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Detection of SARS-CoV-2 virus in saliva samples: lateral flow immunoassay prototype development

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Background

Global SARS-CoV-2 pandemic confirms the importance of diagnostic testing in preventing the transmission of the infection.

Although RT-PCR is the gold standard in SARS-CoV-2 diagnostics, the **development and implementation of easy-to-perform diagnostic methods are of priority.**

Aim of the study: to develop lateral flow assay (LFA)-based test prototype for the qualitative detection of SARS-CoV-2 proteins in saliva samples.

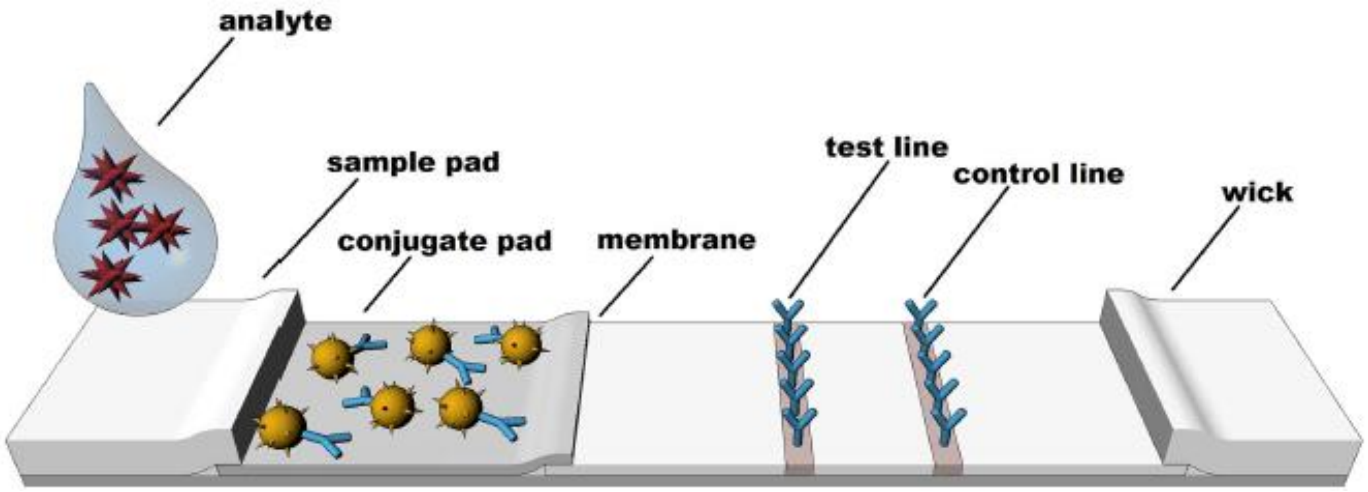


Fig. 1 LFA assay principle (Miocevic et al. 2017)

Methods

Mice immunization

- Polyclonal antibodies (PABs) against recombinant receptor-binding domain (rRBD) **(A)**

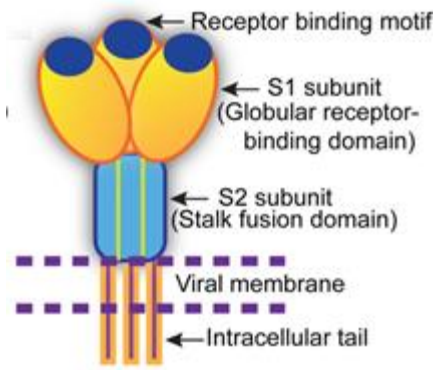


Fig. 2 SARS-CoV-2 spike (S) protein (Mittal et al. 2020)

Sandwich ELISA

- Selection of antibody pairs targeting epitopes of the SARS-CoV-2 spike protein

		Capture antibody				
		A	B	C	D	E
Detection antibody	A	x	x	x		
	B	x	x	x		
	C	x	x	x		
	D				x	x
	E				x	x

Commercial antibodies:
mouse anti-spike S1 MABs (B),
mouse anti-RBD MABs (C),
rabbit anti-spike PABs (D),
rabbit anti-spike/RBD PABs (E)

