# First evaluation of a pioneering proficiency test for all SARS-CoV2 detection methods

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## INTRODUCTION

Controllab, a company that offers quality control solutions for laboratories in several countries, concluded, in April 2020, one month after the declaration of the pandemic by the World Health Organization (WHO), the production and characterization of control materials for the new coronavirus (SARS-CoV2). The following month, in partnership with the Brazilian Society of Clinical Pathology (SBPC/ML), it carried out the first Brazilian Proficiency Test (PT), an initiative of the Controllab provider, free of charge for the group of laboratories that registered through responses to a poll, showing interest in good laboratory practices recommended in technical and regulatory standards.

The Proficiency Test, also known as External Quality Control, or simply External Control, is an effective tool to determine the performance of the analytical phase of the laboratory. Combined with internal control and management committed to quality, it promotes a deep knowledge of the analysis processes and guarantees the reliability of its results.

This quality control solution is offered by Controllab on a continuous and periodic basis, consisting of evaluations of results obtained by the laboratory in the analysis of unknown materials that simulate patients. Each period of this program is called by the provider as a round. Such evaluations result from statistical studies and analyzes by an advisory group, which point out errors and possible causes, successes and considerations on the overall performance of the participants. Reports are made available for the laboratory to verify its performance, identify improvements related to the testing system, equipment and technical staff.

The proficiency test programs offered for coronavirus (SARS-CoV2) in this first round meet:

- 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 (automated 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 - RDT, Rapid Test or POCT (Point-of-care testing), referred to in this document as the **Rapid Diagnostic Test** (RDT) method.

On 5/29/2020, the first evaluation was released and involved the participation of 124 laboratories, among which some participated in more than one of the programs, thus reporting results by different methodologies. Thus, the participations occurred as follows:

- 35,5% with molecular tests
- 7,3% with immunological tests by automated methods
- 73,4% with immunological tests by Rapid Diagnostic Tests (RDT)

At the end of the round, each of the participants (laboratories) received their Individual Evaluation Report, a document containing their performance for each of the reported methods, and a document called the Results Profile, which groups the data of the round and demonstrates the performance systems, within each program.

The purpose of this content is to present the performance of the analytical systems reported in this pilot round for the SARS-CoV2 coronavirus proficiency test, including considerations of the technical-scientific group proposing this work.

It is important to highlight that the Proficiency Test is part of the analytical monitoring of the laboratories with the Internal Quality Control. The results presented here cannot be replaced by reagent validation programs, which aim to ascertain the sensitivity and specificity of kits and reagents available on the market.

## TEST ITEMS

The samples sent in the Proficiency Test are called by the provider "Test Items". These “items”, also known as control materials, were previously chosen by the provider, following a quality control criterion, in which it was defined for this shipment:

### For evaluation of molecular tests

For molecular techniques, 2 (two) items of lyophilized cell suspension were sent, one “reagent/detectable” and the other “non-reagent/non-detectable”. The items were prepared from a viral isolation, where the virus was replicated from a viral strain of clinical isolate, in a cell culture system using cells of Vero lineage and Dulbecco's Modified Eagle's Medium (DMEM) culture medium.

The virus sequence used in the production of the control sample considered as “reagent/detectable” is available in the public GISAID repository under the accession number hCoV-19/Brazil/DFBR-

0001/2020. This sample was provided by the Sabin Medicina Diagnóstica Laboratory, in Brasília, with the consent of the patient for the donation.

### For evaluation of immunological tests

For immunological, automated and RDT methods, 3 (three) items from single donors were sent, with the patients' consent to the donation, whose collection occurred more than 15 days after the onset of symptoms, and two control items developed by Controllab. All control materials were offered to participating laboratories in lyophilized form.

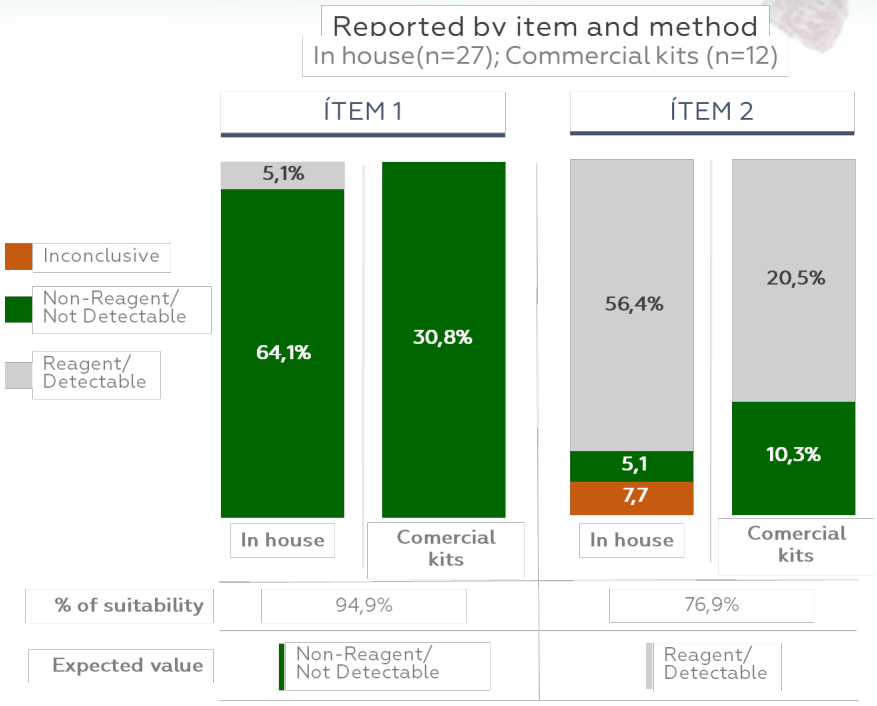
It is also important to highlight that the entire process of preparing the items followed the criteria of Good Manufacturing Practices. The evaluation of the Proficiency Test followed the criteria of ABNT NBR ISO/IEC 17043, a standard which Controllab is accredited with INMETRO in accordance with PEP003. These items were also previously characterized by the Controllab Testing Laboratory, also accredited by INMETRO by ABNT NRB ISO/IEC 17025, according to the scope as CRL0586.

## Molecular Tests

In this first round, 41 laboratories reported their results and, through the information sent, it was possible to observe a great diversity of models, systems and protocols for molecular diagnosis, most of them in the in-house format, a diagnostic test validated by the laboratory itself, using inputs from different manufacturers or not to apply the molecular technique.

It is important to highlight that, of the 41 laboratories that reported using the molecular technique by RT-PCR, two of them did not inform the technique used (in house or commercial kit) and, therefore, were not presented in the statistical analysis below.

Graph 01: Percentage of responses by Molecular Techniques




*Graph 01: Percentage of responses for items 1 and 2 evaluated in the proficiency test program in round 01/2020, for reagents used by the molecular method by RT-PCR in house and commercial kits.*

Regarding the reported data, we can see in Graph 01 - item 1 - that 5.1% of the "false positive" results identified in this item as "reagent/detectable" were reported by laboratories that used the RT-PCR method in house, as well as 10.3% of the "false negatives" were reported in item 2 by the laboratories that used commercial kits reporting values such as "Non-reactive/non-detectable". It is also worth noting that the specificity of commercial kits, when analyzing the results in item 1, was 100%, as also highlighted in graph 03.

