COVID-19

ID Screen®
SARS-CoV-2-N IgG Indirect

APRIL 2020

Loïc COMTET, April 27th 2020,
last update 20-05-04

WITH YOU AT EVERY STEP

Why detect COVID-19 antibodies?

- Perform epidemiological studies
- Determine precise rate of infection
- Identify highly reactive human donors for the generation of convalescent serum as a therapeutic
- Identify viral reservoir hosts
- Patient contact tracing: determine who is immune and who is not
  ⇨very useful for deploying strategic control measures
  BE CAREFUL: HAVING ANTIBODIES ǂ BEING PROTECTED !
- Evaluation of vaccine trials

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ID Screen®
SARS-CoV-2-N IgG Indirect

Semi-quantitative indirect ELISA for IgG antibodies detection against the SARS-CoV-2 virus in human serum and plasma

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ID Screen® SARS-COV-2-N IgG Indirect: FEATURES

METHOD	Semi-quantitative (POS/NEG) indirect ELISA
TARGET	**IgG** antibodies directed against SARS-CoV-2 **Nucleocapsid**
SAMPLE TYPES	• serum • plasma
VALIDATED SPECIES	Human (not for veterinary use).
SPECIMEN VOLUME	10 µl
TEST RUN TIME	95 min
READING WAVELENGHT	450 nm
PRODUCT CODE	SARSCOV2S (note : *S means Screening, NOT for S protein*)
PRODUCT CATEGORY	*In vitro* Diagnostic test. The test is CE marked.
FORMAT	4 plates (480 tests) and 8 plates (768 tests)

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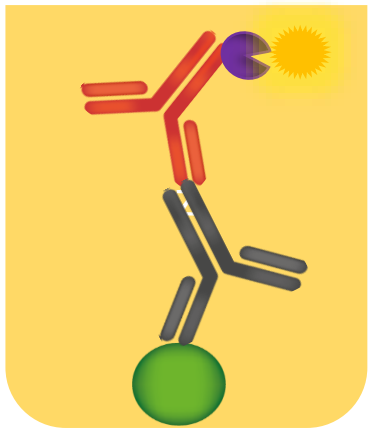
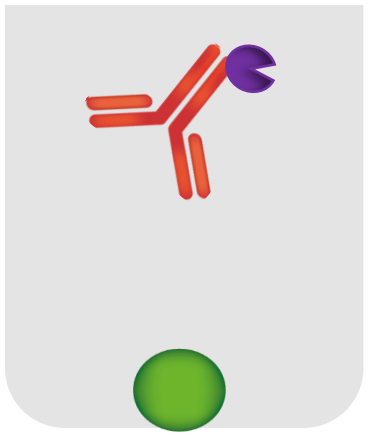
ID Screen® SARS-COV-2-N IgG Indirect: TEST PRINCIPLE

Semi-quantitative test

	Positive sample	**Negative sample**
Substrate Protein G, peroxidase (HRP) labelled conjugate Antibodies anti-SARS-CoV-2 N recombinant protein		

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ID Screen® SARS-COV-2-N IgG Indirect: TEST PRINCIPLE

The microplate is read at 450nm

For each sample, calculate the S/P percentage (S/P):

$$\mathbf{S/P\ \%} = \frac{\mathbf{OD_{Sample} - OD_{NC}}}{\mathbf{OD_{PC} - OD_{NC}}} \mathbf{\ x\ 100}$$

RESULT	STATUS
S/P % ≤ 60	**Negative**
60 % < S/P < 70 %	**Doubtful**
S/P ≥ 70 %	**Positive**

