imegen

# Validation Report

## Imegen® SARS-CoV-2

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Imegen SARS-CoV-2 has been validated by Instituto de Salud Carlos III for the detection of the new betacoronavirus SARS-CoV-2 in respiratory samples

Imegen SARS-CoV-2 Kit is waiting for FDA EUA approval

## 1. Object

The purpose of this document is to describe the clinical and analytical validation method of the Imegen SARS-CoV-2 kit, intended to the diagnosis of SARS-CoV-2 virus from RNA samples extracted from human patients (eg nasopharyngeal swabs).

In accordance with technical guidelines developed by the World Health Organization for the detection of SARS-CoV-2, the **Imegen SARS-CoV-2** kit detects three specific targets in genes common to all betacoronaviruses:

- **The ORF1ab gene**, which encodes most of the enzymatic proteins
- **The S gene**, which encodes the spike glycoprotein
- **The N gene**, which encodes the nucleocapsid protein (N)

Likewise, the kit includes as an endogenous positive control a system that detects the human **RNase P** ribozyme, used as a human endogenous control to confirm the integrity of the RNA sample, is based on the method published by the Centers for Disease Control and Prevention (CDC).

This test enables reverse transcription (RT) of viral RNA and real-time detection via PCR (qPCR) of the target genes through a one-step RT-qPCR, using a combination of multiplexed oligonucleotides and fluorescent hydrolysis probes (FAM and VIC).

The results obtained from this test can be used to confirm the patient's diagnosis. This test is not optimal for the study of the SARS and MERS coronaviruses.

## 2. Design f Imegen SARS-CoV-2

The amplification systems have been designed using 3,123 SARS-CoV-2 genomes deposited in the database of viral sequences commissioned by the Global Initiative on Sharing All Influenza Data (GISAID).

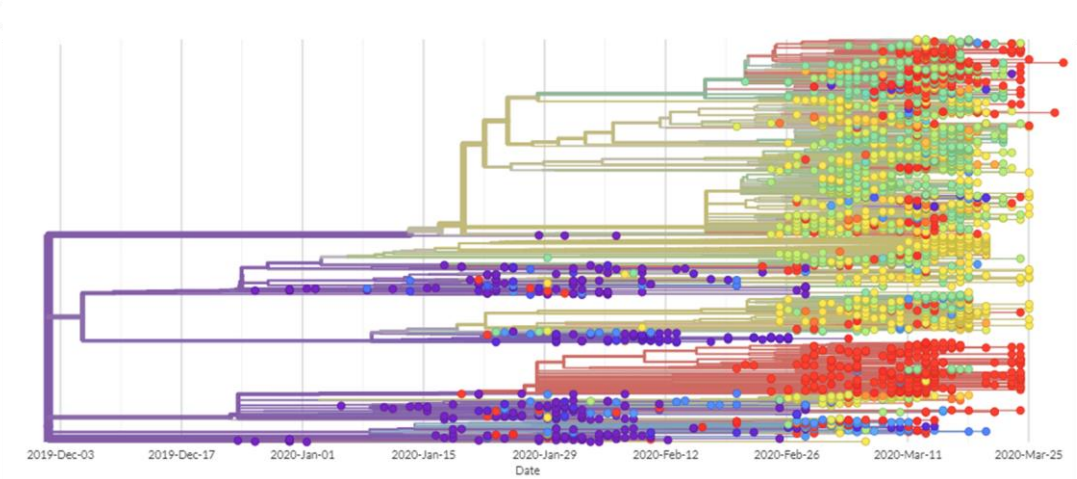


*Figure 1. Graphic of 3,123 genomes analysed between Feb 2020 and April 2020.*

Furthermore, a periodic review confirms and identifies the sensitivity of detection systems taking into account the new mutations detected in new strains of SARS-CoV-2.

## 2.1 INCLUSIVITY

The designs of the S, ORF1ab, and N genes were done using bioinformatics tools and existing genome information in the GISAID database, where all genetic variants are shown and classified according to the country, region, and host they were detected in. The design of oligonucleotides and hydrolysis probes allows for the detection of all genomic variants identified until 29/04/2020.

The results indicate that the diagnostic sensitivity of Imegen-SARS-CoV-2 would not be affected by the described mutations:

- SARS-CoV-2 Genome inclusivity: 3123 / 3123 * 100 = 100%

### Spike Mutation D614G

The Spike D614G mutation is of urgent concern; It began to spread in early February, and when it was introduced to new regions, it quickly became the dominant form. These findings have important implications for SARS-CoV-2 transmission, pathogenesis, and immune interventions.
doi: https://doi.org/10.1101/2020.04.29.069054

The Imegen SARS-CoV-2 S system detects the G strain carrying the D614G mutation, as well as mutations in S943P, V367F, G476F, G476S, V483A, H49Y, Y145H / del, Q239K, A831V, D839Y / N / E and P1263L described by Giorgi et al. 2020.