No participant reported an "inconclusive" result for item 1. Item 2 was reported as "inconclusive" by 7.7% of participants. Even though the molecular technique by RT-PCR in Real Time is considered gold standard for the diagnosis of the infection by Coronavirus (Sars-Cov-2), it is important to observe with caution and attention the reporting of detection failures, causing "false negative" results (non-reactive/non-detectable) in molecular techniques.

For a better analysis of the performance - and to help in the investigation of the possible causes for the inadequate results of the data reported by the laboratories in the Proficiency Test - the performance of the laboratories according to the analytical set used was made available, detailing the classification of the method (in house versus commercial kit), the genetic targets researched and the real-time PCR equipment used in the process. Always considering the accepted result for item 1 as "non-reactive/not detectable" and for item 2 "reagent/detectable".

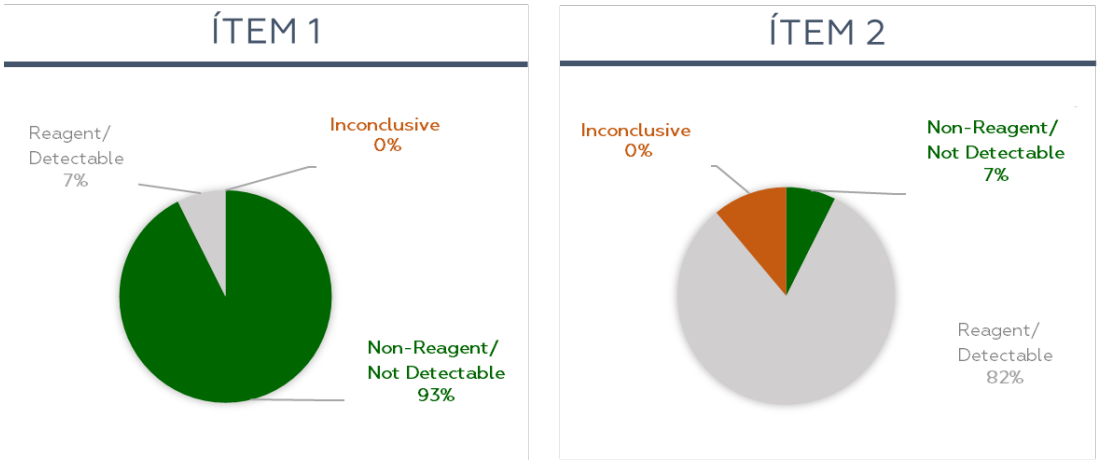
## RT-PCR Method (*in house)*

This method was reported by 27 laboratories, among the 41 that use molecular techniques. It is important to note that, among the participants, some did not identify the system used and therefore were not part of this statistic.

Item 1 presented an adequacy percentage of 92.6%, considering the accepted result as "non-reactive/non-detectable", and item 2 registered an adequacy percentage of 81.5%, considering the accepted result as "reagent/detectable". The percentage of adequacy shows the amount of data presented within the expected value by the provider.

Also noteworthy in item 2, the percentage of 11.1% of inconclusive results and a greater consensus of "reagent/detectable" results, when compared to the performance of commercial kits (Graph 3).

**Graph 02: Percentage of responses by molecular techniques by the RT-PCR method (in house).**



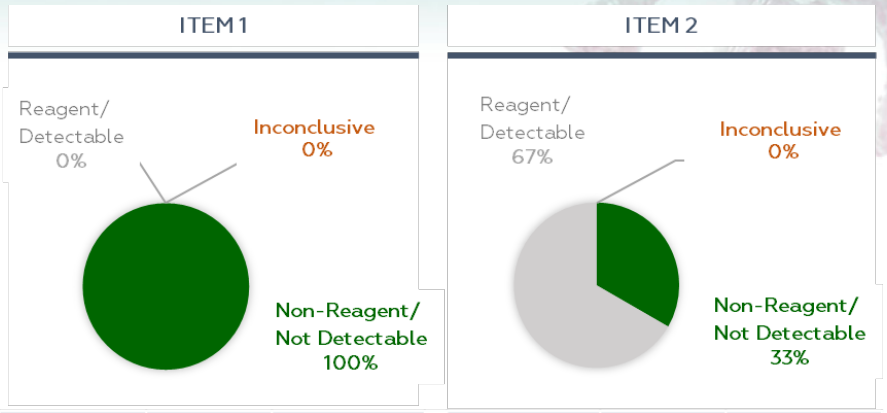

*Graph 02: Percentage of responses for items 1 and 2 evaluated in the SARS-CoV-2 Coronavirus proficiency test program in the 01/2020 round. Only laboratories that reported using the RT-PCR method in house were presented.*

## RT-PCR Method (commercial kits)

There were five different manufacturers presented by the participants:

- Allplex: n= 4
- Cobas: n= 1
- Hybribio: n= 1
- XGEN: n= 4
- Xpert XExpress: n= 2

Graph 03: Percentage of responses by molecular techniques by the RT-PCR method (commercial kits).




| Kit | Qty | Non-Reagent/ Not Detectable | Reagent/ Detectable | Inconclusive | Non-Reagent/ Not Detectable | Reagent/ Detectable | Inconclusive |
|---|---|---|---|---|---|---|---|
| Allplex - RT-PCR in real time | 4 | 100,0% | - | - | 50,0% | 50,0% | - |
| Cobas - RT-PCR in real time | 1 | 100,0% | - | - | - | 100,0% | - |
| Hybribio - RT-PCR in real time | 1 | 100,0% | - | - | 100,0% | - | - |
| XGEN - RT-PCR in real time | 4 | 100,0% | - | - | 25,0% | 75,0% | - |
| Xpert Xpress - RT-PCR in real time | 2 | 100,0% | - | - | - | 100,0% | - |
| **All kits** | **12** | **100,0%** | **0,0%** | **0,0%** | **33,3%** | **66,7%** | **0,0%** |

*Graph 03: Percentage of responses for items 1 and 2 evaluated in the SARS-CoV-2 Coronavirus proficiency test program in the 01/2020 round. Listed only the laboratories that reported RT-PCR method with commercial kits.*

As shown in Graph 03, the adequacy percentage of item 1 was 100%, that is, all of them presented "non-reactive/non-detectable" results.

In item 2, the level of agreement was 67% for the results "reagent/detectable". The 33% of inadequate results with a "false negative" result, that is, "non-reactive/non-detectable", need to be investigated by the program participants.

## Genetic Target

We present the performance of the laboratories according to the genetic target used in the research of the COVID-19 virus. Two tables were made available, in table 01 it was separated between the targets recommended by the World Health Organization (WHO) with the genetic targets (E+N (1 or 2) + RDRP) where 15 laboratories reported for them and the other targets, identified in table 01 as "Others", reported by 21 laboratories.

