Validation Report: SARS-CoV-2 Antigen Rapid Diagnostic Test "2019-nCoV Antigen Test" by Guangzhou Wondfo Biotech Co., Ltd, China

Please find below the summary of the results of our SARS-CoV-2 antigen rapid diagnostic test validation for the "**2019-nCoV Antigen Test**" by **by Guangzhou Wondfo Biotech Co., Ltd, China**, performed by biolytix AG, Witterswil, Switzerland. Executed between the 5th of January 2021 and 28th of January 2021. During the study period a total of 400 nasopharyngeal samples were tested (100 positve and 300 negative). All Antigen Rapid Diagnositc Test results were read after the time indicated by the manufacture. Reference methods for comparison were cycle threshold (Ct) values of the current diagnostic routine SARS-CoV-2 RT-PCR assay (Diagnostic detection of 2019-nCoV by real-time RT-PCR -Protocol and preliminary evaluation as of Jan 17, 2020; Charité Virology, Berlin, Germany) and COVID-19 Rapid Antigen Test from SD Biosensor/Roche.

Assay name: 2019-nCoV Antigen Test
Assay Lot Number: W19601202
Producer: Guangzhou Wondfo Biotech Co., Ltd, China
Distributor: Hammer und Partner GmbH, Hauptstrasse 36, 8840 Einsiedeln

Summary

The "2019-nCoV Antigen Test" has passed the validation criteria according to the "Verordnung 3 vom 19. Juni 2020 über Massnahmen zur Bekämpfung des Coronavirus (Covid-19) (Covid-19-Verordnung 3); Anhang 5a: Kriterien für die technische Validierung von Sars-CoV-2-Antigen-Schnelltests aus Nasen-Rachen-Abstrich». At Ct values of 23, 26 and 29, the "2019-nCoV Antigen Test" assay showed a technical sensitivity of 97%, 90% and 87% compared to the reference standard showing a technical sensitivity of 99%, 97.9% and 97%, respectively. The technical sensitivity was 100%.

Methods

The technical performance was validated in 100 PCR positive and 300 PCR negative samples and in a serial dilution against a reference standard to determine and compare the diagnostic limits of detection. Nasopharyngeal swabs were taken and submerged in viral transport medium (Virus Collection and Preservation System from Kangjiang, China). Swab and viral transport medium were vortexed. 100 µl of the viral transport medium were applied in the 2019-nCoV Antigen Test from Guangzhou Wondfo Biotech Co., Ltd, China, 100 µl of the viral transport medium were applied in the COVID-19 Rapid Antigen Test from SD Biosensor/Roche and 100 µl for RNA extraction for the real time PCR.

The challenged test was the "2019-nCoV Antigen Test". Lot number: W19601202

The reference standard was the Standard Q, COVID-19 Rapid Antigen Test from SD Biosensor/Roche, reference lot number QCO3020081 / Sub:A-4. The PCR System was the the RdRP gene published in: Diagnostic detection of 2019-

nCoV by real-time RT-PCR -Protocol and preliminary evaluation as of Jan 17, 2020; Charité Virology, Berlin, Germany. As a RNA extraction control and PCR control, the human RNase gene was amplified. All measurements were conducted on the real time PCR platform ABI 7900 in double. Master Mix was from Thermo Fisher: Applied Biosystems – TaqMan Fast Virus 1-Step Master Mix, Ref 4444436, Lot 00981925.

Minimal acceptance criteria to successfully pass the validation (according to the Covid-19-Verordnung 3):

Cumulative sensivity: 10'000'000 copies/ml: 95%

Cumulative sensivity: 1'000'000 copies/ml: 90%

Cumulative sensivity: 100'000 copies/ml: 80%

Specificity: 99%

To test the sensitivity, the viral load per reaction was determined by real time RT PCR. This means, that:

10'000'000 copies/ml had a Ct value of 23

1'000'000 copies/ml had a Ct value of 26

100'000 copies/ml had a Ct value of 29

Results

Interpretation of technical sensitivity and specificity

Technical sensitivity at Ct 23, Ct 26 and Ct 29 as well as the overall specificity is shown in Table 1. Figure 1 shows the percentage of antigen positivity in relation to Ct values over a range of 100 PCR positive clinical samples. To detect the limit of 99% of PCR positive samples, the "2019-nCoV Antigen Test" and the reference standard required a Ct value of 29.

