



# Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

## Original article

### Evaluating the performance of two rapid antigen detection tests in diagnosis of SARS- COV- 2 infection

Yasmin A. El Mahdy \*, May S. Soliman, Sahar M. Khairat, Nashwa M. Reda, Nermeen A. El desouky, Noha S. Soliman

Clinical and Chemical Pathology Department, Faculty of Medicine, Cairo University.

#### ARTICLE INFO

##### Article history:

Received 1 January 2022

Received in revised form 26 January 2022

Accepted 4 February 2022

##### Keywords:

SARS-CoV-2

Detection

Antigen assays

RT-PCR

#### ABSTRACT

**Background:** Rapid antigen detection tests for SARS-CoV-2 infection could promote the clinical and public health policies to handle the COVID-19 pandemic. Rapid antigen detection and molecular approaches could expand entry to checking and initial evidence of issues and playing an essential role in public health managing choices that may decrease the transmission. **Objectives:** We evaluated the diagnostic accurateness of couple of rapid antigen recognition tests equated with the molecular-based assays for verdict of SARS-CoV-2 infection. **Methods:** The 100 nasopharyngeal swabs were verified by the SARS-CoV-2 RT-PCR kit as a gold standard for COVID-19 recognition. SARS-CoV-2 antigen (Ag) was evaluated in the nasopharyngeal swabs using iFlash and UNICELL-2019-nCoV antigen methods. The iFlash-2019-nCoV antigen assay, which is a chemiluminescent immunoassay (CLIA), was used to qualitatively determine the nucleocapsid protein antigen, where the other one was used to identify the nucleocapsid protein antigen by lateral flow immunofluorescent test. **Results:** Out of the 100 samples, 62% were positive by RT-PCR. Amongst 62 confirmed COVID-19 cases, 43 (69.4%) were positive by iFlash and 40 samples (64.5%) were positive by the UNICELL-2019-nCoV antigen assay. The specificity of both iFlash-2019-nCoV antigen assay & UNICELL-2019-nCoV antigen assay with RT-PCR were 100% and sensitivity were 69.35 and 64.52%, respectively. This sensitivity was augmented to 100% compared with the PCR with Ct-value of  $\leq 25$  and specificity of 80.28 and 84.51%, respectively. **Conclusion:** Antigen detection rapid diagnostic tests may be motivating in the initial stage of the infection when the viral load is elevated, and the risk of SARS-CoV-2 transmission be high.

#### Introduction

The existing pandemic of coronavirus disease 2019 (COVID-19), which is instigated by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has disseminated swiftly all over the world. As of December 13, 2021, SARS-CoV-2

has diseased 270,458,029 people and caused more than 5,322,978 deaths [1].

Rapid and reliable laboratory diagnosis of COVID-19 along with timely isolation of infectious and infected cases are the foremost key tools for prevention and containment of ongoing community spread throughout a pandemic. The diagnostic

assays for COVID-19 included both molecular and immunological approaches. The molecular checks detected the RNA of SARS-CoV-2, mostly in the nasopharyngeal examples, using nucleic acid augmentation techniques (NAAT) and primarily real time polymerase chain reaction (PCR). Meanwhile, the immunological tests could measure blood's antibodies and/or viral antigens in the respiratory oozes [2,3].

Real time PCR (RT-PCR) is still the key standard and most frequently utilized indicative test in the clinical microbiological laboratories to diagnosis COVID-19. However, it calls for specialized instruments and proficiency, alongside with many shortages of RT-PCR reagents in different countries [4-6].

Antigen detection rapid diagnostic tests (Ag-RDTs) have emerged as supplementary screening tests which could deal with these challenges. Recently, WHO has presented target adduct profiles for such analysis. The most chosen target is the viral nucleocapsid protein, mostly owing to their high abundance in the clinical samples. Ag-RDTs use immune-based technologies as lateral flow sandwich, immunofluorescence, and chromatographic digital immunoassays, with several advantages of simple performance and interpretation, short turnaround time, low cost but less sensitive than NAAT. The WHO, therefore, suggested the utilization of SARS-CoV-2 antigen analyses if NAAT is unavailable and/or when the long reversal times disqualify the clinical usefulness, and within the first 5-7 d ensuing the symptoms onset [7-10].