**Table 01: Percentage of responses by molecular techniques for the genetic target suggested by WHO x Others**

| Genetic Target | Item 1 n (%) | | | Item 2 n (%) | | |
|---|---|---|---|---|---|---|
| | Non reagent/ Not detectable | Reagent/ detectable | Inconclusive | Non reagent/ Not detectable | Reagent/ detectable | Inconclusive |
| (a) OMS | 15 (100,0) | --- | --- | 2 (13,3) | 12 (80,0) | 1 (6,7) |
| (b) Others | 19 (90,4) | 2 (9,6) * | --- | 4 (19,1) ** | 15 (71,4) | 2 (9,5) |

*Table 01: Number and percentage of responses of the items assessed in the SARS-CoV-2 Coronavirus proficiency test program in round 01/2020, separated by groups of genetic target in: (a) suggested by WHO and (b) Others reported target sets.*
** ORF1ab+N*
*** n=1 (E+RDRP) n=3 (ORF1ab+N)*

For item 1, as seen in table 02, only the set of targets ORF1ab and N (n = 2) presented false positive results, that is, "reagent/detectable". For item 2, false negative results, that is, "non-reactive/non-detectable" were observed for the following genetic target groups: E and RdRp (n=1), E, N and RdRp (n=2), and ORF1ab and N (n=3). It is worth mentioning that some of the users did not report information about the genetic target and, therefore, were not listed.

**Table 02: Percentage of responses by molecular techniques to the genetic target**

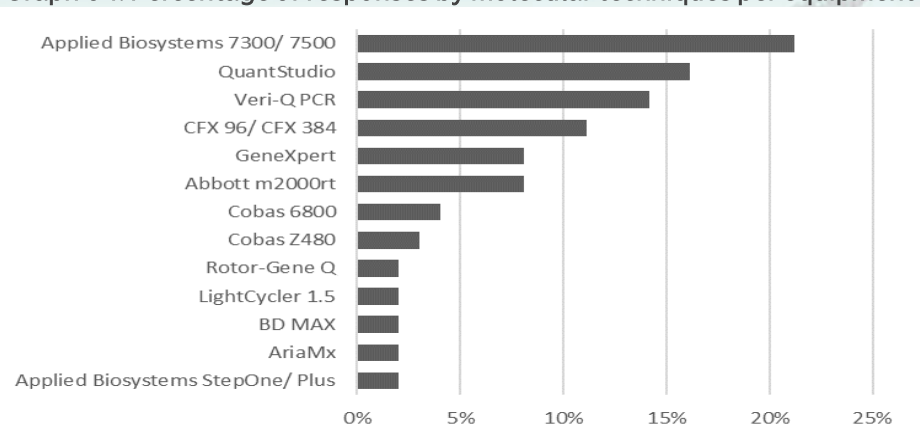
| Genetic Target | Item 1 n (%) | | | Item 2 n (%) | | |
|---|---|---|---|---|---|---|
| | Non reagent/ Not detectable | Reagent/ detectable | Inconclusive | Non reagent/ Not detectable | Reagent/ detectable | Inconclusive |
| gene E | 1 (100,0%) | - | - | - | 1 (100,0%) | - |
| genes E RdRp | 1 (100,0%) | - | - | 1 (100,0%) | - | - |
| genes E, N and RdRp | 8 (100,0%) | - | - | 2 (25,0%) | 6 (75,0%) | - |
| genes N1 and N2 | 3 (100,0%) | - | - | - | 2 (66,6%) | 1 (33,4%) |
| genes N1, N2 and RdRp | 7 (100,0%) | - | - | - | 6 (85,7%) | 1 (14,3%) |
| genes N2 and E | 4 (100,0%) | - | - | - | 4 (100,0%) | - |
| genes ORF1ab and E | 1 (100,0%) | - | - | - | 1 (100,0%) | - |
| genes ORF1ab and N | 9 (81,8%) | 2 (18,2%) | - | 3 (27,3%) | 7 (63,6%) | 1 (9,1%) |

*Table 02: Number and percentage of responses for items 1 and 2, in view of the genetic targets reported in the SARS-CoV-2 Coronavirus proficiency test program in the 01/2020 round.*

## Equipment

In the 01/2020 round, information on the equipment used for Amplification/Detection was requested. Graph 04 shows the equipment used according to their percentage of participation. The Applied Biosystems system with the 7300/7500 series was the most reported, represented by 21% of respondents.

Graph 04: Percentage of responses by molecular techniques per equipment




*Graph 04: Percentage of responses from amplification/detection equipment reported in the SARS-CoV-2 Coronavirus proficiency test program in the 01/2020 round.*

## IMMUNOLOGICAL METHODS

For the evaluation of immunological methods, the same test items were sent, both for automated systems and for Rapid Diagnostic Test (RDT) systems.

### Test Items

- **Items 01 and 02:** single donor who presented positive RT-PCR at the beginning of the infection and performed a new collection 25 days after the onset of symptoms.
- **Items 03**: single donor who presented positive RT-PCR at the beginning of the infection and performed a new collection 15 days after the onset of symptoms.
- **Items 04 and 05**: control material produced by Controllab, with items previously characterized and manipulated.

### Automated immunological methods

The automated systems reported in this round were represented by the chemiluminescence method (Q) in the MagLumi system and by the enzyme immunoassay (EIA) method in the Euroimmun system.

### Enzyme immunoassay method (Euroimmun system)

Analyzing the data individually, we can see in Table 03 that, for the Euroimmun - EIA system, three laboratories reported SARS-Cov2 IgA (Part. A, Part. E, Part. F) and two laboratories reported SARS-Cov2 IgG (Part. A, Part. F), with positive values for all items, according to the reference value presented in the package insert (Ratio ≥ 1.1).

Participant A presented lower Ratio results for the 05 items, in relation to the others who reported IgA in this system. Participant E presented a much higher IgA Ratio value for item 2 in relation to the other participants of this enzyme immunoassay.

For IgG in item 2, a higher value was observed for participant A.

**Table 03: Results of automated immunological methods with individual data (Euroimmum Kit - EIA for IgA and IgG)**

| Ratio | IgA | | | | | Ratio | IgG | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Item 1 | Item 2 | Item 3 | Item 4 | Item 5 | | Item 1 | Item 2 | Item 3 | Item 4 | Item 5 |
| Part. A | 7,79 | 7,16 | 6,95 | 6,70 | 8,63 | Part. A | 7,43 | 13,90 | 7,75 | 5,06 | 14,14 |
| Part. E | 12,53 | 33,65 | 9,70 | 9,53 | 10,72 | Part. F | 5,86 | 9,58 | 5,18 | 5,19 | 9,66 |
| Part. F | 13,14 | 12,41 | 8,77 | 11,74 | 13,86 | | | | | | |

*Table 03: Individual data (Reason) of the items sent in the Coronavirus SARS-CoV-2 proficiency test program in the 01/2020 round, separated by laboratory, item and test. Data represented by the Euroimmum - EIA IgA and IgG kits.*

## Chemiluminescence method (MagLumi system)

The MagLumi system uses the chemiluminescence method and was evaluated in the program in a quantitative (numerical) way. According to table 04, for the IgG antibody, item 1 was a negative material, following the reference value provided in the instructions of the manufacturer (AU / mL <1). Unlike the Euroimmum system, which identified the item as positive. In items 2, 4 and 5, values were considered positive (AU / mL ≥1). For item 3, there was a greater analytical variation. The participant "Part. B " stands out, with a higher value than the other users, except for item 1.