LFA concept

- Artificial spike antigen (rS1/S2) spiked in blank matrix
- Saliva samples from individuals with COVID-19 symptoms and/or confirmed infection

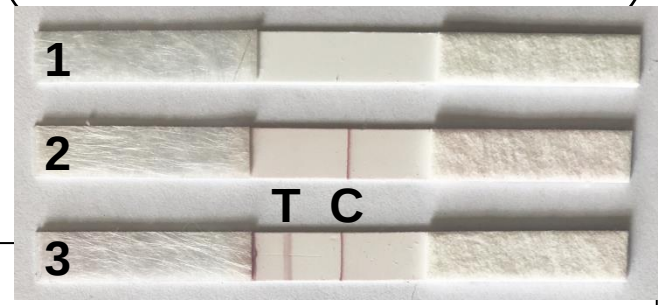
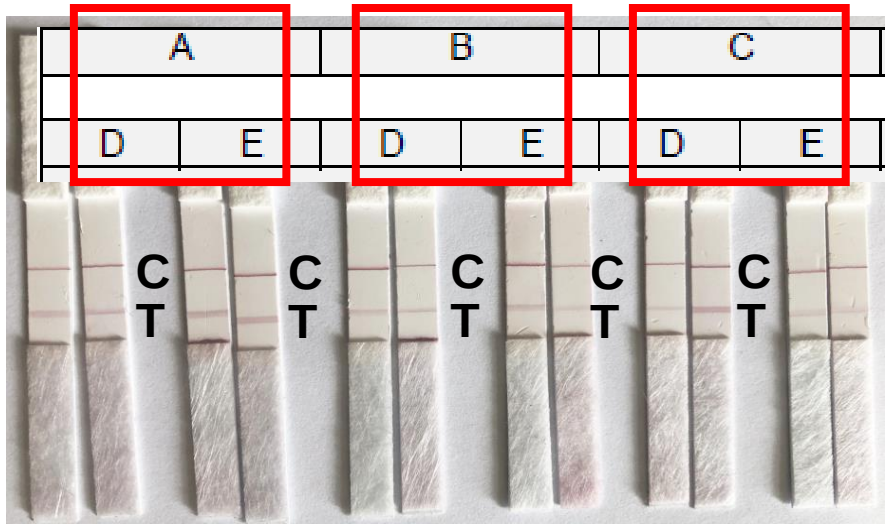


Fig. 3
Representative LFA test strips: test line (T), control line (C); unused strip (1), negative result (2), positive result (3)

Results

rS1/S2 protein concentration (µg/mL)	Capture antibody: A		B		C		D			E		
	Detection antibody: D	E	D	E	D	E	A	B	C	A	B	C
	Measured absorption (A_{450nm})											
10,0	3,637	2,655	3,725	2,199	2,845	2,502	1,805	0,461	0,511	2,248	0,554	0,503
5,00	3,236	2,105	2,904	1,140	2,791	1,440	1,521	0,379	0,376	1,781	0,384	0,412
2,50	2,566	1,816	1,490	0,565	1,769	0,817	1,476	0,318	0,343	1,734	0,264	0,236
1,25	**1,662**	**1,243**	**0,967**	**0,276**	**0,878**	**0,353**	**0,938**	**0,238**	**0,219**	**1,206**	**0,165**	**0,167**
0,63	1,052	0,488	0,354	0,182	0,526	0,167	0,692	0,164	0,150	0,832	0,091	0,092
0,31	0,530	0,360	0,189	0,224	0,279	0,105	0,481	0,101	0,096	0,425	0,069	0,070
0,16	0,335	0,193	0,211	0,126	0,168	0,067	0,349	0,095	0,087	0,248	0,042	0,050

Fig. 4 Sandwich ELISA results



In-house produced antibodies:
mouse anti-rRBD PABs (A)
Commercial antibodies:
mouse anti-spike S1 MABs (B)
mouse anti-RBD MABs (C)
rabbit anti-spike PABs (D)
rabbit anti-spike/RBD PABs (E)

Fig. 5 Performance of the selected antibody pairs in LFA format

Sample No.	LFA result	RT-PCR result
1.1.	-	-
1.2.	+	+
2.	+	+
3.	+	+
4.	-	+
5.	+	+
6.	+	+
7.	+	-
8.	+	+
9.	-	+
10.	+	-
11.	-	+

Table 1 Clinical sample analysis. Comparison of LFA and RT-PCR results

Conclusions

- After passing the validation procedure, the LFA prototype developed in this study has the potential to be applied in screening programs for rapid detection of SARS-CoV-2 in acute patients with high viral loads.
- The advantage of this assay is the use of easy-to-collect saliva as a testing material.

