Cube Scan

Performance Report

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1 - Determination of metrological traceability of calibrator and control values

The Cube Scan equipment detection strategy is based on the principle of magnetic resonance energy absorption associated with magnetic reluctance measurements of the analyzed sample que are converted into phase and magnitude values of the signal applied to a set of frequencies. When a variable electromagnetic field crosses the analyzed sample, absorptions occur at various frequencies. Each of these frequencies is related to the target nano structures, where the electromagnetic field is crossing and interacting with the nano biological structures and other materials.

The Cube Scan has an integrated circuit capable of emitting frequencies between 35 MHz and 4400 MHz, through antennas. To determine the ideal frequency range for antenna operation, a study was conducted as described below:

1 - Three separate samples containing the following solutions:

- 3 ml distilled water with 0.9% sucrose
- 3 ml distilled water with 0.9% NaCl
- 3 ml distilled water

The samples were processed in the Cube Scan, being exposed to electromagnetic waves of different frequencies. Each type of sample had different changes in relation to the amplitude and angle reflected, according to the frequencies that reached them. The objective was to identify which frequency range in which the antennas have the highest sensitivity to detect air the variation of phase and magnitude, which determines which type of sample is being analyzed.

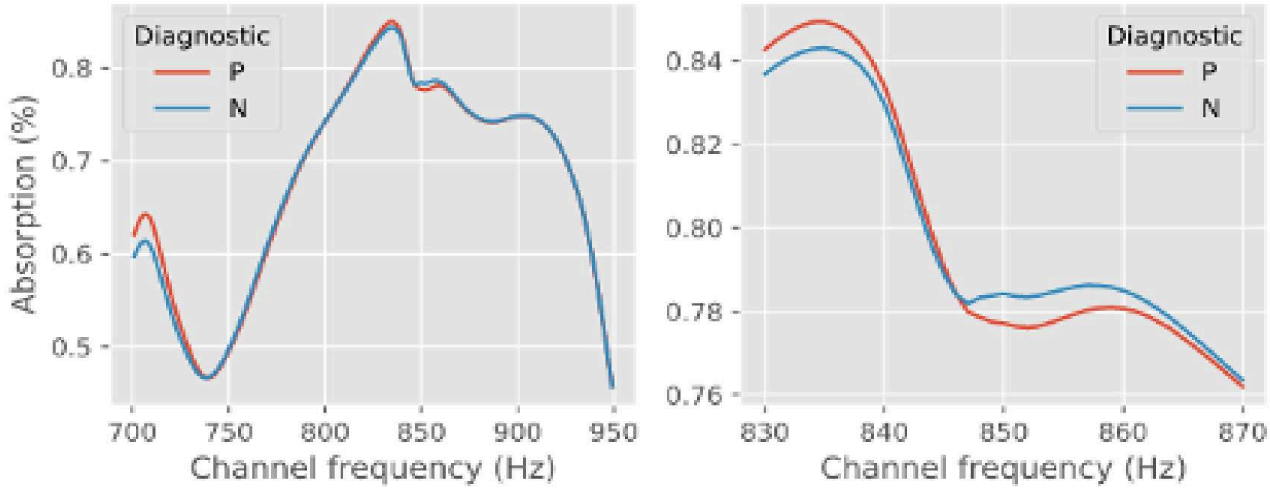
Knowing that the equipment was capable of distinguishing similar samples with a significant sensitivity, the study was initiated using nasal swab samples for covid-19 detection. The study was carried out at Lacen-ES (Central Laboratory of Espírito Santo), with three stages. In the first stage, data were collected from nasal swab samples, in order to feed the artificial intelligence of cube scan. Subsequently, with the results obtained by Lacen-ES, in the RT-PCR test, it was possible to cross-reference the data and measure what absorption behavior and phase difference identified the positive and negative samples. A prediction model was then developed to diagnose samples as positive or negative. This prediction model is used by the software on all Cube Scan equipment.

Because it is an equipment that is based on artificial intelligence, through trained software, the Cube Scan does not require a control sample.

The integrated circuit of the Cube Scan, responsible for generating the frequencies used by the device, is tested and has its efficiency guaranteed by the manufacturer itself, throughout its useful life, a factor that in itself would guarantee the stability necessary for the proper functioning of the equipment.

However, in order to provide even more reliability, every time it is turned on, the Cube Scan performs a self-test on all its components. At that point it will emit a beep and light, indicating this scan. In this self-test, the device as well the difference in phase and absorption of the air inside the receptacle of the cube where the samples are inserted. The result obtained must be within the predetermined range, this way the full operation of the equipment is confirmed, otherwise the equipment will not connect.

Figure 1 - Average absorption in relation to all samples evaluated for a frequency range. The red line indicates positive samples, and the blue line negative samples for Covid-19



2 - Measurement Accuracy

2.1 - Repeatability

Objective: to observe whether there is variation when the same operator analyzes the same sample several times, i.e. the variation due to the measuring equipment.