These data are presented below:

**Table 04: Results of automated immunological methods with individual data (Kit MagLumi IgG - Q)**

| | IgG | | | | |
|---|---|---|---|---|---|
| AU/mL | Item 1 | Item 2 | Item 3 | Item 4 | Item 5 |
| Part. A | 0,12 | 10,87 | 0,65 | 1,89 | 18,97 |
| Part. B | 0,14 | 17,15 | 1,32 | 2,98 | 26,07 |
| Part. C | 0,09 | 13,57 | 0,65 | 1,92 | 25,25 |
| Part. D | 0,15 | 12,90 | 0,61 | 1,98 | 24,12 |
| Average | 0,13 | 13,62 | 0,81 | 2,19 | 23,60 |
| DP | 0,03 | 2,62 | 0,34 | 0,53 | 3,19 |
| CV | 21% | 19% | 42% | 24% | 14% |

*Table 04: Individual data (AU / mL) of items sent in the SARS-CoV-2 Coronavirus proficiency test program in round 01/2020, separated by laboratory, item and test. Data represented by the MagLumi IgG - Q kit*

For IgM in the MagLumi system, (table 05), participants had a negative consensus for item 4 and a positive consensus for item 3. As for the other items (1, 2 and 5), were observed results above and below of reference value provided in the manufacturer's package insert. It is also worth noting that the participant "Part. B " presented lower values for IgM in all items.

**Table 05: Results of automated immunological methods with individual data (Kit MagLumi IgM - Q)**

| | IgM | | | | |
|---|---|---|---|---|---|
| AU/mL | Item 1 | Item 2 | Item 3 | Item 4 | Item 5 |
| Part. A | 1,05 | 1,62 | 6,07 | 0,94 | 1,77 |
| Part. B | 0,26 | 0,94 | 4,28 | 0,54 | 0,92 |
| Part. C | 0,46 | 1,33 | 4,79 | 0,68 | 1,32 |
| Part. D | 0,77 | 1,57 | 5,32 | 0,82 | 1,87 |
| Average | 0,64 | 1,37 | 5,12 | 0,75 | 1,47 |
| DP | 0,35 | 0,31 | 0,77 | 0,17 | 0,44 |
| CV | 55% | 23% | 15% | 23% | 30% |

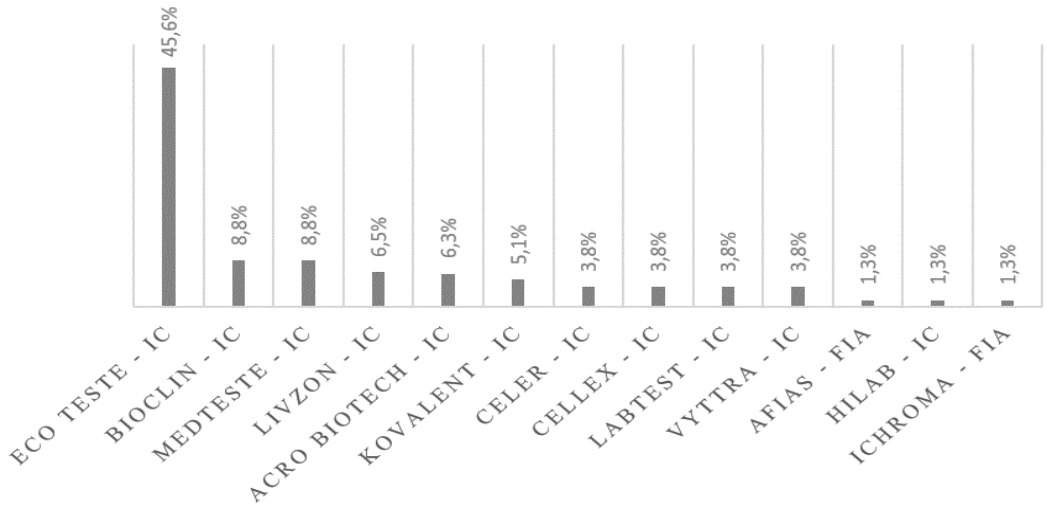
*Table 05: Individual data (AU / mL) of items sent in the SARS-CoV-2 Coronavirus proficiency test program in round 01/2020, separated by laboratory, item and assay. Data represented by the MagLumi - Q IgM kit*

## Immunological method by Rapid Diagnostic Test (RDT)

79 laboratories reported results using immunological methods by Rapid Diagnostic Test (RDT). 13 different types were reported, 11 of which were immunochromatographic (IC) methods, and 02 participants used fluorescence immunoassay (FIA).

The Celer One Step COVID-19 test - Celer Biotecnologia SA evaluates total immunoglobulins. The rest consist of anti IgG and anti IgM antibodies.

**Graph 05: Percentage of responses per RDT kit reported in the Proficiency Test**

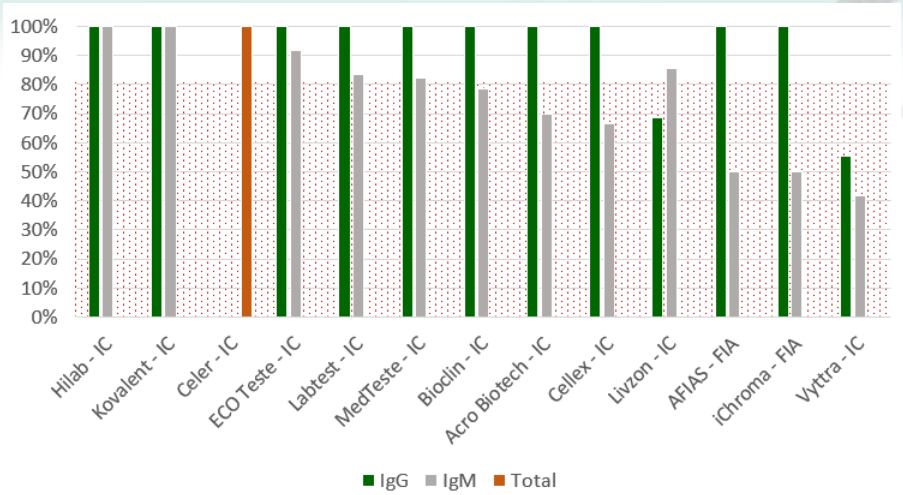



*Graph 05: Percentage of responses from RDT kits in the SARS-CoV-2 Coronavirus proficiency testing program in the 01/2020 round.*

Most participants (63.2%) use only 03 manufacturers brands, with Eco Teste being the most used (45.6%), followed by Bioclin (8.8%) and MedTeste (8.8%); the other 10 brands ranged between 1.3% and 6.5% of the participants in this round.

Taking into account only the items evaluated, the general performance of the Rapid Diagnostic Test kits in the proficiency test is shown below. This percentage was calculated based on the total number of responses reported by them for IgG, IgM and Total (IgG + IgM).

**Graph 06: General percentage of adequacy per RDT kit in the Proficiency Test**




*Graph 06: Percentage of general adequacy of the kits compared to the items evaluated in the SARS-CoV-2 Coronavirus proficiency test program in the 01/2020 round. Overall performance below 80% was highlighted.*

Analyzing graph 06 and table 06, it can be seen that a large part of the RDT kits presented an adequacy percentage close to or equal to 100% for IgG and IgM. Only 02 manufacturers showed adequacy for IgG below 80%, however for IgM six were considered inadequate, that is, below 80%.