We, herein, estimated the implementation of two Ag-RDTs, the UNICELL-2019-nCoV antigen test and iFlash-2019-nCoV antigen assay. The iFlash-2019-nCoV antigen assay (YHLO, China) is a paramagnetic particle chemiluminescent immunoassay (CLIA) for a qualitative determination of the nucleocapsid protein antigen in nasopharyngeal (NP) and nasal (NS) swab specimens using the iFlash Immunoassay Analyzer. The UNICELL-2019-nCoV antigen assay (YHLO, China) detects the nucleocapsid protein antigen by lateral flow immunofluorescent sandwich assay.

## Materials and Methods

### Specimens

Nasopharyngeal swab specimens were collected from SARS-CoV-2 supposed patients admitted to the molecular laboratory at El Kasr El Aini hospitals

(Cairo University, Cairo, Egypt), between April-July 2021. We retrospectively tested 100 PCR-positive clinical samples from 100 different patients for the Ag-RDTs test. The study was agreed and approved by the research ethical committee of clinical and chemical pathology department.

### RT-PCR

The viral RNA was automatically extracted using a chemagic instrument (Perkin Elmer, Hamburg, Germany). The VIASURE SARS-CoV-2 RT-PCR kit (CerTest Biotech SL, Zaragoza, Spain) was utilized to detect SARS-CoV-2 in the nasopharyngeal samples. It is a one-step RT-PCR (applied biosystems 7500 RT-PCR System) targeting the *ORF1ab* and *N*-gene of SARS-CoV-2. In accordance with the manufacturer's guidelines, at first 15 µL of the provided rehydration buffer was added to each well and then 5 µL of the extracted RNA, positive and negative control were added. Thermal cycling was done at 45 °C for 15 min reverse transcript, monitored by 95 °C for 2 min initial denaturation, and then 45 cycles of 95 °C for 10 s denaturation, and 60 °C for 50 s annealing. The Applied Biosystems 7500 Real-Time PCR System was used.

### Rapid antigen detection tests

In the present study we used iFlash-2019-nCoV antigen assay (SHENZHEN YHLO BIOTECH CO., LTD., China) which is a paramagnetic particle chemiluminescent immunoassay (CLIA) for qualitative detection of SARS CoV2 nucleocapsid protein antigen using the iFlash Immunoassay Analyzer. Nasal swab was inserted into an isolation reagent tube provided with the kit, carefully plunged up, and down in the fluid for at least 25 s based on the manufacturer's guidelines. The swab was, then, removed while pressing the tube sides to excerpt the swab's liquid. Specimens were cold stored at 2-8°C for no longer than 4 h. The specimens were centrifuged for 5 min from 2000-4000 rpm and then loaded into I FLASH analyzer. The results were interpreted as either reactive ( $\geq 5$  pg/mL) or non-reactive ( $< 5$  pg/mL).

The second SARS CoV2 antigen assay used in our study was YHLO UNICELL -2019-nCoV Antigen assay (SHENZHEN YHLO BIOTECH CO., LTD., China) which is a Lateral Flow Immunofluorescent Sandwich Assay) for detection of SARS CoV2 nucleocapsid protein antigen. The swabs were inserted inside the provided extraction tube and vertically swung inside the buffer for  $> 15$  s based on the manufacturer's guidelines. The extraction tube was, then, squeezed and the drained swab was removed. After even mixing, 3 drops of the mixing solution were added to the card. The card reading

takes 15 min. As referred to UNICELL YHLO manufacturer, the COI (cut-off index)  $<1.0$  is interpreted as negative and  $\text{COI} \geq 1.0$  as positive.

### Statistical analysis

Data were coded and inserted *via* SPSS, ver. 26 (IBM Corp., Armonk, NY, USA). Data were then outlined by employing interquartile range and median in quantitative data and by employing relative frequency (percentage) and frequency (count) for the categorical data. The quantitative variables were compared *via* non-parametric Kruskal-Wallis and Mann-Whitney tests [11]. Chi square ( $\chi^2$ ) test was done to equate the categorical data. The precise test was utilized rather than the predictable frequency is  $<5$  [12]. Relationships among quantitative variables were achieved by the Spearman correlation coefficient [13]. Standard diagnostic indicators, namely sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic efficacy, were expressed [14]. *p*-values  $<0.05$  were measured as statistically meaningful.