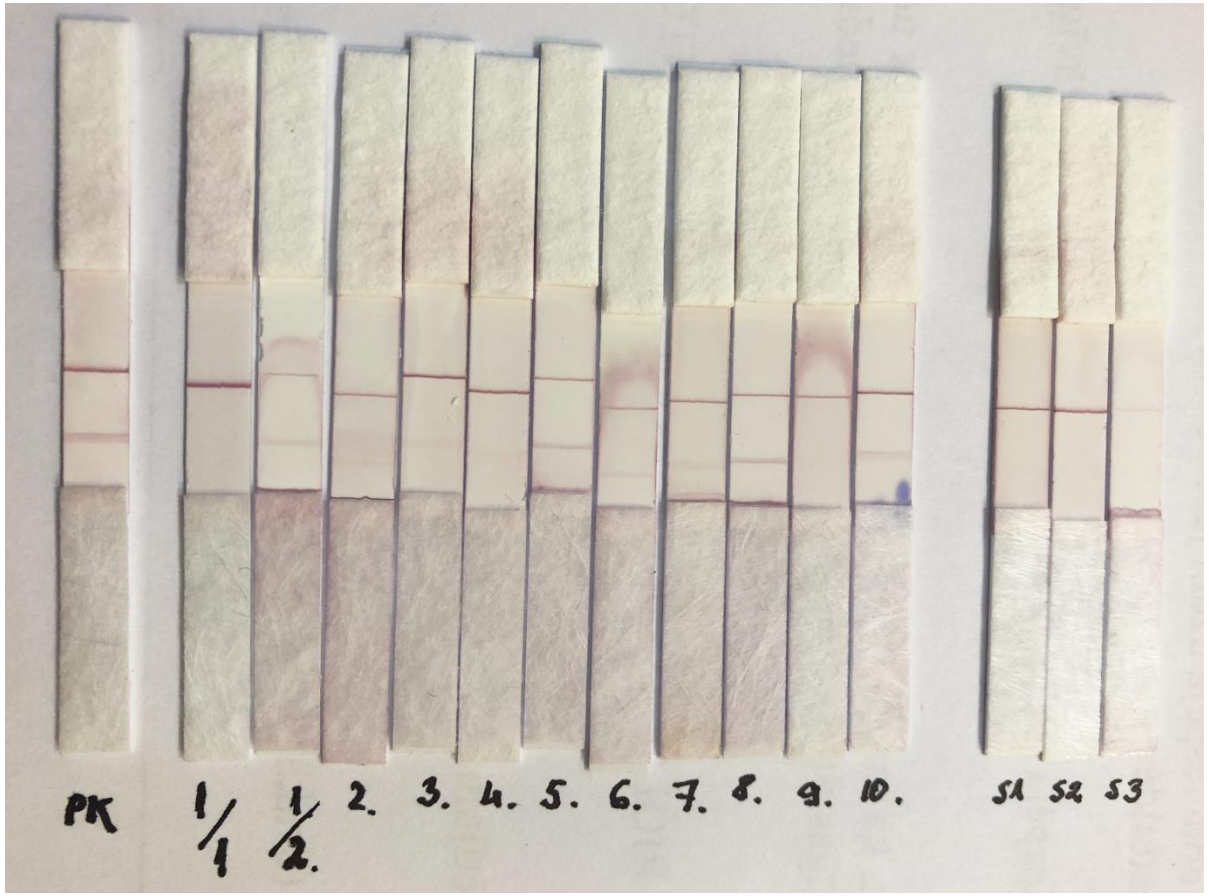


Fig. 6 LFA test strip set of the clinical sample analysis (1-10); PK – positive control; S1, S2, S3 – negative control

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