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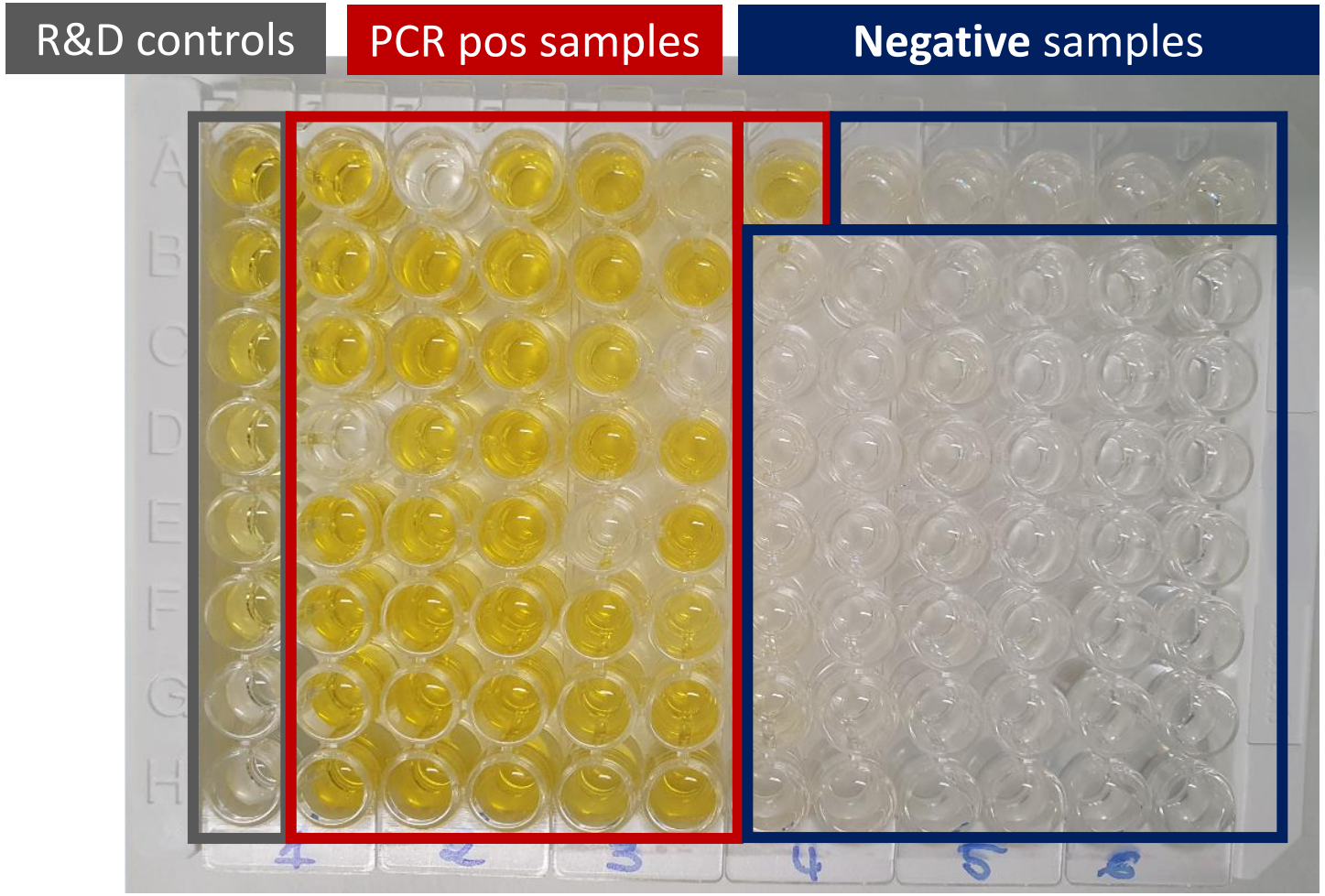
ID Screen® SARS-COV-2-N IgG Indirect: DISCRIMINATORY CAPACITY

Illustration of the excellent discriminatory capacity of the test:



ID Screen® SARS-COV-2-N IgG Indirect: SPECIFICITY

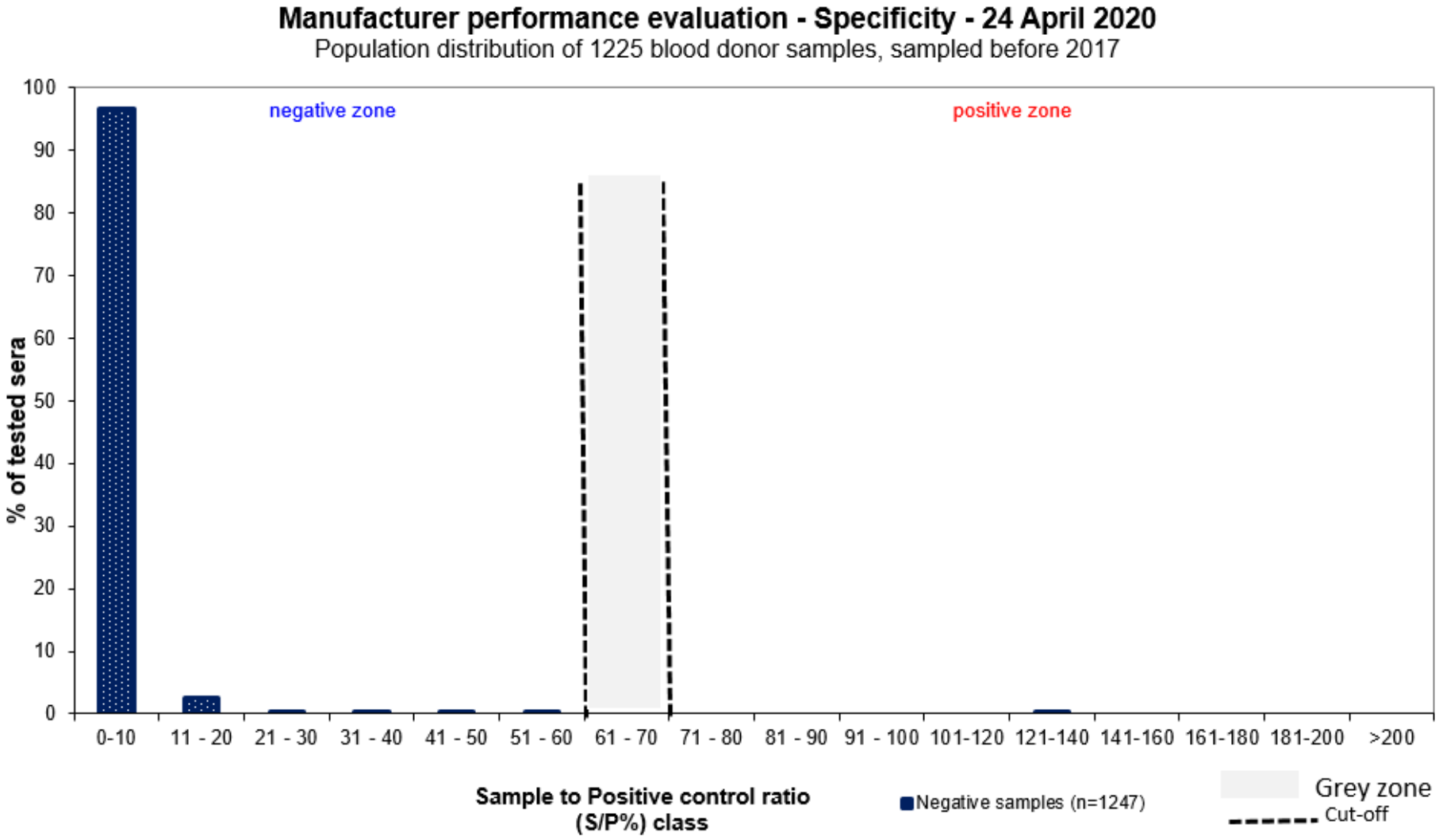
Pre-epidemic sera (2010, 2016, 2017), sampled from healthy blood donors

Tested at IDvet

Age group	Year	Sample type	N=	Tested Pos.	Tested Neg.	Spe
20-33 ans	2016	plasma	51	0	51	100
18-70 ans	2017	plasma	48	0	48	100
18-70 ans	2010	serum	100	0	100	100
18-70 ans	2010	plasma	88	0	88	100
18-70 ans	2017	plasma*	960	1	959	99,9
		TOTAL	1247	1	1246	99,9 [99,6–100]

** Samples kindly provided by EFS (Etablissement Français du Sang) / EFS Occitanie*

ID Screen® SARS-COV-2-N IgG Indirect: SPECIFICITY



1220/1223 sera were =found negative

Measured specificity 99.9 % (IC_{95}: 99.6 % – 100 %)

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ID Screen® SARS-COV-2-N IgG Indirect: SENSITIVITY (1)

Performed at IDvet through the analysis of plasma samples from **41** hospitalized patients **tested positive** for **COVID-19 RNA** by **RT-qPCR** in respiratory samples. For each sample, the **date** of **blood sampling** in relation to **the onset of symptoms** is known.