### Results

There were 100 serum examples collected from COVID-19 patient in Cairo University Hospital (Kasr El-Ainy). The total 100 nasopharyngeal samples were analyzed by gold standard RT-PCR assay, as a national guide line for laboratory diagnosis of COVID-19. It is a one-step RT-PCR targeting the *ORF1ab* and *N*-gene of SARS-CoV-2. The negative RT-PCR results were described as having Ct value  $>38$  for all two target genes (N and ORF).

### Descriptive analysis of our results

From 100 samples, 62% were positive for both genes with Ct-value of  $\leq 38$ . Amongst the 62 confirmed COVID-19 cases, 43 samples (69.4%) were positive by iFlash-2019-nCoV CLIA and 40

samples (64.5%) were positive by the UNICELL-2019-nCoV Antigen assay with a statistical agreement and significant *p*-value  $<0.001$  (Table 1).

### Performance of SARS-CoV-2N Protein Antigen by iFlash chemiluminescent immunoassay and UNICELL-2019-nCoV Antigen assay

The comparison between I Flash-2019-nCoV antigen assay with RT-PCR showed a specificity of 100% (95% Confidence interval CI: 90.75-100.00%), and sensitivity 69.35% (95% CI: 56.35-80.44%). The PPV and NPV were determined as 100 and 66.67% (57.90 to 74.41%), respectively.

The comparison between UNICELL-2019-nCoV antigen assay with RT-PCR showed a specificity 100%, whereas the sensitivity was calculated as 64.52% with CI of 51.34 to 76.26%. This assay had a PPV of 100% and NPV of 63.33% with CI of 55.25 to 70.73% (Table 2).

The sensitivity of iFlash-2019-nCoV antigen assay & UNICELL-2019-nCoV antigen assay were increased to 100% (95% CI :88.06% TO 100.00%) when compared to PCR positive samples with Ct value  $\leq 25$  and specificity 80.28% (95% CI :69.14% to 88.78%), (73.97% to 92.00%) respectively.

### The correlation between the antigen level by I Flash and UNICELL to the Ct-value groups

The SARS-COV19 antigen level in the samples detected by both iFlash-2019-nCoV antigen immunoassay & UNICELL-2019-nCoV antigen assay were correlated with the cycle threshold (Ct) value of RT-PCR. A strong significant relationship with negative correlation coefficient was found (-0.864 & -0.716) and *p*-value  $<0.001$ , respectively between the Ct-value and the level of N-antigen detected (Tables 3).

**Table 1.** Explanation of the results of both I Flash and UNICELL in relation to RT-PCR

		PCR genes				<i>p</i> -value
		+ve		-ve		
		Count	%	Count	%	
<b>Results of I Flash</b>	+ve	43	69.4%	0	0.0%	$< 0.001$
	-ve	19	30.6%	38	100.0%	
<b>Results of UNICELL</b>	+ve	40	64.5%	0	0.0%	$<0.001$
	-ve	22	35.5%	38	100.0%	

**Table 2.** The performance accuracy of both I Flash and UNICELL in relation to RT-PCR.

Statistic	UNICELL		iFlash	
	Value (%)	95% CI (%)	Value (%)	95% CI (%)
Sensitivity	64.52	51.34 to 76.26	69.35	56.35 to 80.44
Specificity	100.00	90.75 to 100.00	100.00	90.75 to 100.00
Positive Predictive Value	100.00		100.00	
Negative Predictive Value	63.33	55.25 to 70.73	66.67	57.90 to 74.41
Accuracy	78.00	68.61 to 85.67	81.00	71.93 to 88.16