**Table 06: General percentage of adequacy per RDT kit in the Proficiency Test**

| Kit | IgG | IgM | Total |
|---|---|---|---|
| Hilab - IC | 100% | 100% | - |
| Kovalent - IC | 100% | 100% | - |
| Celer - IC | - | - | 100% |
| ECO Teste - IC | 100% | 92% | - |
| Labtest - IC | 100% | 83% | - |
| MedTeste - IC | 100% | 82% | - |
| Bioclin - IC | 100% | 79% | - |
| Acro Biotech - IC | 100% | 70% | - |
| Cellex - IC | 100% | 67% | - |
| Livzon - IC | 69% | 86% | - |
| AFIAS - FIA | 100% | 50% | - |
| iChroma - FIA | 100% | 50% | - |
| Vyttra - IC | 56% | 42% | - |

*Table 06: Percentage of adequacy of the RDT immunological kits, evaluated in the SARS-CoV-2 Coronavirus proficiency test program in the 01/2020 round.*

It is important to highlight that the "not evaluated" items are related to the lack of consensus "intra" and "between" kits, which will be presented later in the individual performance of the assay items. Thus, the percentage of adequacy of these kits needs to be compared with the "not evaluated" items.

When analyzing the adequacy percentage of the kits, one must also observe their representativeness in the market in relation to the users who reported the proficiency testing program.

# Individual assessment of items, against immunological methods by Rapid Diagnostic Tests (RDT)

## Item 1

**Table 07: Percentage of responses by immunological method with RDT kit (item 1)**

| KIT | Qty. | IgG | | | IgM | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| Acro Biotech - IC | 5 | 20% | 80% | - | 100% | - | - | | | |
| AFIAS - FIA | 1 | - | 100% | - | 100% | - | - | | | |
| Bioclin - IC | 7 | - | 100% | - | 14% | 86% | - | | | |
| Cellex - IC | 3 | - | 100% | - | 100% | - | - | | | |
| ECO Teste - IC | 37 | 32% | 68% | - | 84% | 16% | - | | | |
| Hilab - IC | 1 | - | 100% | - | 100% | - | - | | | |
| iChroma - FIA | 1 | - | 100% | - | 100% | - | - | | | |
| Kovalent - IC | 5 | 60% | 40% | - | 100% | - | - | | | |
| Labtest - IC | 3 | - | 100% | - | 100% | - | - | | | |
| Livzon - IC | 6 | 83% | 17% | - | 100% | - | - | | | |
| MedTeste - IC | 7 | - | 100% | - | 29% | 71% | - | | | |
| Vyttra - IC | 3 | 100% | - | - | 100% | - | - | | | |
| Celer - IC | 6 | | | | | | | - | 100% | - |
| Total | 79 | 30% | 70% | 0% | 78% | 22% | 0% | 0% | 100% | 0% |
| | | Not evaluated | | | Negative | | | Negative | | |

*Table 07: Behavior of each kit and antibody reported in the Coronavirus SARS-CoV-2 proficiency test program in round 01/2020, for item 1. This item was a single donor, who presented positive RT-PCR at the beginning of the infection and performed a new collection 25 days after the onset of symptoms.*

Analyzing table 07, we can see that the 78% consensus indicated that the item was negative for IgM. In cases where the report was positive, they used only 3 manufacturer brands (Bioclin, Eco teste and Medteste). For IgG, some kits did not show consensus, resulting in an approximate percentage of 30% reporting negative and 70% reporting positive. It is worth noting that some of these kits did not reach consensus within their own group. It should also be noted that for the days between the onset of symptoms and the collection, a "Positive" consensus for IgG was expected

Due to the presence of an antibody identified by 70% of the participants who reported IgG, the kit that reported "total antibodies" was evaluated against this consensus.

## Item 2

According to table 08, this item showed a greater consensus among the kits that detect the IgG antibody, enabling its evaluation. For the IgM antibody, the consensus was lower, resulting in the non-evaluation in the proficiency test. It is noteworthy that the time between the onset of symptoms and the collection of material from the patient was the same as in item 1.

Due to the presence of antibody identified by IgG in the item consensus, the kit that reported "total antibodies" was evaluated against this consensus.

Table 08: Percentage of responses by immunological method with RDT kit (item 2)

| KIT | Qty. | IgG | | | IgM | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| Acro Biotech - IC | 5 | - | 100% | - | 100% | - | - | | | |
| AFIAS - FIA | 1 | - | 100% | - | 100% | - | - | | | |
| Bioclin - IC | 7 | - | 100% | - | 0% | 100% | - | | | |
| Cellex - IC | 3 | - | 100% | - | 33% | 67% | - | | | |
| ECO Teste - IC | 37 | - | 100% | - | 43% | 57% | - | | | |
| Hilab - IC | 1 | - | 100% | - | 100% | - | - | | | |
| iChroma - FIA | 1 | - | 100% | - | 100% | - | - | | | |
| Kovalent - IC | 4 | - | 100% | - | 50% | 50% | - | | | |
| Labtest - IC | 3 | - | 100% | - | 100% | - | - | | | |
| Livzon - IC | 6 | 17% | 83% | - | 50% | 50% | - | | | |
| MedTeste - IC | 7 | - | 100% | - | 0% | 100% | - | | | |
| Vyttra - IC | 3 | 67% | 33% | - | 100% | - | - | | | |
| Celer - IC | 6 | | | | | | | - | 100% | - |
| Total | 78 | 4% | 96% | 0% | 46% | 54% | 0% | 0% | 100% | 0% |
| | | | Positive | | | Not evaluated | | | Positive | |

*Table 08: Behavior of each kit and antibody reported in the Coronavirus proficiency test program SARS-CoV-2 in round 01/2020, for item 2. This item was a single donor, who presented positive RT-PCR at the beginning of the infection and performed a new collection 25 days after the onset of symptoms.*

## Item 3

Regarding the shortest time (days after the onset of symptoms) in relation to items 1 and 2, this item was not evaluated for IgG. As the percentage of positivity between IgG and IgM were close, this period still shows the transition between the drop in IgM and the increase in IgG, being close to the sensitivity between the systems. Due to the presence of antibody identified by IgM, the kit that reported "total antibodies" was evaluated against this consensus.

It should also be noted that for the days between the onset of symptoms and the collection, a "Positive" consensus for IgG was expected.

**Table 09: Percentage of responses by immunological method with RDT kit (item 3)**

| KIT | Qty. | IgG | | | IgM | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| Acro Biotech - IC | 5 | - | 100% | - | 80% | 20% | - | | | |
| AFIAS - FIA | 1 | - | 100% | - | 100% | - | - | | | |
| Bioclin - IC | 7 | - | 100% | - | - | 100% | - | | | |
| Cellex – IC | 3 | 100% | - | - | - | 100% | - | | | |
| ECO Teste - IC | 36 | 22% | 78% | - | 19% | 81% | - | | | |
| Hilab – IC | 1 | - | 100% | - | - | 100% | - | | | |
| iChroma - FIA | 1 | - | 100% | - | 100% | - | - | | | |
| Kovalent - IC | 4 | 100% | - | - | - | 100% | - | | | |
| Labtest – IC | 3 | - | 100% | - | 67% | 33% | - | | | |
| Livzon – IC | 5 | 20% | 80% | - | - | 100% | - | | | |
| MedTeste - IC | 7 | 29% | 71% | - | - | 100% | - | | | |
| Vyttra - IC | 3 | 100% | - | - | 100% | - | - | | | |
| Celer - IC | 6 | | | | | | | - | 100% | - |
| Total | 76 | 28% Not Evaluated | 72% | 0% | 24% | 76% Positive | 0% | 0% | 100% Positive | 0% |

*Table 09: Behavior of each kit and antibody reported in the SARS-CoV-2 Coronavirus proficiency test program in the 01/2020 round, for item 3. This item was a single donor, who presented positive RT-PCR at the beginning of the infection and performed a new collection 15 days after the onset of symptoms.*

## Item 4

For this item, the consensus was greater among the antibodies, allowing its evaluation for both IgG and IgM, and the kit that reports the test as Total. Even so, as noted in the previous items, some kits did not show consensus among their users.

