tensile Properties and ISO 178:2010 Plastics – Determination of Flexural Properties). For tensile testing, swabs were gripped at 10mm from end of the tip. Torsional testing was performed by gripping the swab at 12.7mm from both ends and at a torsional speed of 8 rpm in a clockwise twisting direction. The resulting number of turns (in degrees) when the specimen breaks was reported.

Fluid absorption and release

Two types of medium were used for the testing, including the Universal Viral Transport Medium (Becton, Dickinson and Company, MD, USA #220527) and a viscous fluid that mimics the human mucus[1] – 25% (w/v) Pluronic F127 aqueous solution (Sigma Aldrich #P2443), the viscosity of which ranges from 0.6-24 Pa at 20℃ from an internal pre-test. Fluid (3ml) was transferred to a 15ml centrifuge tube, which was then weighted using an analytical balances (Sartorius Entris, Göttingen, Germany). The swab was immersed in the fluid for 5 – 10 seconds. The tube was weighted again after removing the swab for the calculation of the absorbed liquid,

which is the difference between the weight of the tube before swab immersion and after removing the immersed swab.

Two release methods were performed after fluid absorption, including vortex approach and roll plate approach. The vortex approach is a commonly used procedure to mix an experimental sample and diluent.[2] An empty 15ml centrifuge tube was weighted; after the immersed swab was placed into the tube, it was vortexed on a lab vortex mixer (Vortex Genie 2, model #G-560, Scientific Industries, USA) for 5 sec. The swab was then removed and the tube was weighted again; the difference of the tube weights represents the release liquid. The roll plate approach provides a semi moisture environment that is frequently used in laboratory and clinical testing for swabs.[3] A swab was weighted before the absorption. After the absorption procedure, the swab was transferred and rolled with exerting downward pressure on a 1.5% agar plate (Sigma Aldrich #05040); the swab tip was rolled back and forth across the agar surface. Then, the swab was weighted again. The difference of the weight of the swab before and after the roll plate procedure was used as the release weight of liquid. The volumes of the absorption and release were calculated using the weights and the density of the two liquids, which were 0.00103 g/µl (universal viral transport media) and 0.00102 g/µl (viscous fluid) from an internal pre-test. The experiment was repeated three times for each composition of the swab types, liquids, and release methods.

Murine coronavirus testing

MHV (strain A59) concentrations of 10^6 and 10^4 plaque-forming units (PFU) were each freshly prepared in a microfuge tube containing 1 ml of Dulbecco's modified

Eagle's medium (DMEM). Into each spiked sample was dipped each swab, with swirling and twisting of the swab head for 10 sec, before transferring the infected swab to a fresh tube containing 1 ml DMEM. From the latter, 140 µl was obtained for viral RNA extraction using the QIAamp Viral RNA Mini kit (Qiagen, Germany). Each viral RNA sample (300 ng) was reverse-transcribed to cDNA in a volume of 12.5 µl comprising M-MLV reverse transcriptase (Promega, USA), and MHV-specific primers MHV-NF (5'- ACGCTTACATTATCWACTTC-3') and MHV-NR (5'-GATCTAAATTAGAATTGGTC-3'). Each cDNA sample (1 µl) was subjected to real-time PCR using FastStart Essential DNA Green Master reaction mix (Roche, Singapore) together with MHV-NF and MHV-NR primers targeting a 256-bp fragment of the MHV N gene. Negative controls without cDNA template were also included. Thermal cycling was conducted using the LightCycler 96 Real-Time PCR System (Roche, Singapore), with the following parameters: pre-incubation at 95^{o}C for 5 min, followed by 55 cycles each at 95^{o}C for 10 sec, 40^{o}C for 5 sec, and 72^{o}C for 8 sec. The relative efficiency of the test swabs was compared based on the determined threshold cycle (Ct) values.

References:

1. Lai SK, Wang YY, Wirtz D, Hanes J. Micro- and macrorheology of mucus. *Adv Drug Deliv Rev.* 2009;61(2):86-100.

2. Warnke P, Warning L, Podbielski A. Some are more equal--a comparative study on swab uptake and release of bacterial suspensions. *PLoS One.* 2014;9(7):e102215.

3. Osterblad M, Jarvinen H, Lonnqvist K, et al. Evaluation of a new cellulose sponge-tipped swab for microbiological sampling: a laboratory and clinical investigation. *J Clin Microbiol.* 2003;41(5):1894-1900.

