Second evaluation of the pioneering proficiency test for the main SARS-CoV2 detection methods

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INTRODUCTION

In June, Controllab - laboratory quality control company - in partnership with the Brazilian Society of Clinical Pathology (SBPC/ML), offered the second round of the Proficiency Test (EP) for laboratory tests related to SARS-CoV2, in meeting the requirements of the quality management system.

As in the first round, this round met the exams listed below:

- Molecular Tests using the reverse transcription polymerase chain reaction (RT-PCR) method, referred to in this document as Molecular Techniques;
- Immunological Tests by the Enzyme Immunoassay method, referred to in this document as Automated Immunological Methods;
- Immunological Tests by the Chemiluminescence method referred to in this document as Automated Immunological Methods;
- Immunological Examinations by the Electrochemiluminescence method referred to in this document as Automated Immunological Methods;
- Immunological Examinations by the Chemiluminescence Microparticle Method referred to in this document as Automated Immunological Methods;
- Immunological Tests by the Immunochromatographic and Fluorescence Immunoassay (FIA) methods, also known as Rapid Diagnostic Test - TLR, Rapid Test or POCT (Point-of-care testing), referred to in this document as Rapid Diagnostic Test (TLR) method.

A total of 252 laboratories participated in this second comparison and, among them, 128 were new laboratories that joined this round. On 7/21/2020 the 2nd Individual Evaluation Report was made available. Some laboratories had their proficiency evaluated using more than one methodology, which contributed with different results. The percentage of laboratories participating in each program is listed below:

- 25% with molecular exams;
- 25% with immunological tests by automated methods;
- 70% with immunological tests by Rapid Diagnostic Tests (TLR);

There was an increase in the report of molecular tests (n=08) among the participants in relation to the first round.

Observing an even greater increase for participants of automated immunological methods, which in the first round began to be distributed and made available on the national market.

The Results Profile document, which was made available together with the Individual Evaluation Report, has already allowed laboratories to check the overall performance of their analytical systems according to the programs they participated in. However, this report aims to assist participants in this round (02/2020) in a detailed understanding of the performance of the reported analytical systems.

For this study, data from laboratories that were extended were also considered and reported their results up to one week after the release of the Results Profile.

The general performance (% of adequacy) of the tests and methods used by the laboratories in round 2 is shown in table 01/column "%A1", considering the items evaluated. The "%A2" column shows the percentage of laboratories that achieved adequacy in all items evaluated. The behavior of these methods will be detailed throughout this document.

Table 01: Description of the general performance of all methods shown in the Proficiency Test.

Exams	Test	Method	% A1	% A2	Qty.
Molecular Techniques	RT-PCR commercials kits	PCR In Real Time	92%	80%	25
	RT-PCR kits *in house*	PCR In Real Time	74%	50%	18
Rapid Diagnostic Test	IgG	Fluorescence Immunoassay	98%	86%	14
		Immunochromatography	97%	88%	141
	IgM	Fluorescence Immunoassay	78%	64%	14
		Immunochromatography	78%	72%	141
	Total	Immunochromatography	95%	88%	17
	Antigen	Fluorescence Immunoassay	100%	100%	4
		Immunochromatography	100%	100%	3
Automated immunological	IgG	Enzyme immunoassay	100%	100%	14
		Chemiluminescence	95%	85%	26
	IgM	Enzyme immunoassay	89%	75%	4
		Chemiluminescence	88%	75%	8
	IgA	Enzyme immunoassay	100%	100%	13
	Total	Chemiluminescence	100%	89%	9
		Electrochemiluminescence	98%	94%	16

%A1 – percentage of test adequacy
%A2 – percentage of participants with 100% adequacy of the items evaluated.
Table 01: General percentage of responses of the methods reported in the Coronavirus proficiency test program SARS-CoV-2 in round 02/2020.

Test Items

The samples sent in the Proficiency Test are called by the provider "Test Items". These "items", also known as control materials, were chosen following the quality control criteria previously defined for sending samples from the Proficiency Test programs.

For molecular techniques, 5 (five) items consisting of lyophilized cell suspension were sent, with 1 (one) "not reactive/not detectable" and 4 (four) "reactive/detectable". The reactive items (2, 3, 4 and 5), with the exception of item 4, were prepared from viral isolation with virus replication performed from a viral strain of clinical isolate, in a cell culture system using cells from Vero strain and Dulbecco's Modified Eagle's Medium (DMEM) culture medium. Item 4 consisted of a pool of clinical samples. Item 1, not reactive, was produced from synthetic material with the addition of human cells.

Molecular tests by the real-time polymerase chain reaction method with reverse transcription (RT-PCR)

In this second round, 49 laboratories (18 public and 31 private) reported results using molecular tests. It is worth mentioning that, of these laboratories, 6 were international. Figure 01 shows the general percentage of responses obtained for this exam.

Figure 01: Overall percentage of responses (n=49) from molecular tests

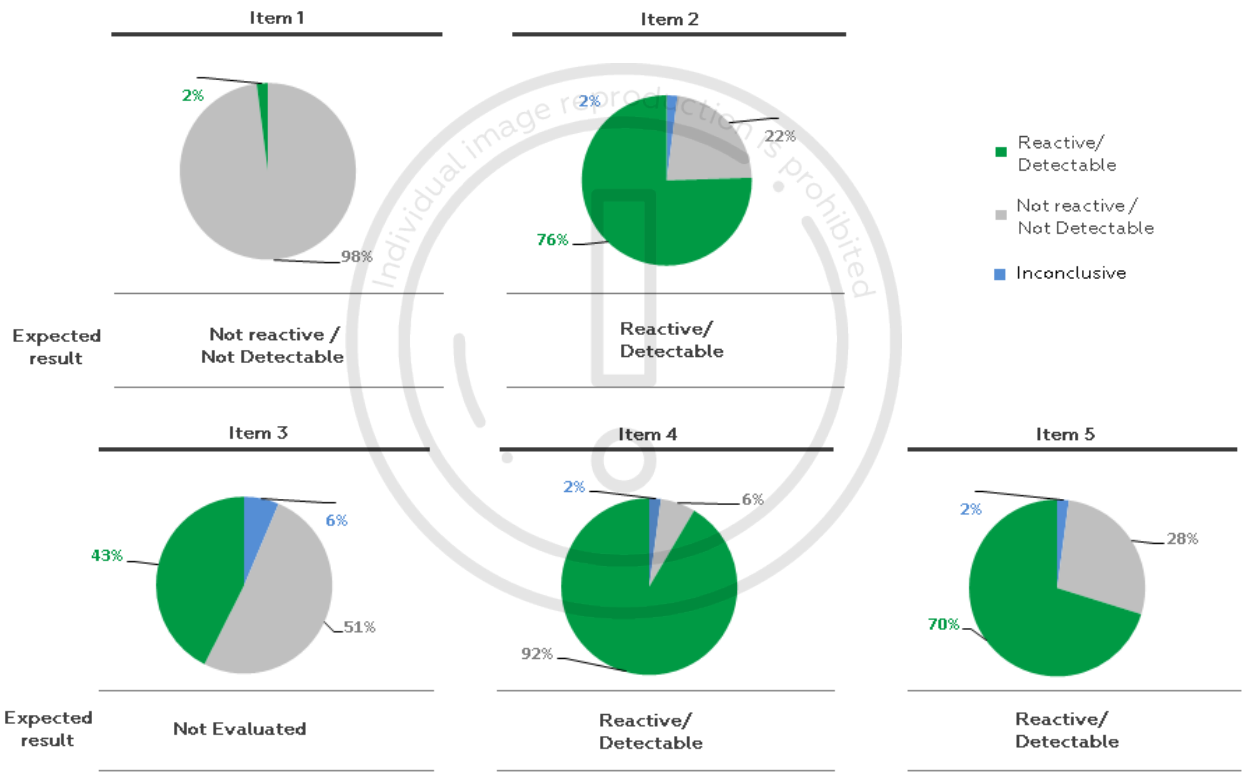

Figure 01: General percentage of responses for items in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020. Presented all laboratories, regardless of the type of method.

When considering all the answers, item 1 presented 98% of the results "Not reactive/Not Detectable". Items 2, 4 and 5 presented, respectively, 76%, 92% and 70% of "Reactive/Detectable" results. Item 3, when compared to the other items, showed no consensus and, for this reason, was not evaluated.

Among the participating laboratories, 6 did not describe the technique used, so they were not included in the statistical evaluation of this document. Thus, the data considered a total of 43 participating laboratories using RT-PCR. Most participants (n=25), that is, 58% used kits distributed commercially. The methods that used reactives prepared and validated by the laboratories themselves (in house) totaled 42% (n=18).

As in the first round, there were reports of reactive kits from different commercial sources. Comparatively, there was a trend towards a smaller number of laboratories reporting in-house methods (down from 27 to 18).

Figure 02: Distribution of results by Molecular Tests according to the type of method (In house and commercial).

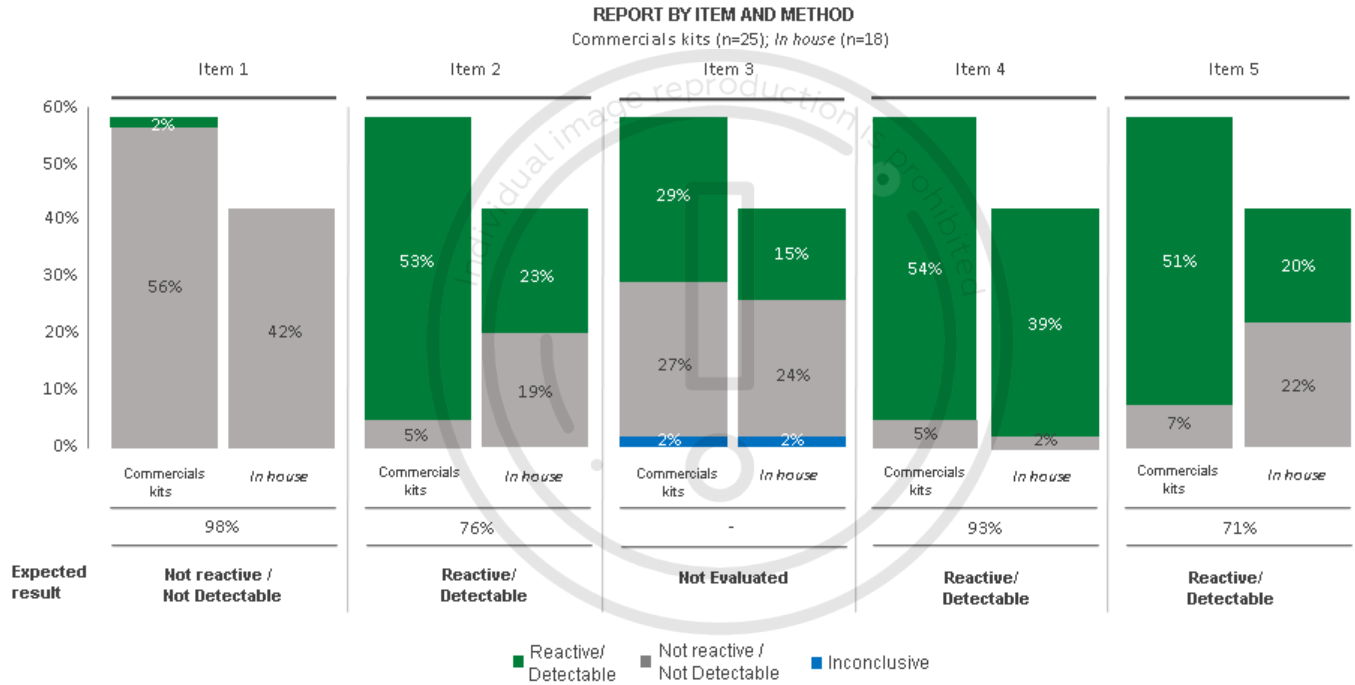

Figure 02: General percentage of responses for each item of the proficiency test program in the 02/2020 round, for reactives used by the molecular method by RT-PCR in house and by commercial kits, compared to the expected result.

Performance of laboratories according to the method (in-house versus commercial kit) and the genetic targets researched

RT-PCR Method (in house)

The percentages of responses from the laboratories that reported the in-house method are shown below in figure 03.

Figure 03: Percentage of responses by molecular techniques by the RT-PCR method (in-house).

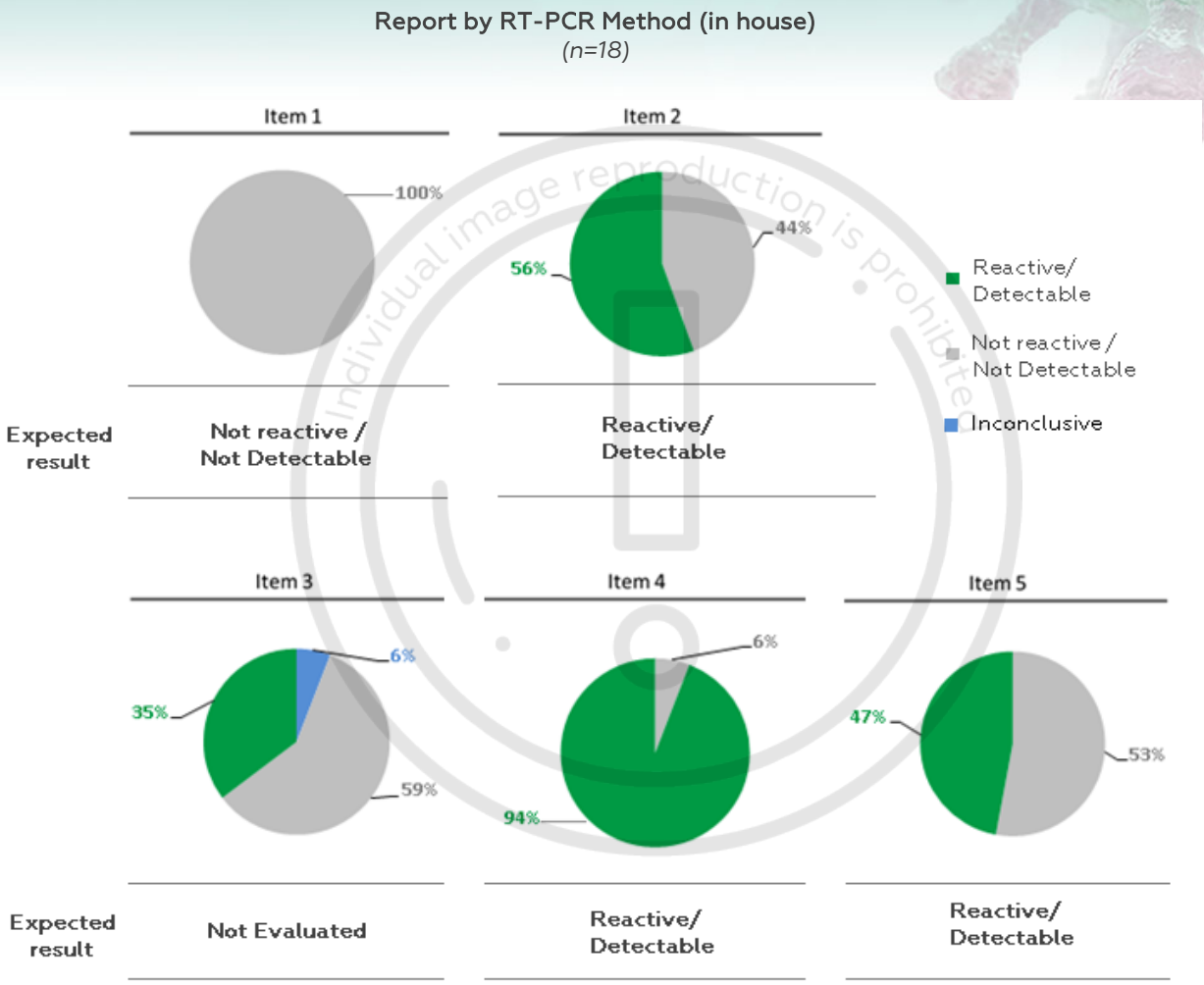

Figure 03: Percentage of responses for items in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round. Presenting only the laboratories that reported using the RT-PCR method in house.