**Table 10: Percentage of responses by immunological method with RDT kit (item 4)**

| KIT | Qty | IgG | | | IgM | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| Acro Biotech - IC | 5 | - | 100% | - | 20% | 80% | - | | | |
| AFIAS - FIA | 1 | - | 100% | - | - | - | 100% | | | |
| Bioclin - IC | 7 | - | 100% | - | - | 100% | - | | | |
| Cellex - IC | 3 | - | 100% | - | 100% | - | - | | | |
| ECO Teste - IC | 36 | 3% | 97% | - | - | 100% | - | | | |
| Hilab - IC | 1 | - | 100% | - | - | 100% | - | | | |
| iChroma - FIA | 1 | - | 100% | - | - | - | 100% | | | |
| Kovalent - IC | 4 | - | 100% | - | - | 100% | - | | | |
| Labtest - IC | 3 | - | 100% | - | - | 100% | - | | | |
| Livzon - IC | 5 | 60% | 20% | 20% | 60% | 40% | - | | | |
| MedTeste - IC | 7 | - | 100% | - | - | 100% | - | | | |
| Vyttra - IC | 3 | - | 100% | - | 33% | 67% | - | | | |
| Celer - IC | 6 | | | | | | | - | 100% | - |
| Total | 76 | 5% | 93% Positive | 0% | 11% | 87% Positive | 0% | 0% | 100% Positive | 0% |

*Table 10: Behavior of each kit and antibody reported in the Coronavirus proficiency test program SARS-CoV-2 in round 01/2020, for item 4. This item was a single donor, who presented positive RT-PCR at the beginning of the infection and performed a new collection 25 days after the onset of symptoms.*

## Item 5

For this item, the consensus was greater among the antibodies, allowing its evaluation for both IgG and IgM, and the kit that reports the test as Total. Even obtaining a higher percentage of adequacy, some kits did not show consensus among their users.

**Table 11: Percentage of responses per RDT kit item 5**

| | | IgG | | | IgM | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|---|
| KIT | Qty | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| Acro Biotech - IC | 5 | - | 100% | - | 20% | 80% | - | | | |
| AFIAS - FIA | 1 | - | 100% | - | - | 100% | - | | | |
| Bioclin - IC | 7 | - | 100% | - | - | 100% | - | | | |
| Cellex - IC | 3 | - | 100% | - | 33% | 67% | - | | | |
| ECO Teste - IC | 36 | - | 100% | - | - | 100% | - | | | |
| Hilab - IC | 1 | - | 100% | - | - | 100% | - | | | |
| iChroma - FIA | 1 | - | 100% | - | - | 100% | - | | | |
| Kovalent - IC | 4 | - | 100% | - | - | 100% | - | | | |
| Labtest - IC | 3 | - | 100% | - | - | 100% | - | | | |
| Livzon - IC | 5 | - | 100% | - | - | 100% | - | | | |
| MedTeste - IC | 7 | - | 100% | - | - | 100% | - | | | |
| Vyttra - IC | 3 | 67% | 33% | - | 100% | - | - | | | |
| Celer - IC | 6 | | | | | | | - | 100% | - |
| Grand Total | 76 | 3% | 97% Positive | 0% | 7% | 93% Positive | 0% | 0% | 100% Positive | 0% |

*Table 11: Behavior of each kit and antibody reported in the Coronavirus proficiency test program SARS-CoV-2 in the 01/2020 round, for item 5. This item was a single donor, who presented positive RT-PCR at the beginning of the infection and performed a new collection 25 days after the onset of symptoms.*

## Conclusion

### Molecular Techniques

It was possible to observe a wide variety of molecular targets being used for amplification and detection, because even with recommendations from international organizations, such as the World Health Organization (WHO) and Pan American Health Organization (PAHO), there is no scientific consensus with those with the best performance and effectiveness. This situation was expected, since it is a new disease (COVID-19), which surprised everyone in the health segment and the world in general.

With regard to inadequate results, there are several reasons for these occurrences, ranging from technical flaws in laboratory procedures, in this case the need for training of technical personnel and possible need for process review - including the quality of the raw materials and kits used and due to the malfunction of the equipment involved - up to the different detection targets and acceptance criteria/CT limits (Cycle Threshold) to consider.

Therefore, it is recommended that each laboratory individually analyze its performance and that it uses the information provided by the provider with the overall market performance (Results Profile documents and this report) to evaluate the performance of the analytical system in the Proficiency Test program. It is also important that these users seek the continuous use of reference material, such as

internal quality control, to daily validate their routines and eliminate any interferences that negatively reflect on the reliability of patient results.

## Immunological Methods

**Automated immunological methods**: as the proficiency test was offered early in the pandemic, automated systems were not yet being offered in large numbers on the market and this explains less participation of these analytical systems in the program. It is expected that, in the next round, in June 2020, there will be a larger number of participants, making it possible to present the behavior of more analytical systems available in the national market. The difference in interpretation between the systems within the same item as "positive" or "negative" will be better analyzed with the entry of new members and systems in the proficiency test.

**Immunological methods by Rapid Diagnostic Test (RDT)**: these methods had a larger number of participants and with different manufacturers, allowing a more robust statistical analysis among the reported systems.

For this method of analysis, some items/antibodies were not evaluated in the proficiency test program due to the lack of consensus “intra” and “inter” kits, which resulted in a higher percentage of adequacy of the kits shown in Graph 6 and table 6. It should be noted, in this case, that these “not evaluated” were single donors, more than 15 days after the beginning of their infection, confirmed by RT-PCR, which demonstrates in this case the lack of standardization of sensitivity between kits and perhaps between their lots.

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 also to 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 and perform a simple analysis between batches and shipments with reference/control materials.

Due to the large number of results report failures for this methodology, we take the opportunity to ask the laboratories, which contributed to this report, to make a critical analysis of their results, seeking to carefully select the suppliers of diagnostic kits. Certainly, all other precautions regarding technical procedures must be performed; as an example, we suggest making good practices the object of permanent attention and review.

Controllab is committed to this cause and already has internal quality control for all methodologies. The company also offers the services of Performance Panel and Validation batch by batch, which can be used by manufacturers/suppliers of kits and also by laboratories.

A new date for the second round of the SARS-CoV-2 Coronavirus Proficiency Test is already set: next 6/15/2020.

We also highlight some sites that are disclosing their analysis regarding the sensitivity of the and specificity tests. One of them is the ANVISA website http://portal.anvisa.gov.br, which discloses the previous analyzes carried out by INCQS (https://www.incqs.fiocruz.br); and the other site is the link https://www.testecovid19.org/, an initiative of several entities in the laboratory sector united in the program of evaluation of diagnostic kits for Covid-19.