Days after symptoms onset	N=	Tested Pos.	Tested Neg.	Mean S/P% value	Se
0 – 4 days	1	1	0	3	0
5 – 9 days	2	1	1(*)	42	50
10 – 14 days	8	7	1	169	87,5
15 – 19 days	20	19	1(*)	195	95,0
>19 days	10	9	1(*)	175	90,0

Samples were provided by the Biological Resource Montpellier Hospital, CHU of Montpellier
(COVIDthèque collection, n°. 2020-A00935-34)

For samples >15 days after offset of symptoms(-)

Measured sensitivity: 93,3% (IC_{95} 78,8% – 98,2%), n=30

(*) Non-reactive samples on different prototypes based on the detection of the N protein of SARS-CoV-2 using an anti IgG-A-M conjugate, and tested negative on another commercially available SARS-CoV-2 IgG ELISA, S protein based.

(-) Guidelines for evaluation of performance of SARS-Cov-2 serological tests in France, « Haute Autorité de Santé », April 2020 16th

ID Screen® SARS-COV-2-N IgG Indirect: SENSITIVITY (2)

Performed at Montpellier's Hospital (CHU Montpellier), on samples from hospitalized patients COVID-19 RNA positive (by RT-qPCR) with the ID Screen® ELISA. Results were kindly provided by the hospital.

Days after symptoms onset	N=	Tested Pos.	Tested Neg.	Mean S/P% value	Se
0 – 4 days	10	0	10	7	0
5 – 9 days	9	1	8	14	11,1
10 – 14 days	20	15	5	187	75,0
15 – 19 days	22	21	1	279	95,5
>19 days	32	31	1	280	96,9

For samples >15 days after offset of symptoms(-)

Measured sensitivity: 96,3% (IC_{95} 87,58% – 99,0%), n=54

(-) Guidelines for evaluation of performance of SARS-Cov-2 serological tests in France, « Haute Autorité de Santé », April 2020 16th

ID Screen® SARS-COV-2-N IgG Indirect: SENSITIVITY (3)

In summary :

Days after symptoms onset	Study	N =	Tested Pos.	Tested Neg.	Mean S/P value	Se
15 – 19 days	IDvet	20	19	1	195	95,0
	Montpellier hospital	22	21	1	279	95,5
>19 days	IDvet	10	9	1	175	90,0
	Montpellier hospital	32	31	1	280	96,9

For samples >15 days after ofset of symptoms(-)

Measured sensitivity: 95,2 (IC_{95} 88,4 – 98,1), n=84

(-) Guidelines for evaluation of performance of SARS-Cov-2 serological tests in France, « Haute Autorité de Santé », April 2020 16th

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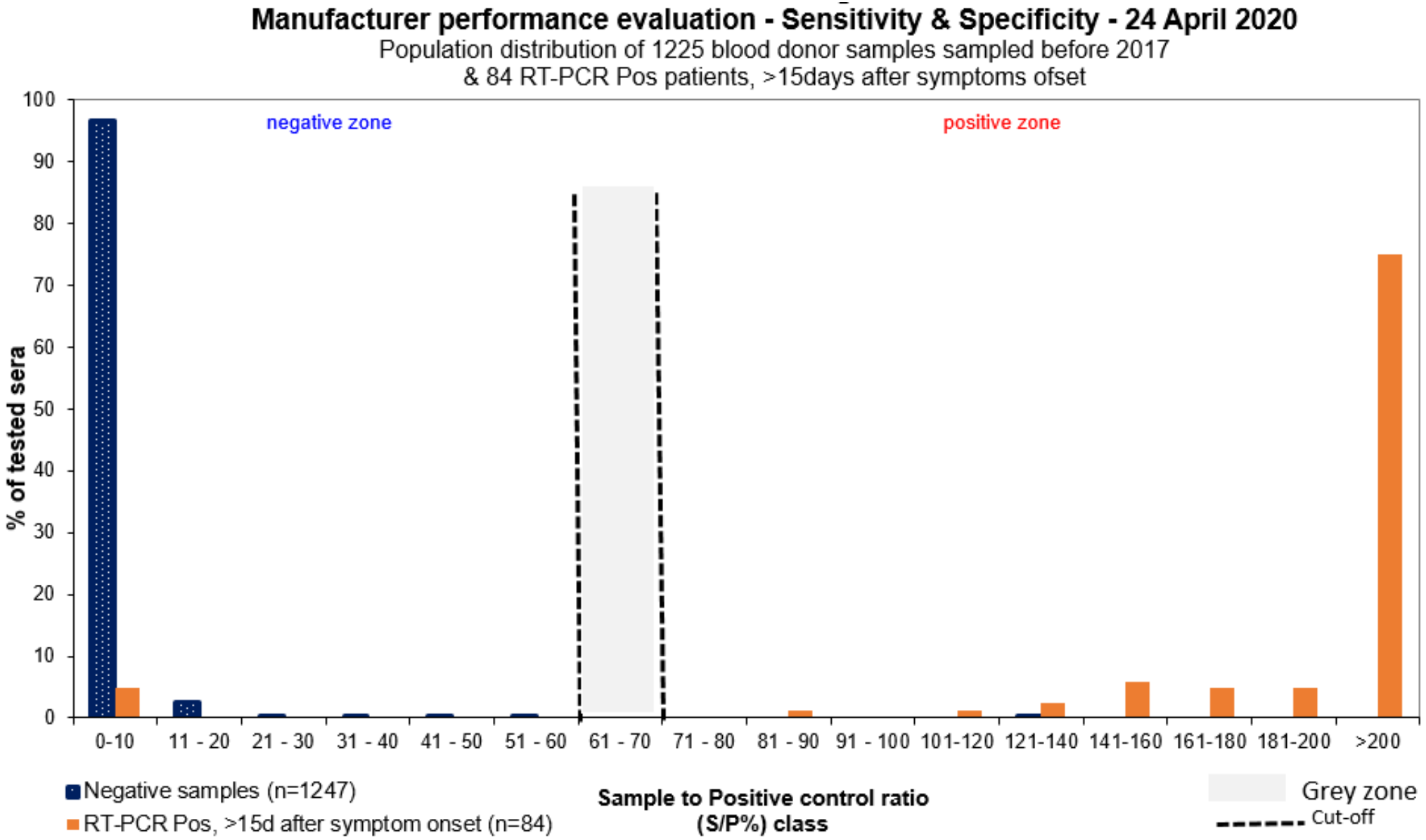
ID Screen® SARS-COV-2-N IgG Indirect: Test performance