Methodology: The study was conducted with 10 samples of patients tested for Covid-19 in the state of Espírito Santo, in June 2021; provided by Lacen- ES. Each sample was tested 4 times, totaling 40 analyses. The results were obtained conform and then:

Table 1: Repeatability Results

Sample / **Test**	1	2	3	4	5	6	7	8	9	10
1	P	N	P	N	P	N	P	N	P	N
2	P	N	P	N	P	N	P	N	P	N
3	P	N	P	N	P	N	P	N	P	N
4	P	N	P	N	P	N	P	N	P	N

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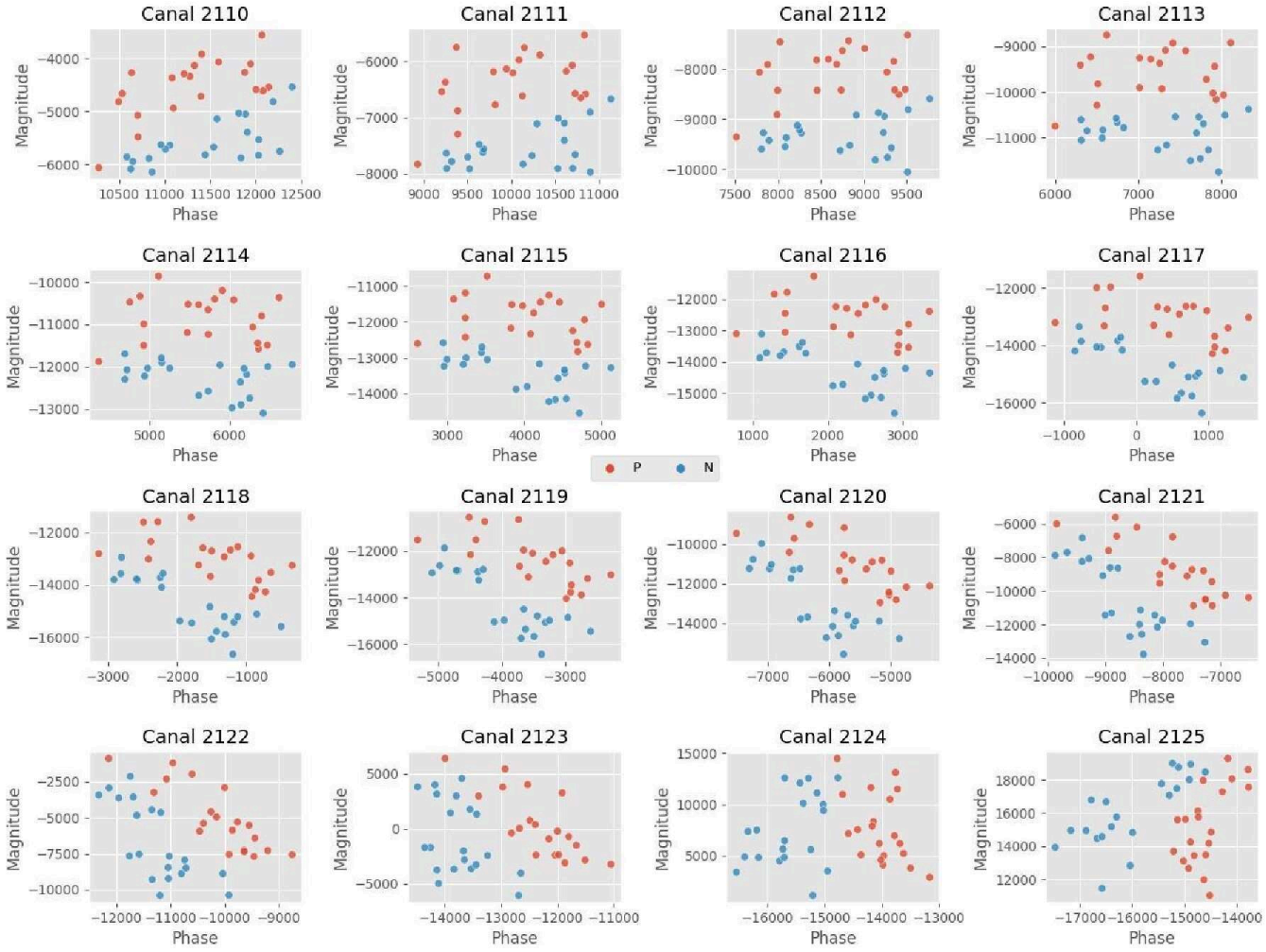
The graphs below show the data of the distinction observed between the positive samples and the negative samples that were used in the repeatability test.

Each channel corresponds to a frequency of signal radiated in the sample. The results are clasified according to magnitude and phase values found in each sample analyzed. The magnitude represents the degree of energy absorbed by the sample, while the phase represents the degree of molecular agitation of the sample.

Graph 1 - Repeatability test: positive samples x negative samples



Conclusion:

The result obtained for the 10 samples demonstrated uniformity in the 4 tests performed for each sample. Therefore, no variations were recorded due to the measuring equipment.

