Statistical Analysis Plan (SAP)

SARS-CoV-2 Nucleic Acid Detection Kit

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1. SYNOPSIS OF STUDY DESIGN

1.1 Purpose of Statistical Analysis Plan

This statistical analysis plan (SAP) is intended to provide a detailed and comprehensive description of the planned methodology and analysis to be used for CPSP CW008, the SARS-CoV-2 Nucleic Acid Detection Kit Clinical Performance Study clinical performance investigation. This plan is based on the Version 2.0, 10-Jun-2022 Clinical Performance Study Plan.

1.2 Clinical Investigation Objectives

The main objective of this clinical performance study (CPS) is to evaluate the SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR) diagnostic accuracy, defined in terms of sensitivity and specificity.

1.3 Clinical Investigation Design

The CPS to assess the diagnostic accuracy of the SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR) has been designed as non-interventional, retrospective, non-randomized, open-label and single-center. A total number of 600 samples specimens collected from nasopharyngeal swabs are required for the completion of this CPS. These samples will be distributed as follows:

- 100 positive samples of SARS-CoV-2 to test sensitivity
- 500 negative samples of SARS-CoV-2 to test specificity

No comparisons with the gold-standard method or other additional activities will be performed, and therefore no additional data will be collected or analyzed.

This CPS will be concluded when all samples have been analyzed. The expected duration of the study is 6 months, and no follow-up activities are required since the CPS only uses leftover specimens.

1.4 Endpoints

The primary endpoint of this CPS is to evaluate the sensitivity and specificity of the SARS-CoV-2 Nucleic Acid Detection Kit in the diagnosis of COVID-19. Their analysis will be performed independently:

- The sensitivity of the SARS-CoV-2 NAD Kit is defined as the capacity to correctly detect confirmed positive specimens of SARS-CoV-2 infection (True Positive, TP).

  To comply with the MDCG 2021-21, sensitivity will be assessed in 100 leftover samples determined as SARS-CoV-2 RNA positive by the laboratory standard method (gold-standard). Since different SARS-CoV-2 genotypes/subtypes and mutants are considered, specimens from different stages (first semester 2020, first semester 2021, and December 2021 - March 2022) and outbreak clusters of the COVID-19 pandemic will be selected from the leftover material.

- The specificity of the SARS-CoV-2 NAD Kit is the ability to correctly classify specimens with no SARS-CoV-2 infection as negative (True Negative, TN).

  To comply with the MDCG 2021-21, specificity will be assessed in 500 SARS-CoV-2 RNA negative human specimens defined so as to reflect the target population for which the device is intended, such as blood donors, hospitalized patients or pregnant women. These specimens must have been previously confirmed as negative by the gold-standard.

1.5 Randomization

There is no planned randomization for this CPS.

1.6 Blinding

There is no blinding in this CPS.

2. ANALYSIS CONSIDERATIONS

2.1 Analysis Populations

All specimens that meet the selection criteria are included in the per-protocol (PP) population, which will be analyzed as per the CPS Protocol (CPSP) and this Statistical Analysis Plan (SAP).

2.2 Statistical Methods

2.2.1 Descriptive statistics for categorical variables

For the categorical variables of sensitivity and specificity, results will be summarized with specimens counts and percentages/rates, and where specified in the table mockups, with exact 95% Clopper-Pearson confidence intervals.

2.2.2 Regression

No regression analyses are planned for this CPS.

2.3 Endpoint Analysis

The primary endpoint of this CPS evaluates the diagnostic accuracy of the SARS-CoV-2 Nucleic Acid Detection Kit in the diagnosis of COVID-19, which will be assessed through the definition of the sensitivity and specificity of the test:

- The sensitivity is the ability to detect a target marker (in this case, genetic material) associated with SARS-CoV-2. The sensitivity means the capacity to detect positive samples from COVID-19 patients. Therefore, sensitivity will be analyzed as the result of the ratio of the True Positive measurements (TP) to total known positive measurements (True Positive + False Negative).

$$Sensitivity = \frac{True\ Positive}{(True\ Positive + False\ Negative)}$$

- The specificity is the ability to recognize the absence of a target marker (for this kit, genetic material), associated with SARS-CoV-2, which means the capacity to detect true negative samples of COVID-19. Consequently, specificity will be determined as the ratio of the True Negative measurements (TN) to total known negative measurements (True Negative + False Positive).

$$\text{Specificity} = \frac{\text{True Negative}}{(\text{True Negative} + \text{False Positive})}$$

The analysis of the primary endpoint will be performed using the PP population.