- Item 1 presented an adequacy percentage of 100% considering the accepted result as “not reactive/not detectable”.
- Item 2 presented a percentage of 56% for “Reactive/Detectable”.
- In item 3, 35% of the laboratories identified the presence of the virus.
- Item 4 presented the 94% consensus for “Reactive/Detectable”.
- Item 5 showed a lower percentage among the items evaluated, being 47% for "Reactive/Detectable".

RT-PCR Method (commercials kits)

In this second round, kits from ten different manufacturers were used by the participants and are shown in table 02.

Table 02 - Distribution of commercial kits by origin

Brand	Number of participants (n)
Abbott RealTime	01
Allplex	05
Bio-Manguinhos Molecular	01
GeneFinder Plus	01
Hybribio	01
TaqPath CE-IVD	02
Veri-Q 316	02
Viasure S	02
XGEN	03
Xpert Xpress	07

Table 02: List of commercial kits used in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020.

The percentages of responses from laboratories that reported the method with commercial kits are shown below.

Figure 04: Percentage of responses by molecular techniques by the RT-PCR method (commercial kits)

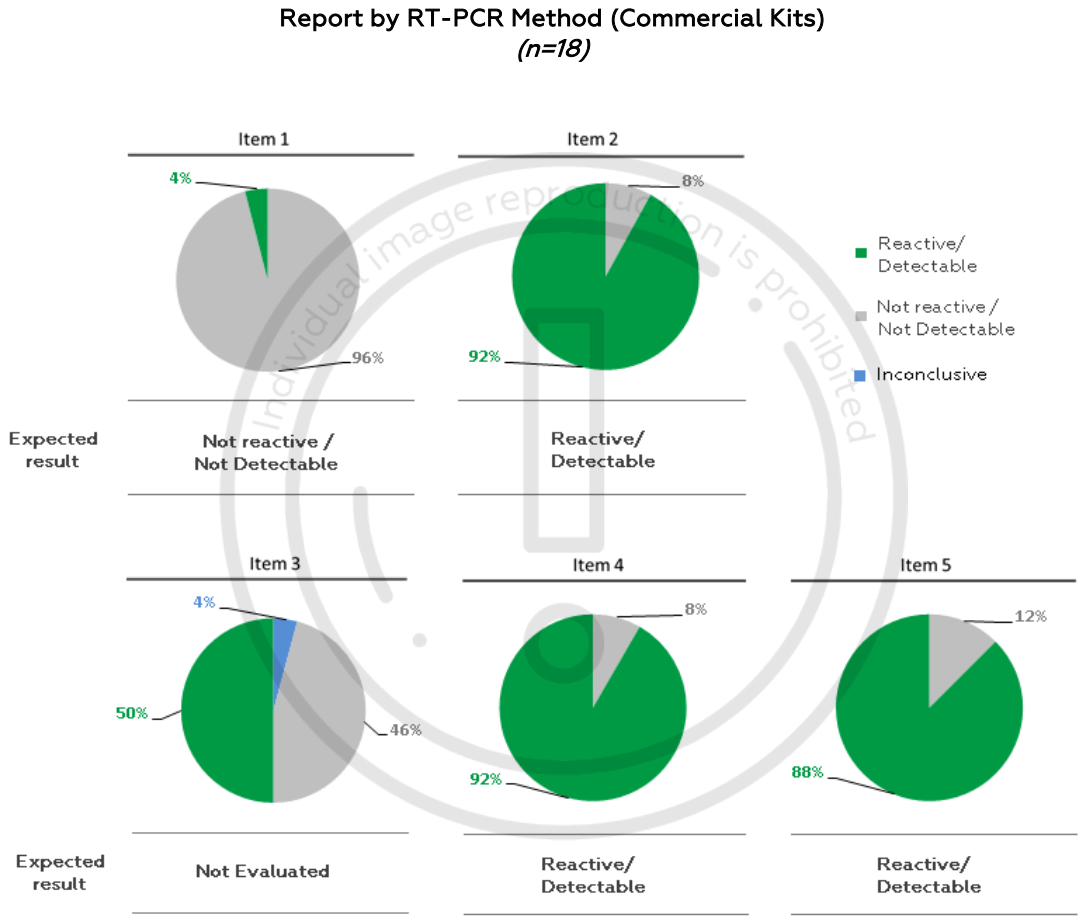

Figure 04: Percentage of responses for items in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round. Listed only laboratories that reported RT-PCR method with commercial kits.

- Item 1: The adequacy percentage was 96% for "Not reactive/Not detectable" results".
- Item 2: Presented a percentage of 92% for "Reactive/Detectable" results".

- Item 3: A lower consensus of responses was observed (50%), in addition to an Inconclusive result (4%). It is worth noting that this item showed a greater consensus for "Reactive/Detectable" results when compared to the in-house method (Figure 3).
- Items 4 and 5 presented, respectively, percentages of 92% and 88% for "Reactive/Detectable" results".

Table 03 shows the commercial kits reported in relation to the number of responses.

Table 03: Number of responses per commercial kit of molecular techniques using the RT-PCR method.

Commercial Kits (RT-PCR In Real Time)	Qty	Item 1			Item 2			Item 3			Item 4			Item 5		
		N	R	I	N	R	I	N	R	I	N	R	I	N	R	I
Abbott RealTime	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
Allplex	5	5	-	-	1	4	-	3	2	-	-	5	-	1	4	-
Bio-Manguinhos Molecular(E)	1	1	-	-	-	1	-	1	-	-	-	1	-	-	1	-
GeneFinder Plus	1	1	-	-	-	1	-	1	-	-	-	1	-	-	1	-
Hybribio	1	1	-	-	1	-	-	1	-	-	-	1	-	-	1	-
TaqPath CE-IVD	2	2	-	-	-	2	-	2	-	-	-	2	-	-	2	-
Veri-Q 316	2	1	1	-	-	2	-	-	1	1	-	2	-	-	2	-
Viasure S	2	2	-	-	-	2	-	1	1	-	1	1	-	1	1	-
XGEN	3	3	-	-	-	3	-	2	-	-	1	1	-	1	1	-
Xpert Xpress	7	7	-	-	-	7	-	-	7	-	-	7	-	-	7	-
Grand total	**25**	**24**	**1**	**0**	**2**	**23**	**0**	**11**	**12**	**1**	**2**	**22**	**0**	**3**	**21**	**0**
expected result		**Not reactive / Not Detectable**			**Reactive/ Detectable**			**Not Evaluated**			**Reactive/ Detectable**			**Reactive/ Detectable**		

N Not reactive / Not Detectable R - Reactive/Detectable I - inconclusive

Table 03: Number of responses per commercial kit for items in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round.

Genetic Target

Table 04 shows the performance of the laboratories according to the genetic target used in the research of the Covid-19 virus.

Table 04: Number of responses by molecular techniques by the genetic target

Genetic Targets	Qty.	Item 1			Item 2			Item 3			Item 4			Item 5		
		N	R	I	N	R	I	N	R	I	N	R	I	N	R	I
E	3	3	-	-	2	1	-	3	-	-	1	2	-	2	1	-
E+ N	1	1	-	-	1	-	-	1	-	-	-	1	-	1	-	-
E+ N+ N1+ N2+ RdRp	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-
E+ N+ ORF1ab+ RdRp	1	1	-	-	-	1	-	1	1	-	-	1	-	-	1	-
E+ N+ RdRp	8	8	-	-	6	5	-	8	2	-	1	8	-	4	4	-
E+ N1	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
E+ N1+ N2	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
E+ N2	6	6	-	-	-	6	-	3	6	-	-	6	-	-	6	-
E+ RdRp	3	3	1	-	3	3	-	3	1	1	1	3	-	3	2	-
N	4	3	1	-	-	3	1	2	2	-	-	3	1	-	4	-
N+ ORF1ab	6	6	-	-	3	4	-	5	2	-	1	4	-	3	3	-
N+ ORF1ab+ S	2	2	-	-	1	2	-	2	-	-	-	2	-	1	2	-
N+ RdRp	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-

Genetic Targets (continuation)	Qtd.	Item 1			Item 2			Item 3			Item 4			Item 5		
		N	R	I	N	R	I	N	R	I	N	R	I	N	R	I
N1	2	2	-	-	-	2	-	-	1	1	-	2	-	-	2	-
N1+ N2	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
N1+ N2+ ORF1ab	1	1	-	-	1	-	-	1	-	-	-	1	-	1	-	-
N1+ N2+ RdRp	2	2	2	-	-	2	-	-	1	-	-	1	-	-	1	-
N2	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
ORF1ab	1	1	-	-	1	-	-	1	-	-	-	1	-	1	-	-
RdRp	3	3	-	-	-	3	-	1	2	-	-	3	-	-	2	1
S	1	1	-	-	-	1	-	1	-	-	-	1	-	-	1	-
expected result		Not reactive / Not Detectable			Reactive/ Detectable			Not Evaluated			Reactive/ Detectable			Reactive/ Detectable		

Qty. – Quantity N Not reactive / Not Detectable R - Reactive/Detectable I - inconclusive

Table 04: Number of responses for the items of the 02/2020 round, against the genetic targets reported in the SARS-CoV-2 Coronavirus proficiency test program.

- It is observed that item 1 presented only false positive results in the groups of genes E + RdRp, N and N1 + N2 + RdRp (Reactive/Detectable - R/D).
- Item 2 presented eight sets of genes with false negative results (Not reactive/Not Detectable - NR/ND), with the groups E+N+RdRp, E+RdRp and N+ORF1ab with a higher percentage.
- For item 3, it was possible to observe 13 sets with false negative result data, of which three were groups with no consensus (E+N+ORF1ab+RdRp/N/N1). It is important to mention that the E+N+RdRp group presented a significant number of false negative results in relation to the number of responses.
- Item 4 presented NR/ND results in groups E, E+N+RdRp, E+RdRp and N+ORF1ab.
- Item 5 demonstrated the same behavior in nine groups, three groups with a significant number of NR/ND data (E + N + RdRp, E + RpRd and N + ORF1ab).

Table 05 shows the genetic targets reported by methods (commercial kits and In house).

Table 05: Number of responses from genetic targets by method (commercial and in-house kits).

Genetic Targets Commercial kits	Qty.	Item 1			Item 2			Item 3			Item 4			Item 5		
		N	R	I	N	R	I	N	R	I	N	R	I	N	R	I
E	1	1	-	-	-	1	-	1	-	-	-	1	-	-	1	-
E+ N+ N1+ N2+ RdRp	1	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-
E+ N+ RdRp	6	6	-	-	4	5	-	6	2	-	-	6	-	2	4	-
E+ N2	6	6	-	-	-	6	-	3	6	-	-	6	-	-	6	-
N	3	2	1	-	-	2	1	2	1	-	-	2	1	-	3	-
N+ ORF1ab	6	6	-	-	3	4	-	5	2	-	1	4	-	2	3	-
N+ ORF1ab+ S	2	2	-	-	1	2	-	2	-	-	-	2	-	1	2	-
N+ RdRp	1	2	-	-	-	1	-	-	1	-	-	1	-	-	1	-
N1	1	1	-	-	-	1	-	-	-	1	-	1	-	-	1	-
N2	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
ORF1ab	1	1	-	-	1	-	-	1	-	-	-	1	-	1	-	-

Genetic Targets Commercial kits (continuation)	Qty.	Item 1			Item 2			Item 3			Item 4			Item 5		
		N	R	I	N	R	I	N	R	I	N	R	I	N	R	I
RdRp	3	3	-	-	-	3	-	1	2	-	-	3	-	-	2	1
S	1	1	-	-	-	1	-	1	-	-	-	1	-	-	1	-

Genetic Targets *In house*	Qty.	Item 1			Item 2			Item 3			Item 4			Item 5		
		N	R	I	N	R	I	N	R	I	N	R	I	N	R	I
E	2	2	-	-	2	-	-	2	-	-	1	1	-	2	-	-
E+ N	1	1	-	-	1	-	-	1	-	-	-	1	-	1	-	-
E+ N+ ORF1ab+ RdRp	1	1	-	-	-	1	-	1	1	-	-	1	-	-	1	-
E+ N+ RdRp	2	2	-	-	2	-	-	2	-	-	1	2	-	2	-	-
E+ N1	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
E+ N1+ N2	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
E+ RdRp	3	3	1	-	2	2	-	3	1	1	1	3	-	3	2	-
N	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
N1	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
N1+ N2	1	1	-	-	-	1	-	-	1	-	-	1	-	-	1	-
N1+ N2+ ORF1ab	1	1	-	-	1	-	-	1	-	-	-	1	-	1	-	-
N1+ N2+ RdRp	2	2	2	-	-	2	-	-	1	-	-	1	-	-	1	-
RdRp	1	-	-	-	-	1	-	-	1	-	-	1	-	-	-	1
Expected Result		**Not reactive/ Not Detectable**			**Reactive/ Detectable**			**Not evaluated**			**Reactive/ Detectable**			**Reactive/ Detectable**		

Qty. – Quantity N Not reactive / Not Detectable R - Reactive/Detectable I - inconclusive

Table 05: Number of responses of genetic targets by method reported in the 02/2020 round in the SARS-CoV-2 Coronavirus proficiency test program.