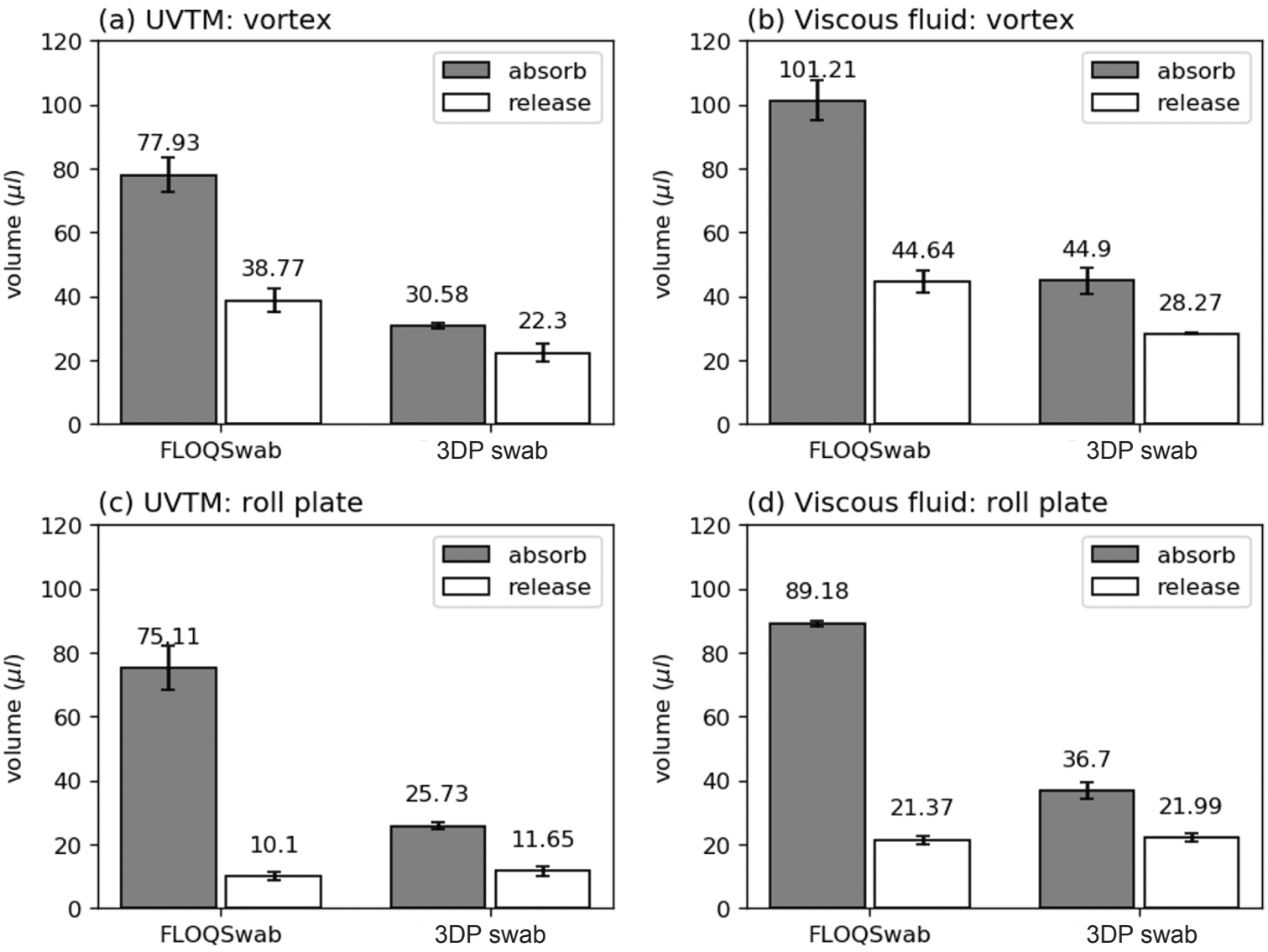
Sample_ID_masked	Group	Reference swab	Odd_or_Even	Day_of_illness	ORF1a_Reference_sw
C1	control	FLOQSwab	1		Neg
C2	control	FLOQSwab	2		Neg
C3	control	FLOQSwab	1		Neg
C4	control	FLOQSwab	2		Neg
C5	control	FLOQSwab	1		Neg
C6	control	FLOQSwab	2		Neg
C7	control	FLOQSwab	1		Neg
C8	control	FLOQSwab	2		Neg
C9	control	FLOQSwab	1		Neg
C10	control	FLOQSwab	2		Neg
P1	case	FLOQSwab	2	12	31.6
P2	case	FLOQSwab	2	5	27.22
P3	case	FLOQSwab	1	3	25.07
P4	case	FLOQSwab	2	6	30.78
P5	case	FLOQSwab	2	2	29.9
P6	case	FLOQSwab	2	11	Neg
P7	case	FLOQSwab	1	10	27.66
P8	case	FLOQSwab	2	7	Neg
P9	case	FLOQSwab	1	6	Neg
P10	case	FLOQSwab	1	14	Neg
P11	case	FLOQSwab	1	13	31.82
P12	case	FLOQSwab	2	8	Neg
P13	case	FLOQSwab	2	13	30.29
P14	case	FLOQSwab	2	9	26.1
P15	case	FLOQSwab	1	13	Neg
P16	case	FLOQSwab	2	2	20.92
P17	case	FLOQSwab	1	3	21.84
P18	case	FLOQSwab	1	8	32.21
P19	case	FLOQSwab	1	8	Neg
P20	case	FLOQSwab	2	6	31.78
P21	case	FLOQSwab	1	7	25.15
P22	case	FLOQSwab	1	14	28.97
P23	case	FLOQSwab	1	4	31.87
P24	case	FLOQSwab	2	12	32.55
P25	case	FLOQSwab	1	11	25.84
P26	case	FLOQSwab	2	11	Neg
P27	case	FLOQSwab	2	9	17.39
P28	case	FLOQSwab	1	8	29.6
P29	case	FLOQSwab	1	4	19.14
P30	case	FLOQSwab	1	4	26.86
P31	case	FLOQSwab	1	8	Neg
P32	case	FLOQSwab	1	8	Neg
P33	case	FLOQSwab	2	9	32.98
P34	case	FLOQSwab	2	10	33.06
P35	case	FLOQSwab	1	14	33.76
P36	case	FLOQSwab	1	10	29.77