Excellent discriminatory capacity

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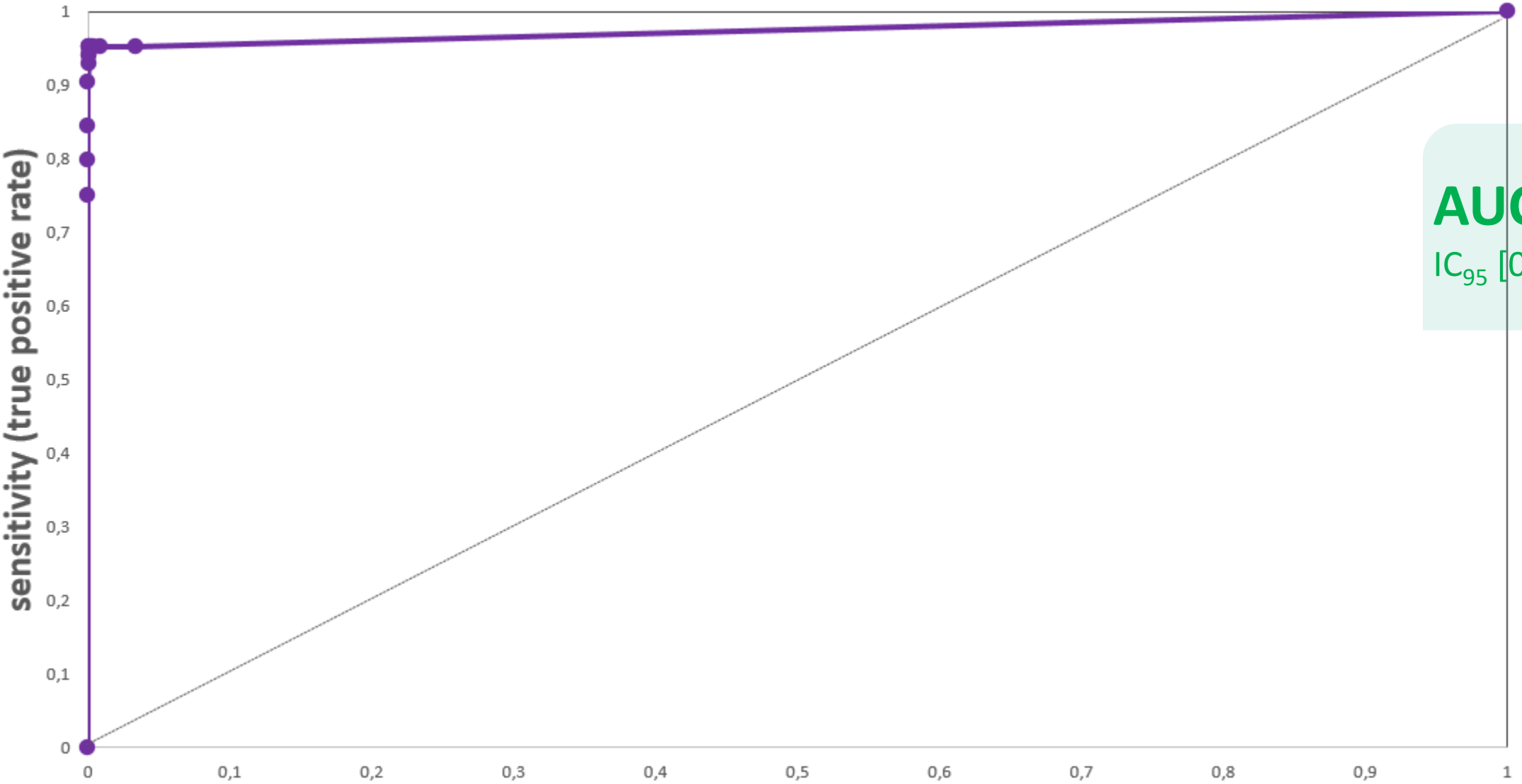
ID Screen® SARS-COV-2-N IgG Indirect: ROC analysis (1)



