



TECHNICAL BULLETIN: SARS-CoV-2 / COVID-19

Unlike the SARS Coronavirus of 2003 and the MERS Coronavirus of 2014, the novel Coronavirus (SARS-CoV-2) has quickly spread to six continents and has generated self-sustaining outbreaks in many of the major cities, all with catastrophic effects. This is particularly true for patients with co-morbidities and high-risk groups that include the elderly. Although travel bans and quarantines can slow or halt the spread of the virus, this outcome is viewed as unlikely since there is a substantial exportation and circulation of asymptomatic and 'pre-symptomatic cases'.¹ Much like the Coronaviruses that are currently circulating in the general population (OC43 and 229E of the 1960s and HKU1 and NL63 of the post-SARS outbreak of 2004), this new Coronavirus may become an integral part of the seasonal landscape of respiratory tract infections; an opinion that is shared by a number of epidemiologists.²

As was the case for the H1N1 of 2009, Eurofins Diatherix has validated the SARS-CoV-2 TEM-PCR™ assay using standard CLIA guidelines. This viral pathogen will be a part of the new COVID-19 test. Key test performance indicators are shown in Table 1 and copies of the full Performance Evaluation are available on request. The COVID-19 test will also include the primary bacteria that are associated with community acquired pneumonia and exacerbations of COPD; e.g. *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. In addition, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* bacteria are often a part of the physician's differential diagnosis of these respiratory infections and are included in this test.³

COVID-19 Test Components:

SARS-CoV-2 (HPCPS-U0003, CPT 87635)	<i>Moraxella catarrhalis</i> (CPT 87798)
<i>Streptococcus pneumoniae</i> (CPT 87798)	<i>Chlamydia pneumoniae</i> (CPT 87486)
<i>Haemophilus influenzae</i> (CPT 87798)	<i>Mycoplasma pneumoniae</i> (CPT 87581)
<i>Haemophilus influenzae</i> (type B) (CPT 87798)	

Table 1. SARS-CoV-2 target performance indicators

Assay Sensitivity:
The sensitivity or limit of detection (LoD95) of the SARS-CoV-2 TEM-PCR™ assay is 1 copies/μL.*
Assay Specificity:
All gene alignments show 100% identity to the primer sequences utilized by the SARS-CoV-2 TEM-PCR™ test. No positive signals for SARS-CoV-2 were observed in cross-reactivity evaluations with both viruses and bacteria found in both symptomatic and asymptomatic patients.
Assay Inclusivity:
No positive signals for SARS-CoV-2 were observed in the negative samples (100% agreement). Percent agreement for contrived samples was 100% at 2X LoD and 100% for the remainder of the samples.
Assay Exclusivity:
No positive signals for SARS-CoV-2 were observed, indicating that the diverse microflora and genomic material found within the human respiratory tract will not cross-react with the assay.
* Specimens with low viral loads that are at or below the limit of detection (LOD) may produce variable results upon retesting.

Viral-bacterial interactions that cause more severe infections in both the upper and lower respiratory tract have been documented since the 1918 Influenza pandemic. The Spanish flu pandemic of 1918, the deadliest in history, infected an estimated 500 million people worldwide—about one-third of the planet’s population—and killed an estimated 20 million to 50 million victims, including some 675,000 Americans.⁴ This devastating pandemic was likely the result of the interaction of the Influenza virus and one of more of the bacteria that are a part of the upper respiratory tract’s microbiome (see Figure 1).⁵ Establishing the presence of a viral-bacterial co-infection will provide actionable information to the clinician by identifying patients that have the COVID-19 infection.