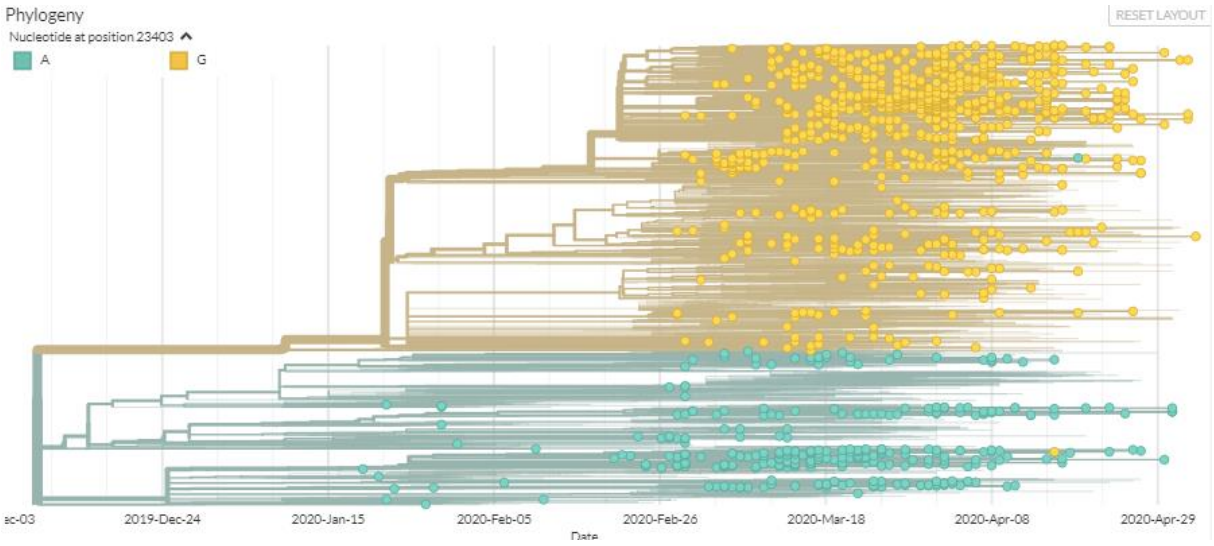


*Figure 2. Distribution of the D614G mutation (nucleotide position 23403 A> G) in USA (Cladogram shows 1245 SARS-CoV-2 genomes sampled between January and April 2020).*

### 2.2 CROSS-REACTIVITY

The genome sequences suggest the presence of a virus associated with severe acute respiratory syndrome (SARS) that is closely related to the members of a viral species called CoV, a species defined by the agent of the 2002/03 outbreak of SARS in humans. Because of this, each system's specificity has been evaluated to confirm its analytical specificity via BLAST in the NCBI and GISAID public databases.

FDA defines *in silico* cross-reactivity as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism. There were no probes in the three SARS-CoV-2 targets included in Imegen SARS-CoV-2 kit with homology ≥ 80%, with the exception of Bat SARS-CoV which presented a sequence homology > 80%.

## 3. CLINICAL PERFORMANCE

For the SARS-CoV-2 detection analysis, the recommendations described in the IFUs instructions are followed. Validation tests are aimed at confirming that the assay is specific, sensitive, repeatable and reproducible.

### 3.1 DIAGNOSTIC SENSITIVITY AND SPECIFICITY

The clinical evaluation of the trial has been carried out to assess the predictive diagnostic capacity of the Imegen SARS-CoV-2 kit using nasopharyngeal swabs from patients for whom a report of presence / absence of SARS-CoV-2 had previously been issued after PCR testing in a reference laboratory.

Forty-three (43) positive samples and forty (40) negative samples were analysed to evaluate the diagnostic specificity and sensitivity:

| Clinical Diagnostic | ARN Samples | Results |
|---|---|---|
| Positive (Sensitivity) | 42/43 | 97.7% |
| Negative (Specificity) | 40/40 | 100% |

SAMPLE RESULTS:

| Samples SARS-CoV-2 | S | | N | | ORF1ab | | RNaseP | |
|---|---|---|---|---|---|---|---|---|
| | Average Ct | Range | Average Ct | Rango | Average Ct | Rango | Average Ct | Rango |
| Positive | 28.0 | 20.1--34.0 | 27.9 | 20.5--36.3 | 28.0 | 20.9--35.4 | 25.8 | 19.1--30.8 |
| Negative | N.D. | - | N.D. | - | N.D. | - | 28.2 | 21.9--36.5 |

*Table 1. Cycle threshold (Ct) results obtained for negative and postiive SARS-CoV-2. N.D., not detected.*

The values obtained for the samples included in the clinical validation show a very wide viral load range, ranging from thousands of copies to values close to the detection limit.