P37	case	FLOQSwab	2	7	Neg	
P38	case	FLOQSwab	2	9		29.64
P39	case	FLOQSwab	2	8		32.49
P40	case	FLOQSwab	2	8		19.54
T1	case	Dacron	2	8	Neg	
T2	case	Dacron	2	2		31.98
T3	case	Dacron	1	11		31.45
T4	case	Dacron	1	11		33.46
T5	case	Dacron	2	4	Neg	
T6	case	Dacron	1	4		23.48
T7	case	Dacron	2	5		22.65
T8	case	Dacron	2	3		18.91
T9	case	Dacron	1	5		29.92
T10	case	Dacron	2	6		28.08
T11	case	Dacron	2	8		34.22
T12	case	Dacron	1	6		22.04
T13	case	Dacron	1	5		25
T14	case	Dacron	2	7		28.19
T15	case	Dacron	1	3	Neg	
T16	case	Dacron	2	5		30.93
T17	case	Dacron	1	5	Neg	
T18	case	Dacron	2	7		27.44
T19	case	Dacron	2	3	Neg	
T20	case	Dacron	2	3	Neg	
T21	case	Dacron	1	14	Neg	
T22	case	Dacron	1	2		24.5
T23	case	Dacron	1	8	Neg	
T24	case	Dacron	2	7		19.19
T25	case	Dacron	1	13		32.49
T26	case	Dacron	1	5		24.35
T27	case	Dacron	1	5	Neg	
T28	case	Dacron	1	4		31.82
T29	case	Dacron	2	13		32.81
T30	case	Dacron	2	6		19.99
T31	case	Dacron	1	9		30.9
T32	case	Dacron	2	5		25.6
T33	case	Dacron	1	11		31.06
T34	case	Dacron	2	2		17.78
T35	case	Dacron	2	1		24.73
T36	case	Dacron	2	7		19.88
T37	case	Dacron	2	6		32.86
T38	case	Dacron	1	4		21.2
T39	case	Dacron	1	12	Neg	

ORF1a_Python_swab	Egene_Reference_swa	Egene_Python_swab
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
31.65	33.36	33.9
26.55	27.44	26.77
26.74	24.87	26.87
32.16	33.34	34.33
28.92	30.89	29.06
Neg	Neg	Neg
32.54	27.55	33.07
Neg	Neg	Neg
Neg	Neg	Neg
Neg	Neg	Neg
Neg	34.07	35.88
Neg	Neg	Neg
31.51	32.73	33.99
28.62	26.56	29.16
Neg	Neg	Neg
22.38	20.68	22.28
23.08	22.1	23.37
32.1	25.64	24.98
Neg	37.76	Neg
31.8	33.82	34.14
24.68	25.32	24.79
33.38	30.67	34.98
31.68	33.28	32.72
Neg	34.93	35.98
29.65	25.91	30.2
Neg	38.12	Neg
19.35	17.05	19.19
31.89	30.43	33.11
21.32	19.1	21.29
30.34	26.97	31.5
Neg	Neg	Neg
Neg	37.48	Neg
Neg	35.6	38.08
31.56	34.49	32.44
Neg	35.86	37.02
30.73	30.6	31.03