When analyzing table 05, it is observed that the false negatives obtained are related to gene E (isolated or analyzed with another gene).

For the group of genes E+N+RdRp, there is a greater false negative report for items 2, 3 and 5, both for commercial kits and for the in-house method. The E+RdRp group presented results only in the in-house method, also with a higher index of false negative results for items 2, 3 and 5.

The same is observed for N+ORF1ab, but with data presented only for commercial kits, and a greater number of false negatives in items 2 and 3.

Table 06 shows the percentage of responses from genetic targets considering the possible protocols used.

Table 06: Performance of genetic targets against the protocols used

Protocols	Genetic targets	Commercial Kits				*In house*			
		VN	VP	FN	FP	VN	VP	FN	FP
CDC (China)	N+ ORF1ab	100	65%	35%	0%	-	-	-	-
CDC (China) or (Thermofisher)*	ORF1ab	100	33%	67%	0%	-	-	-	-
CDC (EUA)	N1	100	100%	0%	0%	100%	100%	0%	0%
CDC (EUA)	N2	100	100%	0%	0%	-	-	-	-
CDC (EUA)	N1+ N2	-	-	-	-	100%	100%	0%	0%
CDC (EUA) + CDC (China) or Thermofisher*	N1+ N2+ ORF1ab	-	-	-	-	100%	33%	67%	0%
CDC (EUA) + Charité or Pasteur	N1+ N2+ RdRp	-	-	-	-	50%	100%	0%	50%
Charité	E	100%	100%	0%	0%	100%	17%	83%	0%
Charité	E+ N	-	-	-	-	100%	33%	67%	0%
Charité	E+ N+ RdRp	100	71%	29%	0%	100%	29%	71%	0%
Charité	E+ RdRp	-	-	-	-	75%	54%	46%	25%
Charité + CDC (China) or Thermofisher*	E+ N+ ORF1ab+ RdRp	-	-	-	-	100%	100%	0%	0%
Charité + CDC (EUA)	E+ N+ N1+ N2+ RdRp	-	50%	50%	-	-	-	-	-
Charité + CDC (EUA)	E+ N2	100%	100%	0%	0%	-	-	-	-
Charité + CDC (EUA)	E+ N1	-	-	-	-	100%	100%	0%	0%
Charité + CDC (EUA)	E+ N1+ N2	-	-	-	-	100%	100%	0%	0%
Charité or HKU or NIH (Thailândia)*	N	67%	78%	0%	33%	100%	100%	0%	0%
Charité or Pasteur*	RdRp	100	89%	0%	0%	-	67%	0%	-
Charité or Pasteur*	N+ RdRp	100	100%	0%	0%	-	-	-	-
Thermofisher	N+ ORF1ab+ S	100	75%	25%	0%	-	-	-	-
Thermofisher*	S	100	100%	0%	0%	-	-	-	-

VP - True negative VP - True positive FN - False negative FP - False positive

Table 06: General percentage of responses of the items evaluated in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round, in view of the possible protocol.

Data from items 2, 4 and 5 were considered, due to item 3 known to have a lower virus concentration.

It is observed that for commercial kits, groups based on the Charité and CDC protocols (China) have a higher percentage of false negatives.

Among the participating laboratories using the Charité protocol, a higher proportion of false negatives was verified for the in-house method.

Table 07 shows the percentages of responses by molecular techniques that used the genetic targets recommended by the World Health Organization (WHO) with genes (E+N (1 or 2) + RDRP).

Table 07: Percentage of responses by molecular techniques for the genetic target suggested by WHO x Others

Genetic Target	Item 1			Item 2			Item 3			Item 4			Item 5		
	N	R	I	N	R	I	N	R	I	N	R	I	N	R	I
(a)WHO	95%	5%	-	29%	69%	2%	57%	41%	2%	5%	94%	1%	30%	69%	1%
(b)Outros	100%	-	-	36%[1]	64%	-	85%[2]	15%	-	8%[3]	92%	-	39%[1]	61%	-
Expected Result	Not reactive/ Not Detectable			Reactive/ Detectable			Not Evaluated			Reactive/ Detectable			Reactive/ Detectable		

N Not reactive / Not Detectable R - Reactive/Detectable I - inconclusive

Table 07: Percentage of responses for items assessed in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020, separated by groups of genetic targets in: (a) suggested by WHO and (b) Other target sets reported.

[1] n=1 (S); n=4 (OF1ab)

[2]n=3 (S); n=8 (ORF1ab)

[3]n=1 (ORF1ab)

Table 08 shows the statistics of the CTs (Cycle Threshold) that were reported by the users of the program for each genetic target, broken down by item: quantities, averages, standard deviations and the coefficient of variation.

Table 08: Statistics of CTs reported by laboratories according to the reported gene.

Gene	Item 1				Item 2				Item 3				Item 4				Item 5			
	Qty	Med	DP	CV	Qty	Med	DP	CV	Qty	Med	DP	CV	Qty	Med	DP	CV	Qty	Med	DP	CV
E	-	-	-	-	13	33,9	3,1	9,3%	5	37,4	2,7	7,3%	19	24,4	6,8	27,7%	11	33,8	2,6	7,8%
N	-	-	-	-	16	33,5	4,4	13,1%	6	34,1	6,0	17,5%	17	26,4	3,8	14,4%	16	34,0	3,9	11,4%
N1	-	-	-	-	5	29,1	3,8	12,9%	4	31,2	2,6	8,3%	6	22,6	4,5	19,8%	6	29,8	2,1	7,1%
N2	-	-	-	-	6	30,6	5,0	16,2%	6	34,3	4,6	13,4%	7	23,0	4,8	21,0%	7	32,4	3,3	10,2%
ORF1ab	-	-	-	-	8	30,8	8,9	28,7%	2	21,9	18,6	85,1%	10	27,1	6,0	22,4%	7	35,3	2,8	7,8%
RdRp	-	-	-	-	9	29,5	7,8	26,3%	6	31,4	6,1	19,5%	13	26,6	5,6	21,2%	10	31,6	7,5	23,7%
S	-	-	-	-	2	32,2	1,5	4,6%	-	-	-	-	3	26,5	0,9	3,2%	2	34,3	0,3	0,8%
	Not reactive/ Not Detectable				Reactive/ Detectable				Not Evaluated				Reactive/ Detectable				Reactive/ Detectable			

Qty. – Quantity Med – Mean DP – Standard deviation CV – Coefficient of variation

Table 08: Statistics of CTs reported by laboratories in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020, separated by gene.

- As item 1 was Not Reactive, the aforementioned calculations were not made.
- In item 2, the greatest data dispersion occurred in the ORF 1 ab gene (28.7%), followed by the RdRp gene (26.3%).
- In item 3, the coefficients of variation (CV) of ORF 1 ab were much higher than the others with 85.1%, followed by the RdRp gene (19.5%) and the N gene (17.5%). It is worth mentioning that this item had a lower concentration of viruses than the other reactive items.
- For item 4, the highest observed CV was for gene E with 27.7%. The N2, ORF 1 ab, RdRp genes were matched with CV of 21.0%, 22.4% and 21.2%, respectively.
- In item 5, the RdRp gene had a CV of 23.7%, standing out with greater inaccuracy than the others.

Equipment

Figure 05 shows the equipment used for Amplification/Detection according to their percentage of participation.

Figure 05: Percentage of responses by molecular techniques by equipment

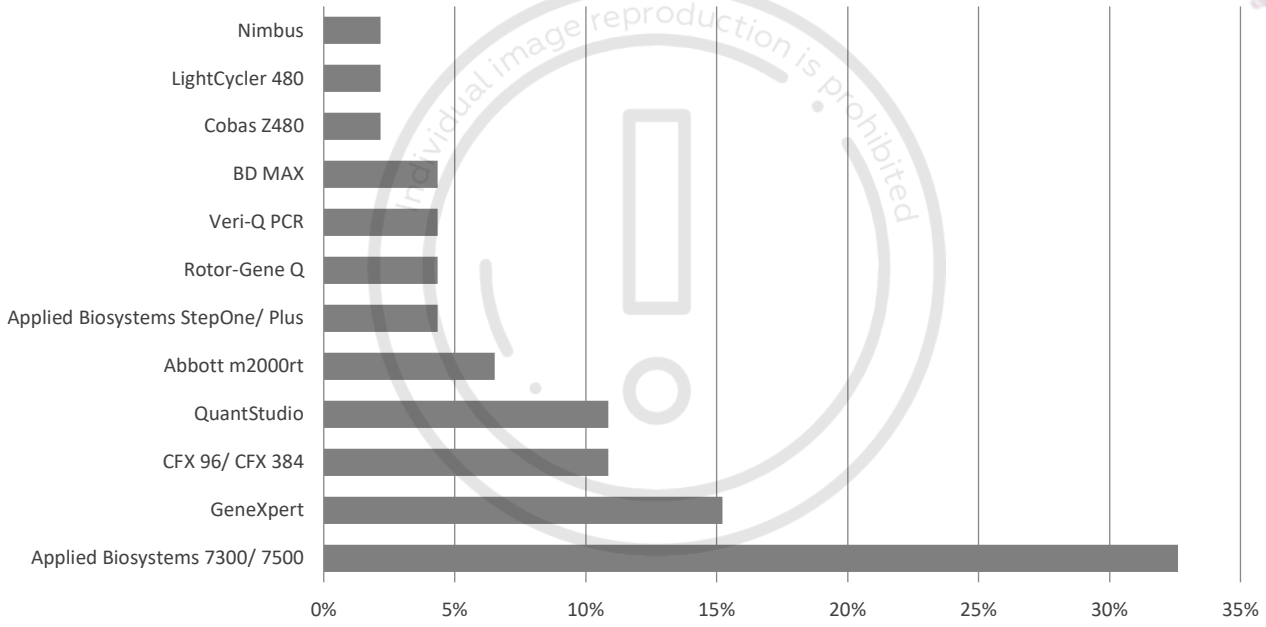

Figure 05: Percentage of responses from amplification/detection equipment reported in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round.

We observed that the Applied Biosystems system with the 7300/7500 series was again the most reported, represented by 33% of respondents.

Extraction kits and extraction method

In this round (02/2020), information was requested on the kits, methods and equipment used for extraction. Below, the data presented according to the percentage of participation.

The different brands of kits used by participating laboratories for RNA extraction can be seen in figure 06.

Figure 06: Percentage of responses of molecular techniques per kit for RNA extraction

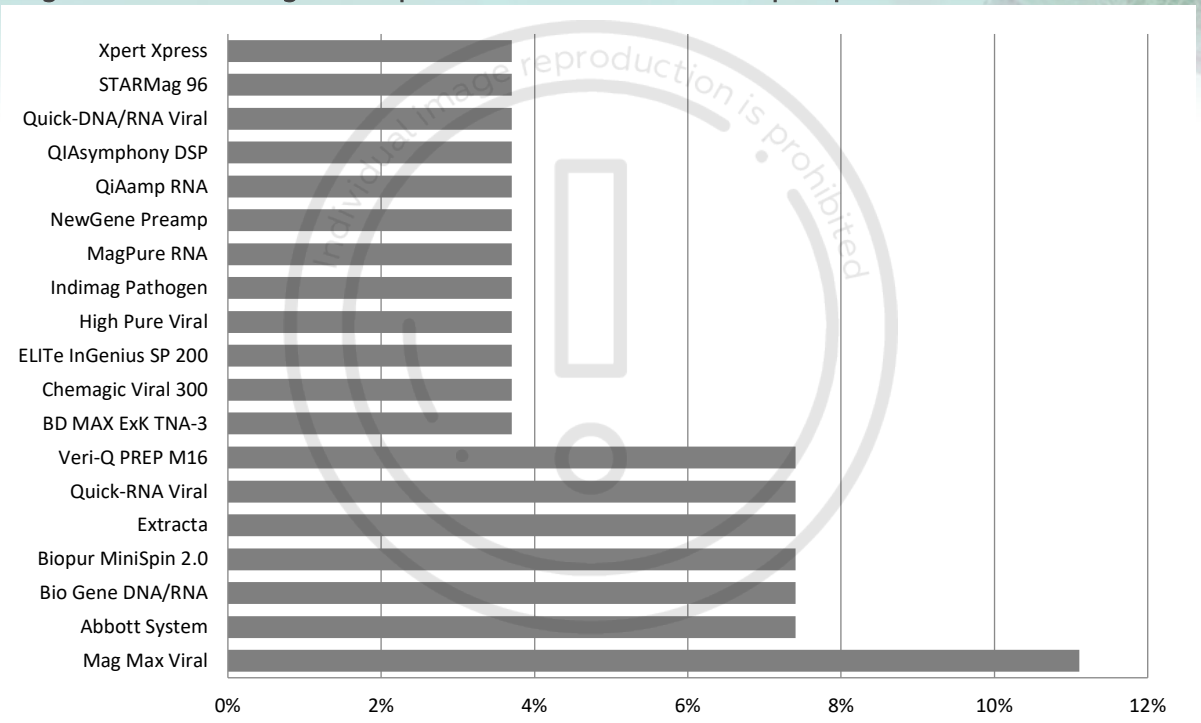

Figure 06: Percentage of responses from extraction kits reported in the Coronavirus proficiency test program SARS-CoV-2 in round 02/2020.

For the extraction method, three were reported by the laboratories and are shown in figure 07.

Figure 07: Percentage of responses of molecular techniques by extraction methods.