## Thanks

We register our thanks to:

- ✓ Laboratories that joined the initiative, who participated and committed to the credibility and quality of their reports, supporting the diagnosis for SARS-CoV2 with reliable information. The data were fundamental to demonstrate to the market the performance of the analytical systems used.
- ✓ The board and staff of the Sabin Laboratory, Dr. Gustavo Barcelos Barra, Dr. Ticiane Henriques Santa Rita, Dr. Pedro Goes Mesquita and Dr. Rafael Henriques Jácomo, for their partnership and donation of Molecular Biology material.

## Attachment 1

Below are listed the Kits and lots (batches), respectively, reported in the proficiency 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 testing program.

Behavior between batches

| Acro Biotech - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| NCP20030273 | 1 | 2 | | | 3 | | | 3 | | | 3 | | | 3 | |
| NCP20030323 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| NPC20030287 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| **IgM** | | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | |
| 20030323 | 1 | | | 1 | | | | 1 | | 1 | | | | 1 | |
| NCP20030273 | 3 | | | 3 | | | 3 | | | | 3 | | | 3 | |
| NPC20030287 | 1 | | | 1 | | | 1 | | | | 1 | | 1 | | |

| AFIAS - FIA | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| WHQDA11G | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| **IgM** | | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | |
| WHQDA11G | 1 | | | 1 | | | 1 | | | | | 1 | | 1 | |

| Bioclin - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| 5 | | 2 | | | 2 | | | 2 | | | 2 | | | 2 | |
| 6 | | 2 | | | 2 | | | 2 | | | 2 | | | 2 | |
| 7 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| 8 | | 2 | | | 2 | | | 2 | | | 2 | | | 2 | |
| **IgM** | | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | |
| 5 | | 2 | | | 2 | | | 2 | | | 2 | | | 2 | |
| 6 | | 2 | | | 2 | | | 2 | | | 2 | | | 2 | |
| 7 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| 8 | 1 | 1 | | | 2 | | | 2 | | | 2 | | | 2 | |

| Celer - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **Total** | | ↓ | | | ↓ | | | ↓ | | | ↓ | | | ↓ | |
| W195 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| W19500329 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| W19500335 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| W19500337 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| W195004117 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |

| Cellex - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| 20200404WI5513C-3 | | 2 | | | 2 | | 2 | | | | 2 | | | 2 | |
| 20200406W15513C-3 | | 1 | | | 1 | | 1 | | | | 1 | | | 1 | |
| **IgM** | | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | |
| 20200404WI5513C-3 | 2 | | | | 2 | | | 2 | | 2 | | | 1 | 1 | |
| 20200406W15513C-3 | 1 | | | 1 | | | | 1 | | 1 | | | | 1 | |

Behavior between batches

| ECO Teste - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| 202004011 | 3 | 2 | | | 5 | | 1 | 4 | | 1 | 4 | | | 5 | |
| 202004012 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202004013 | 2 | | | | 2 | | 1 | 1 | | | 2 | | | 2 | |
| 202004015 | 1 | | | | 1 | | 1 | | | | 1 | | | 1 | |
| 202005003 | 1 | | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202005004 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202005007 | 1 | 1 | | | 2 | | | 2 | | | 2 | | | 2 | |
| 202005009 | 1 | 3 | | | 4 | | 1 | 3 | | | 4 | | | 4 | |
| 202005012 | 1 | | | | 1 | | 1 | | | | 1 | | | 1 | |
| 202005015 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202005016 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202005017 | | 1 | | | 1 | | | | | | | | | | |
| 202005019 | 1 | 13 | | | 14 | | 2 | 12 | | | 14 | | | 14 | |
| 202005026 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202004019P | 1 | | | | 1 | | 1 | | | | 1 | | | 1 | |
| **IgM** | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | | |
| 202004011 | 5 | | | 2 | 3 | | 2 | 3 | | | 5 | | | 5 | |
| 202004012 | 1 | | | 1 | | | 1 | | | | 1 | | | 1 | |
| 202004013 | 2 | | | 1 | 1 | | 1 | 1 | | | 2 | | | 2 | |
| 202004015 | 1 | | | 1 | | | 1 | | | | 1 | | | 1 | |
| 202005003 | 1 | | | 1 | | | 1 | | | | 1 | | | 1 | |
| 202005004 | 1 | | | | 1 | | 1 | | | | 1 | | | 1 | |
| 202005007 | 2 | | | 2 | | | | 2 | | | 2 | | | 2 | |
| 202005009 | 4 | | | 2 | 2 | | | 4 | | | 4 | | | 4 | |
| 202005012 | 1 | | | 1 | | | | 1 | | | 1 | | | 1 | |
| 202005015 | 1 | | | 1 | | | | 1 | | | 1 | | | 1 | |
| 202005016 | 1 | | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202005017 | 1 | | | 1 | | | | | | | | | | | |
| 202005019 | 8 | 6 | | 2 | 12 | | | 14 | | | 14 | | | 14 | |
| 202005026 | 1 | | | | 1 | | | 1 | | | 1 | | | 1 | |
| 202004019P | 1 | | | 1 | | | | 1 | | | 1 | | | 1 | |

| Hilab - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| COV3D200513 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| **IgM** | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | | |
| COV3D200513 | 1 | | | 1 | | | | 1 | | | 1 | | | 1 | |

| iChroma - FIA | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| whqda50 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| **IgM** | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | | |
| whqda50 | 1 | | | 1 | | | 1 | | | | | 1 | | 1 | |

| Kovalent - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| 11877207 | | 1 | | | 1 | | 1 | | | | 1 | | | 1 | |
| 111877202 | 1 | | | | 1 | | 1 | | | | 1 | | | 1 | |
| 111877206 | 1 | | | | 1 | | 1 | | | | 1 | | | 1 | |
| 111879202 | 1 | 1 | | | 1 | | 1 | | | | 1 | | | 1 | |
| **IgM** | ↓ | | | | | | ↓ | | | ↓ | | | ↓ | | |
| 11877207 | 1 | | | | 1 | | | 1 | | | 1 | | | 1 | |
| 111877202 | 1 | | | 1 | | | | 1 | | | 1 | | | 1 | |
| 111877206 | 1 | | | | 1 | | | 1 | | | 1 | | | 1 | |
| 111879202 | 2 | | | 1 | | | | 1 | | | 1 | | | 1 | |

Behavior between batches

| Labtest - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| 2630695 | | 2 | | | 2 | | | 2 | | | 2 | | | 2 | |
| 2630965 | | 1 | | | 1 | | | 1 | | | 1 | | | 1 | |
| **IgM** | ↓ | | | | | | | ↓ | | | ↓ | | | ↓ | |
| 2630695 | 2 | | | 2 | | | 2 | | | | 2 | | | 2 | |
| 2630965 | 1 | | | 1 | | | | 1 | | | 1 | | | 1 | |

| Livzon - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| CK2003030410 | 3 | 1 | | 1 | 3 | | | 3 | | 2 | 1 | | | 3 | |
| CK2003070410 | 1 | | | | 1 | | 1 | | | 1 | | | | 1 | |
| CK2003090410 | 1 | | | | 1 | | | 1 | | | | 1 | | 1 | |
| **IgM** | ↓ | | | | | | | ↓ | | | ↓ | | | ↓ | |
| CK2003030410 | 4 | | | 1 | 3 | | | 3 | | 2 | 1 | | | 3 | |
| CK2003070410 | 1 | | | 1 | | | | 1 | | 1 | | | | 1 | |
| CK2003090410 | 1 | | | 1 | | | | 1 | | | 1 | | | 1 | |