**Table 3.** The relation between level of antigen by iFlash, UNICELL, and the Ct-groups.

		Ct-groups						p-value
		15-20	20-25	25-30	30-35	35-40	>40	
Results of I Flash	Median	3213.60	1013.63	63.13	2.33	0.22	0.21	< 0.001
	1 <sup>st</sup> quartile	1243.60	168.26	11.20	0.38	0.18	0.18	
	3 <sup>rd</sup> quartile	15424.69	9341.38	518.56	9.76	0.38	0.25	
Results of UNICELL	Median	87.45	24.46	2.38	0.42	0.43	0.48	< 0.001
	1 <sup>st</sup> quartile	19.74	5.55	0.94	0.26	0.30	0.38	
	3 <sup>rd</sup> quartile	192.31	145.24	23.96	0.75	0.62	0.63	

## Discussion

It is needed to utilize the appropriate diagnostic analysis for SARS-CoV-2 in the existing ongoing COVID-19-pandemic to control the virus scattered and properly handle COVID-19 patients. The employment of RDTs in the identification of COVID-19 could have considerable advantages by improving the efficacy of huge testing tactics [15]. Corona virus disease-19 RDTs detect either SARS-CoV-2 antigen in respiratory specimen or anti-SARS-CoV-2 antibodies in the whole blood, plasma, and/or serum.

Rapid diagnostic tests are useful devices that facilitate testing outside of laboratory settings, a capability needed for hard to reach populations [16]. Additionally, RTDs convey speed results than RT-PCR. This keeping time is a vital for the

detection, separation, and facility of suitable clinical nursing to COVID-19-patients. Rapid diagnostic tests also decrease the overworks in emergency circuits [17]. Rapid antigen immunoassays with corresponding sensitivity and specificity to RT-PCR methods will aid to accelerate the disease examination. Herein, we evaluated the performance of two Ag-RDTs, the UNICELL -2019-nCoV antigen, and iFlash-2019-nCoV antigen assays equated with RT-PCR for the recognition of SARS-CoV-2 infection.

By evaluating the iFlash which is a chemiluminescence assay for detecting SARS CoV2 antigen against gold standard RT-PCR, it showed a specificity of 100% (95% CI: 90.75 to 100.00% and sensitivity 69.35% (95% CI: 56.35 to 80.44%). Our

findings showed lower sensitivity than that of the results of **Qiaoling et al.**, in which a total of 914 serum samples were utilized to quantify N-protein antigen quantities by iFlash-2019-nCoV antigen. The author found that the sensitivity and specificity of serologic N-protein antigen were 76.27 and 98.78%, separately [18]. This disparity may be due to the variation in the sample type, where we used a nasopharyngeal swab instead of serum sample. The sampling timing in relation to the symptoms play a key role in antigen levels which was missed in our study as it was retrospectively done on the collected samples.

As regard SARS CoV2 antigen detection by UNICELL lateral immunofluorescence assay, the sensitivity and specificity were reported by the manufacturer as 85.3% and 100%, respectively in a study done on 249 direct nasopharyngeal swabs. The sensitivity and specificity of this test were evaluated in our study, and we found that the specificity was 100%, whereas the sensitivity was calculated as 64.52% with CI: (51.34 to 76.26%). Our results showed lower sensitivity (64.52% vs 85.3%) and the same specificity. The difference in our results could be due to several factors namely the lower number of tested samples. The collection of clinical specimens might have lower viral load (high Ct-value) compared with that of the manufacturer's samples.

The sensitivity of iFlash-2019-nCoV antigen assay & UNICELL-2019-nCoV antigen assay were increased to 100% when compared to PCR positive samples with Ct value  $\leq 25$  and specificity was 80.28%, this mean that sensitivity was high in those with high viral load samples.

In a recent review done by **Dinnes et al.**, to estimate the indicative accurateness of Ag-RDTs and molecular-based analysis for indicative of SARS-CoV-2-infection [19]. A 48-finding stated 58 assessments of antigen tests. Estimations of sensitivity noticeably differed among experiments. Regarding the antigen test assessments in symptomatic contributors, a substantial heterogeneity in sensitivities (and to a smaller degree the specificities). Whereas the average sensitivity was 72.0% (95% CI 63.7 to 79.0%) and specificity was 99.5% (95% CI 98.5 to 99.8%), alongside with regular sensitivity which was declined with period since the symptom's onset, being greater in the first week (78.3%, 95% CI 71.1 to 84.1%) than when done delayed (51.0 95% CI 40.8 to 61.0%). Sensitivity was superior in those

with greater viral loads described by Ct-values of 25 (94.5%, 95% CI 91.0 to 96.7%) equated to those with smaller viral loads (40.7%, 95% CI 31.8 to 50.3%). As referred to the systematic review by Dinnes et al. 2021, 3 studies evaluated the fluorescence immunoassays in SARS Cov-2 antigen detection with reported sensitivities and specificities of 67%-94% and 93%-100%, respectively [19]. On the other hand, another study evaluated chemiluminescence immunoassay in SARS CoV2 antigen detection with reported sensitivity and specificity of 73% and 100%, respectively [20].