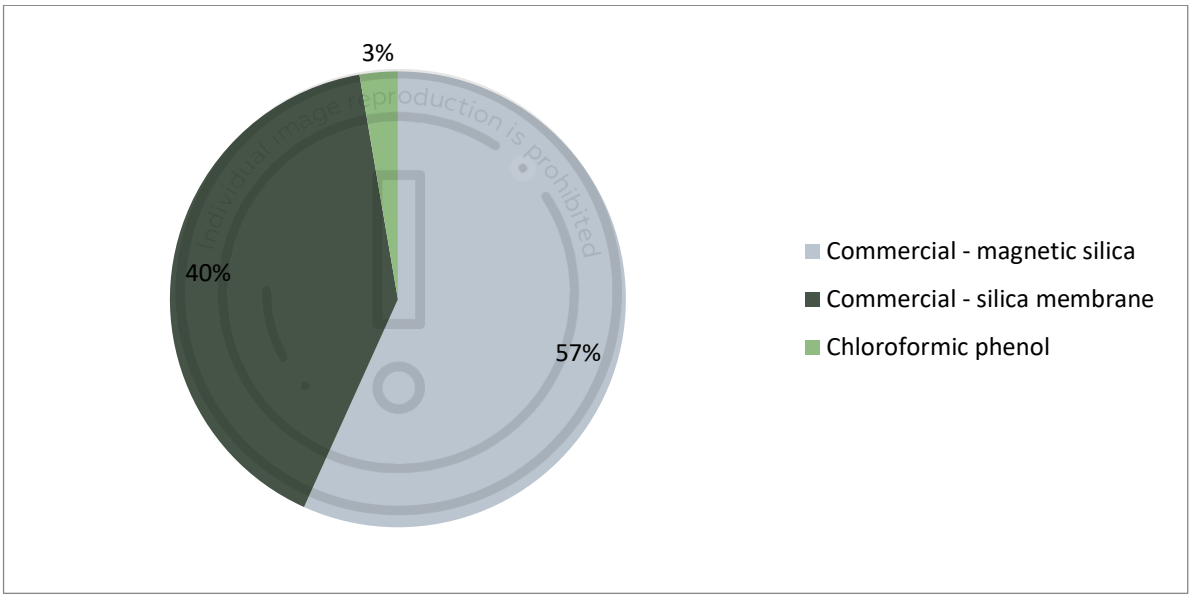

Figure 07: Percentage of responses from extraction methods reported in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round.

- Commercial - magnetic silica (57%);
- Commercial - silica membrane (40%);
- Chloroformic phenol (3%).

Some participants did not report the extraction kit and/or the method. Therefore, this statistical analysis was not considered.

Extraction equipment

Figure 08 shows the percentages of equipment used for RNA extraction as reported by participating laboratories.

Figure 08: Percentage of responses by molecular techniques for equipment for extraction

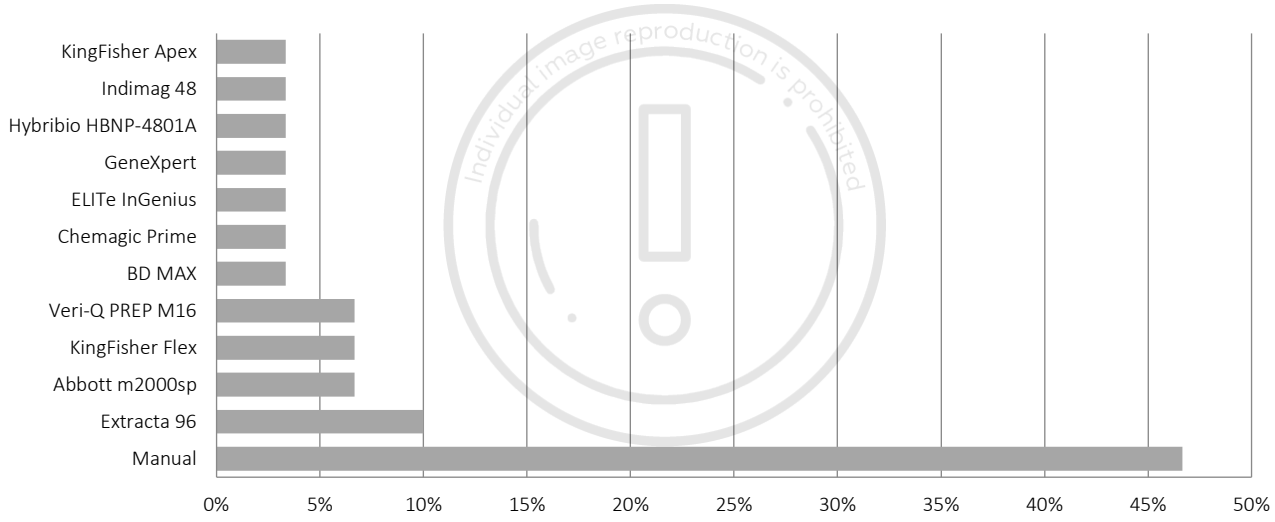

Figure 08: Percentage of responses from extraction equipment reported in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round.

Manual extraction was the most reported, being represented by 47% of laboratories, followed by Extracta 96.

IMMUNOLOGICAL METHODS

Test Items

For the evaluation of the immunological methods used in the search for antibodies, the materials sent in items 3 and 4 were the same both for the evaluation of the automated analytical systems and for the analysis of the Rapid Diagnostic Test (TLR) - Antibodies systems.

Automated immunological methods

In this round, 58 laboratories (55 Private and 3 Public) reported results with automated analytical systems. Of these laboratories, 3 were international. The systems used and reported in this round were represented by the chemiluminescence (Q), electrochemiluminescence (EQ), chemiluminescence microparticles (CMIA) and enzyme immunoassay (EIA) methods. The results were evaluated qualitatively (Positive/Negative), but in this document the quantitative data are presented.

The general performance of the participants who reported the automated kits in the proficiency test is shown below in figure 09. Only the items evaluated were considered and the percentage was calculated in relation to the total number of responses reported by them for IgA, IgG, IgM and Total (IgG + IgM + IgA).

Figure 09: General percentage of suitability for automated immunological kit of the Proficiency Test for IgG, IgM, IgA and Total.

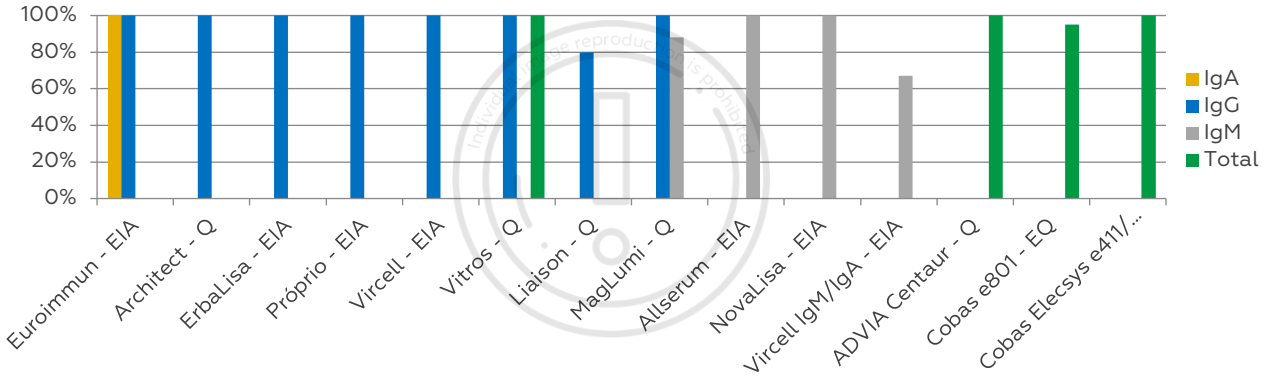

Figure 09: Percentage of general suitability of kits compared to items assessed in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round.

Enzyme Immunoassay method (kit Euroimmun)

Table 09 describes the automated immunological methods for IgG and IgA by the Euroimmun kit.

Table 09: Results of automated immunological methods with individual data (Euroimmun-EIA Kit for IgG and IgA)

	IgG - Kit Euroimmun- EIA					IgA - Kit Euroimmun- EIA				
	Item 1	Item 2	*Item 3	Item 4	Item 5	Item 1	Item 2	*Item 3	Item 4	Item 5
Part. A	3,90	6,53	0,15	**12,50**	7,83	4,06	6,29	0,46	9,94	5,12
Part. C	2,10	4,26	0,47	7,00	4,69	4,62	5,81	0,36	5,89	4,26
Part. D	2,59	5,16	0,13	**12,23**	6,48	6,08	6,17	0,64	6,18	5,88
Part. E	2,88	2,16	0,07	9,87	7,31	3,05	5,19	0,12	7,39	3,65
Part. F	3,26	6,08	0,17	6,94	5,25	6,59	6,12	0,20	8,10	8,10
Part. H	2,69	6,08	0,11	10,37	5,52	5,91	5,67	0,16	5,08	4,10
Part. I	2,49	5,31	0,07	8,44	5,76	3,73	7,41	0,14	5,34	4,68
Part. J	2,94	5,32	0,05	8,75	5,70	3,39	7,74	0,15	7,74	3,27
Part. K	3,39	4,97	0,25	**13,51**	6,84	3,27	7,13	0,28	7,13	4,51
Part. L	2,88	5,82	0,06	8,38	6,71	4,49	8,49	0,22	9,28	4,78
Part. M	2,87	5,41	0,04	8,21	5,45	4,46	4,81	0,24	**17,37**	4,35
Part. L	-	-	-	-	-	4,12	6,98	0,18	7,53	5,45
Part. M	-	-	-	-	-	3,57	5,41	0,06	6,45	3,60
Qty	11	11	11	11	11	13	13	13	13	13
Mean	2,91	5,19	0,14	9,65	6,14	4,41	6,40	0,25	7,96	4,75
DP	0,48	1,18	0,13	2,25	0,96	1,13	1,08	0,16	3,17	1,25
CV	16,6%	22,8%	87,6%	23,3%	15,7%	25,7%	16,9%	64,1%	39,8%	26,3%
Expected result	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 09: Individual data (reason) of the items sent in the Coronavirus SARS-CoV-2 proficiency test program in the 02/2020 round, separated by laboratory, item and test. Data represented by the Euroimmun - EIA IgG and gA kits. Metrics recalculated after the round was released with the inclusion of extended participants.
**Only for item 3 a negative result was expected for IgG and IgA antibodies.*

Observing the data reported for the Euroimmun kit in table 09, among the participants of the round there was a report by 11 laboratories with results for anti-SARS-CoV-2 IgG. For this test, all reported positive values for items 1, 2, 4 and 5 according to the reference value on the kit insert (Ratio ≥ 1.1). Despite being in accordance with the qualitative value expected for the item (Positive), it is worth noting that the participating laboratory K was the one that presented the highest value for item 4 (13,51), followed by participants A (12,5) and D (12,23). In item 3, all laboratories reported negative values, being less than 0,8 according to the instructions.

For anti-SARS-CoV-2 IgA, similar behavior was observed, with positive values for items 1, 2, 4 and 5 reported by the 13 participating laboratories and negative values for item 3. It was observed that the participating laboratory M, in item 4, it presented a higher ratio value (17.37) compared to data reported by other laboratories that used the same analytical system.

Enzyme Immunoassay method (Other kits)

Table 10 describes the automated immunological methods for IgG and demonstrates the results reported by two participating laboratories with two different reactive kits for the measurement of IgG by EIA.

Table 10: Individual results of automated immunological methods (Other IgG kits by EIA)

	IgG – Other kits by Enzyme immunoassay					
KIT	Participants	Item 1	Item 2	Item 3	Item 4	Item 5
ErbaLisa - EIA	Part. B	1,205	1,330	0,171	3,157	1,516
Vircell - EIA	Part. N	*4,46	*4,08	0,360	*5,03	3,110
Expected Result	Positive	Positive	Negative	Positive	Positive	Positive

*Table 10: Individual data (Index) of the items sent in the Coronavirus SARS-CoV-2 IgG proficiency test program in the 02/2020 round, separated by laboratory and item. Data represented by ErbaLisa, Vircell kits. *Index >6*

The participant who reported with the Vircell-EIA system answered index results between 4.0 to 6.0 (“Doubtful”, according to the kit's instructions) for items 1, 2 and 4. However, the interpretation of the results reported by this user was “Positive”. The ErbaLisa-EIA kit showed “Positive” results in all items, except for item 3, in agreement with the Euroimmun kit.

Table 11 describes the automated immunological methods for IgM.

Table 11: Individual results of automated immunological methods (IgM kits by EIA)

	IgM – kits by Enzyme Immunoassay					
KIT	Participants	Item 1	Item 2	Item 3	Item 4	Item 5
Allserum - EIA	Part. X	2,00	0,40	0,20	1,70	0,20
	Part. L	1,12	1,03	0,86	1,82	0,39
NovaLisa - EIA	Part. B	1,80	1,03	0,10	1,75	0,23
Vircell IgM/IgA - EIA	Part. N	*0,64	*1,76	*0,03	*0,07	*0,01
Expected result	-	Not evaluated	Not evaluated	Negative	Positive	Negative

*Table 11: Individual data (Index) of the items sent in the Coronavirus SARS-CoV-2 IgM proficiency test program in round 02/2020, separated by laboratory and item. Data represented by the Allserum, Novalisa and Vircell kits. *Index >6*

There were 4 participating laboratories that reported the Allserum, Novalisa and Vircell kits to detect IgM by EIA.

Items 1 and 2 showed positive and negative values. Item 02 presented values corresponding to positive, negative and indeterminate results (Allserum 0,9 – 1,1).

Microparticle chemiluminescence method (Architect System)

Table 12 describes the results reported by participants who used the Architect analytical system to measure IgG (n=08).

Table 12: Results of automated immunological methods with individual data. (Kit Architect IgG – Q)

	IgG – KIT Architect - Q				
	Item 1	Item 2	Item 3	Item 4	Item 5
Part. Q	6,05	6,10	0,02	8,91	4,33
Part.R	5,72	5,99	0,02	8,38	4,52
Part. S	5,70	5,72	0,02	8,47	4,68
Part. T	6,10	6,11	0,02	8,98	4,59
Part. D	5,94	6,06	0,02	9,13	4,61
Part. A	5,76	6,13	0,02	9,05	4,65
Part. V	4,96	4,40	0,01	6,90	3,66
Part. K	5,38	5,51	0,02	8,40	4,35
Qty	8	8	8	8	8
Mean	5,70	5,75	0,02	8,53	4,42
DP	0,38	0,59	0,00	0,72	0,34
CV	6,6%	10,2%	18,9%	8,5%	7,6%
Expected Result	Positive	Positive	Negative	Positive	Positive

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 12: Individual data of the items sent in the SARS-CoV-2 IgG Coronavirus proficiency test program in round 02/2020, separated by laboratory, item and kit. Data represented by the Architect -Q kit.

When analyzing the data reported for the Architect system, results with positive values for anti-SARS-CoV-2 IgG are observed in all items, except item 3, which presented results with negative values and in agreement with the reference value present in the package insert (Architect Index ≥ 1.4).

The highest coefficients of variation were observed for items 2 and 3 respectively (10,2% e 18,9%).