300 samples were tested by real time PCR negative, as well by the reference and "2019-nCoV Antigen Test".

Table 1: Technical sensitivity and specificity, expressed in percentage. For sensitivities at Ct 23, 26 and 29 a threshold of 95%, 90% and 80% must be reached. Overall specificity needed to be at least 99%.

	Sensitivity			Specificity
	Ct 23	Ct 26	Ct 29	
Reference	99.0%	97.9%	97.0%	100%
Guangzhou Wondfo Biotech	97.0%	90.0%	87.0%	100%

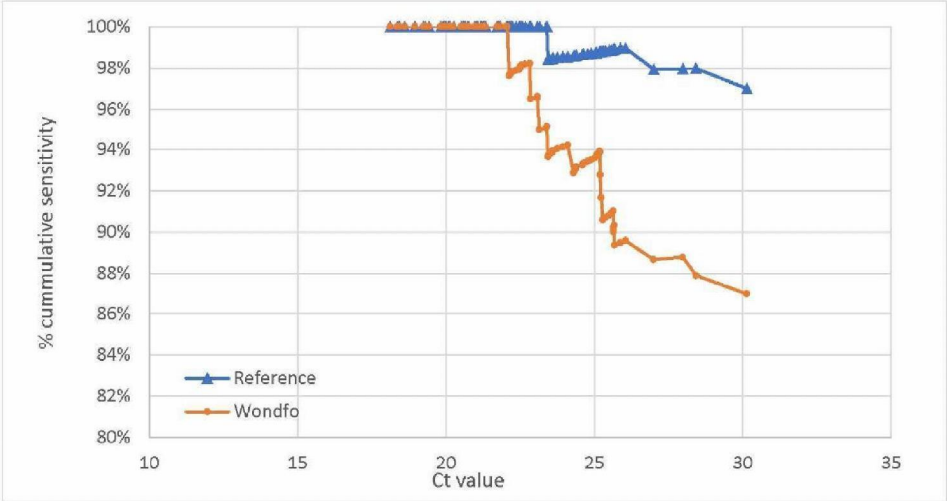

Figure 1: Percentage of antigen positivity compared to Ct values of samples.

The median Ct values in antigen positive samples were 22.2 for the "2019-nCoV Antigen Test" assay and 22.5 for the reference standard, shown in Figure 2.

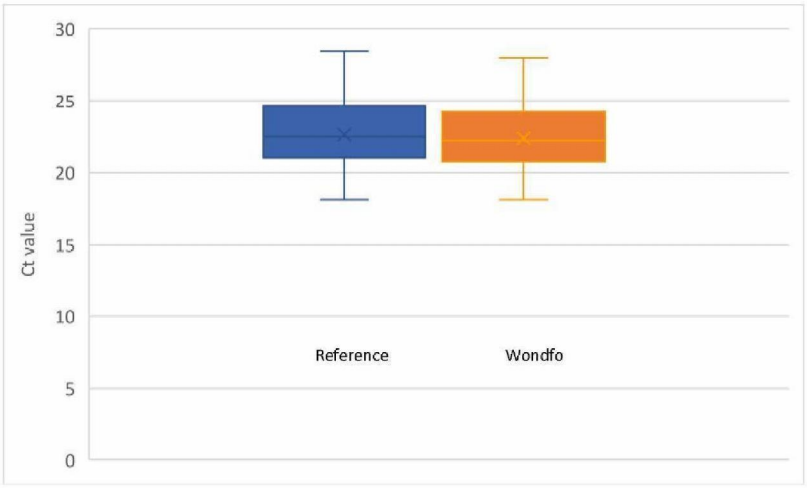

Figure 2: Ct values of SARS_CoV-2 specific antigen positive tested samples. Boxes show median and interquartile range, whiskers show the 10-90th percentile.

Samples tested from serial dilution

The serial dilution from a positive sample indicates that "2019-nCoV Antigen Test" has a higher sensitivity than the reference assay. The minimal positive of Ct 24.9 has been reached (see Table 2)

Table 2: serial dilution of a positive sample in a back-to-back comparison. + = clear positive reaction, (+) = faint band, - = negative. Green shade indicates the range within a test must be positive.

Ct	22.70	23.6	24.3	24.9	26.4	27.3	27.9
Wondfo	+	+	+	+	(+)	-	-
Reference	+	+	+	-	-	-	-