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2.2 - Reproducibility

Objective: To observe if there is variation between operators, i.e. variation due to the measurement method.

Methodology: We analyzed 14 samples, provided by Lacen/ES, from patients tested in the state of Espírito Santo, Brazil, in June 2021. The reproducibility analyses were performed by two different operators, one at a time, which analyzed according to the sequence provided, positive and negative samples for Covid-19.

- **Table 2: Operator 1 - Viviane Domingos**

Sample / **Test**	A	B	C	D	E	F	G	H	I	J	L	M	N	O
RESULT	P	N	P	P	P	N	N	N	P	N	N	P	N	N

- **Table 3: Operator 2 - Jan Jensen**

Sample / **Test**	A	B	C	D	E	F	G	H	I	J	L	M	N	O
RESULT	P	N	P	P	P	N	N	N	P	N	N	P	N	N

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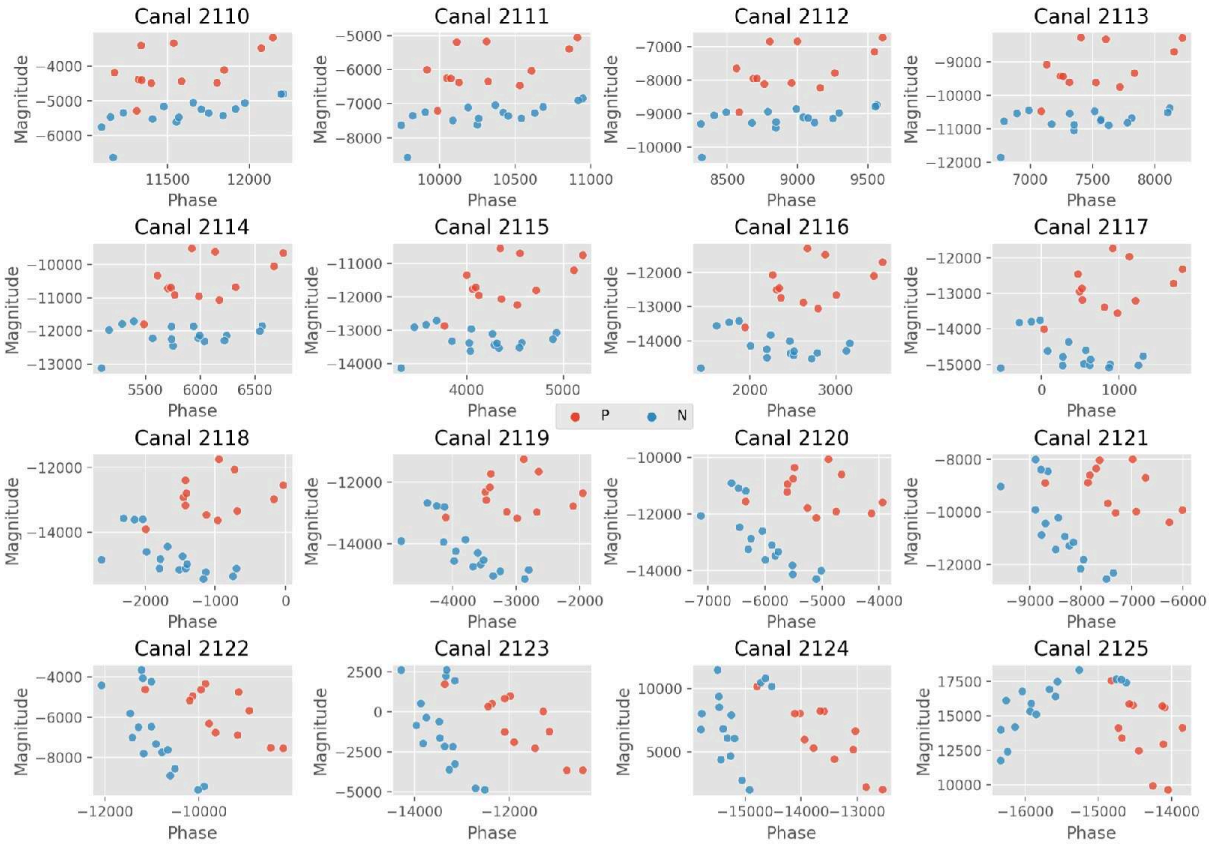
The graphs below show the data of the distinction observed between the positive samples and the negative samples that were used in the reproducibility test.

Graph 2 - Reproducibility test: positive samples x negative samples



Conclusion:

Data obtained in the reproducibility tests show that no discrepancies were recorded in the results by different operators for the analyzed samples.

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3 - Analytical Sensitivity or Detection Limit

Objective: To establish the lowest viral concentration that can be detected by the equipment.

Methodology: The tests to stipulate the limit of detection of the equipment were carried out at the Molecular Biology Laboratory of Lacen/ES, in June 2021. The method of dilution of the Positive Control of the Kit TaqPath™ COVID-19 CE-IVD RT- PCR was used. At first followed the step by step preparation for solution containing 25 copies / µL, provided in the instructions for use of the kit:

"1. If frozen, thaw the reagents on ice.