Neg		Neg		Neg			
	29.11		30.19		29.92		
	32		33.9		33.19		
	24.16		19.14		23.85		
Neg		Neg			36.86		
	34.25		32.26		34.87		
	33.4	Neg			36.63		
	35.91	Neg		Neg			
Neg		Neg		Neg			
	23.8		23.36		23.72		
	23.08		23.68		24.14		
	19.12		20.92		21		
	31		30.73		32.38		
	28.67		30.62		31.36		
	36.57	Neg			37.08		
	22.4		23.24		23.43		
	25.05		29.06		29.62		
	28.91		30.08		31.03		
Neg		Neg		Neg			
	32.9		29.33		29.93		
Neg		Neg		Neg			
	28.21		24.19		24.85		
Neg		Neg			36.64		
Neg			32.51		33.89		
Neg		Neg		Neg			
	25.04		28.05		28.55		
Neg		Neg		Neg			
	19.58		18.9		19.09		
	35.6		31.37		33.19		
	24.94		24.9		25.61		
Neg		Neg		Neg			
	33.83		32.88		34.7		
	34.06		34.46		35.7		
	20.53		25.99		26.54		
	33.01		35.27		36.4		
	26.01		23.52		24.11		
	32.86		31.46		33.96		
	17.89		19.25		19.71		
	25.26		24.23		24.67		
	20.52		20.89		21.52		
	35.2		32.57		35.06		
	21.53		21.75		22.33		
	37.58		35.19	Neg			

eFigure 1. Fluid release and absorption. a-b, Fluid absorption and release for the FLO-QSwab and 3DP swab for (**a**) universal viral transport media (UVTM) and (**b**) viscous fluid (25% w/v Pluronic F127 aqueous solution), tested using the vortex approach. **c-d,** Fluid absorption and release for the FLOQSwab and 3DP swab for (**c**) UVTM and (**d**) viscous fluid, tested using the roll plate approach.



eFigure 2. a-c, Categorical results comparing the 3DP swab and FLOQSwab (n = 40) by combined testing for both ORF1ab and E-gene targets, and for each target alone. **d-f,** Categorical results comparing the 3DP swab and Dacron swab, n = 39.

a

FLOQSwab	3DP swab: Combined testing	Positive	Negative
	Positive	29	3
	Negative	0	8

OA 0.925 (0.796 - 0.984)
PPA 0.906 (0.750 - 0.980)
NPA 1.00 (0.631 - 1.00)
Kappa 0.794

b

FLOQSwab	3DP swab: ORF1ab	Positive	Negative
	Positive	25	4
	Negative	0	11

OA 0.900 (0.763 - 0.972)
PPA 0.862 (0.683 - 0.961)
NPA 1.00 (0.715 - 1.00)
Kappa 0.774

c

FLOQSwab	3DP swab: E-gene	Positive	Negative
	Positive	29	3
	Negative	0	8

OA 0.925 (0.796 - 0.984)
PPA 0.906 (0.750 - 0.980)
NPA 1.00 (0.631 - 1.00)
Kappa 0.794

d

Dacron swab	3DP swab: Combined testing	Positive	Negative
	Positive	29	1
	Negative	3	6

OA 0.897 (0.758 - 0.971)
PPA 0.967 (0.828 - 0.999)
NPA 0.667 (0.299 - 0.925)
Kappa 0.689

