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# SARS-CoV-2 RT-PCR Assay (COVID-19)

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## Molecular Testing for **SARS-CoV-2 (COVID-19)**

Coronaviruses (CoV) are a large family of viruses which cause illnesses ranging from common cold to more severe diseases, including Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The novel coronavirus (SARS-CoV-2) is a new viral strain which has not been previously identified in humans but has been causing pandemic worldwide.

Sema4 is offering the qualitative real time PCR assay (RT-PCR) for the detection of active infection with the SARS-CoV-2 virus, consistent with the diagnosis of COVID-19. This testing is recommended for use on respiratory specimens (human oropharyngeal swab and nasopharyngeal swab) from patients suspected to have COVID-19 by their healthcare providers. The test utilizes the Perkin Elmer (PE) New Coronavirus Nucleic Acid Detection Kit (Reference 3) which has been granted emergency use authorization (EUA) by the FDA, and it will be available until FDA revokes its use. This assay is designed to target two specific regions of SARS-CoV-2: nucleocapsid (*N*) gene and *ORF1ab* gene. For additional information on the EUA process please refer to the Reference 7. For additional information on the PE's fact sheets for the providers and the patients regarding this qualitative RT-PCR assay detecting SARS CoV-2, please refer to References 8 and 9.

### Testing Methods, Sensitivity, and Limitations-Qualitative RT- PCR

Viral RNA isolation is accomplished using the Perkin Elmer (PE) chemagic™ viral DNA/RNA 300 kit executed on the chemagic™ 360 instrument. Nucleic acid isolation is followed by quantitative RT-PCR utilizing the Perkin Elmer Coronavirus New Nucleic Acid Detection Kit. This method uses *in vitro* reverse transcription of the SARS-CoV-2 viral RNA resulting in the synthesis of cDNA. The resulting cDNA undergoes PCR amplification followed by fluorescent detection of the nucleocapsid (*N*) gene and *ORF1ab* gene of the SARS-CoV-2 virus. In addition to the two virus specific probe sets, an internal control (IC) probe set is included with each reaction to monitor the internal extraction, amplification and fluorescence detection as a control that is spiked-in to each specimen. The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products which otherwise would result in false positivity, in addition to positive and negative internal controls to prevent reporting of incorrect results. Results are established by reviewing the Ct values of the probe sets for each sample, and the IC. There will be four types of reports generated: Not-Detected, Detected, Failed, and TNP (test not performed) using the criteria described per manufacturer's package insert v3 (Reference 5).

The sensitivity established by PE was 95% at the limit of detection of 25 copies/mL for *N* gene target and 9.3 copies/mL for the *ORF1ab* target. This assay is used as a means of qualitative detection and does not directly reflect the viral load present in the original specimen. The assay is 100% specific, and no cross reactivity has been observed with other DNA or pathogenic organisms found in the respiratory tract (Reference 5).

A positive test result for COVID-19 indicates that the SARS-CoV-2 RNA is detected, and there is active infection. Health care providers should use this result to inform their patients for the diagnosis of COVID-19 and discuss their management options, including, but not limited to, social distancing and self-quarantining. All results may be reported to regulatory bodies as required.

A negative test result means that SARS-CoV-2 RNA is not present in the specimen above the limit of detection and does not necessarily exclude the possibility of COVID-19. Because, a negative result does not rule out COVID-19, it should not be used solely to manage the patient. Rarely, mutations may occur in the regions of the virus where the primers and probes are expected to anneal, with resultant false negative results despite the presence of the virus. Should there be continued suspicion for COVID-19, the assay should be re-ordered following collection of a new specimen that needs to be submitted for repeat testing within 1-2 days. The same recommendation holds for the failed result.

Inappropriately collected, transported, stored or processed specimens will not be tested as they may lead to inaccurate results, and a test not performed report will be provided, requesting a new specimen to be collected.

## Turnaround Time

Results for samples received during hours of laboratory operation (Monday- Saturday, 7AM-5PM) are reported to the referring physician within 24 hours of the receipt of the specimen.

## Specimen and Shipping Requirements

- **Specimen Types:**
  - **Oropharyngeal swab:** Minimum 1 oropharyngeal swab specimen collected from the patient and submitted in 3mL universal transport medium is required.
  - **Nasopharyngeal swab:** Minimum 1 nasopharyngeal swab specimen collected from the patient and submitted in 3mL universal transport medium is required.
- **Specimen Rejection Criteria:**
  - Any samples that show up in the following state will not be processed:
    - Tube is broken or leaking
    - No swab is present
    - Tube is not labeled
    - Use of calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and/or inhibit PCR testing.
    - Use of metal or organic material swabs.

- **Specimen Storage:**
  - **Up to 72 hours after collection:** Store specimens at 2-8˚C
  - **Any delay in shipping past 72 hours:** Store specimens at -70˚C or below
- **Shipping:** *Please contact laboratory before shipping samples directly*
  - **Specimen stored at 2-8˚C:** Ship overnight to the lab on ice pack
  - **Specimen stored at -70˚C or below:** Ship overnight to the lab on dry ice
  - **Laboratory Contact Information:**

    **Tel:** 800-298-6470

    **Fax:** 646-859-6870

## References

1. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/policy-diagnostic-tests-coronavirus-disease-2019-during-public-health-emergency
2. https://www.who.int/health-topics/coronavirus
3. https://perkinelmer-appliedgenomics.com/home/products/new-coronavirus-2019-ncov-nucleic-acid-detection-kit/
4. https://www.cdc.gov/coronavirus/2019-ncov/faq.html
5. http://www.fda.gov/media/136410/download
6. https://connect.medrxiv.org/relate/content/181
7. https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization - 2019-ncov

## Perkin Elmer Fact Sheets

8. Fact Sheet for Patients-https://www.fda.gov/media/136409/download
9. Fact Sheet for Healthcare Providers-https://www.fda.gov/media/136408/download

## Disclaimer

This test was developed by Perkin Elmer and approved for emergency use testing by the FDA.