Rapid antigen detection test had high specificity in our result, thus in symptomatic population (where prevalence is possible to be extreme), the risk of false positives is minimal. At 69.3%, 64.5% sensitivity for iFlash & UNICELL, the possibility that affected entities are lost is 30.6 & 35.5% greater than for RT-PCR. The probability of incorrect undesirable results is highly in those with high clinical thought of COVID-19 and tested several days after the onset of signs when the viral load stages may have dropped. This is considered a general limitation in rapid antigen tests which may lead to missed diagnosis of COVID-19 patients and consequent built decisions for proper isolation.

The SARS-CoV2 antigen level in the samples detected by both iFlash-2019-nCoV antigen immunoassay & UNICELL-2019-nCoV antigen assay were correlated with the cycle threshold (Ct) value of RT-PCR. We observed a strong significant relationship with negative correlation coefficient (-0.864 & -0.716) and  $p$ -value  $< 0.001$ , respectively between the Ct value and the level of N-antigen detected in samples.

This means that the AG rapid detection test are most possible to well operate in a patient with superior viral load (Ct-value of  $< 25$ ) which typically seem in the pre-symptomatic (1-3 d before the sign onset) and initial sign forms of the disease within the first 5-7 d of illness [21-22]. These proposals the chance for initial diagnosis and disruption of transmission through targeted isolation of the most virulent cases and their close contacts. Patients who show  $> 5-7$  d after the onset of signs are more expected to have lower viral loads, and the probability of incorrect adverse outcomes with Ag-RDTs [21].

Despite these limits in performance of Ag-RDTs, it could be a considerable role in directing patient managing, public health ruling creation, and

in observation of COVID-19. The overall efficacy of diagnostic tactics is not only exemplified by the intrinsic operations of *in vitro* attempts, which are mostly expected by the sensitivity and specificity, but also by their convenience, efficiency, speed of process, and period to obtain the findings. Additionally, diagnosis can gain from a scanning algorithm based on successive steps of triage, screening, and confirmation [17]. We conceded that our study has some barriers which include that the sample timing in relation to symptoms were unknown. Furthermore, the serum level of antibodies in relation to the symptoms timing were not tested, and these two points play an indispensable role in the evaluation of our Ag-RDTs.

### Conclusion

The antigen tests differ in sensitivity, only those indicated to convene the lowest performing needs of  $\geq 80\%$  sensitivity and  $\geq 97\%$  specificity could be deemed as a rational substitute for RT-PCR of SARS-CoV-2 [23]. As the data on SARS-CoV-2 RNA suggested that the viral overload summits within the first days after the onset of symptoms [24].

Hence, Ag RDTs may be exciting in the initial stage of the disease when the viral capacity be high, and the threat of SARS-CoV-2 transmission is at its highest. A combination of RDTs that assessing SARS-CoV-2 antigen as well as antibodies would increase the rate of COVID-19 validation equated with COVID-19 testing utilizing antigen RDT alone [25].

