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August 20, 2021

CDPH-Laboratory Field Services
320 West 4th Street, Suite 890
Los Angeles, CA 90013
Attention: Catherine Tolentino, Examiner II

Dear Ms. Tolentino:

I am writing in response to Ms. Elsa Eleco's June 08, 2021, letter requesting additional information from the CDPH Branch Laboratory operated by PerkinElmer Genetics, Inc. in Valencia, California ("VBL"). Please find the additional information you requested below and enclosed.

I have provided for your reference process and procedure improvements to further ensure changes to medical interpretations, i.e., interpretation with respect to Ct count, are appropriately justified with laboratory and epidemiological data as well as relevant clinical published literature. **(Reference Exhibit A, Policy for Changes to Test Interpretation.)** As you know, changes to interpretation of results depending on Ct cut-off values were implemented for the LDT in use at VBL. The laboratory acknowledges that supporting data and justification for changing Ct interpretations was not formally documented at the time these decisions were made, and the improvements will ensure that laboratory directors timely execute required documentation.

In addition, I have attached a new and enhanced validation package. **(Reference Exhibit B, Complete Package for LDT Validation.)** While I do not agree that right-to-reference is unavailable to support LDT validation, we have nevertheless completed a full validation. The attached package includes the original validation report which is now reorganized and labeled to indicate application to each performance specification requirement listed in 493.1253(b)(2)(i-vii). In addition, development data from alternate media types and conditions are now included as Appendix G to the validation report.

D5423 Establishment of Performance Specifications

A. Clinical Performance in Asymptomatic Individuals (D5423 1.a., page 62 of the report)

To correct the deficiency, the laboratory must submit the following evidence:

1. Timeline for the study.

   The letter was issued on January 12, 2021, and per the initial statement, "Perkin Elmer MUST further evaluate the clinical performance from ASYMPTOMATIC individuals in an FDA agreed

upon post authorization clinical evaluation study within 30 calendar days of the date of this letter. Labeling updates must be made after submission to FDA."

2. Status of the clinical evaluation. If the evaluation is still in progress, provide the protocol for the study and its initial evaluation.

Response to A:

1. Per the manufacturer of the PerkinElmer New Coronavirus Nucleic Acid Detection Kit, the asymptomatic study is currently ongoing. **(Reference Exhibit C, Asymptomatic Study Protocol and Timeline.)**
2. The clinical evaluation is in progress and the current study proposal is attached. The manufacturer has not released any data and there is no timetable. **(Reference Exhibit C, Asymptomatic Study Protocol and Timeline.)**

B. Interpretation of Test Results (D5423 1.f., page 67 of the report)

To correct the deficiency, the laboratory must submit the following evidence:

1. A step-by-step validation protocol to support the claim that Ct values between 37-42 are “presumptive positive” and not true negative or true positive. Please submit information that pertains specifically to the CDPH Branch Laboratory. Submit as requested, and do not cross-reference information from an existing IFU of manufacturer’s EUA.
2. Documentation from current as well as historical data generated at the CDPH Branch Laboratory (Valencia Branch Laboratory) to validate the laboratory’s current interpretation of results for “presumptive positive,” when the Ct values are between 37 – 42, including:
    a. Historical data (N=study population) to support that 37-42 cycle thresholds are “presumptive positive,” and not true negative or true positive.
    b. Original numbers planned/selected, the number used in the final analysis, and the number omitted from the final analysis.
    c. A description of the selection process for the study population.
    d. A list of potential sources of bias. Did you minimize them to avoid inaccurate estimates in your study design and data analysis?
    e. Histograms of results by condition status (if known).
3. The instrument printouts for the study population used to determine that Ct values between 37-42 are “presumptive positive.”
4. The final patient test reports that support the instrument printouts.
5. The actual calculation that supports the validation that Ct values between 37-42 are “presumptive positive.”
6. A description of the mechanism for data analysis, including:

a. How was the data imported or processed in the system in order to determine that Ct values between 37-42 are "presumptive positive?"
b. Was manual import considered or performed during the preparation of the grid?

7. The summary and conclusion of the historical data, including the study population selected in the determination that Ct values between 37-42 are "presumptive positive."

Response to B:

1. The lab's analytically validated COVID test is designed to detect the presence or absence of the virus via RT-qPCR; a semiquantitative method. The laboratory initially used the manufacturer's Ct cut-off of 42. Lab directors appointed by the California Department of Public Health ("CDPH") approved a change in interpretation within the range of > 37 to ≤ 42 based on best available professional consensus and the published literature. There is no specific recommended language for interpretation of high Ct values in molecular viral testing. The designation "presumptive positive" is a clinical designation, assigned by the laboratory director to counsel patients on the appropriate clinical management, and reflects uncertainty about the presence of virus in the sample.

Analytical Validation

We have conducted retrospective analysis and determined that interpretation within the range of > 37 to ≤ 42 as presumptive positive were appropriate based on the data below:

- Sequencing data generated at VBL demonstrating the presence of SARS-CoV-2 viral sequence from samples with Ct values > 37 to ≤ 42. **(Reference Exhibit D, Sequencing Analysis Supporting Ct cut-offs.)**
- A review of amplification curves from results interpreted as "Presumptive Positive." **(Reference Exhibit E, Amplification Curve Analysis Supporting Ct cut-offs.)**

Clinical Interpretation

Prior to January 27, 2021, the VBL laboratory directors in collaboration with state health officials determined that Ct values > 37 to ≤ 42 be interpreted as presumptive positive to best serve the public health policies.

2. Exhibits D and E provide the requested information. **(Reference Exhibit D, Sequencing Analysis Supporting Ct cut-offs; Reference Exhibit E, Amplification Curve Analysis Supporting Ct cut-offs.)**

3. Instrument printouts from a representative 252 cases with Ct values between > 37- ≤ 42 are provided. These data illustrate that the amplification curves with Ct values 37-42 show the necessary exponential amplification to show a true signal. **(Reference Exhibit F, Instrument Print Out With Ct > 37- ≤ 42; Reference Exhibit G, Data Analysis SOP CA-RPT- SOP-002.)**

4. See patient test reports for the representative 252 cases with Ct values > 37- ≤ 42. **(Reference Exhibit H, Patient Test Reports With Ct > 37- ≤ 42.)**

5. Cycle Threshold (Ct) values are produced by the thermocycler algorithm which is agnostic to the test that it is running. **(Reference Exhibit E, Amplification Curve Analysis Supporting Ct cut-offs.)** The interpretation of Ct values is assigned by LIMC according to the laboratory SOP. **(Reference Exhibit G, Data Analysis SOP CA-RPT- SOP-002.)**

6. LIMC assigns the interpretation based on the Ct value as described in the Data Analysis Procedure. No manual import of data was performed. **(Reference Exhibit G, Data Analysis SOP CA-RPT- SOP-002.)**

7. The historical studies presented in the Sequencing Analysis Supporting Ct Cut-off **(Reference Exhibit D, Sequencing Analysis Supporting Ct cut-offs)** and the Raw Data Study **(Reference Exhibit E, Amplification Curve Analysis Supporting Ct cut-offs)**, indicate that samples with Ct range of >37 to ≤ 42 contain the SARS-CoV-2 virus and support a clinical interpretation of "presumptive positive".

Thank you, and we look forward to working with LFS to address any additional questions that might arise, and to continuing to offer accurate and timely COVID testing for the people of California and beyond.

Sincerely,

Adam Rosendorff, MD
Laboratory Director