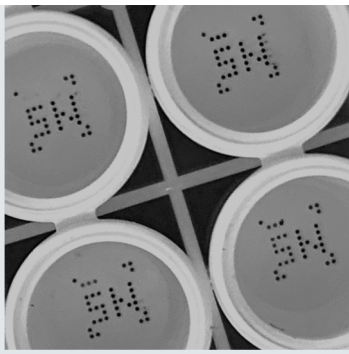
CoViDiag

APPLICATION NOTE

CoViDiag®: HD diagnostic device for COVID-19 serological testing and immune status characterization.

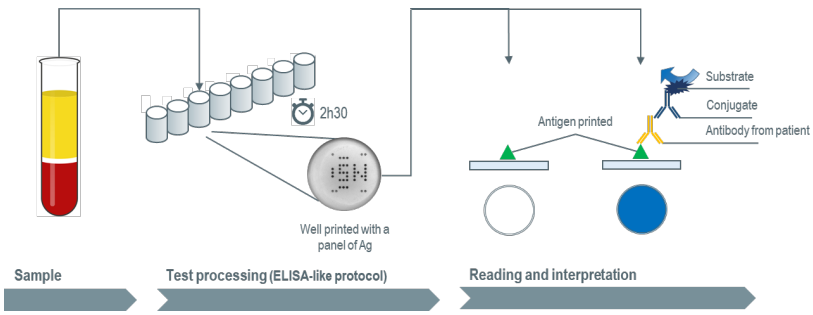
Key points



- ✓ **Multiplex ELISA-like** test for the serodetection of different **anti-SARS-CoV-2 IgG antibodies***.
- ✓ **Using 5 antigens printed side by side** in the same well: Nucleocapside (N), Spike 1 subunit (S1), receptor binding domain of the S1 (S1-RBD), N-terminal domain of the S1 (S1-NTD), and Spike 2 subunit (S2) to give a comprehensive and educated response.
- ✓ The combination of N and S1 antigens increases diagnostic performances[1,2]: **99,6 % specificity and 95,9 % sensitivity***. The addition of the S1-RBD, S1-NTD and S2 antigens enlarge again the **comprehensive picture of the humoral response diversity**.
- ✓ Standard lab equipment. Quick visual interpretation***.

* CoViDiag is dedicated to IgG detection (IgM and IgA can be detected if needed). ** Diagnostic sensitivity on plasma samples collected over 8 days after symptoms onset and infection confirmed by rt-PCR. *** Automated interpretation is available on a specific reader

MATERIAL & METHODS



MATERIAL

- ✓ CoViDiag kits
- ✓ Blood plasma samples known as positive (n=69) and negative (n=121)
- ✓ Precision micropipettes with suitable tips
- ✓ Distilled or deionized water
- ✓ Microplate washer (recommended)
- ✓ Microplate shaker & incubator (recommended)
- ✓ Colorimetric biochips reader (optional)

METHODS

The whole process has been performed according to the IFU. Samples were diluted at 1:100 in diluent buffer (DB_CVD). Plates were incubated 1 h at 37°C on a microplate shaker at 300 rpm. After 3 washing cycles (WB_CVD), conjugate (CA_CVD) was diluted at 6:1000 in diluent buffer (DB_CVD) and added, followed by 1 h incubation at 37°C. After 3 washing cycles, substrate (SU_CVD) was incubated at room temperature for 15 min. The wells were rinsed and dried 15 min at 37°C before pictures acquisition.

RESULTS

Diagnostic performances are assessed on 106 positive samples collected from symptomatic patients and confirmed by RT-PCR and 240 negative samples: 190 collected before the Covid-19 outbreak and 50 samples from symptomatic patients presenting a negative RT-PCR. All tests are interpreted visually by the operator as well as automatically using a colorimetric biochips reader and a dedicated algorithm for comparison.

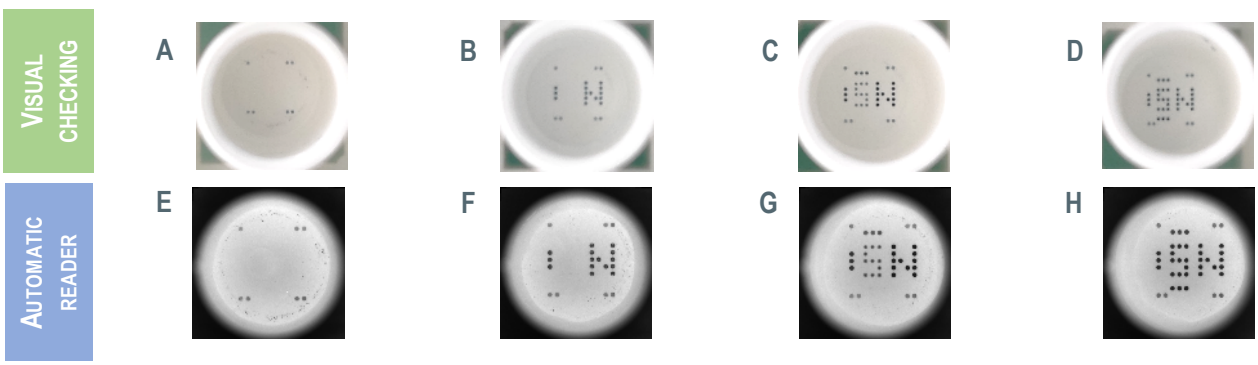


Figure 1 *- Pictures of wells after samples testing acquired with a classic smartphone (A to D) or biochips reader (E to H). Images A and E are related to negative samples. Images B, C, D, F, G and H are related to positives samples.*

Corner spots are positive controls ensuring the technical validation of the process (fig. 1). For negative samples, only positive controls are visible (fig.1-A/E). For positives samples, letters shapes are clearly visible according to the position of the printed antigens. Figures 1B and F show the presence of anti-N Ab only in the patient's blood sample. Figure 1C, D, G and H show a set of various specific antibodies in other samples.