### References

- 1- **Worldometer.** Available at: <https://www.worldometers.info/coronavirus/> Accessed 15 December 2021.
- 2- **Ferrari D, Motta A, Strollo M, Banfi G, Locatelli M.** Routine blood tests as a potential diagnostic tool for COVID-19. *Clinical chemistry and laboratory medicine (CCLM)* 2020;58(7):1095-9.
- 3- **Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguín-Rivera Y, Escalera-Antezana JP, et al.** Clinical, laboratory and imaging features of COVID-19: A systematic review and meta-analysis. *Travel medicine and infectious disease* 2020; 34:101623.
- 4- **Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al.** Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance* 2020;25(3):2000045.
- 5- **WHO.** Laboratory testing strategy recommendations for COVID-19: Interim guidance, 21 March 2020. World Health Organization, 2020.
- 6- **Kobia F, Gitaka J.** COVID-19: Are Africa's diagnostic challenges blunting response effectiveness? *AAS open research* 2020;3.
- 7- **WHO.** Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays. 2020.
- 8- **Mattiuzzi C, Henry BM, Lippi G.** Making sense of rapid antigen testing in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnostics. *Diagnosis* 2021;8(1):27-31.
- 9- **Simmons S, Saguil A.** Rapid Point-of-Care Antigen and Molecular Tests for Diagnosis of SARS-CoV-2 Infection. *American Family Physician* 2021;104(1):29-30.
- 10- **Ndwandwe D, Mathebula L, Kamadjeu R, Wiysonge CS.** Cochrane corner: rapid point-of-care antigen and molecular-based tests for the diagnosis of COVID-19 infection. *The Pan African Medical Journal* 2020;37(Suppl 1).
- 11- **Chan YH.** *Biostatistics102: Quantitative Data –Parametric & Non-parametric Tests.* Singapore Med J 2003a;44(8): 391-396.
- 12- **Chan YH.** *Biostatistics 103: Qualitative Data – Tests of Independence.* Singapore Med J 2003b;44(10): 498-503.
- 13- **Chan YH.** *Biostatistics 104: Correlational Analysis.* Singapore Med J 2003c;44(12): 614-619.

- 14-**Galen RS**. Predictive values and efficiency of laboratory testing. *Pediatr J Clin North Am* 1980; 27:861-69.
- 15-**Helgouach N, Champigneux P, Schneider FS, Molina L, Espeut J, Alali M, et al**. EasyCOV: LAMP based rapid detection of SARS-CoV-2 in saliva. *Infect Dis*.2020. <https://doi.org/10.1101/2020.05.30.20117291>.
- 16-**WHO**. Advice on the use of point-of-care immunodiagnostic tests for COVID-19: scientific brief. 2020. Available at: <https://www.who.int/publications-detail-redirect/advice-on-the-use-of-point-of-care-immunodiagnostic-tests-for-COVID-19-scientific-brief>.
- 17-**Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, et al**. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ* 2020;27(5):1451-1454.
- 18-**Qiaoling D, Guangming Y, Yunbao P, Wen X, Gui Y, Zhiqiang L, et al**. High Performance of SARS-Cov-2N Protein Antigen Chemiluminescence Immunoassay as Frontline Testing for Acute Phase COVID-19 Diagnosis: A Retrospective Cohort Study *Frontiers in Medicine* 2021; 8:676560.
- 19-**Dinnes J, Deeks JJ, Adriano A, Berhane S, Davenport C, Dittrich S, et al**. Rapid, point-of-care antigen, and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database of Systematic Reviews* 2020(8).
- 20- **Matsuzaki N, Orihara Y , Kodana M, Kitagawa Y, Matsuoka M, Kawamura R, et al**. Evaluation of a chemiluminescent enzyme immunoassay-based high-throughput SARS-CoV-2 antigen assay for the diagnosis of COVID-19: The VITROS® SARSCoV-Antigen Test . *Journal of Medical Virology* 2021;1-4.
- 21-**Weiss A, Jellingsø M, Sommer MOA**. Spatial and temporal dynamics of SARS-CoV-2 in COVID-19 patients: A systematic review and meta-analysis. *EBioMedicine* 2020;58.
- 22-**Arons MM, Hatfield KM, Reddy SC, Kimball A, James A, Jacobs JR, et al**. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. *New England Journal of Medicine* 2020;382(22):2081-90.
- 23-**WHO**. Antigen-detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays: Interim guidance WHO reference number: WHO/2019-nCoV/Antigen\_Detection/2020.1.
- 24-**Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al**. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;581(7809):465-469.
- 25-**Veyrenche N, Bolloré K, Pisoni A, Bedin AS, Mondain AM, Ducos J, et al**. Diagnosis value of SARS-CoV-2 antigen/antibody combined testing using rapid diagnostic tests at hospital admission. *Journal of medical virology* 2021;93(5), 3069-3076.