AUC = 0,968

IC_{95} [0,958 - 0,978]

Receiver Operating Curve analysis
max Area Under Curve (AUC)=1 « chance line » : 0,5

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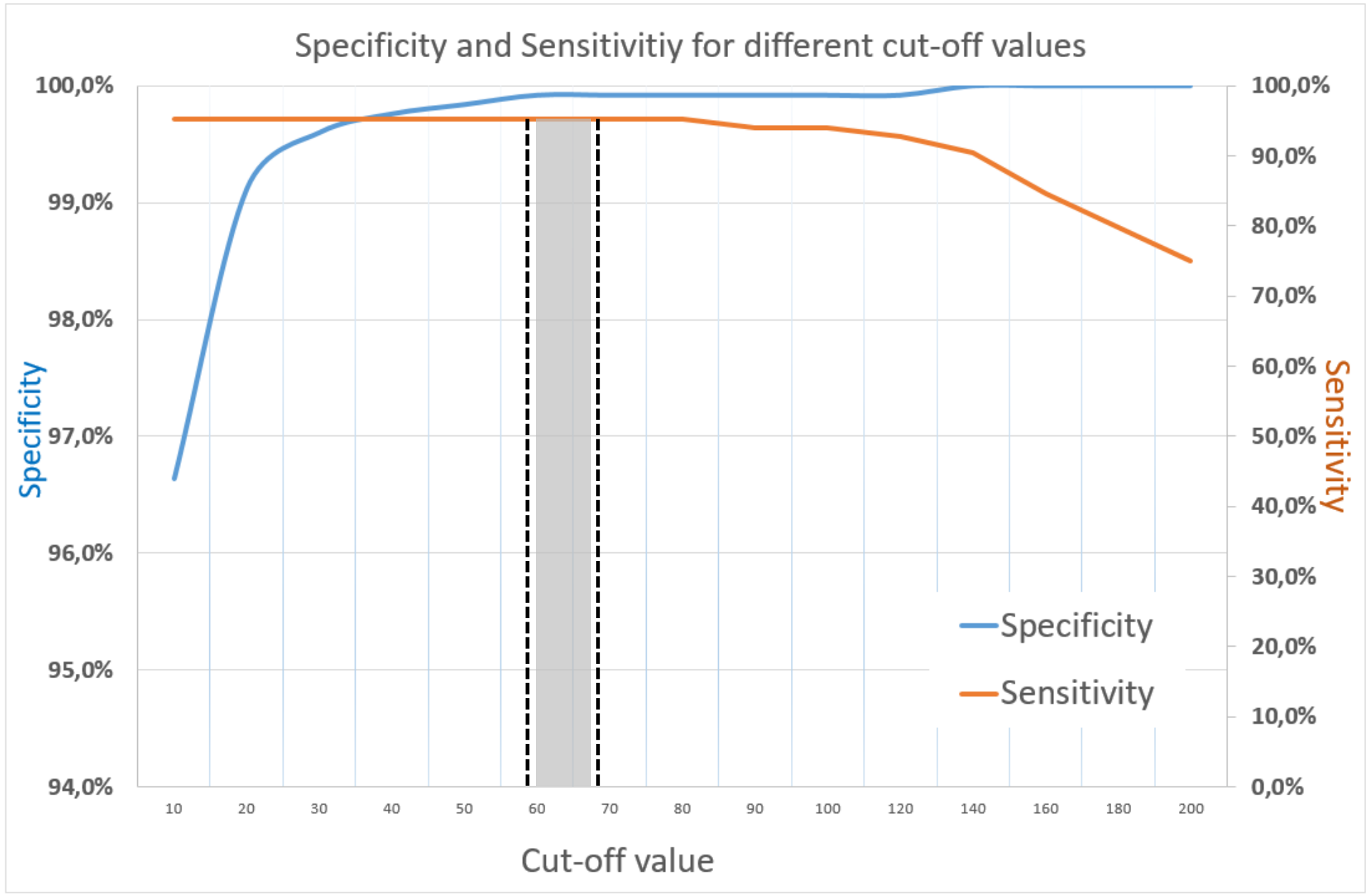
ID Screen® SARS-COV-2-N IgG Indirect: ROC analysis (2)