2. Gently vortex the reagents, then centrifuge briefly to collect liquid at the bottom of the tube.

3. Dilute TaqPath™ COVID-19 control (1 × 10^4 copies/µL) to a working stock of 25 copies /µL on ice:

a) Pipet 98 µL of TaqPath™ COVID-19 Control Dilution Buffer into a microcentrifuge tube, then add 2 µL of TaqPath™ COVID-19 Control. Mix well, then centrifuge briefly.

b) Pipet 87.5 µL of the TaqPath™ COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then add 12.5 µL of the dilution created in substep 3a. Mix well and then centrifuge briefly."

Three final solutions of 200 µL each were prepared, containing 25 copies / µL to satisfy the test demand, with some values multiplied by 2. Three vials of active control were used to ensure the accuracy of the results. The preparation occurred as follows:

a) Were pipetted 98 µL of TaqPath™COVID-19 Control Dilution Buffer into a microcentrifuge tube and then 2 µL of TaqPath™COVID-19 Control was added. Mixed well, then centrifuged briefly. This step was performed 3 times, producing 3 samples, each from different bottles of TaqPath TM COVID-19 Control.
b) Were pipetted 175 µL (87.5 µL x 2) of the TaqPath™COVID-19 Control Dilution Buffer into a second microcentrifuge tube, then added 25 µL (12.5 µL x 2) the dilution created in substep 3a. Mixed well, then centrifuged briefly. This step was performed 3 times, producing 3 samples.

The Cube Scan equipment was designed to analyze samples packed in Falcon Tubes of 15 mL, containing the swab in 3 mL of viral transport media. The next step was the preparation of solutions with different concentrations. In this, tubes containing viral transport media were used. The following dilutions were made:

1. 30 µL of solution 3b (corresponding to 750 copies of virus) was added to the tube containing viral transport media. Three tubes were prepared with this concentration.
2. 40 µL of solution 3b (corresponding to 1000 copies of virus) was added to the tube containing viral transport media. Three tubes were prepared with this concentration.

3. 48 µL of solution 3b (corresponding to 1200 copies of virus) was added to the tube containing viral transport media. Three tubes were prepared with this concentration.
4. 52 µL of solution 3b (corresponding to 1300 copies of virus) was added to the tube containing viral transport media. One tube was prepared with this concentration.
5. 56 µL of solution 3b (corresponding to 1400 copies of virus) was added to the tube containing viral transport media. One tube was prepared with this concentration.
6. 60 µL of solution 3b (corresponding to 1500 copies of virus) was added to the tube containing viral transport media. One tube was prepared with this concentration.
7. 70 µL of solution 3b (corresponding to 1750 copies of virus) was added to the tube containing viral transport media. One tube was prepared with this concentration.

NOTE: The objective of the test was to determine the detection limit of the Cube Scan, for this reason solutions with lower viral concentration were prepared in greater quantity. In order to detect the minimum possible viral load, a sample of 20 µL of type 3b solution was prepared, with 500 copies of SARS-CoV-2 for 3 mL of solution, for this sample the equipment showed instability in reading.

Table 4: Detection limit

		Viral concentration for 3 mL of solution						
		1750 copies	1500 copies	1400 copies	1300 copies	1200 copies	1000 copies	750 copies
Samples	1	+	+	+	+	+	+	+
	2					+	+	+
	3					+	+	+

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☐ **Images related to the test performed:**