Visual interpretation checks for the presence of the "N" and/or "S" letters at the bottom of the well to identify the sample as positive. Visual checking of the wells finds 95 positive samples among the 106 tested: 3 different operators found the same results. 3 negative samples were identified as positive by operators.

In order to confirm visual checking, pictures were acquired with a dedicated reader and analyzed using a software and interpretation algorithm. Depending of signal acquired on pictures and antigens giving signals, algorithm is able to identify a sample as "POSITIVE", "BORDERLINE" or "NEGATIVE". In the present study, Borderline are considered as Negative for Statistics. Use of automatic reading is improving the whole performances of the test, specially in complex cases.

		n =	Tested Pos.	Tested Neg.	Diagnostic performances
Expected as positive	< 8 days after symptoms	9	7	2	78 % sensitivity
	8 to 14 days after symptoms	40	36	4	90 % sensitivity
	> 14 days after symptom	57	57	0	**100 % sensitivity**
Expected as negative (collected before june 2019)		240	1	239	**99,6 % specificity**

***Figure 2** - observed results after 346 samples testing and comparison with expected results.*

We obtained a global sensitivity of 95,9% for samples > 8 days after symptoms onset when using the automatic reading. Visual checking is presenting a sensitivity of 92,8% for the same samples. Visual checking leads to identify 3 negative samples as positive due to low signal (98,8% specificity). The combination of Ag used in automatic reading is able to decipher low unspecific signals to real positivities. When using the interpretation algorithm and automatic reading, the whole specificity reaches 99,6%%.

Repeatability has been assessed using 4 samples tested 48 times on two different plates. Pictures were acquired with colorimetric biochips reader and the data were extracted. Mean and CV were calculated for each Ag printed in the different wells.

	Nucleocapside								S1 Spike							
	Sample #1		Sample #2		Sample #3		Sample #4		Sample #1		Sample #2		Sample #3		Sample #4	
	n = 46		*n = 46*		*n = 48*		*n = 48*		*n = 46*		*n = 46*		*n = 48*		*n = 48*	
Mean signal	**84,34**		**56,00**		**76,65**		**0,11**		**72,33**		**70,38**		**67,97**		**0,06**	
Range of signal	*78,3*	*93,1*	*46,5*	*61,9*	*72,3*	*82,5*	*0,009*	*0,514*	*68,7*	*77,6*	*65,8*	*75,1*	*64,2*	*74,4*	*0,004*	*0,187*
Overall result	**Positive**		**Positive**		**Positive**		**Negative**		**Positive**		**Positive**		**Positive**		**Negative**	
%Positive	100%		100%		100%		0%		100%		100%		100%		0%	
%Questionable	0%		0%		0%		0%		0%		0%		0%		0%	
%negative	0%		0%		0%		100%		0%		0%		0%		100%	
	SD	***CV***	SD	***CV***	SD	***CV***	SD	***CV***	SD	***CV***	SD	***CV***	SD	***CV***	SD	***CV***
Repeatability	3,474	***4,12%***	2,976	***5,31%***	2,657	***3,47%***	0,102	***91,6%***	1,680	***2,32%***	2,076	***2,95%***	2,361	***3,47%***	0,039	***66,7%***

***Figure 3** - Repeatability study results.*

CONCLUSION

CoViDiag allows high performance COVID-19 antibody testing. The combined detection of Nucleocapside and Spike 1 antibodies increases the chance to detect the immune response, especially under 14 days after symptoms onset [1,2]. The multiplex approach enables user to get a more comprehensive picture of the patient immune profile in a single assay: it brings a high added value to understand how the patient is protected[3].

PERSPECTIVES

CoViDiag is evolutive and can almost instantaneously include additional antigens, isotypes (IgA, IgM). The intent is to develop a differential respiratory diagnostic tool by the end of 2020 to fit specific demands.

1. Analysis of SARS-COV-2 Antobodies in COVID-19 Convalescent Blood using a Coronavirus Antigen Microarray, Assis et al., *bioRxiv* preprint, 2020
2. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients, Sun et al., *Emerging Microbes and Infections*, 2020
3. Anti-Spike, anti-Nucleocapsid and neutralizing antibodies in SARS-CoV-2 hospitalized patients and asymptomatic carriers, Brochot et al., *medRxiv* preprint,2020

CoViDiag was developped by

innobiochips GD Biotech

CoViDiag has been awarded

DGA MINISTÈRE DES ARMÉES AGENCE INNOVATION DÉFENSE