Performance of Lateral Flow Assays for SARS-CoV-2 compared to RT-qPCR

Supplementary Material

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Supplementary materials 1. Detailed description of Methods

Methods

Study setting

All clinical specimens were anonymized nasopharyngeal swabs performed by trained personnel and obtained during routine diagnostic testing at the University Hospital Krems between May and November 2021. Due to anonymization, correlation of the results to sex, age or medical conditions of the tested individuals was not possible. We selected 119 PCR-positive and 55 PCR-negative samples delivered in universal transport medium (UTM-RT, Copan, Italy), which is commonly used for the evaluation of LFAs (Kobayashi et al., 2021; Porte et al., 2020; Weitzel et al., 2021).

Aliquots of 100 µL of each sample were directly pipetted onto the sample pad of each LFA. All tests were performed in parallel using aliquots of the same samples. Samples were stored for a maximum of 48 h at 4 °C before performing the LFA. The test lines were evaluated by visual inspection.

Lateral flow assays

We selected the LFAs based on their common distribution among hospitals, care facilities for elderly, pharmacies, governmental test facilities, and general practitioners in Austria. The following tests were used: Abbott (Panbio, Abbott Rapid Diagnostics Jena GmbH, Jena, Germany), Acon (Flowflex, Acon Laboratories Inc., CA, USA), Clungene (Hangzhou Clungene Biotech Co., Ltd., Tianjin, China), Joysbio (Joysbio (Tianjin) Biotechnology Co., Ltd., Tianjin, China), Lepu Medical (Beijing Lepu Medical Technology Co., Ltd., Peking, China), Nadal (nal von minden GmbH, Moers, Germany), Orient (Zhejiang Orient Gene Biotech Co., Ltd., Zhejiang, China), Realy Tech (Hangzhou Realy Tech Co., Ltd., Zhejiang, China), Roche (SD Biosensors, Gyeonggi-do, Republic of Korea), Siemens (Clinitest, Healgen Scientific Ltd., TX, USA).

RT-qPCR setup

Samples were subjected to routine RT-qPCR testing before and after applying the samples to LFAs. First, RNA was extracted using the NucleoSpin 96 Virus kit (Macherey-Nagel, Düren, Germany) using a vacuum chamber system (Macherey-Nagel) according to the manufacturer's manual. Subsequently, extracts were tested for the presence of the E- and S- gene using the CE-IVD RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) according to the instructions provided by the manufacturer. The lowest cT-value of the RT-qPCR performed after analysis by LFA was used for data analysis.

Data Analysis

Data analysis and graphic design were performed using R (Version 4.1) and the tidyverse package. Binomial logistic regression analysis was applied to model the probability of obtaining a positive LFA result depending on the cT-value. In order to integrate negative PCR-negative samples into the analysis, we imputed a cT-value of 40 for PCR-negative tests. Additionally, we provide a sensitivity analysis in which we exclusively considered samples with PCR-positive samples. We estimated the confidence intervals (CI) via nonparametric bootstrapping with 100.000 iterations. We plotted the predicted probability for a positive test result and the 95% CI depending on the cT-value (ranging from the lowest cT-value of our dataset to up to 40) for each test.

Finally, we provide the sensitivity and specificity of each LFA across all samples, as well as the sensitivity for a subset of samples with cT-values < 25. Sensitivity was calculated by dividing the sum of correct positive LFAs results by the sum of PCR-positive samples for each LFAs. Specificity was calculated by dividing the sum of correct negative LFA results by the sum of PCR-negative samples for each LFA.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Supplementary materials 2. Figures and Tables

Supplementary Table S1. Diagnostic performance of the Lateral Flow Assays used in this study.