Figure 2 - Test Performed

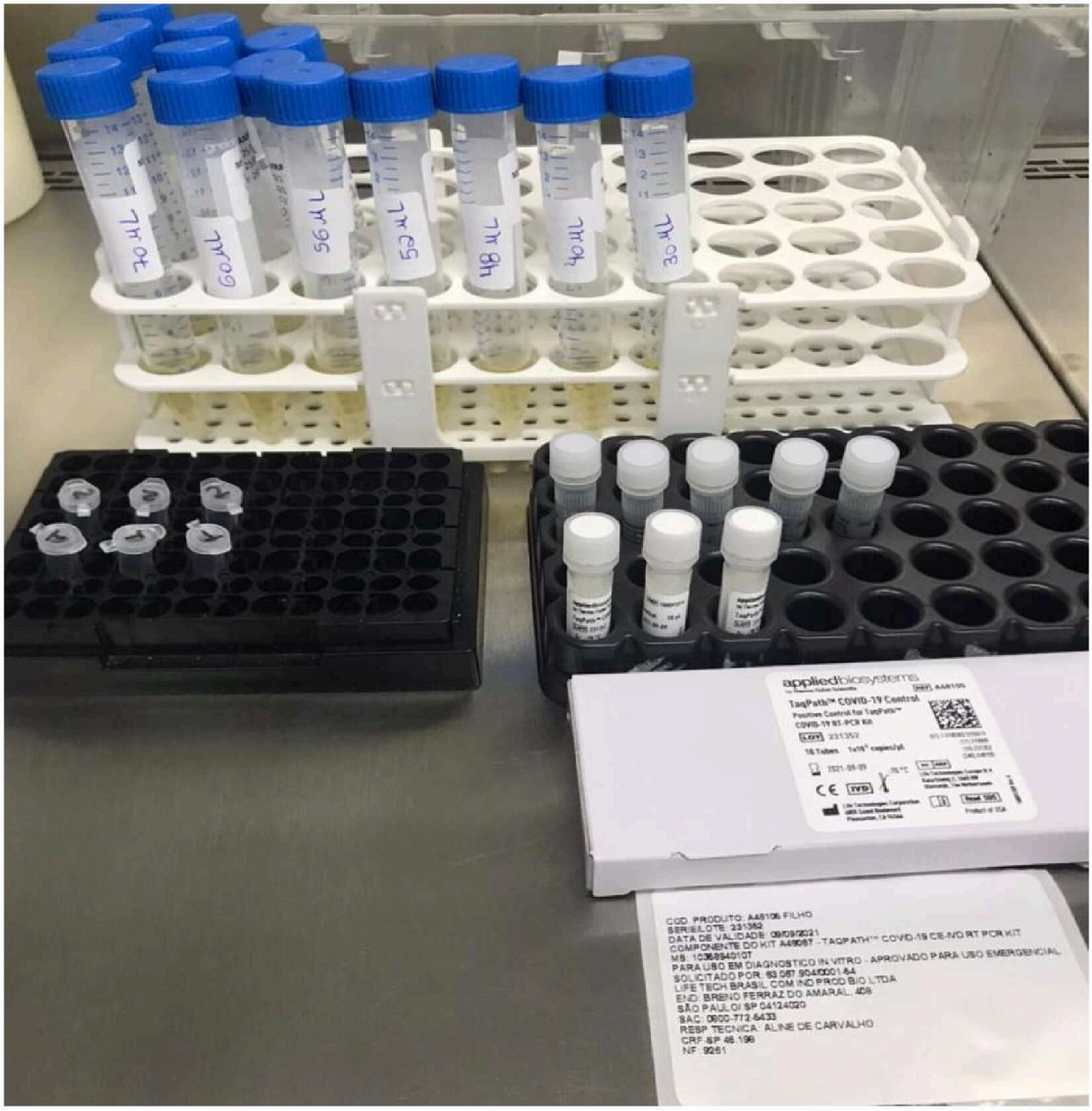
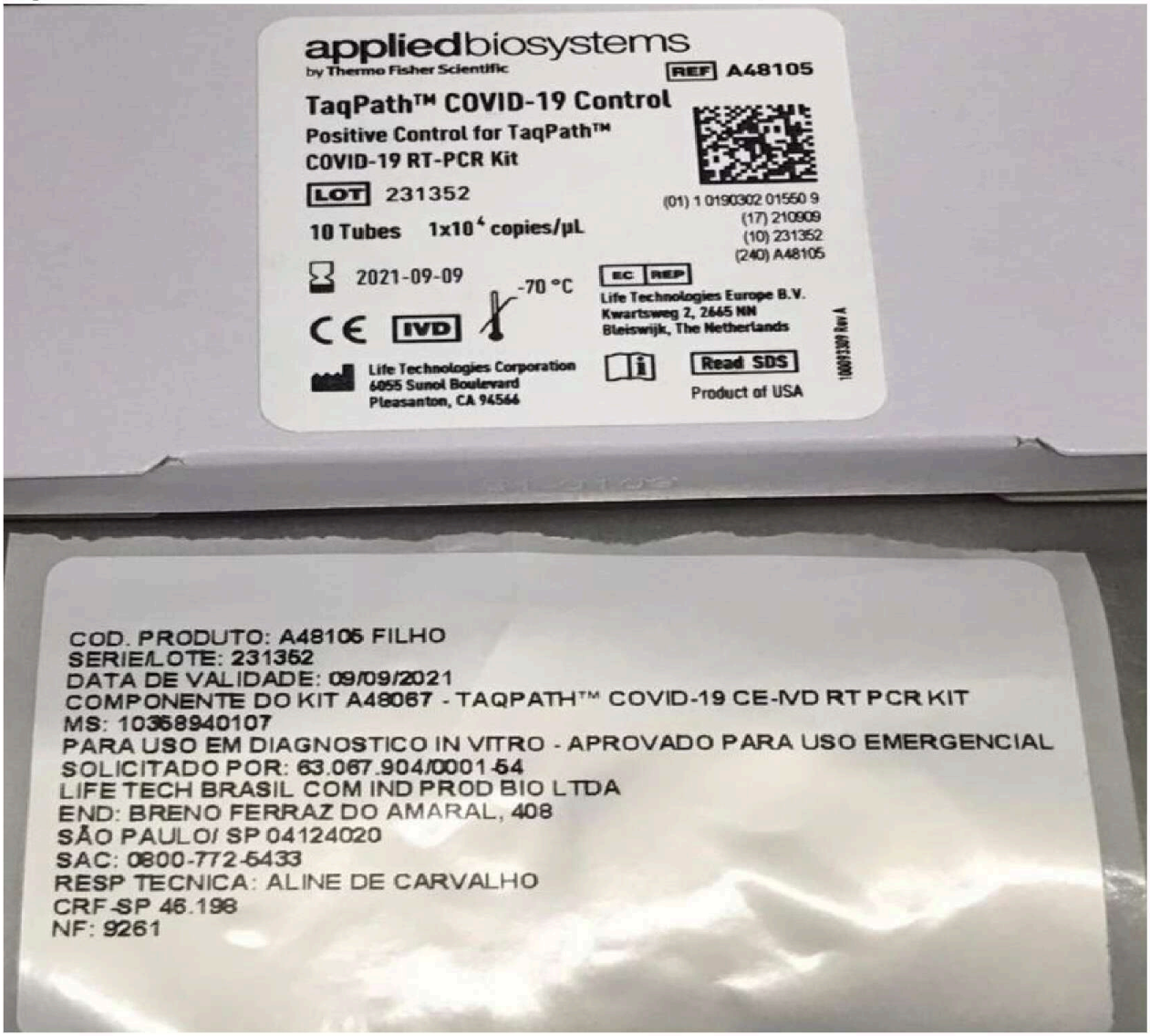