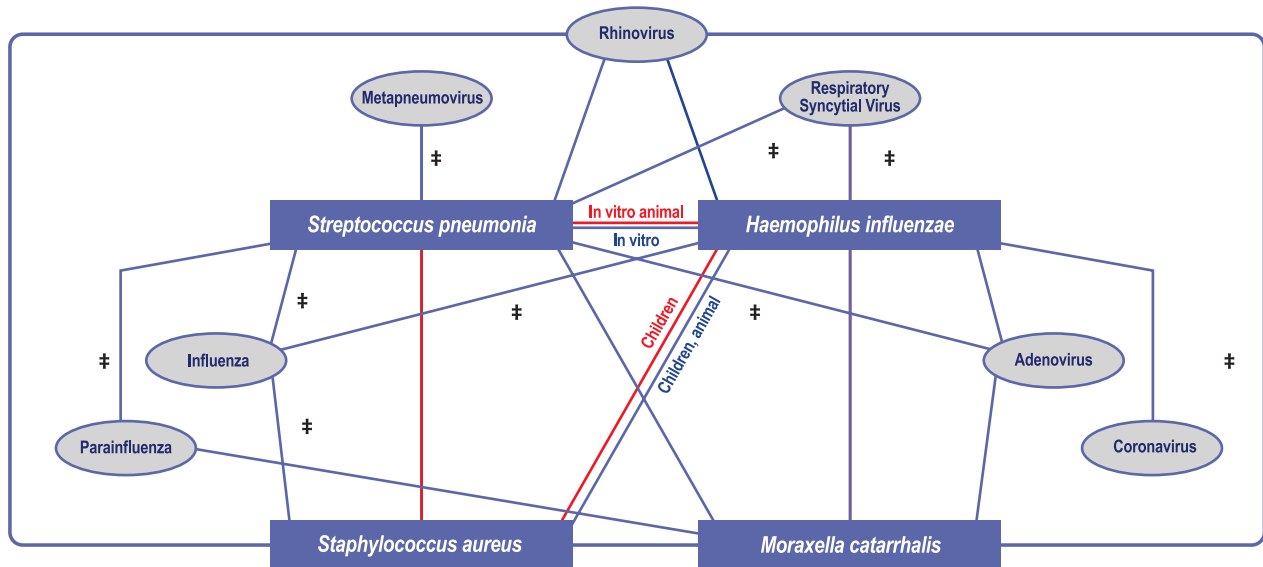


Figure 1. Virus and bacterial interactions. Although it is clear that certain viruses, such as respiratory syncytial and influenza, can cause disease on their own, the illustration demonstrates the synergistic effect in their persistent colonization or infection (blue lines).¹⁰ The red lines indicate antagonism. There is accumulating evidence in animal models as well as *in vitro* studies (⊕) that these positive associations may influence the progression and intensity of the disease.⁶

With Emergency Use Authorization granted to Eurofins Diatherix in 2009 by the FDA for the H1N1-09 Pandemic, 10,624 patient specimens were studied. 29.3% of these specimens were found to be positive for the pandemic strain. For those specimens with greater than 1 positive result, 53% contained a viral pathogen without co-detection of bacterial pathogens. For these patients where only viruses were detected, antimicrobial drugs that are often prescribed on clinical findings alone could be avoided, thus reducing drug resistance by eliminating selective pressure on normal flora.⁷ Although there are few studies that have reported on the complications of bacterial co-infection with the H1N1 virus, a retrospective study by MacIntyre et al. found that *Streptococcus pneumoniae* was present in nearly 25% of patients with a bacterial co-infection.⁸

In a recent study by Xia et al.,⁹ a 40% co-infection rate was seen in children with SARS-CoV-2 where fever and cough were common findings. In 80% of the children studied, elevations in procalcitonin levels (*a marker for bacterial infection in severe pneumonia and sepsis*) were observed which was not present in SARS-CoV-2 infected adults or were simply undetected. This finding suggests that routine antibacterial treatment may be appropriate when evidence of co-infection can be established. The use of a comprehensive multiplex molecular assay such as the COVID-19 test in patients who are symptomatic of COVID-19 will provide actionable information to the clinician.

The viral load seen in patients with COVID-19 infections is variable. These variations were due to the stage of the illness, the severity of the infection, and the methods used to collect the specimen. In Figure 2, the viral load measured by PCR testing is shown for specimens taken from the posterior oropharynx in non-intubated patients and from tracheal aspirates of those who required respirator support.