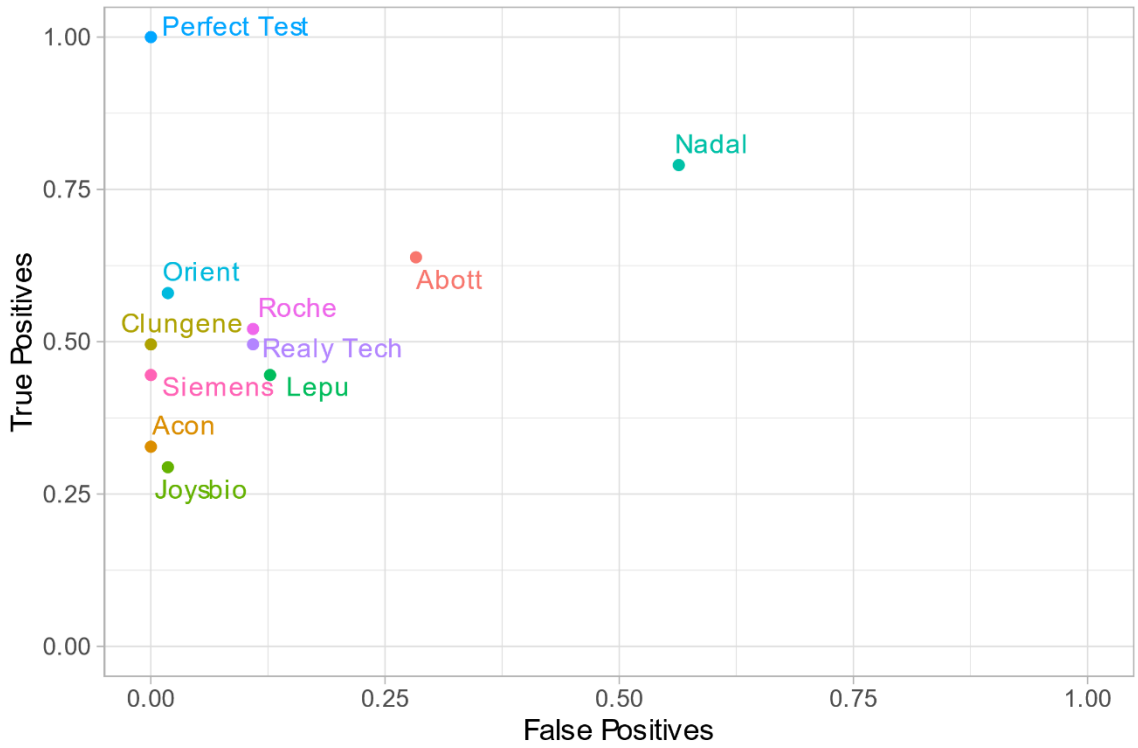
Company	n	TP	FP	FN	TN	Sensitivity	Specificity	PPV	NPV	Accuracy
Abbott	172	76	38	15	43	64	72	84	47	66
Acon	174	39	55	0	80	33	100	100	41	54
Clungene	169	57	54	0	58	50	100	100	48	66
Joysbio	174	35	54	1	84	29	98	97	39	51
Lepu	174	53	48	7	66	45	87	88	42	58
Nadal	174	94	24	31	25	79	44	75	49	68
Orient	174	69	54	1	50	58	98	99	52	71
Realy Tech	174	59	49	6	60	50	89	91	45	62
Roche	174	62	49	6	57	52	89	91	46	64
Siemens	174	53	55	0	66	45	100	100	45	62

TP = true positive, FP = false positive, FN = false negative, TN = true negative, PPV = positive predictive value, NPV = negative predictive value.

Supplementary Table S2. Diagnostic performance of the Lateral Flow Assays in samples with a cT-value < 25

Company	n	TP	FN	Sensitivity
Abbott	47	42	5	89
Acon	47	36	11	77
Clungene	47	43	4	91
Joysbio	47	32	15	68
Lepu	47	36	11	77
Nadal	47	44	3	94
Orient	47	40	7	85
Realy Tech	47	42	5	89
Roche	47	40	7	85
Siemens	47	40	7	85

TP = true positive, FN = false negative.



Supplementary Figure S1. Sensitivity and specificity of the Lateral Flow Assays used in this study.