Chemiluminescence method (Vitros System)

Table 13 describes the performance of the Vitros system for IgG and Total.

Table 13: Results of automated immunological methods with individual data. (Kit Vitros IgG and Total – Q)

	IgG – Kit Vitros - Q					Total – Kit Vitros - Q				
	Item 1	Item 2	Item 3	Item 4	Item 5	Item 1	Item 2	Item 3	Item 4	Item 5
Part. X	7,56	14,90	0,02	20,00	14,70	36,70	52,40	0,03	245,00	68,00
Part. Y	8,67	14,80	0,01	19,50	15,90	32,30	44,90	-	219,00	86,80
Part. Z	11,40	20,70	0,01	25,10	21,10	-	-	-	-	-
Part. AA	10,60	19,90	0,01	24,80	19,90	-	-	-	-	-
Part. AB	12,40	23,00	0,01	27,80	23,70	-	-	-	-	-
Part. AC	9,72	17,80	0,01	21,20	18,60	40,30	55,60	0,11	283,00	116,00
Part. AD	9,14	16,50	0,01	21,00	17,60	39,40	58,20	0,15	283,00	107,00
Part. AE	10,50	18,40	0,01	22,70	18,60	48,80	69,80	0,05	341,00	136,00
Part. AF	8,77	14,90	0,01	18,60	16,40	42,40	57,80	0,12	296,00	-
Qty.	9	9	9	9	9	6	6	6	6	5
Mean	9,86	17,88	0,01	22,30	18,50	39,98	56,45	0,09	277,83	102,76
DP	1,51	2,91	0,00	3,05	2,79	5,55	8,17	0,05	42,22	26,27
CV	15,3%	16,3%	30,0%	13,7%	15,1%	13,9%	14,5%	56,9%	15,2%	25,6%
Expected Result	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Positive

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 13: Individual data of the items sent in the SARS-CoV-2 IgG and Total Coronavirus proficiency test program in the 02/2020 round, separated by laboratory, item and assay. Data represented by the Vitros -Q kit.

For the Vitros system, a behavior similar to that presented in the Architect - IgG system is observed, with positive values for anti-SARS-CoV-2 IgG in all items, except for item 3, which also presented negative values, according to the reference value of the package insert (Vitros IgG Index ≥ 1.0).

For anti-SARS-CoV-2 Total, 6 of the 9 participating laboratories that reported results for IgG with the Vitros kit, also presented their data for the anti-SARS-CoV-2 Total assay. Except for the participating laboratory Y, which did not report a result for item 3. All values reported for items 1, 2, 4 and 5 were positive according to the package insert (Vitros - Total Q ≥ 1.0). The participating laboratory AE reported higher positive values compared to the other participants.

The highest coefficients of variation occurred in Item 3, IgG (30%) and Total (56%).

Chemiluminescence method (ADVIA Centaur System)

Table 14 shows the three results obtained with the ADVIA Centaur system for anti-SARS CoV-2 Total.

Table 14: Results of automated immunological methods with individual data. (Kit ADVIA Centaur - Q)

	Total – Kit ADVIA Centaur - Q				
	Item 1	Item 2	Item 3	Item 4	Item 5
Part. J	2,27	5,53	0,05	10,00	10,00
Part. AG	2,95	6,27	0,05	10,00	10,00
Part. AH	2,07	5,06	0,05	10,00	9,05
Qty.	3	3	3	3	3
Mean	2,43	5,62	0,05	10,00	9,68
DP	0,46	0,61	0,00	0,00	0,55
CV	19,0%	10,9%	0,0%	0,0%	5,7%
Expected Result	Positive	Positive	Negative	Positive	Positive

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 14: Individual data for items sent in the Coronavirus proficiency test program SARS-CoV-2 Total in round 02/2020, separated by laboratory, item and test. Data represented by the ADVIA Centaur –Q kit (Index ≥ 1.0)

In agreement with the other systems, the laboratories reported positive results for items 1, 2, 4 and 5 (Index ≥1.0). Only item 3 is left with the negative consensus. There was no inaccuracy for items 3 and 4.

Chemiluminescence method (MagLumi System)

Table 15 shows the results obtained with the MagLumi system for anti-SARS-CoV-2 IgG.

Table 15: Results of automated immunological methods with individual data (Kit MagLumi IgG – Q)

	IgG – Kit MagLumi - Q				
	Item 1	Item 2	Item 3	Item 4	Item 5
Part.BA	4,77	5,69	0,50	12,95	1,96
Part.BB	7,25	9,44	0,03	52,00	1,79
Part.BC	12,62	10,41	0,06	22,22	5,59
Part.BD	7,99	11,06	0,01	53,48	1,90
Part.BE	9,03	14,32	0,04	63,47	2,07
Part.BF	7,91	9,99	0,04	**1,66**	**57,05**
Part.BG	7,60	9,50	0,10	53,20	1,60
Qty.	7	7	7	7	7
Mean	8,17	10,06	0,11	42,89	2,49
DP	2,36	2,55	0,17	20,24	1,53
CV	28,9%	25,4%	155,8%	47,2%	61,6%
Expected result	Positive	Positive	Negative	Positive	Positive

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 15: Individual data (AU / mL) of items sent in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020, separated by laboratory, item and test. Data represented by the MagLumi IgG - Q kit.

The MagLumi system uses the chemiluminescence method and in this round, it was evaluated in the program in a qualitative way (Positive/Negative). According to table 15, for the IgG antibody, item 3 was the only one that was a negative material, following the reference value provided in the manufacturer's instructions (AU / mL> 1) and in agreement with the Euroimmum, Vitros and Architect systems . In items 1, 2, 4 and 5, results of values considered positive were observed (AU / mL ≥1). Item 5 presented a greater analytical variation. The participant "Part. BF "that reported a result with a higher value than the other laboratories for this item. This participating laboratory obtained a lower value for item 4, suggesting an

incorrect transcription and, therefore, it was disregarded for the calculation of the metrics presented at the end of the table.

Table 16 shows the individual results for the MagLumi system for the anti-SARS-CoV-2 IgM.

Table 16: Results of automated immunological methods with individual data (Kit MagLumi IgM – Q)

	IgM – Kit MagLumi - Q				
	Item 1	Item 2	Item 3	Item 4	Item 5
Part. G	0,69	0,97	0,56	1,95	0,71
Part. BA	0,61	0,78	0,37	1,50	0,42
Part. BC	0,77	0,76	0,60	1,64	0,73
Part. BD	0,84	0,99	0,77	1,82	0,75
Part. BE	1,08	1,62	0,86	2,59	1,01
Part. BF	0,72	0,86	0,48	0,70	1,84
Part. BG	0,70	0,80	0,60	1,70	0,70
Part. BJ	0,57	0,77	0,43	1,42	0,62
Qty.	8	8	8	8	8
Mean	0,75	0,94	0,58	1,67	0,85
DP	0,16	0,29	0,17	0,53	0,43
CV	21,2%	30,5%	28,4%	32,0%	51,0%
Expected result	Negative	Not Evaluated	Negative	Positive	Negative

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 16: Individual data (AU / mL) of the items sent in the SARS-CoV2 Coronavirus proficiency test program in the 02/2020 round, separated by laboratory, item and test. Data represented by the MagLumi - Q IgM kit.

The laboratories showed a negative consensus for item 3. The participating laboratories BE (items 1 and 5) and G (item 2) presented values for the flagged items between 0.9 to 1.1 AU/mL, which according to the manufacturer , can be considered indeterminate. Item 4 presented values considered as positive (AU/mL ≥1), except for the participating laboratory BF.

For item 5 there was a high inaccuracy (CV=51%).

Chemiluminescence method (Liaison system)

Table 17 shows the individual results obtained with the Liaison system for anti-SARS-CoV-2 IgG.

Table 17: Results of automated immunological methods with individual data (Kit Liaison – Q IgG)

	IgG – Kit Liaison - Q				
	Item 1	Item 2	Item 3	Item 4	Item 5
Part.BH	36,20	69,80	56,70	150,00	79,20
Part.BI	38,20	79,60	52,50	194,00	87,50
Part.BJ	39,20	78,10	57,30	159,00	79,80
Qty.	3	3	3	3	3
Mean	37,87	75,83	55,50	167,67	82,17
DP	1,53	5,28	2,62	23,25	4,63
CV	4,0%	7,0%	4,7%	13,9%	5,6%
Expected result	Positive	Positive	Negative	Positive	Positive

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 17: Individual data (AU/mL) of items sent in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020, separated by laboratory, item and test. Data represented by Liaison - Q IgG

The Liaison system also uses the chemiluminescence method and was evaluated qualitatively in the program. All items showed positive behavior. Item 3 stands out, which presented results contrary to the one presented for the MagLumi system, which identified the item as negative. This behavior obtained in item 3

was investigated with the manufacturer and the laboratories involved were not harmed. The greatest variation occurred in item 4 (Cv=13,9%).

Electrochemiluminescence Method (Cobas system)

Table 18 presents the individual results obtained with the Cobas series systems for anti-SARS-CoV-2 Total.

Table 18: Results of automated immunological methods with individual data - Cobas Series.

	Total - Kit Cobas e801 - EQ				
	Item 1	Item 2	Item 3	Item 4	Item 5
Part. K	45,90	27,30	0,10	21,50	2,93
Part. AJ	69,00	20,35	0,51	2,52	17,05
Part. AK	43,20	13,30	0,10	19,60	2,96
Qty.	3	3	3	3	3
Mean	52,70	20,32	0,24	14,54	7,65
DP	14,18	7,00	0,24	10,45	8,14
CV	26,9%	34,5%	100,3%	71,9%	106,5%
Expected result	Positive	Positive	Negative	Positive	Positive

	Total - Kit Cobas e411/ e601/ e602 - EQ				
	Item 1	Item 2	Item 3	Item 4	Item 5
Part. AL	34,74	18,65	0,05	20,20	2,44
Part. AM	35,16	16,84	0,07	18,10	2,52
Part. AN	33,97	19,37	0,06	21,02	2,24
Part. AO	38,23	16,23	0,06	18,53	2,43
Part. AP	37,04	12,21	0,08	17,62	2,64
Part. AQ	41,20	14,25	0,08	18,95	2,83
Part. AR	42,84	19,51	0,08	19,57	2,39
Part. AS	41,01	13,26	0,08	16,93	2,54
Part. AT	37,08	18,04	0,07	19,37	-
Part. AU	33,89	11,68	0,07	15,99	2,10
Part. AV	40,59	13,76	0,08	18,74	2,91
Part. AX	34,62	11,05	0,08	15,05	2,27
Part. AY	33,52	14,93	0,05	16,85	**47,4**
Part. AZ	-	13,86	0,06	-	1,94
Qty.	13	14	14	13	13
Mean	37,22	13,28	0,06	15,95	2,11
DP	3,25	2,00	0,01	1,27	0,23
CV	8,7%	15,1%	19,5%	8,0%	11,1%
Expected result	Positive	Positive	Negative	Positive	Positive

Qty. – Quantity DP – Standard deviation CV – Coefficient of variation

Table 18: Individual data of the items sent in the Coronavirus proficiency test program SARS-CoV-2 Total in the 02/2020 round, separated by laboratory, item and test. Data represented by the Cobas series kits.

When analyzing the results obtained with the Cobas series systems, the same behavior is observed for the chemiluminescence method with positive values for anti-SARS-CoV-2 Total in items 1, 2, 4 and 5, according to the value reference sheet of the kit (Index ≥ 1.0). The AJ laboratory presented inverted values for items 4 and 5, when compared to the participating laboratories K and AK, for the Cobas e801 system.

For the Cobas Elecsys e411/e601/e602 series, it was observed that the participant AY obtained a higher value for item 5 (47.4) when compared with the other participating laboratories in the same group. Therefore, it was disregarded from the calculation of the metrics presented at the end of the table.

Items 2, 3 and 5 showed greater dispersion of data with coefficients of variation of 15.1%, 19.5%, and 11.1%, respectively.

Immunological method by Rapid Diagnostic Test (TLR) - Antibodies

In this round, 167 laboratories (148 private and 19 public) reported results using immunological methods by Rapid Diagnostic Test (TLR) to detect antibodies. Of these laboratories, 3 of them were international.

Contrary to what was presented for the automated methods (individual data), the data will be made available by kit due to the nature of the data being only qualitative

Figure 10 shows the percentage of responses obtained with the TLR kits. It was observed that there were responses to 18 different kits, 16 of which were immunochromatographic (IC) methods and 2 for the fluorescence immunoassay method (FIA).

The kits Celer one step COVID-19 test - Celer Biotecnologia AS and Coronavirus Rapid Test (both produced by the company Wondfo) detect the total antibodies without differentiation. The others consist of discriminated detection of IgG and IgM antibodies.

Figure 10: Percentage of responses per TLR kit reported in the Proficiency Test

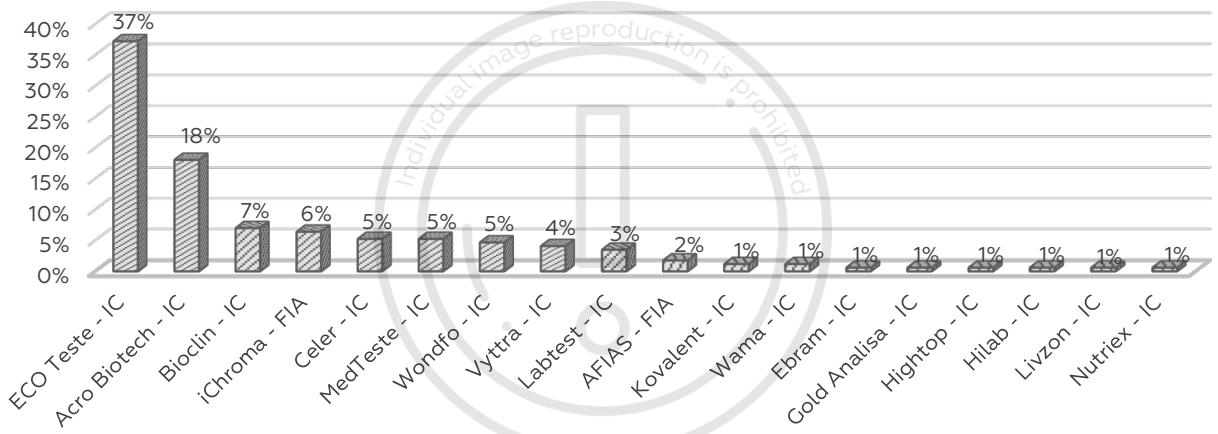

Figure 10: Percentage of responses from TLR kits in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020.