| MedTeste - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| COV20030060 | | 5 | | | 5 | | 1 | 4 | | | 5 | | | 5 | |
| COV20030081 | | 2 | | | 2 | | 1 | 1 | | | 2 | | | 2 | |
| **IgM** | ↓ | | | | | | | ↓ | | | ↓ | | | ↓ | |
| COV20030060 | 1 | 4 | | | 5 | | | 5 | | | 5 | | | 5 | |
| COV20030081 | 1 | 1 | | | 2 | | | 2 | | | 2 | | | 2 | |

| Vyttra - IC | 1 | | | 2 | | | 3 | | | 4 | | | 5 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind | Neg | Pos | Ind |
| **IgG** | | | | | ↓ | | | | | | ↓ | | | ↓ | |
| 2004313 | 2 | | | 2 | | | 2 | | | | 2 | | 2 | | |
| FJN29201 | 1 | | | | 1 | | 1 | | | | 1 | | | 1 | |
| **IgM** | ↓ | | | | | | | ↓ | | | ↓ | | | ↓ | |
| 2004313 | 2 | | | 2 | | | 2 | | | | 2 | | 2 | | |
| FJN29201 | 1 | | | 1 | | | 1 | | | 1 | | | 1 | | |

## Attachment 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.

**Table 12: List of participating laboratories**

### BAHIA (4)

- Hospital Santa Izabel - Santa Casa de Misericórdia da Bahia
- Instituto Análise de Pesquisas Clínicas
- Labchecap - Laboratórios de Análises Clínicas
- Laboratório Análise - Instituto Análise de Pesquisas Clínicas

### CEARÁ (3)

- DASA - Laboratório Hospital Unimed CE
- Labluz - Instituto da Primeira Infância - IPREDE
- LACEN CE

### DISTRITO FEDERAL (3)

- DASA - Hospital Brasília - DF
- Instituto Hospital de Base do Distrito Federal - IHBDF
- Laboratório Sabin de Análises Clínicas

### ESPÍRITO SANTO (1)

- Hospital UNIMED Sul Capixaba

### GOIÁS (1)

- Laboratório INGOH - Instituto Goiano de Oncologia e Hematologia

### MARANHÃO (2)

- Laboratório Gaspar
- Rede Sarah de Hospitais de Neuroreabilitação - Unidade São Luís – MA

### MINAS GERAIS (22)

- Check-Up Laboratório de Análises Clínicas
- Fundação Ezequiel Dias
- GMN Nefrolab
- Hematologia e Patologia Clínica Souza Assunção
- Hospital Mater Dei
- Hospital Unimed - Campo Grande MS Cooperativa de Trabalho Médico
- I9MED LABORATÓRIO TESTES RÁPIDOS
- Instituto de Análises Clínicas Carlos Chagas
- Instituto Hermes Pardini
- José Alair Couto Laboratório de Análises Clínicas
- Laboratório Cortes Villela Ltda
- Laboratório da UPA Barreiro
- Laboratório de Patologia Clínica Hospital Márcio Cunha - Unidade I
- Laboratório Distrital Norte/Venda Nova
- Laboratório Monte Sinai
- Laboratório Santa Lúcia
- Laborclínica Análises e Pesquisas Clínicas
- Lab-Rede - Laboratório de Referência em Diagnósticos Especializados
- Lamel - Laboratório Médico Especializado
- Quibasa - Bioclin
- Santa Casa de Misericórdia de Juiz de Fora - Laboratório Análises Clínicas
- Tecsa - Laboratórios

### PARÁ (1)

- Laboratório Central do Estado do Pará

### PERNAMBUCO (1)

- Marcelo Magalhães Diagnósticos

### PIAUÍ (1)

- Bioanálise

## PARANÁ (10)

• DB - Diagnósticos do Brasil
• DASA - Frischmann Aisengart Medicina Diagnóstica
• Hi Technologies Ltda
• Hospital Infantil Pequeno Príncipe
• Hospital Nossa Senhora das Graças - Laboratório Análises Clínicas
• LABMED - Laboratório Médico de Londrina
• Laboratório de Patologia Bom Jesus
• Laboratório Pasteur
• Unimed Curitiba Participações S/A
• Unimed de Londrina Cooperativa de Trabalho Médico

## SÃO PAULO (28)

• Associação Fundo de Incentivo à Pesquisa
• Biomega Medicina Diagnóstica
• Centro de Genomas
• CIPAX Medicina Diagnóstica
• Cura Centro de Ultrassonografia e Radiologia
• DB Diagnósticos do Brasil
• DASA - Laboratório Central Alphaville
• Fleury Centro de Medicina Diagnóstica
• Fundação Faculdade Regional de Medicina
• Hemodiag Laboratório de Análises Clínicas
• Hospital Santa Cruz
• Hospital Santa Joana
• Hospital São Camilo - Ipiranga
• Instituto de Análises Clínicas de Santos
• Instituto de Assistência Médica ao Servidor Público Estadual.
• Laboratório Central de Patologia Clínica do Hospital das Clínicas FMRP USP
• Laboratório Central do Hospital São Paulo
• Laboratório de Análises Clínicas Unimed São Roque
• Laboratório Lemelab de Análises Clínicas
• Laboratório São Francisco
• Laboratório Unimed - Santa Barbara d'Oeste e Americana Participações
• LABVIVALLE
• PBS Pesquisas e Serviços Biomédicos
• Senne Líquor Diagnóstico
• Serviço Social da Indústria e da Construção Civil do Estado de São Paulo - SECONCI
• Sociedade Campineira de Educação Instrução
• UNIMED São José do Rio Preto Cooperativa de Trabalho Médico
• Universidade Estadual de Campinas - UNICAMP - Divisão de Patologia Clínica/HC

## RIO DE JANEIRO (7)

• Centro de medicina Nuclear da Guanabara
• Contraprova Doping e Toxicologia
• DASA - Rio de Janeiro
• Hospital dos Servidores do Estado
• Laboratório de Histocompatibilidade e Criopreservação HLA-UERJ
• Laboratório Richet
• Controllab

## RONDÔNIA (1)

• Laboratório Unimed Vilhena

## RIO GRANDE DO SUL (6)

• Aspeur - Associação Pró Ensino Superior em Novo Hamburgo
• Laboratório Amplicon
• Laboratório de Análises Clínicas Carlos Franco Voegeli
• Serviço de Diagnóstico Laboratorial - Hospital de Clínicas de Porto Alegre
• Sociedade Dr. Bartholomeu Tacchini
• Unimed POA - Laboratório Cachoeirinha

## SANTA CATARINA (5)

• Laboratório Biomédico - Laboratório de Pesquisas Clínicas e Bromatológicas
• Laboratório de Análises Clínicas Dr. Willy Carlos Jung
• Laboratório de Análises Clínicas Unimed Litoral
• Laboratório Médico Santa Luzia
• Laboratório Santa Isabel de Análises Clínicas

## SERGIPE (1)

• CEMISE - Centro de Medicina Integrada de Sergipe

## TOCANTINS (1)

• PHD Laboratório Clínico

## INTERNATIONAL (2)

• Ecuador: Synlab Sociedad Anónima

• Portugal: Laboratório Fernanda Galo Lda