ID Screen® SARS-COV-2-N IgG Indirect: ANALYTICAL SPECIFICITY

- Due to the low homology of the antigen used with other pathogens that can affect humans, cross-reactions are unlikely.
- A high proportion of the population (up to 90%) have antibodies against other coronaviruses of the genus Betacoronavirus(8.13) affecting humans (HCoVs), such as seasonal coronaviruses (HCoV-HKU1,HCoV-OC43, HCov-NL63, HCoV-229E). Diagnostic specificity (>99,8%), measured on panels of samples probably containing antibodies against these HcoVs, even if their presence could not be established, suggests that cross-reactions are very unlikely.
- Due to the high sequence homology of the antigen used between SARS-CoV-1 and SARS-CoV-2 (>98%), cross-reactions are certain but could not be tested.
- Due to the low sequence homology (<50%) of the antigen used between SARS-CoV-1 and -2, and the Middle East Respiratory Syndrome virus (MERS), cross-reactions are unlikely but could not be tested.

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ID Screen® SARS-COV-2-N IgG Indirect: ANALYTICAL SPECIFICITY

- In addition, 92 blood samples (12) taken simultaneously along with an oropharyngeal sample on which at least one respiratory pathology was confirmed by RT-qPCR, were tested on this kit (samples provided by Montpellier Hospital, CHU Montpellier).
- Of these samples:
  - 79 had a unique positivity by RT-PCR against Rhinovirus (n=47); Coronavirus 229 (n=1); Adenovirus (n=2); Bocavirus (n=1); Enterovirus (n=6); Metapneumovirus (n=2); Coronavirus NL63 (n=3); Coronavirus OC43 (n=2); Parainfluenza type 1 (n=7); or VRS (n=5).
  - 12 samples had PCR positivity against two pathogens, of which 2 showed Coronavirus 229 positivity.
  - 1 sample showed PCR positivity against 3 pathogenic viruses, other than coronaviruses.
- Although it was not possible to determine the presence of antibodies for each of the viruses identified, all samples were found to be negative (maximum S/P %= 32% ; cut-off 60/70 for a Rhinovirus positive sample).

The kit demonstrates excellent analytical specificity

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ID Screen® SARS-COV-2-N IgG Indirect: ANALYTICAL SENSITIVITY

In the absence of both an analytical sensitivity referential sample for SARS-CoV-2 antibodies and a national or international standard, the limit of detection (LoD) was measured by end-point dilution, to determine the dilution for which there is no longer antibody detection (sub-threshold; last positive dilution), on 30(-) samples patients hospitalized and tested positive for COVID-19 RNA by RT-qPCR. (2-fold serial dilutions),

Days after symptoms onset	Last positive dilution							
	neat	1 :2	1 :4	1 :8	1 :16	1 :32	1 :64	>1 :128
Number of samples	2	3	7	6	9	2	1	1

The median titer was 1:8.