The Eco Teste brands with 37%, Acro Biotech (18%), Bioclin (7%) and iChroma (6%) represent the majority of participating laboratories (68%). The other brands ranged from 1% to 5% of users.

The general performance of the participants who reported the TLR kits in the proficiency test will be shown below in figure 11. Only the items evaluated were considered and the percentage was calculated in relation to the total number of responses obtained for IgG, IgM and Total (IgG + IgM).

Figure 11: Overall percentage of adequacy per TLR kit in the Proficiency Test for IgG, IgM and Total.

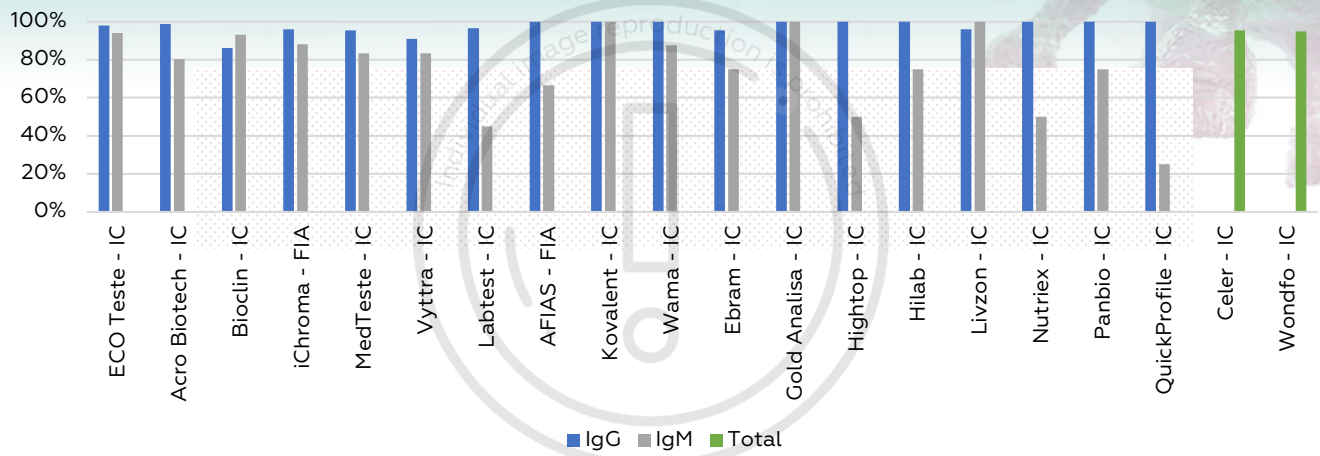

Figure 11: Percentage of general suitability of the kits compared to the items evaluated in the SARS-CoV-2 Coronavirus proficiency test program in the 02/2020 round.

Table 19 shows the general percentage of adequacy with the amount of participation per TLR kit. It is also possible to check the total number of responses, considering all the items evaluated.

Table 19: General percentage of adequacy per TLR kit in the Proficiency Test

Kit	Qty. Participants	IgG		IgM		Total	
		Qty. Answers	% Adequacy	Qty. Answers	% Adequacy	Qty. Answers	% Adequacy
ECO Teste - IC	64	313	98%	239	94%	-	-
Acro Biotech - IC	31	150	98%	98	80%	-	-
Bioclin - IC	12	46	86%	39	93%	-	-
iChroma - FIA	11	51	96%	38	88%	-	-
MedTeste - IC	9	41	91%	30	83%	-	-
Celer - IC	9	-	-	-	-	43	96%
Wondfo - IC	8	-	-	-	-	38	95%
Vyttra - IC	7	28	91%	10	83%	-	-
Labtest - IC	6	28	93%	20	45%	-	-
AFIAS - FIA	3	15	100%	8	67%	-	-
Kovalent - IC	2	10	100%	8	100%	-	-
Wama - IC	2	10	100%	7	88%	-	-
Ebram - IC	1	5	100%	3	75%	-	-
Gold Analisa - IC	1	5	100%	4	100%	-	-
Hightop - IC	1	5	100%	2	50%	-	-
Hilab - IC	1	5	100%	3	75%	-	-
Livzon - IC	1	5	100%	4	100%	-	-
Nutriex - IC	1	5	100%	2	50%	-	-
Panbio - IC	1	5	100%	4	75%	-	-
QuickProfile - IC	1	5	100%	1	25%	-	-

Qty - Quantity

Table 19: Percentage of adequacy of the immunological of TLR kits for detection of antibodies evaluated in the SARS-CoV-2 Coronavirus Proficiency Test program in the 02/2020 round.

When analyzing figure 11 and table 19, it is observed that for IgG all kits have a general percentage of adequacy greater than 80%. However for IgM, 8 kits showed a percentage below 80%. It is worth noting that these kits had a low percentage of (between 1% to 5%) responding users, as can be seen in Figure 10. For detection of total antibodies (IgG + IgM), both kits had an overall percentage above 90%.

Individual evaluation of items against immunological methods by Rapid Diagnostic Test (TLR) - Antibodies

The data presented below include the percentage of responses obtained with the TLR kits per test item provided.

Item 1

Table 20: Percentage of responses by immunological method with TLR kit (item 1)

		IgG			IgM			Total		
KIT	Qty	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
Acro Biotech - IC	31	3,2%	96,8%	-	61,3%	38,7%	-	-	-	-
AFIAS - FIA	3	-	100,0%	-	-	100,0%	-	-	-	-
Bioclin - IC	12	41,7%	58,3%	-	0,0%	100,0%	-	-	-	-
Celer - IC	9	-	-	-	-	-	-	-	100,0%	-
Ebram - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
ECO Teste - IC	64	1,6%	98,4%	-	10,9%	87,5%	1,6%	-	-	-
Gold Analisa - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
Hightop - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
Hilab - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
iChroma - FIA	11	-	100,0%	-	9,1%	90,9%	-	-	-	-
Kovalent - IC	2	-	100,0%	-	-	100,0%	-	-	-	-
Labtest - IC	6	-	100,0%	-	66,7%	16,7%	16,7%	-	-	-
Livzon - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
MedTeste - IC	9	-	100,0%	-	-	100,0%	-	-	-	-
Nutriex - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
Panbio - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
QuickProfile - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
Vyttra - IC	7	14,3%	85,7%	-	100,0%	-	-	-	-	-
Wama - IC	2	-	100,0%	-	-	100,0%	-	-	-	-
Wondfo - IC	8	-	-	-	-	-	-	-	100,0%	-
Grand Total	172	5,2%	94,8%	-	25,3%	73,4%	1,3%	-	100,0%	-
		Positive			Not Evaluated			Positive		

Qty. Quantity Neg - Negative Pos. - Positive Ind – inconclusive

Table 20: Behavior of each kit and antibody reported in the Coronavirus SARS-CoV-2 proficiency test program in round 02/2020, for item 1. This item was a single donor.

NOTE: The AFIAS and iChroma kits have a different method and were evaluated for IgM.

This item showed a satisfactory consensus among the results obtained by most kits presented in this round for the IgG antibody, but there is a kit mark (Bioclin) with a higher percentage of negative results. For the result regarding the IgM antibody, the consensus was lower, which resulted in the non-assessment of this marker in the proficiency test. Three brands of kits (Acro Biotech, Labtest and Vyttra) showed a negative consensus for this antibody.

In view of the consensus of 94.8%, positive presented for the IgG marker, it was possible to evaluate the kits that detect the total antibodies (Celer and Wondfo).

Item 2

Table 21: Percentage of responses by immunological method with TLR kit (item 2)

		IgG			IgM			Total		
KIT	Qty	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
Acro Biotech - IC	31	3,2%	96,8%	-	29,0%	71,0%	-	-	-	-
AFIAS - FIA	3	-	100,0%	-	66,7%	33,3%	-	-	-	-
Bioclin - IC	11	9,1%	90,9%	-	9,1%	90,9%	-	-	-	-
Celer - IC	9	-	-	-	-	-	-	-	100,0%	-
Ebram - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
ECO Teste - IC	64	-	100,0%	-	6,3%	92,2%	1,6%	-	-	-
Gold Analisa - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
Hightop - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
Hilab - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
iChroma - FIA	11	-	100,0%	-	9,1%	81,8%	9,1%	-	-	-
Kovalent - IC	2	-	100,0%	-	-	100,0%	-	-	-	-
Labtest - IC	6	-	100,0%	-	16,7%	83,3%	-	-	-	-
Livzon - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
MedTeste - IC	9	-	100,0%	-	-	100,0%	-	-	-	-
Nutriex - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
Panbio - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
QuickProfile - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
Vyttra - IC	7	-	100,0%	-	100,0%	-	-	-	-	-
Wama - IC	2	-	100,0%	-	-	100,0%	-	-	-	-
Wondfo - IC	8	-	-	-	-	-	-	-	100,0%	-
Grand Total	154	1,3%	98,7%	-	17,0%	81,7%	1,3%	-	100,0%	-
		Positive			Positive			Positive		

Qty. Quantity Neg - Negative Pos. - Positive Ind – inconclusive

Table 21: Behavior of each kit and antibody reported in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020 for item 2. This item was control material developed by Controllab.

As noted in table 21, among all items, item 2 performed better for the IgG marker (98.7%), however very close to item 4, with 98.6%. Only results obtained through two brands of kits (Acro Biotech and Bioclin) showed a negative result. For IgM, although the consensus presented is satisfactory, 17% of the laboratories that reported results obtained through 9 different kit brands, reported a negative result.

Item 3

Table 22: Percentage of responses by immunological method with TLR kit (item 3)

		IgG			IgM			Total		
KIT	Qty	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
Acro Biotech - IC	31	100,0%	-	-	96,8%	3,2%	-	-	-	-
AFIAS - FIA	3	100,0%	-	-	100,0%	-	-	-	-	-
Bioclin - IC	11	81,8%	18,2%	-	81,8%	18,2%	-	-	-	-
Celer - IC	9	-	-	-	-	-	-	88,9%	11,1%	-
Ebram - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
ECO Teste - IC	64	96,9%	3,1%	-	96,9%	1,6%	1,6%	-	-	-
Gold Analisa - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
Hightop - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
Hilab - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
iChroma - FIA	11	81,8%	9,1%	9,1%	100,0%	-	-	-	-	-
Kovalent - IC	2	100,0%	-	-	100,0%	-	-	-	-	-
Labtest - IC	6	83,3%	16,7%	-	83,3%	16,7%	-	-	-	-
Livzon - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
MedTeste - IC	9	77,8%	22,2%	-	77,8%	22,2%	-	-	-	-
Nutriex - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
Panbio - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
QuickProfile - IC	1	100,0%	-	-	100,0%	-	-	-	-	-
Vyttra - IC	7	85,7%	14,3%	-	100,0%	-	-	-	-	-
Wama - IC	2	100,0%	-	-	100,0%	-	-	-	-	-
Wondfo - IC	8	-	-	-	-	-	-	87,5%	12,5%	-
Grand Total	154	93,5%	5,8%	0,6%	94,8%	4,6%	0,7%	88,2%	11,8%	-
		Negative			Negative			Negative		

Qty. Quantity Neg - Negative Pos. - Positive Ind – inconclusive

Table 22: Behavior of each kit and antibody reported in the SARS-CoV-2 Coronavirus Proficiency Test program in round 02/2020 for item 3. This item was a single donor.

For item 3, the expected result was negative. Most kits obtained a percentage above 80% for the IgG and IgM markers, but we highlight that the Bioclin, Eco Teste, Labtest and Medteste kits demonstrated positive results for both antibodies, although in a low percentage.

Item 4

Table 23: Percentage of responses by immunological method with TLR kit (item 4)

KIT	Qty	IgG			IgM			Total		
		Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
Acro Biotech - IC	30	-	100,0%	-	10,0%	90,0%	-	-	-	-
AFIAS - FIA	3	-	100,0%	-	33,3%	33,3%	33,3%	-	-	-
Bioclin - IC	10	-	100,0%	-	-	100,0%	-	-	-	-
Celer - IC	9	-	-	-	-	-	-	-	100,0%	-
Ebram - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
ECO Teste - IC	63	1,6%	98,4%	-	1,6%	93,7%	4,8%	-	-	-
Gold Analisa - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
Hightop - IC	1	-	100,0%	-	-	-	100,0%	-	-	-
Hilab - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
iChroma - FIA	10	-	100,0%	-	10,0%	80,0%	10,0%	-	-	-
Kovalent - IC	2	-	100,0%	-	-	100,0%	-	-	-	-
Labtest - IC	6	16,7%	83,3%	-	16,7%	83,3%	-	-	-	-
Livzon - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
MedTeste - IC	9	-	100,0%	-	11,1%	88,9%	-	-	-	-
Nutriex - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
Panbio - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
QuickProfile - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
Vyttra - IC	5	-	100,0%	-	40,0%	60,0%	-	-	-	-
Wama - IC	2	-	100,0%	-	-	100,0%	-	-	-	-
Wondfo - IC	8	-	-	-	-	-	-	-	100,0%	-
Grand Total	**148**	**1,4%**	**98,6%**	**-**	**7,4%**	**88,5%**	**4,1%**	**-**	**100,0%**	**-**
			Positive			**Positive**			**Positive**	

Qty. Quantity Neg - Negative Pos. - Positive Ind – inconclusive

Table 23: Behavior of each kit and antibody reported in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020 for item 4. This item was control material developed by Controllab.

As already mentioned, this item presented results with a percentage of positive results for IgG, close to item 2, also with negative results only for 2 brands of kits. For IgM, 8 brands of kits showed a negative result. In a brand (AFIAS) there is no consensus.

Given the consensus of 98.6% and 88.5% positive for the IgG and IgM markers, respectively, it was possible to evaluate the kits that detect the total antibodies (Celer and Wondfo).