2.4 Sample Size Calculations

The sample size calculation is based on the purpose to determine the diagnostic accuracy (in terms of sensitivity and specificity) of the SARS-CoV-2 Nucleic Acid Detection Kit (Multiplex Real Time RT-PCR). According to the requirements defined in the MDCG2021-21, a total number of 600 samples is sufficient to prove these parameters, which will be divided considering different purposes:

- 100 positive samples of SARS-CoV-2 to test sensitivity.
- 500 negative samples of SARS-CoV-2 to test specificity.

No drop-out ratio is expected in this CPS.

2.5 Interim Analysis

No formal interim analyses are planned for this study. As such, no formal statistical rule for early termination of the investigation is defined. Interim study reports with descriptive analysis may be produced for regulatory or reimbursement purposes.

2.6 Learning Curve

No learning curve is required for the adequate evaluation of the primary endpoint of this CPS.

2.7 Timing of Analysis

The final analysis of the primary endpoints will be completed when all specimens have been analyzed by the investigational device.

2.8 Study/ Investigation Success

Success will be declared when the primary endpoint is met.

2.9 Subgroups of Analysis

No subgroup analyses are planned for this CPS. Nevertheless, stratification analyses according to different characteristics of the sample (viral load, storage time, freeze/thaw cycles, etc.) might be performed.

2.10 Handling of Missing Data

There will be no imputation for any missing data, and the variables will be analyzed as recorded in the database.

2.11 Poolability Issue

Pooling strategies do not apply to this CPS.

2.12 Multiplicity Issues

There is no simultaneous multiple hypothesis testing in this clinical investigation.

2.13 Adjustments for Covariates

Unless otherwise specified, no adjustments for covariates will be made for any of the variables in the analyses.

2.14 Exploratory Analysis

No exploratory analyses are considered in this CPS.

3. DESCRIPTIVE ENDPOINTS AND ADDITIONAL DATA

3.1 Baseline and Demographic Characteristics

The following demographic variables will be summarized for the subjects enrolled: gender, and age.

3.2 Adverse Events

This CPS has been designed to use leftover specimens only, which will pose none additional risks to the subjects from whom the sample is collected. Therefore, no adverse events will be reported during this study.

3.3 Subject Early Termination

There is no formal statistical rule for early termination of the CPS for insufficient performance of the tested device defined.

3.4 Protocol Deviation

Protocol deviations will be summarized by major and minor categories for those cases in which a protocol deviation was reported.

Any major changes to the statistical plan will be documented in an amendment to the statistical plan. Less significant changes to the planned analyses will be documented in the final report.

3.5 Number of Subject Imbalance

This CPS will be conducted at only 1 site in Spain, and therefore no strategies for handling potential imbalance are designed.

3.6 Descriptive Endpoints or Additional Data

No descriptive or exploratory endpoints are considered in this CPS.

4. DOCUMENTATION AND OTHER CONSIDERATIONS

All analyses will be performed using IBM SPSS Statistics Base for Windows, version 18.0 or higher, or R software for Windows, version 4.1.2 or higher.

5. ACRONYMS AND ABBREVIATIONS

Acronym or Abbreviation	Complete Phrase or Definition
CPS	Clinical Performance Study
CPSP	Clinical Performance Study Protocol
FN	False Negative
FP	False Positive
RT-PCR	Reverse Transcription - Polymerase Chain Reaction
TN	True Negative
TP	True Positive
SAP	Statical Analysis Plan

APPENDIX A: MOCK-UP TABLES

Table 1. COVID-19 diagnostic accuracy

Parameter	(%)	[95% CI]
Sensitivity		
Specificity		

Abbreviations: n: counts, N: total, %: percentage

Table 2. Protocol deviations

Parameter		n/N (%)
Any protocol deviation	Yes	
	No	
Protocol deviation detection	Specimen collection procedure	
	RNA extraction procedure	
	Nucleic acid amplification procedure	
Type of protocol deviation	Inclusion/Exclusion criteria not met	
	Mishandling of samples	
	Assays not performed according to IFU	
	Prospective collection of specimens	
	Other	

Abbreviations: n: counts, N: total, %: percentage

Table 3. Device deficiencies

Parameter		n/N (%)
Any device deficiency	Yes	
	No	
Type of device deficiency	A malfunction or deterioration in the characteristics or performance	
	Contamination with other substances or products	
	Degradation/destruction of the device	
	An inaccuracy in the labelling or in the instructions for use	
	Other	

Table 4. Study completion

Parameter		n/N (%)
Specimens without screening failure	Yes	
	No	
Completed study	Yes	
	No	

Abbreviations: n: counts, N: total, %: percentage

Table 5. Specimen information

Specimen information	n/N (%) [95% CI]
Specimens´ origin for analysis	
Biobank	
Other	
Specimen storage conditions	
4ºC	
-20ºC	
-70ºC	

Abbreviations: CI: Confidence Interval n: counts; N: total; %: percentage