Figure 3 - The insum used in the test



Conclusion:

Therefore, after the above-mentioned analyses, it is concluded that the lowest viral concentration detected by Cube Scan is 250 copies/mL, or 0.25 copies/µL.

4 - Analytical Specificity

Objective: To demonstrate the ability of Cube Scan to identify only the target in question: SARS-CoV-2, with no cross-reactivity with other pathogens.

A validation study was conducted in Lacen-ES, Annex 1, in which the following data were obtained:

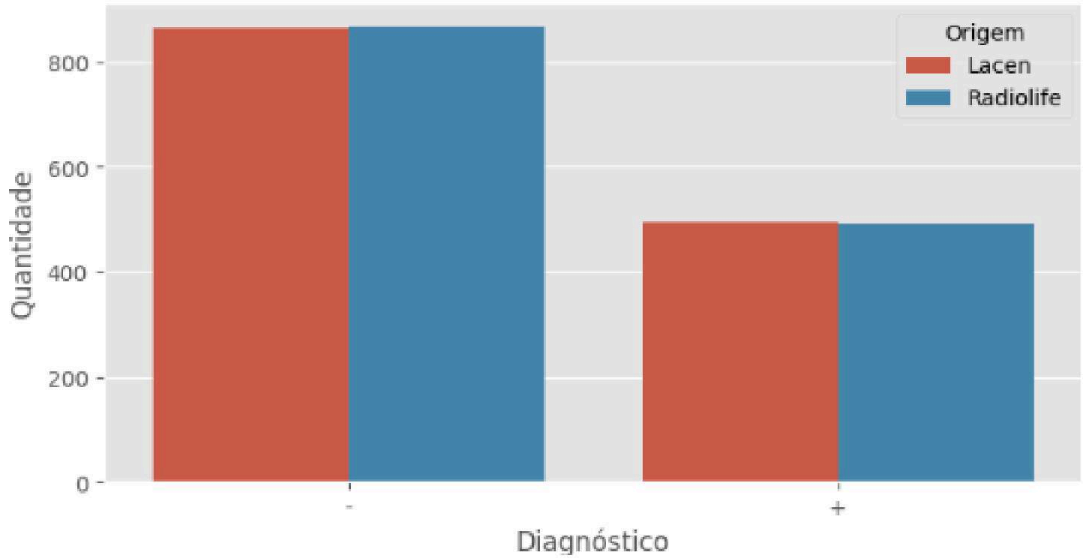
Table 5: Cube Scan Results Agreement/ PCR Results

	CUBE SCAN POSITIVE	CUBE SCAN NEGATIVE	TOTAL
PCR Positive	490	3	493
Negative PCR	0	864	864
Total	490	867	1357

Positive agreement rate: 490/493 (99.39%)
Negative agreement rate: 864/864 (100%) Total
agreement rate: 1354/1357 (99.77%)

Positive judgment value analysis
Detection result
'+' means positive result, '-' means negative result

Grific 3 - Comparison from Findings Lacen-ES e Radiolife



Sensitivity= 0.9939
Specificity= 1.0

Cube Scan used a frequency broadband to train classifiers and learn various metrics that will be applied to determine and distinguish positive and negative tests. Its classification operates in three stages:

1) The first is deterministic and takes advantage of the differences in signal absorption between positive and negative samples for SARS-CoV-2, identifying the best frequencies for virus detection;

2) Then, using sensor data, the information needed to be processed is extracted;
3) Finally, you choose a machine learning classifier to combine this information and interactively build decision trees, combining resources for the correct prediction of the result.

The successful learning phase allowed a 99.39% level of agreement between RT-PCR results and Cube Scan results to be reached.

