Over the counter Rapid Antigen Test for detection of SARS-CoV-2 virus: Clinical Evaluation Statistical Analysis Plan

December 1, 2021

Protocol Number: EDP-SOP-TNC-014

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Statistical Analysis Plan for EDP-SOP-TNC-014

Data Evaluation and Accessibility

A report tabulating and analyzing the data will be prepared by the Clinical Investigator including tables of the results and a description of any deviations from the protocol.

Acceptability of Data for Analysis

Valid individual specimen results generated in invalid runs are not acceptable for inclusion in final data analysis. Samples tested in invalid runs must be re-tested if the remaining specimen volume is available. Only valid results from specimens in valid runs will be included in the final data analysis.

Exclusion of Data from Analysis

Data from invalid runs results will be excluded. All excluded results will be documented with rationale for exclusion and a summary of these cases will be provided.

Data Entry and Corrections to Study Documents

All entries must be legible and made in indelible ink (preferably black); do not use pencil. Strike incorrect entries with a single line (do not obliterate or put "white-out" on the original entry). Then enter the correct information next to the original entry, initial, and date the correction.

Assay Run Data

Any Printouts corresponding to assay runs will be maintained and a copy placed in a study binder. A record will be maintained regarding assay reagents and calibrators by lot number and expiration date.

Discordant Results

If any discordant results are generated during the clinical study, further testing using a second high-sensitivity EUA PCR method, CDC's Influenza SARS-CoV-2 (Flu SC2) Multiplex Assay performed by Premier Laboratories, will be performed with specimens yielding discordant results obtained with the initial PCR methodology. If the NP swab sample has been retained and stored properly, and there is sufficient sample available, the same sample will be run on the second PCR assay. Both the AN specimen and the nasopharyngeal swab specimen can be repeat tested if possible. The results from the discrepant analysis will be reported as a footnote in the performance table. The discordancy will not be used to alter the performance data.

Acceptance Criteria for Method Comparison

The study sponsor or designee will ensure that all specimens are analyzed appropriately, and results are accurately transcribed to an excel spreadsheet for analysis. Qualitative results from the Antigen Test and quantitative results from the PCR Test may be evaluated for concordance between the two test methods using standard statistical methods.

Sensitivity and Specificity Analyses

Analysis will be conducted according to the Clinical and Laboratory Standards Institute EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures: Approved Guideline – Third Edition. Formulas used to calculate sensitivity (PPA) and specificity (NPA) are as follows:

- Positive Percent Agreement (PPA) = True Positive/(True Positive + False Negative)
- Negative Percent Agreement (NPA) = True Negative/(False Positive + True Negative)

95% confidence intervals for sensitivity and specificity are as follows:

- 95% Confidence Interval for PPA:
  - $Q_{1PPA} = 2 \times True\ Positive + 3.84$
  - $Q_{2PPA} = 1.96 \times \sqrt{3.84 + 4\frac{\text{True Positive}\times\text{False Negative}}{\text{True Positive}+\text{False Negtaive}}}$
  - $Q_{3PPA} = 2 \times (True\ Positive + False\ Negative) + 7.68$
  - Lower bound $= 100 \times \frac{Q_{1PPA}-Q_{2PPA}}{Q_{3PPA}}$
  - Upper bound $= 100 \times \frac{Q_{1PPA}+Q_{2PPA}}{Q_{3PPA}}$
- 95% Confidence Interval for NPA:
  - $Q_{1NPA} = 2 \times TN + 3.84$
  - $Q_{2NPA} = 1.96 \times \sqrt{3.84 + 4\frac{True\ Negative\times False\ Positive}{True\ Negative+False\ Positive}}$
  - $Q_{3NPA} = 2 \times (True\ Negative + False\ Positive) + 7.68$
  - Lower bound $= 100 \times \frac{Q_{1NPA}-Q_{2NPA}}{Q_{3NPA}}$
  - Upper bound $= 100 \times \frac{Q_{1NPA}+Q_{2NPA}}{Q_{3NPA}}$

- Analysis for sensitivity, specificity, and 95% confidence intervals based on days post-symptom onset will be conducted as follows:
  - Subjects considered symptomatic according to the November 9, 2021 FDA EUA guidance will be separated into the following categories based on the number of days after symptom onset that they report when they present for testing:
    - 0-1
    - 0-2
    - 0-3
    - 0-4
    - 0-5
    - 0-6
    - 0-7
    - 0-14
    - 0-15+
    - Reported exposure, not symptomatic

  - The sensitivity, specificity, and 95% confidence intervals for each category of days post-symptom onset will be calculated based on the cumulative number of subjects who fit into that category.
  - The subjects considered symptomatic will also be separated into the following categories in order to calculate the sensitivity and its 95% confidence interval based on non-cumulative reported days post-symptom onset:
    - 0
    - 1
    - 2
    - 3
    - 4
    - 5
    - 6
    - 7
    - 8 to 14
- Analysis for sensitivity, specificity, and 95% confidence intervals based on presence of symptoms or reported exposure and based on absence of symptoms or reported exposure.
  - Subjects considered symptomatic according to the November 9, 2021 FDA EUA guidance will be collectively analyzed for sensitivity and specificity using the above formulas.
  - Subjects considered asymptomatic according to the November 9, 2021 FDA EUA guidance will be collectively analyzed for sensitivity and specificity using the above formulas.
- Analysis will also be conducted for all subjects (total sample size of 188), regardless of categorization, using the same above formulas for sensitivity, specificity, and the 95% confidence intervals.
- Overall positivity rate for the subject population will be calculated based on age group and reported gender.
  - Age groups will be separated based on the November 9, 2021 FDA EUA guidance into the following categories:
    - 2 to 13 years of age
    - 14 to 24 years of age
    - 25 to 64 years of age
    - 65+ years of age
  - Gender will be separated into male and female. No consideration will be given to gender identity or categories other than male and female.
  - Positivity rate will be calculated based on the results of the comparator method(s) used to gauge the efficacy of the candidate device.
    - This will be calculated for each of the above age groups, as well as for the subject population overall. The total number of subjects in each age group who test positive on the candidate device will also be calculated.
    - The total number of males and females in the study population will be calculated, and further separated by the above age groups.
- Analysis for sensitivity and 95% confidence intervals based on comparator cycle threshold (Ct) value will be conducted as follows:

  - Cycle threshold values will be separated into the below categories. These are based on the number of PCR cycles taken by the comparator method to detect enough viral material to report a positive result. The higher the Ct value is, the lower the viral load of the sample:
    - 10 to 14
    - 15 to 19
    - 20 to 24
    - 25 to 29
    - 30 to 34
    - 35+
  - Sensitivity and its 95% confidence interval will be calculated for each category.
- The mean, median, and minimum and maximum Ct values will be calculated for each category of subjects based on reported days post-symptom onset.
- Sensitivity and its 95% confidence interval for positive samples determined to have a low viral load (samples with Ct values greater than or equal to 32) will be calculated using the formulas above for each of the following categories:
  - 10% of the 12 low-positive samples
  - 15% of the 12 low-positive samples
  - 20% of the 12 low-positive samples