e

Dacron swab	3DP swab: ORF1ab	Positive	Negative
	Positive	26	3
	Negative	2	8

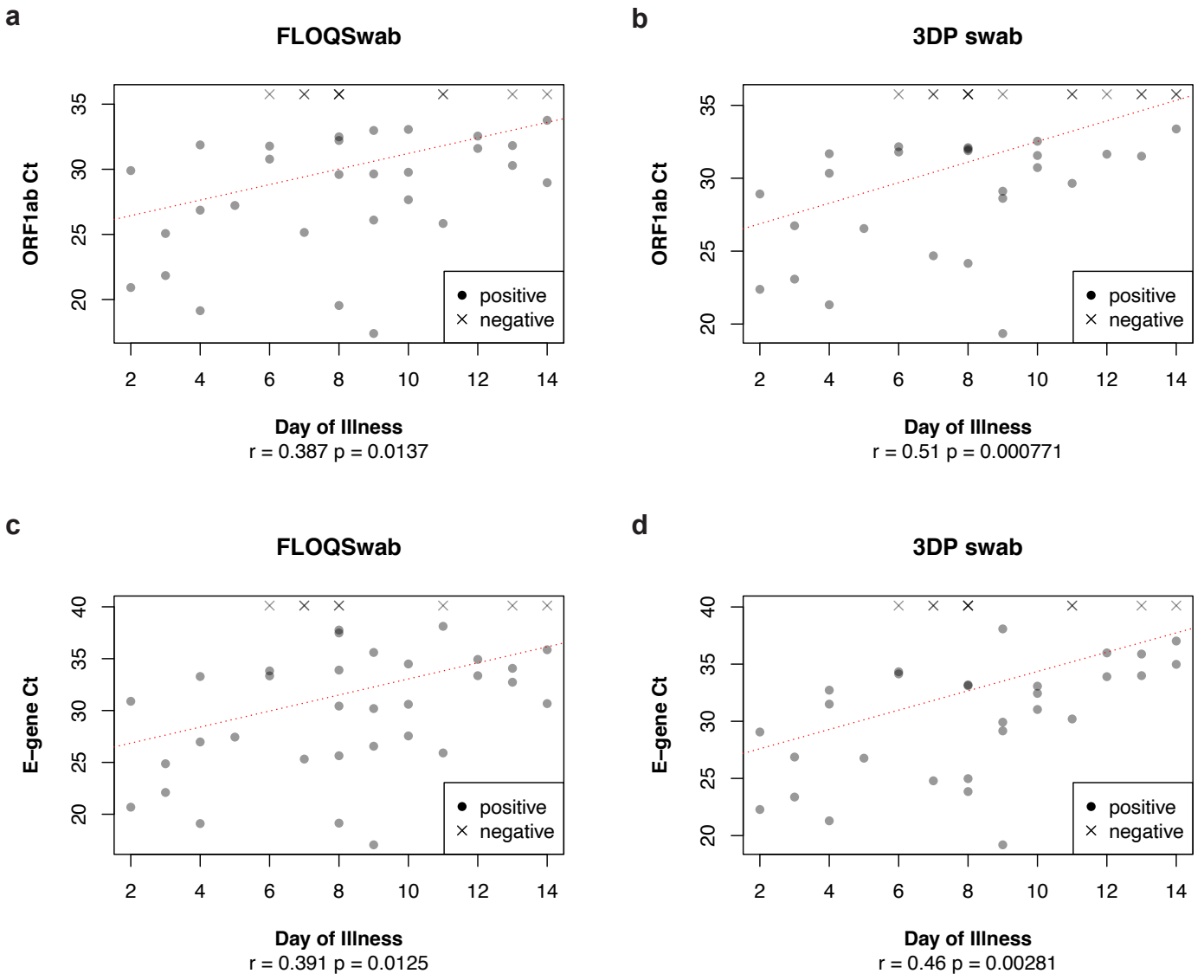
OA 0.872 (0.726 - 0.957)
PPA 0.897 (0.726 - 0.978)
NPA 0.800 (0.444 - 0.975)
Kappa 0.674

f

Dacron swab	3DP swab: E-gene	Positive	Negative
	Positive	28	2
	Negative	3	6

OA 0.872 (0.726 - 0.957)
PPA 0.933 (0.779 - 0.992)
NPA 0.667 (0.299 - 0.925)
Kappa 0.624

eFigure 3.
a-b, Ct values and day of illness for the ORF1ab target for the FLOQSwab and the paired 3DP swab. **c-d,** Ct values and day of illness for the E-gene target for the FLOQSwab and the paired 3DP swab. n = 40, red lines represents line of best fit in a linear model.



eFigure 4. Bland-Altman plots for the comparison of Ct values between paired positive swabs for the ORF1ab and E-gene when comparing the 3DP swab with the FLOQSwab (**a,b**) and the Dacron swab (**c,d**). The horizontal axis shows the mean Ct value for each pair of swabs, while the vertical axis shows the difference in Ct value between the swabs (Ct value for 3DP swab - Ct value for reference swab). The mean difference in Ct value is represented by the blue bar and the shaded area represents the boundaries of the 95% confidence interval.