Item 5

Table 24: Percentage of responses by immunological method with TLR kit (item 5)

		IgG			* IgM			Total		
KIT	Qty	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
Acro Biotech - IC	30	3,3%	96,7%	-	36,7%	63,3%	-	-	-	-
AFIAS - FIA	3	-	100,0%	-	66,7%	33,3%	-	-	-	-
Bioclin - IC	10	-	100,0%	-	-	100,0%	-	-	-	-
Celer - IC	9	-	-	-	-	-	-	11,1%	88,9%	-
Ebram - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
ECO Teste - IC	63	1,6%	98,4%	-	6,3%	93,7%	-	-	-	-
Gold Analisa - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
Hightop - IC	1	-	100,0%	-	-	-	100,0%	-	-	-
Hilab - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
iChroma - FIA	10	-	100,0%	-	20,0%	30,0%	50,0%	-	-	-
Kovalent - IC	2	-	100,0%	-	-	100,0%	-	-	-	-
Labtest - IC	6	-	100,0%	-	16,7%	83,3%	-	-	-	-
Livzon - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
MedTeste - IC	9	22,2%	77,8%	-	33,3%	66,7%	-	-	-	-
Nutriex - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
Panbio - IC	1	-	100,0%	-	-	100,0%	-	-	-	-
QuickProfile - IC	1	-	100,0%	-	100,0%	-	-	-	-	-
Vyttra - IC	5	20,0%	80,0%	-	80,0%	20,0%	-	-	-	-
Wama - IC	2	-	100,0%	-	50,0%	50,0%	-	-	-	-
Wondfo - IC	8	-	-	-	-	-	-	12,5%	87,5%	
Grand total	**148**	**3,4%**	**96,6%**	**-**	**21,6%**	**74,3%**	**4,1%**	**11,8%**	**88,2%**	**-**
		Positive			**Positive**			**Positive**		

Qty. Quantity Neg - Negative Pos. - Positive Ind – inconclusive

Table 24: Behavior of each kit and antibody reported in the SARS-CoV-2 Coronavirus proficiency test program in round 02/2020 for item 5. This item was control material developed by Controllab.

NOTE: FIA method kits showed no consensus and were not evaluated for IgM in item 5.

In this item there was a high consensus for IgG, although 4 brands of kits (Acro Biotech - IC, ECO Teste - IC, MedTeste - IC and Vyttra - IC) have presented negative results. For IgM, the percentage presented during data analysis allowed the item to be evaluated. The percentage of positive results for both antibodies contributed to the evaluation of kits that detect total antibodies.

Immunological method by Rapid Diagnostic Test (TLR) – Antigen

In this round (02/2020), 7 Brazilian laboratories participated in the program using reactive kits to detect the SARS-CoV-2 antigen. The reactive item (item 1) was prepared from viral isolation and the not reactive item (item 2) was produced from synthetic material with the addition of human cells.

The percentage of responses per TLR kit for antigen detection for each item is shown below.

Table 25: Percentage of responses by immunological method with TLR kit for antigen.

		Item 1			Item 2		
Kit	Qty.	Neg	Pos	Ind	Neg	Pos	Ind
Eco Diagnóstica - FIA	3	-	100%	-	100,0%	-	-
Eco Teste - IC	4	50,0%	50,0%	-	100,0%	-	-
Grand Total	7	28,6%	71,4%		100,0%	-	-
		Not Evaluated			Negative		

Qty. Quantity Neg - Negative Pos. - Positive Ind – inconclusive

Table 25: Behavior of each kit reported in the SARS-CoV-2 Coronavirus Proficiency Test program for antigen detection in round 02/2020.

Three participants reported results obtained with the Eco Diagnóstica kit by the fluorescence immunoassay method (FIA) and the other four laboratories also used the kit from the same brand, but by the Immunochromatography (IC) method. This last methodology showed an absence of consensus among the participating laboratories, which together with the reduced number of participation, contributed to the non-evaluation of the item. Item 2 presented 100% negativity allowing the assessment

CONCLUSION

In view of the evolution of the Covid-19 pandemic in different countries, which has provided the publication of numerous studies - among which some referring to the evaluation of reactives for in vitro diagnosis - we consider it opportune to publish the 2nd round of evaluation of the proficiency of brazilian and international laboratories in examinations related to SARS-VOC-2, since we are faced with a situation of global proportion.

The participation of an increasing number of clinical laboratories in proficiency test programs demonstrates the acquired maturity in relation to quality assurance practices, without which, there can be no security regarding the accuracy of the results obtained.

For the lack of consensus within the same kit, it is suggested that users check the way in which they are carrying out their analysis in relation to the procedure indicated by the manufacturer. It is also important to comment that in Annex 1 the consensus of these kits is verified in relation to the reported lots, which may indicate - for some of them - a possible change in sensitivity between the lots.

Such behavior underscores the need for manufacturers and importers to validate their kits with reference materials and to also continuously monitor the sensitivity between batches of kits that will be made available to the market. It is also important to highlight the responsibility of the laboratories to carry out

the validation of the kit as soon as it is implanted in the routine and perform a simple analysis between batches and shipments with reference/control materials.

Molecular Tests

It was observed that the number of laboratories using commercial kits increased compared to the first round. However, the variety of molecular targets used in the amplification and detection of RNA remained large.

Although the number of participating laboratories using commercial kits (n=25) was greater in relation to laboratories using in-house method (n=18), the number presented for in-house draws attention.

The in-house method consensus for the items "Reactive/Detectable" was lower in three of the items (2, 3 and 5). It is worth mentioning that item 3 also had a low consensus (50%) for commercial kits. However, when comparing with the in-house method (35%), a higher percentage was still observed.

However, when analyzing the genetic targets reported for both methods (commercial and in-house kits), a higher prevalence of false negatives for the E gene is observed, being analyzed alone or with other genes.

When observing the data presented in table 06, with the performance of the genetic targets against the source protocol, it is clear that false negatives are associated with the protocol used and not with the use of the in-house method or commercial kits.

We highlight here the possible reasons for the inadequate results, both for commercial kits and for in-house kits:

- Different detection targets and with different sensitivities;
- Acceptance criteria - cutoff points in different CTs (Cycle Threshold);
- Unfamiliarity in validating in-house methods;
- Quality of inputs and kits used;
- Malfunction of the equipment involved.

It is notable that about 50% of participants use non-automated extraction systems, which contributes to the likelihood of false positive or negative results. At the same time, it shows the dysfunctional infrastructure in routine laboratory tests, which has not been established in high processing volumes.

Immunological Methods

Automated immunological methods: In this round, the number of participants increased and new brands of Chemiluminescence and Enzyme immunoassay reactives were used by the participants, even if some brands with few responses (Tables 9 and 10). The test for detecting total antibodies was added to this round, making it possible for laboratories to participate with the Electro-chemiluminescence method.

It is evident that electrochemiluminescence and chemiluminescence present inaccuracies under reproducibility conditions inferior to those evidenced by ELISA.

For IgM antibodies, there was less consensus among the results reported by the laboratories when compared to the results of IgG antibodies.

Immunological methods by Rapid Diagnostic Test (TLR): Again, this method presented a greater number of participants, having also increased in this round. For IgG, it was possible to evaluate all items for the Immunochromatographic (CI) and Fluorescence Immunoassay (FIA) methods. With the inclusion of specific fields in the form for reporting quantitative results, new participants signed up for the program with the iChroma brand.

In TLRs, the performance of immunochromatographic methods is presented for IgM with a percentage of false negatives ranging between 20% and 80% (kits with only one participant were not considered). The percentage of false negatives is more significant compared to false positives, where it varied between 3 and 20%. As these tests are mostly dependent on the observer, it is not recommended that the observer be the only source for determining the diagnosis.

When implementing this method in the laboratory, it is recommended to train the technique by observers.

It is worth mentioning that for the correct use of the information in this report, it is necessary to:

1. Observe the representativeness of the systems (kits and equipment) against the market. There are systems with few respondents in the interlaboratory comparison.
2. Analyze the percentage of adequacy of the system adopted by the laboratory in each item, observing the number of respondents facing the market.
3. Check the percentage of inadequacy of some items by the respondents.
4. Investigate the performance of kits against items not evaluated by the provider.
5. Examine the quantity of batches that manufacturers make available on the market and if there is a difference in sensitivity between batches.
6. Consider that some data were not evaluated because they presented very different results from the other participants

For methods that detect antigens, although results have been presented (Table 25), it is expected that in the 03/2020 round there will be a greater number of participants, making it possible to present the behavior of more analytical systems available on the market and the evaluation of possible items positive.

ANNEXES

Annex 1

Below are listed the Kits and lots, respectively, reported in the proficiency test for Rapid Diagnostic Test. The columns highlighted with "↓" represent the results accepted in the proficiency test. Items that do not have this mark have not been evaluated. It is important to highlight that the lots are presented as informed by the users of the proficiency test program.

Antibody

Acro Biotech - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
20030272		1			1			1			1			1	
NCP 20030273		1			1			1			1			1	
NCP 20030322		1			1			1			1			1	
NCP 20050071		1			1			1			1			1	
NCP20030237	1	21		1	21			21			21		1	20	
NCP20030272		2			2			2			2			2	
NCP20030322		2			2			2			2			2	
NCP20050065		1			1			1			1			1	
IgM					↓			↓			↓			↓	
20030272	1			1				1			1		1		
NCP 20030273	1			1				1		1			1		
NCP 20030322	1			1				1			1		1		
NCP 20050071		1			1			1			1			1	
NCP20030237	13	9		2	20			22		1	20		4	17	
NCP20030272	1	1		2				1	1		2		2		
NCP20030322	2			2				2			2		2		
NCP20050065		1			1			1		1				1	

Bioclin - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
7		2			2			2			2			2	
8	1	3			4			4			4			4	
10	4	1		1	3			3	1		3			3	
9		1			1				1		1			1	
IgM					↓			↓			↓			↓	
7		2			2			2			2			2	
8		4			4			4			4			4	
10		5		1	3			3	1		3			3	
9		1			1				1		1			1	

Ebram - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
05893A0616		1			1			1			1			1	
IgM					↓			↓			↓			↓	
05893A0616		1			1			1			1		1		

ECO Teste - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
202005012		2			2			2			2			2	
202005017		1			1			1			1			1	
202005019		2			2			1	1		1			1	
202005026		1			1			1			1			1	
202005028		7			7			7			7			7	
202005029		1			1			1			1			1	
202005031		4			4			4			4			4	
202005032		2			2			2			2			2	
202005033		2			2			2			2			2	
202005043		3			3			3			3			3	
202006002		9			9			9			9			9	
202006005		2			2			2			2			2	
202006006		1			1			1			1			1	
202006009		2			2			2			2			2	
202006010		14			14			14			14			14	
202006011		6			6			5	1		6		1	5	
202006012		1			1			1			1			1	
202007009	1	3			4			4		1	3			4	
IgM					↓			↓			↓			↓	
202005012	1		1		2			2			2			2	
202005017		1			1			1			1			1	

202005019		2			2			1	1			1		1	
202005026	1				1			1				1		1	
202005028		7			7			7				7		7	
202005029	1				1			1				1		1	
202005031		4			4			4				4		4	
202005032		2			2			2				2		2	
202005033		2			2			2				2		2	
202005043		3			3			3				3		3	
202006002		9			9			9				9		9	
202006005		2			2			2				2		2	
202006006	1			1				1					1	1	
202006009	1	1		2				2				1	1	1	1
202006010		14			14			14				14			14
202006011		6			5	1		5	1			5	1	1	5
202006012	1			1				1				1		1	
202007009	1	3			4			4			1	3			4

Gold Analisa - IC

	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
200655		1			1			1			1			1	
IgM					↓			↓			↓			↓	
200655		1			1			1			1			1	

Hightop - IC

	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
COV1252004C		1			1			1			1			1	
IgM					↓			↓			↓			↓	
COV1252004C		1			1			1				1			1

Hilab - IC

	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
COVV0200604		1			1			1			1			1	
IgM					↓			↓			↓			↓	
COVV0200604		1			1			1			1		1		

Kovalent - IC

	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
111877205		1			1			1			1			1	
1118772020		1			1			1			1			1	
IgM					↓			↓			↓			↓	
111877205		1			1			1			1			1	
1118772020		1			1			1			1			1	

Labtest - IC

	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
2630695		1			1			1			1			1	
3120322		5			5			4	1	1	4			5	
IgM					↓			↓			↓			↓	
2630695	1				1			1			1			1	
3120322	3	1	1	1	4			4	1	1	4		1	4	

Livzon - IC

	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
CK2003030410		1			1			1			1			1	
IgM					↓			↓			↓			↓	
CK 2003030410		1			1			1			1			1	

MedTeste – IC

	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
10120600835		1			1			1			1			1	
COV20030060		1			1				1		1		1		
COV20030081		5			5			4	1		5		1	4	
COV20030122		1			1			1			1			1	

	COV20050007		1			1			1			1			1	
IgM						↓			↓			↓			↓	
	10120600835		1			1			1			1			1	
	COV20030060		1			1		1				1		1		
	COV20030081		5			5		4	1			5		1	4	
	COV20030122		1			1		1				1			1	
	COV20050007		1			1		1			1			1		

Nutriex - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
P46200427A1A		1			1			1			1			1	
IgM					↓			↓			↓			↓	
P46200427A1A	1			1				1			1		1		

Panbio - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
COV0042020		1			1			1			1			1	
IgM					↓			↓			↓			↓	
COV0042020		1			1			1			1			1	

QuickProfile - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
20052080A		1			1			1			1			1	
IgM					↓			↓			↓			↓	
20052080A	1			1				1					1		

Vyttra - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
2005442		1			1			1			1			1	
2006486		2			2			2							
2006511		1			1			1			1			1	
FJFB16201	1				1				1		1		1		
FJFB24201		2			2			2			2			2	
IgM					↓			↓			↓			↓	
2005442	1			1				1			1			1	
2006486	1			1				1							
2006511	1			1				1			1		1		
FJFB16201	1			1				1		1			1		
FJFB24201	2			2				2		1	1		2		

Wama - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
20e 018		1			1			1			1			1	
20F001		1			1			1			1			1	
(vazio)		3			3		2	1			3			3	
20050002		2			2		2				2			2	
20CG2504X		1			1			1			1			1	
IgM					↓			↓			↓			↓	
20e 018		1			1			1			1		1		
20F001		1			1			1			1			1	
20050002		2			2			2			2		1	1	
20CG2504X		1			1			1			1			1	

Celer - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
Total		↓			↓			↓			↓			↓	
w19500335		5			5		5				5			5	
w19500336		1			1			1			1		1		
W19500341		1			1		1				1			1	
W195004116		1			1		1				1			1	
W195004140		1			1		1				1			1	

Wondfo - IC	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
Total		↓			↓			↓			↓			↓	
NI		1			1			1			1		1		
w19500335		5			5		5				5			5	

	w195004117		1			1			1			1			1	
	W195004140		1			1			1			1			1	

AFIAS - FIA	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
WHQDA11G		1			1			1			1			1	
WHQEA36G		2			2			2			2			2	
IgM		↓			↓			↓			↓				
WHQDA11G		1			1			1			1			1	
WHQEA36G		2		2				2		1		1	2		

iChroma - FIA	Item 1			Item 2			Item 3			Item 4			Item 5		
	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind	Neg	Pos	Ind
IgG		↓			↓			↓			↓			↓	
WHQDA50		2			2			2			2			2	
WHQDA51		1			1			1			1			1	
WHQDA66		1			1			1			1			1	
WHQDA67		1			1			1			1			1	
WHQDA68		1			1			1			1			1	
WHQDA70		1			1			1			1			1	
WHQEB30		1			1			1			1			1	
WHQEB31		1			1			1			1			1	
WHQEB56		1			1			1							
WHQEB58		1			1				1		1			1	
IgM		↓			↓			↓			↓				
WHQDA50		2			2			2			2			1	1
WHQDA51		1			1			1			1			1	
WHQDA66		1			1			1			1			1	
WHQDA67		1			1			1			1				1
WHQDA68		1			1			1			1				1
WHQDA70		1			1			1				1			1
WHQEB30		1				1		1			1		1		
WHQEB31	1			1				1		1			1		
WHQEB56		1			1			1							
WHQEB58		1			1			1			1				1

Antigen

Eco Diagnóstica - FIA	Item 1			Item 2		
	Neg	Pos	Ind	Neg	Pos	Ind
				↓		
202006013		1		1		
202006014		1		1		
FCO302005153		1		1		

Eco Teste - IC	Item 1			Item 2		
	Neg	Pos	Ind	Neg	Pos	Ind
				↓		
20205038	1			1		
202005038	1			1		
202005046		1		1		
202006007		1		1		

Annex 2

Below, we list the laboratories that enabled this work to be carried out and made available to the market. It was necessary to have agility to incorporate this proficiency test in the analytical routines and the laboratories endeavored to demonstrate their commitment to the reliability of their data, participating in the program.