The studies presented by the Carlos III Health Institute (ISCIII) indicate that by means of the RT-PCR technique it has been observed that the infected patients mostly present a high viral load (between $10^4$ and $10^8$ copies of genome / ml in nasopharyngeal sample or saliva) during the first days of appearance of the symptoms and probably during the pre-symptomatic phase.

## 3.2 LIMIT OF DETECTION (LOD)

The detection limit repreesnts the minimum number of copies that the system is capable of repeatably detecting. To do this, we have used a certified SARS-CoV-2 genomic RNA control. To evaluate the detection limit, 11 replicates have been analysed for each of the systems:

LOD using the StepOne Plus Real-time PCR cycler.

| Analyte | Average Cycle threshold (Ct) | | | Result | Diagnostic sensitivity at LOD |
|---|---|---|---|---|---|
| | ORF1ab | N | S | | |
| **20 ARN copies** | 33.9 | 34.6 | 35.3 | Positive | **100%** |
| **10 ARN copies** | 34.4 | 33.7 | 34.7 | Positive | **100%** |

*Table 2. Limit of detection of Imegen SARS-CoV-2. Threshold has been set up at 0.02.*

LOD using the CFX96 Touch Real-time PCR cycler.

| Analyte | Average Cycle threshold (Ct) | | | Result | Diagnostic sensitivity at LOD |
|---|---|---|---|---|---|
| | ORF1ab | N | S | | |
| **20 ARN copies** | 35.6 | 34.2 | 35.8 | Positive | **100%** |
| **10 ARN copies** | 36.3 | 34.5 | 36.9 | Positive | **91%** |

*Table 3. Limit of detection of Imegen SARS-CoV-2. Threshold has been set up at 100.*

LOD using the LightCycler 480 Real-time PCR cycler

| Analyte | Average Cycle threshold (Ct) | | | Result | Diagnostic sensitivity at LOD |
|---|---|---|---|---|---|
| | ORF1ab | N | S | | |
| **20 ARN copies** | 34.5 | 34.0 | 34.4 | Positive | **100%** |
| **10 ARN copies** | 31.8 | 31.1 | 31.3 | Positive | **100%** |

*Table 4. Limit of detection of Imegen SARS-CoV-2. Threshold has been set up using the manual noise-band at 1 (FAM) and 3 (HEX).*

LOD using the 7500 FAST Real-time PCR cycler

| Analyte | Average Cycle threshold (Ct) | | | Result | Diagnostic sensitivity at LOD |
|---|---|---|---|---|---|
| | ORF1ab | N | S | | |
| **20 ARN copies** | 34.6 | 34.2 | 35.2 | Positive | **100%** |
| **10 ARN copies** | 35.3 | 35.0 | 36.7 | Positive | **82%** |

*Table 5. Limit of detection of Imegen SARS-CoV-2. Threshold has been set up at 0.02.*

The sensitivity of the kit for the detection of 5 total copies was evaluated, indicating that 50% of the samples with 5 total copies were detected as positive, while the rest were identified as inconclusive, by detecting the presence of a single target.

