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1 **Rapid SARS-CoV-2 antigen detection by immunofluorescence – a new tool to detect**
2 **infectivity**
3
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16
17 **Abstract**
18 The evaluated SARS-CoV-2 antigen rapid fluorescence immunoassays reliably identified
19 patients within the first 5 days of symptom onset, when respiratory secretions carried high viral
20 loads. This high performance suggests that these tests might play an important role for future
21 PCR-independent strategies to detect early or infective cases.
22
23 **Key words:** SARS-CoV-2; Covid-19; Diagnosis; Rapid diagnostic test; Antigen detection

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24 **Introduction**

25 Since its emergence in 2019, the SARS-CoV-2 pandemic has resulted in over 30 million
26 confirmed cases and almost 1 million deaths worldwide, as of September 2020
27 (https://covid19.who.int). Early detection of cases by highly sensitive and specific real-time
28 reverse-transcription polymerase chain reaction (RT-PCR) is the currently recommended
29 diagnostic strategy [1]. However, the high cost of RT-PCR, shortage of reagents, and need for
30 trained personnel have limited the testing capacities of laboratories to provide results in a timely
31 manner [2]. Thus, alternative diagnostic tools allowing the fast testing of large numbers of
32 samples are of high priority [3]. In addition, new aspects of SARS-CoV-2 testing include the
33 ability to evaluate infectivity to help tailor control measures of known or suspected Covid-19
34 cases [4].

35 Rapid antigen detection tests (Ag-RDT) using immunochromatographic tests (ICT) or
36 fluorescent immunoassays (FIA) have recently become available; many of which are CE-IVD
37 licensed and some have received FDA emergency use authorization (EUA) [5]. As previously
38 suggested, FIAs are highly specific and can reach remarkably high sensitivities, if applied in
39 samples from early phases of infection or with high viral loads [6,7]. Here we present the
40 performance of two novel FIA automated antigen detection systems in samples from Covid-19
41 patients presenting within 5 days of symptom onset.

42

43 **Material and methods**

44 Samples derived from patients attending Clínica Alemana in Santiago, Chile, for Covid-
45 19 testing. Specimens consisted of naso-oropharyngeal flocked swabs obtained by trained
46 personnel and placed in universal transport media (UTM-RT® System, Copan Diagnostics,

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47 Murrieta, CA, USA). Samples were examined for SARS-CoV-2 RNA by RT-PCR assay
48 (COVID-19 Genesig®, Primerdesign Ltd., Chander´s Ford, UK). Samples exhibiting exponential
49 amplification curves and cycle thresholds (Ct) values ≤40 were considered positive.
50 RT-PCR characterized UTM samples were aliquoted and kept at -80° C until analysis by
51 the two FIA kits, "SOFIA SARS Antigen FIA" (Quidel Corporation, San Diego, CA, USA) and
52 "STANDARD® F COVID-19 Ag FIA" (SD Biosensor Inc., Gyeonggi-do, Republic of Korea).
53 Both tests detect SARS-CoV-2 nucleocapsid protein by lateral flow immunofluorescence, which
54 is interpreted by automated analysers (SOFIA 2, Quidel Corporation; F2400, SD Biosensor Inc.).
55 Both kits are CE-IVD labelled; Quidel recently received FDA EUA. Manufacturers state that
56 both tests should be performed using nasopharyngeal swabs collected from symptomatic
57 individuals within 5 days of symptom onset. The use of samples stored in certain brands of
58 transport media (including Copan UTM) is permitted for the SD Biosensor assay; the Quidel test
59 initially also allowed using UTM, but a recent instruction update discourages the use of
60 prediluted samples [8].
61 For the evaluation, 32 RT-PCR positive UTM samples, all collected within the first 5
62 days after symptom onset, and 32 negative specimens were selected. All positive samples were
63 from symptomatic patients, 12 negative samples were from asymptomatic patients screened
64 before surgery. Some of the positive (n = 27) and negative samples (n = 19) had been used in a
65 previous evaluation [7]. Assays were performed using the same sample aliquot, following
66 manufacturers´ instructions, by the same laboratory personnel, who were blinded to RT-PCR
67 results. In brief, specimens were mixed with an extraction reagent, dispensed into the cassette´s
68 sample well, and read after incubation by an instrument. All procedures, except the reading, were
69 performed under a BSL2 cabinet. Results were compared to those of RT-PCR as reference

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70 method; in case of discordant results, tests were repeated. Demographic and clinical data were
71 obtained from mandatory notification forms and analysed in an anonymized manner. Statistical
72 analysis considered the calculation of sensitivity, specificity, and accuracy using standard
73 formulas, and Wilson score Confidence Interval at 95% (OpenEpi version 3.01). Test
74 performance was evaluated for all samples and for those with high viral loads (Ct ≤25), as
75 previously described [9]. Kits and analysers were provided by manufacturers at reduced costs for
76 evaluation purposes. The study was approved by the institutional review board (Comité Etico
77 Científico, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, Chile)
78 and a waiver of informed consent was granted.

79

80 **Results**

81 The study included a total of 64 samples, 32 were RT-PCR positive and 32 RT-PCR
82 negative. The median age was 39 years (IQR 36.7-57) and 52% were male. Median days from
83 symptom onset to RT-PCR testing of positive and negative cases were 2 (IQR 1-3) and 1 (IQR
84 0.75-4), respectively. Ct values had a median of 17.95 (IQR, 16.4-22.4); 29/32 samples (90.6%)
85 had a Ct ≤25.