Samples were provided by the Biological Resource Montpellier Hospital, CHU of Montpellier
(COVIDthèque collection, n°. 2020-A00935-34)

(-) Guidelines for evaluation of performance of SARS-Cov-2 serological tests in France, « Haute Autorité de Santé », April 2020 16th

ID Screen® SARS-COV-2-N IgG Indirect: CONCORDANCE

Results obtained for 41 samples from the diagnostic sensitivity study panel, as well as for 8 samples from the diagnostic specificity study panel, were compared with those obtained with commercially-available ELISA kits based on the S protein, specifically detecting IgG or IgA.

ELISA test used	PCR Pos.		Pre-epidemic	
	Tested Pos.	Tested Neg	Tested Pos.	Tested Neg
ID screen® SARS-CoV-2-N IgG Indirect	36	5	0	8
Kit A, ELISA IgG indirect	36	5	0	8
Kit B, ELISA IgA indirect	38	3	2	6

Performance of the ID Screen® was comparable in terms of sensitivity, whereas specificity issues were reported with kit A and kit B in published studies(*) (93 or 96%, and 96%, respectively).

Samples were provided by the Biological Resource Montpellier Hospital, CHU of Montpellier
(COVIDthèque collection, n°. 2020-A00935-34)

(*) Evaluation of nine commercial SARS-CoV-2 immunoassays, Ria Lassaunière, et al. , pre-print paper. medRxiv 2020.04.09.20056325
Comparison of four new commercial serologic assays for determination of SARS-CoV-2 IgG. Kruttgen Aet al. Pre print paper , Journal of Clinical Virology (2020)

ID Screen® SARS-COV-2-N IgG Indirect: CUT-OFF ADAPTATION

Seroconversion after the onset of symptoms was followed at Montpellier's Hospital (CHU de Montpellier) in 4 patients hospitalized and tested positive for COVID-19 by RT-PCR.

Patient	Time after onset of symptoms (days)										
	1	2	3	5	6	7	9	11	13	15	18
# 1	3	-	17	61	121	-	153	151	-	-	-
# 2	-	3	-	-	-	3	5	39	160	258	318
# 3	-	-	-	2	-	120	-	-	-	332	-
# 4	-	-	-	-	86	186	236	-	317	309	-

For the 4 patients tested here, IgG antibodies were detected between **5 and 13 days** after the onset of symptoms.

«Alternative cut-off» : the cut-off (60/70%) has been set in order to favor specificity.
In the event of suspected COVID-19 infection, it is advisable to re-sample and retest 48h later any patient whose sample has an S/P%> 30% (possible seroconversion in progress)

ID Screen® SARS-COV-2-N IgG Indirect: CUT-OFF ADAPTATION

Cut-off modification impact:

Sensitivity / Specificity for different cut-off values, with 95 Confidence Interval:

Cut-off value	Specificity (95 CI)			Sensitivity (95 CI=)		
	Sp			Se		
10	96,6	95,5	97,5	95,2	90,3	98,9
20	99,1	98,4	99,5	95,2	94,1	99,9
30	99,6	99,1	99,8	95,2	94,9	100
40	99,8	99,3	99,9	95,2	95,2	100
50	99,8	99,4	100	95,2	95,3	100
60	99,9	99,5	100	95,2	95,5	100
70	99,9	99,5	100	95,2	95,5	100
80	99,9	99,5	100	95,2	95,5	100
90	99,9	99,5	100	94,0	95,5	100
100	99,9	99,5	100	94,0	95,5	100
120	99,9	99,5	100	92,9	95,5	100
140	100	99,7	100	90,5	95,6	100

« Alternative cut-off » : The Spe remains high even at 30% cut-off

Cut-off recommended for screening purposes

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ID Screen® SARS-COV-2-N IgG Indirect: REPEATABILITY

Intra-test accuracy was determined by measuring the coefficient of variation (CV%) on intra-plate repetitions (n=60 or 36) for a series of four samples for which the reactivity is distributed over the measurement range.

Sample	Negative	Positive sub-threshold	Average positive	Strong positive
n =	60	36	60	36
Average S/P	**8**	**33**	**94**	**154**
SD	0,006	0,011	0,020	0,042
CV%	**3,5**	**2,7**	**3,6**	**5,3**

The test shows excellent repeatability.

Reproducibility is under evaluation.

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Acknowledgments

VIROLOGY LABORATORY (Pr Philippe **VANDE PERRE)**

Dr Edouard **TUAILLON**
Pr Vincent **FOULONGNE**

EFS / EFS Occitanie

Laurent **BARDIAUX**
Chantal **FOURNIER-WIRTH**
Magali **RONDEAU**