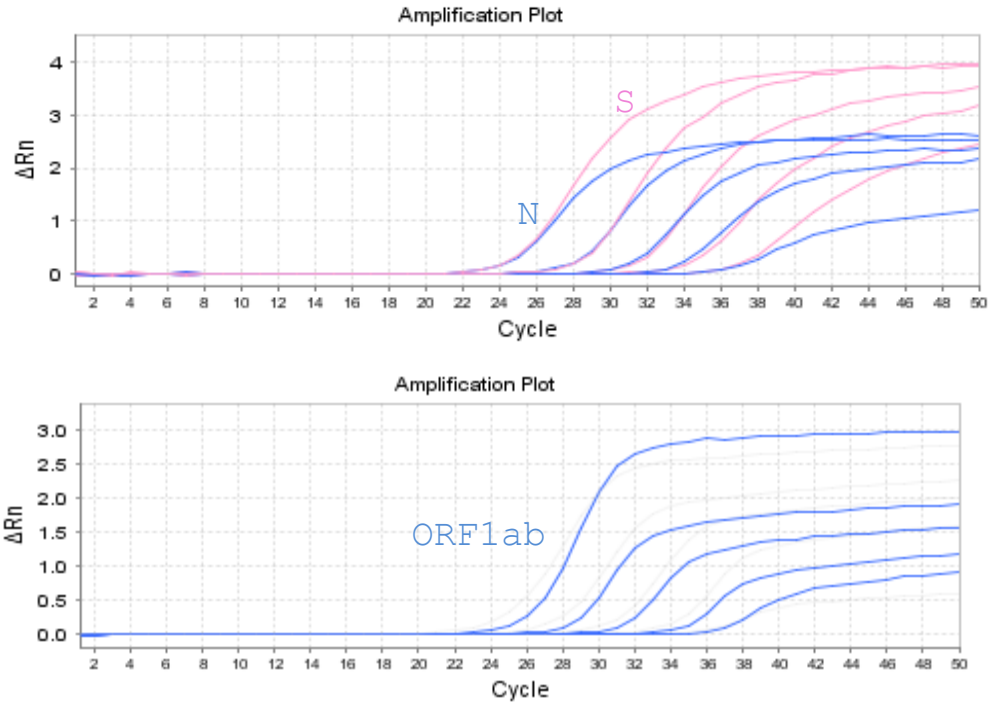


*Figure 3. Resutls obtained for systems N, S, ORF1ab using from 15,000 to 1 total copies per reaction. Results obtained with the StepOne Plus Real-time PCR cycler*

## 3.3 REPEATABILITY

Repeatability studies the robustness of the assay in successive measurements of the same magnitude, carried out under the same measurement conditions: origin of the sample, the operator performing the assay, the reagents used and the measurement equipment with which the assay is performed.

Eleven replicates of the LOD are analysed in each real-time PCR kit.

| Equipment | ORF1ab (10 copies) | | | N (10 copies) | | | S (10 copies) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Average | DESVEST | %CV | Average | DESVEST | %CV | Average | DESVEST | %CV |
| **CFX96 Touch** | 36.3 | 0.9 | 2.5% | 34.5 | 0.5 | 1.6% | 36.9 | 0.9 | 2.6% |
| **StepOne Plus** | 34.4 | 0.5 | 1.5% | 33.7 | 0.7 | 1.9% | 34.7 | 0.6 | 1.7% |
| **LightCycler 480** | 35.2 | 0.6 | 1.8% | 34.4 | 0.8 | 2.2% | 35.5 | 1.2 | 3.3% |
| **7500 FAST** | 35.3 | 0.7 | 2.0% | 35.0 | 0.8 | 2.3% | 36.7 | 0.6 | 1.7% |

*Table 6. Repeatability of Imegen SARS-CoV-2. DESVEST, standard deviation; CV, coefficient of variation.*

The assay indicates that the repeatability of the LOD when the test parameters (eg reagents, PCR kits) are not modified results in a coefficient of variation of less than 5%, showing its high repeatability.

## 3.4 REPRODUCIBILITY

Reproducibility studies the robustness of the test in successive measurements of the same magnitude, carried out under different measurement conditions. Conditions that change in a reproducibility assay include: the source of the sample, the operator performing the assay, the reagents used, and the measurement equipment with which the assay is performed.

Eleven replicates of the LOD are analysed in each real-time PCR kit.

| Equipment | ORF1ab (10 copies) | | | N (10 copies) | | | S (10 copies) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Average | DESVEST | %CV | Average | DESVEST | %CV | Average | DESVEST | %CV |
| **CFX96 Touch** | 35.3 | 1.0 | 2.8% | 34.4 | 0.8 | 2.3% | 35.9 | 1.2 | 3.3% |
| **StepOne Plus** | | | | | | | | | |
| **LightCycler 480** | | | | | | | | | |
| **7500 FAST** | | | | | | | | | |

*Table 7. Reproducibility of Imegen SARS-CoV-2. DESVEST, standard deviation; CV, coefficient of variation.*

The assay indicates that the reproducibility of the LOD when the test parameters are modified (eg reagents, PCR kits) results in a coefficient of variation of less than 5%, showing its high reproducibility.

## 4. Conclusions

The kit exhibits the clinical and analytical characteristics suitable for the intended use described.

The assay is characterised by:

- Diagnostic sensitivity 97.5%
- Diagnostic specificity 100%
- Repeatability and reproducibility with %CV<5%
- Analytical sensitivity (Limit of detection) of 10 total ARN copies

The detection systems used offer a repeatable and reproducible result.

Imegen is a biotechnology company certified to the **UNE EN ISO 13485:2018 Sanitary products** norm by AEMPS (Agencia Española del Medicamento y Producto Sanitario) for the design, development, fabrication, and commercialization of genetic analysis kits for *in vitro* diagnosis and for the development of software for bioinformatics analysis of genetic data.