86 Both assays demonstrated an overall sensitivity >90%, reaching 100% for samples with
87 high viral loads (Table 1). False negative results were observed with the Quidel and SD
88 Biosensor assays in two and three samples, respectively, which had Cts of 30.89 to 32.57 and
89 were taken on the fourth or fifth day after symptom onset. Specificity was 96.7% for both tests,
90 i.e. both kits displayed a single false positive result, from two distinct symptomatic RT-PCT
91 negative cases. Both assays were user friendly, included ready-to-use reagents and required little
92 hands-on time. Moreover, analysers were easy-to-use, stored the results, and included options for
93 QR coding, printing, and connection to laboratory information systems.

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Discussion

At present, RT-PCR is the recommended diagnostic method in patients with suspected SARS-CoV-2 infection [1]. However, material shortages and laboratory capacity limitations, especially during high transmission situations, have caused significant problems and led to the emergence of various new PCR-independent diagnostics [10]. Antigen-based assays are among the most recent developments, but peer-reviewed evaluations of their diagnostic performance are scarce. Hence, their role within the routine diagnostic workup is yet not defined [9,11]. Since antigen detection per se has a lower sensitivity than RT-PCR, it will most likely not replace it [9]. However, the results of this and former studies indicate that antigen detection by immunofluorescence, especially when used with an automated reader, has an excellent sensitivity to detect SARS-CoV-2 in samples with estimated viral loads above ~10^6 copies/mL (Ct values ≤25) [9], which are found in pre-symptomatic (1-3 days before symptom onset) and early symptomatic Covid-19 cases (5-7 days after symptom onset) [9,12-14]. According to recent modelling studies, elevated viral titers are associated to infectivity [15]. This is in accordance with *in vitro* experiments, which showed no viral growth from samples with Cts >24 or taken >8 days after symptom onset [16,17]. A viral load of 10^6 copies/mL has therefore been suggested as the limit of infectivity for clinical practice [18]. However, until the exact threshold of contagiousness is known, other authors have considered a more conservative approach (1,000 copies/mL) [19].

For samples with high viral loads both evaluated tests were 100% sensitive. In our panel of positive samples, false negatives only occurred with Cts >30, which translates to viral loads <10^4 for the used RT-PCR protocol [20], although this finding has to be confirmed with a larger number of specimens. The high-performance value coincides with recent studies of a similar FIA

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117 with automated reading (BioEasy), which demonstrated sensitivities of 100% for samples with
118 Cts ≤25 [6,7] and of 98% for samples with Cts ≤30 [21]. In contrast, immunochromatographic
119 SARS-CoV-2 antigen tests demonstrated lower sensitivity values of 74%-85% for samples with
120 Cts ≤25 [7,22,23].

121 Although additional studies with larger numbers of samples are needed, the excellent
122 performance data of FIA Ag-RDTs suggest their potential use in the following scenarios, when
123 RT-PCR is unavailable or impractical: 1) closed or semi-closed remote communities such as
124 cruise ships or military camps [9], 2) High-risk congregate facilities including schools, care-
125 homes, dormitories, etc., when testing daily or every other day could reduce secondary infections
126 by 100% or 90%, respectively [24], and 3) screening of asymptomatic attendees at potential
127 superspreader events, like conferences, weddings, and sports or cultural events. In the future, due
128 to their high sensitivity to detect infective patients, FIA Ag-RDTs might also play an important
129 role within "test-out" strategies, i.e. the early release of suspected cases from self-isolation or
130 shortening quarantine for proven cases.

131

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135

136 **CRediT authorship contribution statement**

137 **L. Porte:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology,
138 Project administration, Supervision, Validation, Writing - original draft, Writing - review &
139 editing. **P. Legarraga:** Formal analysis, Supervision, Validation, Writing - review & editing. **M.**

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140 **Iruretagoyena:** Formal analysis, Validation, Writing - review & editing. **G. Pizarro:** Data
141 curation, Investigation. **V. Vollrath:** Supervision, Validation, Writing - review & editing. **J.M.**
142 **Munita:** Validation, Writing - review & editing. **R. Araos:** Validation, Writing - review &
143 editing. **T. Weitzel:** Conceptualization, Formal analysis, Methodology, Project administration,
144 Validation, Writing - original draft, Writing - review & editing.
145

146 **Declaration of competing interest**

147 There is no conflict of interest.
148

149 **Table 1.** Performance of two automated SARS-CoV-2 antigen detection assays compared to RT-PCR

Antigen detection test		RT-PCR			Sensitivity				Specificity		Accuracy
		Positive		Neg.	All		High VL[a]				
Assay	**Result**	**All**	**High VL[a]**		**%**	**CI95%**	**%**	**CI95%**	**%**	**CI95%**	**%**
Sofia SARS Antigen FIA	Positive	30	27	1	93.8	79.9-98.3	100	87.5-100	96.9	84.3-99.4	95.3
	Negative	2	0	31							
Standard F COVID-19 Ag FIA	Positive	29	27	1	90.6	75.8-96.8	100	87.5-100	96.9	84.3-99.4	93.8
	Negative	3	0	31							

150 VL, viral load; CI95%, confidence interval 95%; Neg., negative

151 [a]Samples with Ct ≤25

152

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