ALAGOAS (2)

- Laboratório Sabin de Patologia Clínica de Alagoas
- Proclínico Diagnóstico Laboratorial

BAHIA (7)

- LPC Medicina Laboratorial
- Biocenter - Centro de Hematologia e Patologia Clínica Ltda
- "Hospital Santa Izabel - Santa Casa de Misericórdia da
- Bahia"
- LACEN-SESAB - Laboratório Central Gonçalo Muniz
- Hemocenter Laboratório de Análises Clínicas
- Hospital Universitário Professor Edgard Santos
- Labchecap - Laboratórios de Análises Clínicas Ltda.

CEARÁ (3)

- Laboratório Clementino Fraga
- Laboratório Emílio Ribas
- LACEN CE

DISTRITO FEDERAL (7)

- Laboratório Sabin de Análises Clínicas
- Centro de Patologia Clínica do Hospital Universitário de Brasília
- Hospital Anchieta
- Laboratório Fleury - Hospital Santa Luzia
- Fleury S/A - Hospital Santa Helena
- Instituto Hospital de Base do Distrito Federal - IHBDF
- Fleury S/A - Hospital Sírio Libanês Brasília

ESPIRÍTO SANTO (2)

- Laboratório Henrique Tommasi Netto
- Hospital Meridional

GOIÁS (5)

- Padrão Laboratório Clínico

- Laboratório Médico CAPC
- Laboratório INGOH - Instituto Goiano de Oncologia e Hematologia S/S Ltda
- Base Laboratório Médico
- Laboratório Núcleo

MARANHÃO (1)

- Lacmar - Laboratório de Análises Clínicas do Maranhão

MINAS GERAIS (70)

- Instituto Hermes Pardini
- Laboratório Geraldo Lustosa
- São Paulo Patologia Clínica
- Laboratório de Patologia Clínica Hospital Márcio Cunha - Unidade I - Fundação São Francisco Xavier
- FAEPU - Fundação Assistência Pesquisa
- Biocor Instituto
- Labtest Diagnóstica
- José Alair Couto Laboratório de Análises Clínicas
- Santa Casa de Misericórdia de Juiz de Fora - Laboratório Análises Clínicas
- Laboratório Análises Clínicas São José
- Lemos Laboratório de Análises Clínicas
- Laboratório Santa Lúcia
- Lab-Rede - Laboratório de Referência em Diagnósticos Especializados S/C
- Laboratório Monte Sinai
- Check-Up Laboratório de Análises Clínicas
- Gold Analisa Diagnóstica
- Laboratório de Citoanálise
- Laborclínica Análises e Pesquisas Clínicas
- Hermes Pardini - Núcleo Técnico Belo Horizonte
- Laboratório Distrital Leste/Nordeste
- Laboratório Distrital Noroeste
- Laboratório Distrital Norte - Venda Nova
- Laboratório Distrital Oeste/Barreiro
- Laboratório da UPA Barreiro
- Laboratório da UPA Leste
- Laboratório da UPA Norte
- Laboratório da UPA Oeste
- Laboratório da UPA Pampulha
- Laboratório da UPA Venda Nova
- Hospital Mater Dei
- I9med - Serviços Médicos e Laboratório de Testes Rápidos LTDA
- Codon Biotécnologia
- Unimed Juiz de Fora
- Laboratório Santa Lúcia
- Laboratório Humberto Abrão
- I9med
- I9med Domiciliar
- I9MED Centenário
- I9MED Cristiano Machado
- I9MED Gutierrez
- I9MED Matriz
- I9MED Miguel Perrela
- I9MED Savassi
- I9MED Coração Eucarístico
- I9MED Guaicui
- I9MED Life Center

- I9MED Mater Dei
- I9MED Praça Da Bandeira
- I9MED Alameda Da Serra
- I9MED Raja Drive
- I9MED Rubens Caporali
- I9MED Santa Catarina
- I9MED São Araujo São Pedro
- I9MED São Lucas
- I9MED Sion
- I9MED Tancredo Neves
- I9MED Vitório Marçola
- I9MED Andradas
- I9MED Padre Eustáquio
- I9MED Pronto Socorro
- I9MED Alfredo Noronha
- I9MED Alipio de Melo
- I9MED Cidade Nova
- I9MED Estoril
- I9MED Gustavo Ayala
- I9MED Palmares
- I9MED Filial Planalto
- I9MED Mc Donald'S
- I9MED Antonio Araujo
- I9MED Via Brasil

MATO GROSSO (1)
- Laboratório Santa Mônica

PARÁ (1)
- Laboratório Central do Estado do Pará

PERNAMBUCO (4)
- Fleury S/A - a+ Pernambuco
- Genomika Diagnósticos
- Hospital Unimed Recife III
- DB - Diagnósticos do Brasil

PIAUÍ (1)
- Bioanálise Laboratório de Análises Clínicas

PARANÁ (7)
- Laboratório Paraná Clínicas
- Laboratório Pasteur
- Complexo do Hospital de Clínicas da UFPR
- Hospital Ministro Costa Cavalcanti - Fundação de Saúde Itaiguapy
- DB - Diagnósticos do Brasil
- Unimed Curitiba Participações
- Hi Technologies

RIO DE JANEIRO (19)
- Laboratório Morales
- Labormed Laboratórios Médicos
- Controllab Controle de Qualidade para Laboratórios
- Laboratório Central Noel Nutels
- Hemorio - Sorologia - Serviço de Hemoterapia
- Bio- Manguinhos - Instituto de Tecnologia em Imunobiológicos / FIO CRUZ
- Laboratório Unimed Volta Redonda
- Hospital Quinta D'Or - Rede D'Or São Luiz
- Laboratório Richet
- Fleury - Rio de Janeiro
- Laboratório Moisés Alvim
- Hospital Caxias D'Or
- Hospital Oeste D'or
- Contraprova Doping e Toxicologia
- Hospital Copa D'or Star
- Eliel Figueiredo Diagnósticos Médicos
- Instituto Hermes Pardini S.A - Centro de medicina Nuclear da Guanabara
- Hemoclin Clínica Hematológica
- Controllab - Laboratório de Bacteriologia

RIO GRANDE DO NORTE (1)
- Laboratório DNA Center

RONDÔNIA (1)
- Laboratório Unimed Vilhena

RORAIMA (1)
- Masterclin

RIO GRANDE DO SUL (10)
- Labimed - Análises Clínicas
- Laboratório de Análises Clínicas Carlos Franco Voegeli
- Laboratório de Análises Clínicas do Hospital Nossa Senhora da Conceição
- Núcleo Técnico Alfa
- Laboratório Unimed Nordeste
- Laboratório Amplicon
- EBSERH - Hospital Universitário de Santa Maria
- Laboratório de Microbiologia Clínica-LMC - Fundação Universidade de Caxias do Sul
- Lac - Laboratório Escola de Análises Clínicas
- Fleury S/A - Weinmann Laboratório

SANTA CATARINA (7)
- Laboratório de Análises Clínicas Unimed Litoral
- Laboratório de Análises Clínicas Dr. Willy Carlos Jung

- Hemos Laboratório Médico
- Lacen Florianópolis - Fundo Estadual de Saúde
- Laboratório Biomédico
- Laboratório de Análises Clínicas Verner Willrich
- Sabin Medicina Diagnóstica - Laboratório Bioclínico Porto

SERGIPE (4)

- Fundação de Saúde Parreiras Horta - FSPH
- CEMISE - Centro de Medicina Integrada de Sergipe Ltda
- Laboratório do Hospital Primavera
- Solim Laboratórios - Laboratório de Patologia Cirúrgica e Citologia

SÃO PAULO (71)

- Instituto de Análises Clínicas de Santos
- Fleury Centro de Medicina Diagnóstica
- DASA - Laboratório Central Alphaville
- Universidade Estadual de Campinas - UNICAMP- Divisão de Patologia Clínica/HC
- Laboratório Médico Dr. Maricondi
- Patologia Clínica Franceschi
- Laboratório Central do Hospital São Paulo
- Laboratório de Análises Clínicas Marlene Spir
- Laboratório Dr. Tajara
- Laboratório Central de Patologia Clínica do Hospital das Clínicas FMRP USP
- Laboratório Unimed - Seclin
- Rebouças Laboratório de Análises Clínicas
- HEMAT - Instituto de Hematologia de São José do Rio Preto L
- Notrelabs Lapa
- Laboratório Médico Ramos de Souza
- Sociedade Campineira de Educação Instrução
- Tecnolab Análises Clínicas
- Associação Fundo de Incentivo à Pesquisa
- Allmed Serviços Médicos
- Laboratório de Análises Clínicas Céllula Mater
- Laboratório Clínico Raul Dias dos Santos
- São Joaquim Hospital e Maternidade
- Senne Líquor Diagnóstico
- Laboratório C.M.L - C.M.L Centro Médico Laboratorial
- Divisão de Lab. Central do Hospital das Clínicas da FMUSP
- EBRAM Produtos Laboratoriais
- Hospital do Coração - Associação Beneficente Síria
- Cientificalab Produtos Laboratoriais e Sistemas
- Laboratório Confiance Medicina Diagnóstica
- Hospital Novo Atibaia
- Centro de Genomas
- Cura Centro de Ultrassonografia e Radiologia
- Laboratório Cepac - Centro de Patologia e Diagnóstico Clínico
- Instituto de Biomedicina do ABC
- WAMA Diagnósticos
- LCA Laboratórios Clínicos Associados
- Laboratório Fleury - Hospital Samaritano
- Laboratório Fleury - Hospital Sírio Libanês
- Laboratório Fleury - Hospital Santa Catarina
- Fleury - Hospital Alemão Oswaldo Cruz
- Laboratório São Francisco
- Precision Centro de Diagnósticos
- CIPAX Medicina Diagnóstica
- Fleury - Hospital São Luiz Morumbi
- Fleury - Hospital São Luiz Anália Franco
- Fleury - Hospital São Luiz Itaim
- Hospital Brasil
- Fleury - Hospital São Luiz Jabaquara
- Fleury - Hospital Assunção
- CRM Líquor
- Hermes Pardini - NTO
- Tecnolab Patologia Clínica
- Laboratório Unimed - Unimed Santa Barbara d'Oeste e Americana Participações
- DB Diagnósticos do Brasil
- Hospital 10 de Julho - Unimed Pindamonhangaba Cooperativa de Trabalho Médico
- DB - Diagnósticos do Brasil
- Hospital São Camilo - Pompéia
- Hospital São Camilo - Santana
- Fleury - Hospital Beneficência Portuguesa
- Hospital São Luiz São Caetano - Laboratório Fleury
- Medicina Laboratorial e Diagnósticos Clínicos Eireli
- Fleury S/A - Instituto Brasileiro de Controle de Câncer - HIMO
- Hospital AC Camargo Câncer Center
- Laboratório Lemelab de Análises Clínicas
- Hospital Vera Cruz
- Master Vida Laboratório de Análises Clínicas
- Vyttra Diagnósticos Importação e Exportação
- Fleury - Hospital Vila Nova Star
- Hospital Santa Cruz - Sociedade Brasileira e Japonesa de Beneficência Santa Cruz
- Euroimmun Brasil

- Laboratorio BIOLAB

TOCANTINS (3)

- Laboratório Central de Referência em Saúde Pública Estado do Tocantins
- Analisys Laboratório Clínico
- PHD Laboratório Clínico

INTERNATIONAL (23)

- Synlab Sociedad Anónima
- Hospital de Los Valles S.A. Hodevalles - Laboratório Hospital de Los Valles
- Instituto Nacional de Salud-Laboratório de Microbiologia
- Hospital de Clínicas Dr. Manuel Quintela
- LAC - Laboratorio de Análisis Clínicos
- Laboratorio Clínico Hospital Naval Almirante Nef
- Hospital Roberto del Río
- Hospital Eugenio Espejo - Laboratorio de analisis clinicas
- Pontificia Universidad Católica Del Ecuador - PUCE
- Centro Nacional de Enfermedades Tropicales - CENETROP - Ministerio de Salud de Bolivia
- Unilabs Perú - Sede Basadre
- Laboratorio de Analises Clinicas Vale do Sousa
- Laboratorio de Diagnóstico Molecular Fundación Arturo López Perez
- Laboratorio Central Hospital Clínico Universidad de Chile
- Bupa Integramedica
- Synlab Centro
- Laboratorio Clínico Synlab Noroccidente
- Synlab Suroccidente-Angel Diagnostica
- Synlab Caribe -Falab
- M.I.C Central - Microbiologia Industria Clinica
- Departamento de Investigación en Virología y Biotecnología - Instituto Conmemorativo Gorgas de Estudios de la Salud
- Laboratorio Clínico - Hospital Santo Tomás
- The Panama Clinic Medical, S.A.