Cross-reactivity

Methodology: Positive samples were used for other infectious microorganisms of patients tested in the state of Espírito Santo, provided by Lacen/ES in June 2021, according to the tables below. Four positive samples for ***Mycobacterium tuberculosis and*** 5 samples with respiratory **syncytial virus were tested.** The interpretation of the results was made considering (-) to negative, i.e., there was no cross-reaction and (+) for positive, in case of cross-reaction.

Table 6: Batch of Cross Reaction samples

Samples	1	2	3	**4**	**5**
Mycobacterium tuberculosis	-	-	-	-	
Respiratory Syncytial Virus	-	-	-	-	-

The following graphs show the distinction of the results obtained in the samples of patients with Covid-19, patients without any type of infection (healthy negative control) and patients with tuberculosis or respiratory syncytial virus, demonstrating that each organism responds in a unique way for each frequency Applied.

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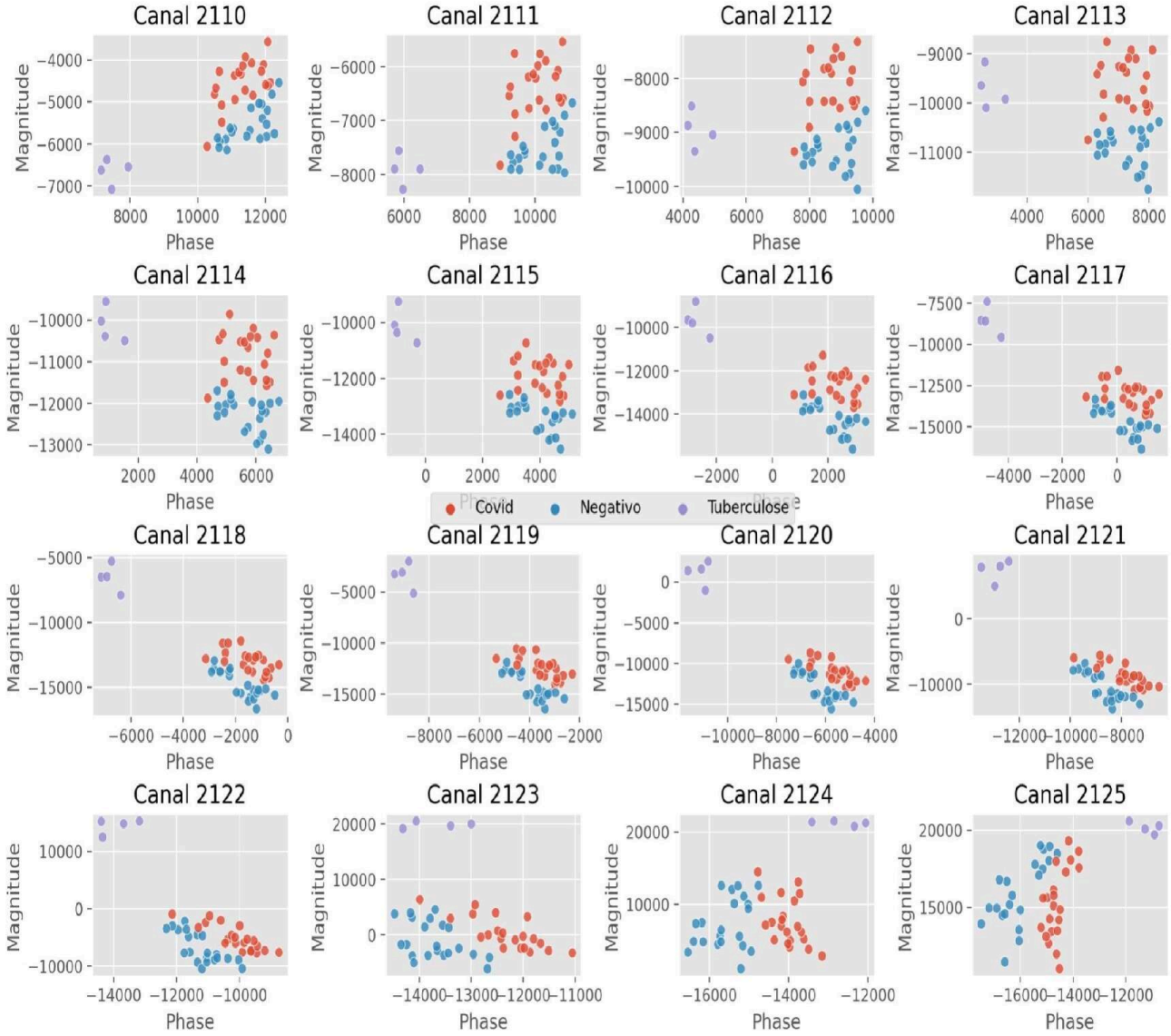
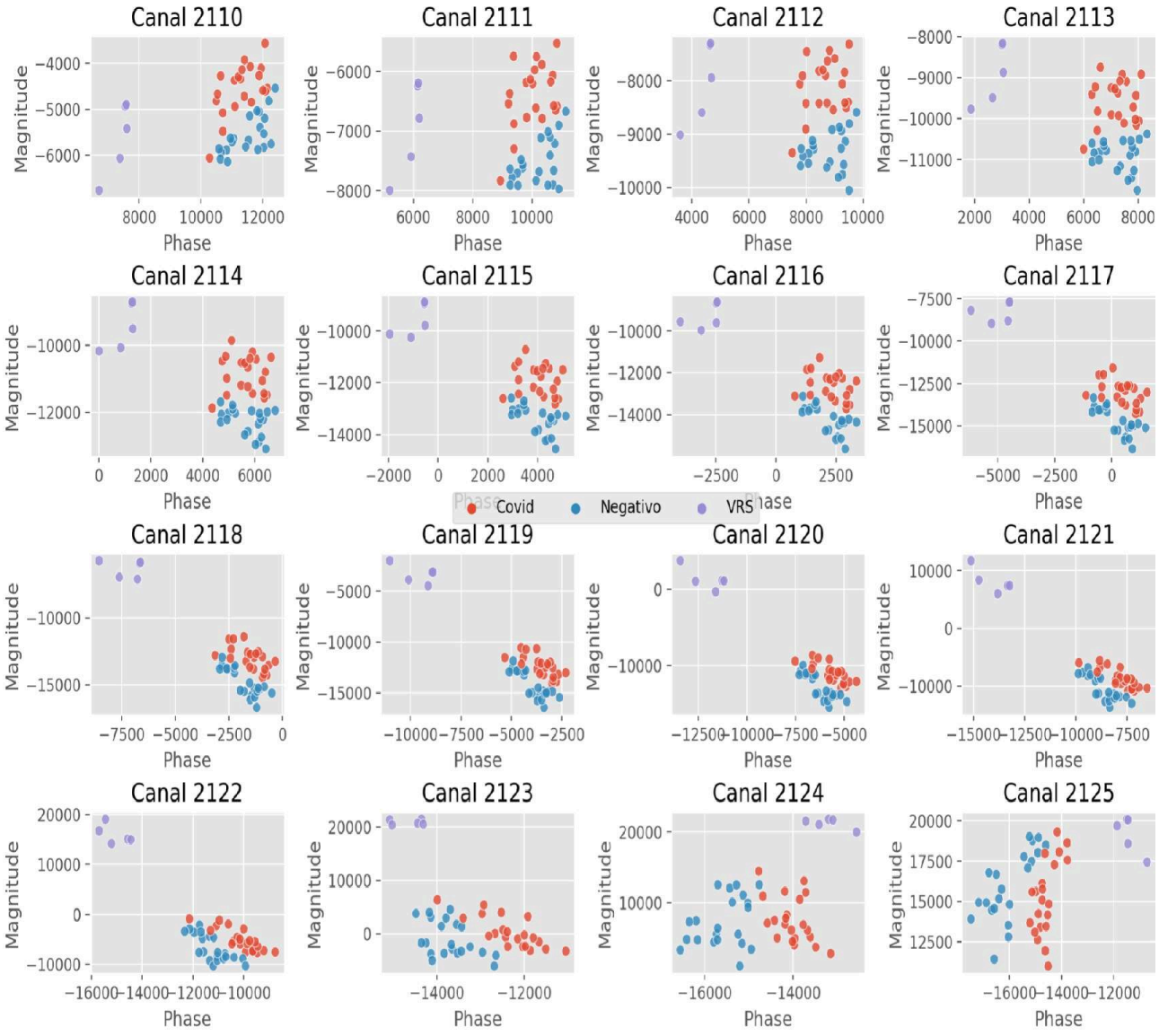
Graph 4 - Behavior of samples with Tuberculosis:



Graph 5 - Behavior of samples with Respiratory Sincicial Virus:



Conclusion: There was no cross-reactivity between the tested organisms, proving that because it is adjusted for detection of SARS-CoV-2, the Cube Scan does not erroneously identify other organisms present in the samples.

5 - Cut-Off value definition

Cube Scan makes use of an algorithm for predicting results, not applying a cut-off value.

Because it does not use chemical reagents or reactions that depend on thermal cycles applied to the sample, Cube Scan does not present ct reading. To determine the result, Cube Scan was trained to diagnose Covid-19 in samples with RT-PCR results with Ct values between 14 and 37.

6 - Test Procedure Validation Report

Report d the validation of the Test procedure is found in the Lacen Report, annexed to this report.

7 - Cleaning and disinfection procedure validation report

There is no specific procedure for cleaning and disinfecting the Cube Scan, however, we recommend due care:

- Keep the work environment clean and airy
- For cleaning the external surface of the instrument, a clean cloth and a neutral cleaning product should be used (do not allow the equipment to come into contact with solvents or corrosive material).
- Always check that the sample tube socket is clean, without the presence of objects, clean the socket with a dry cloth only when necessary.

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