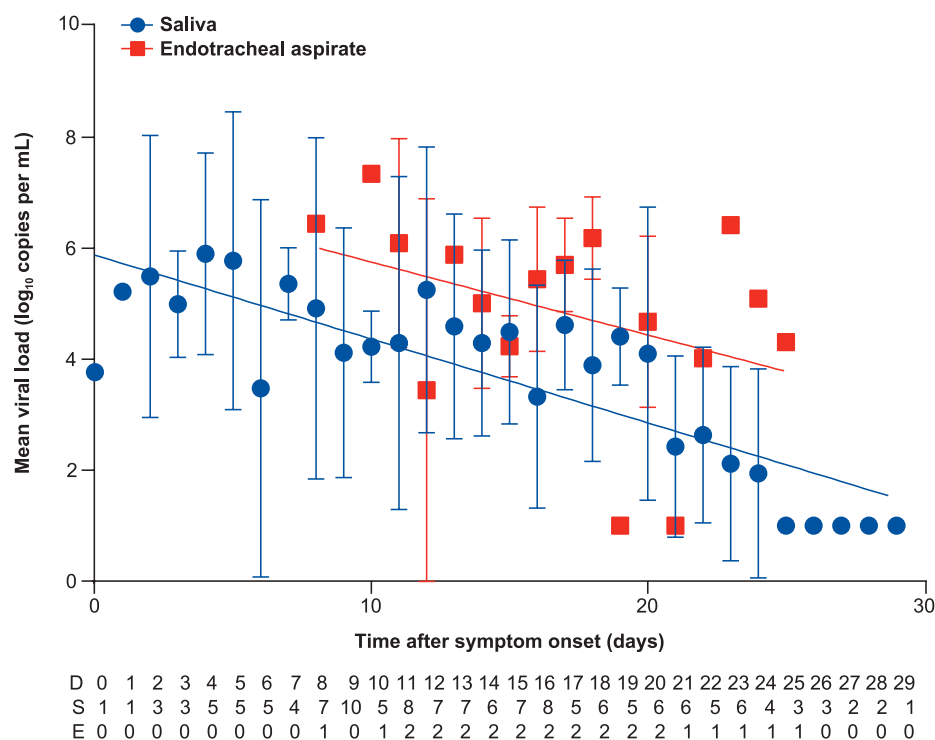


Figure 2. Temporal profile of serial viral load from all patients (n=23). The highest viral loads were seen in specimens of posterior oropharyngeal saliva samples with the exception of three patients who were intubated, in whom the viral load data were from endotracheal aspirates and are plotted separately. Data points denote the mean; error bars indicate SD; slope represents best fit line. The number of patients who provided a sample on each day is shown in the table below the plot. D=days after symptom onset. S=saliva. E=endotracheal aspirate.¹⁰

From March 17th through April 15th, 2020, Eurofins Diatherix has performed tens of thousands of COVID-19 tests. SARS-CoV-2 has been the only pathogen detected in 2,177 (8.0%) of them, but 17.8% of the SARS-CoV-2 positives had one or more bacterial pathogens detected as well. See Figure 3 for a breakdown of SARS-CoV-2 detection alone and in combination with bacteria present in the oropharyngeal microbiome.

SARS-CoV-2 Positive (2,177) in 27,349 Tests Plus Bacterial Co-detections

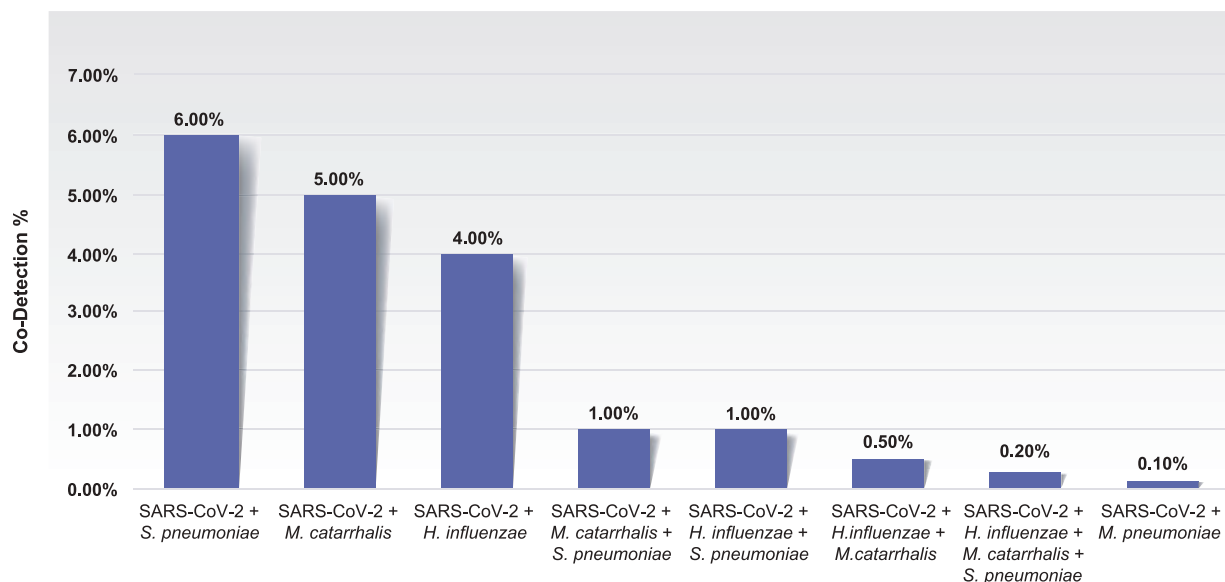


Figure 3. Detection of SARS-CoV-2 in 27,349 tests (with and without pathobionts of the Oropharynx)

The COVID-19 test developed by Eurofins Diatherix also incorporates controls that identify samples that contain PCR inhibitors that may contribute to false-negative results. A more difficult problem, and the more likely source of false negative test results, is the apparently low titer of SARS-CoV-2 shed in specimens collected early in illness on patients who may have variable symptoms. Given that SARS-CoV-2 transmission is not completely worldwide, the probability that a positive test result will be a “false positive” is high. To decrease the possibility of a false-positive result, testing should be limited to patients with a high index of suspicion for having COVID-19 disease. This alone may make it difficult to confirm a diagnosis because of a low viral load present in the specimen and it emphasizes the demand for good quality specimens.

Specimen collection procedures for the COVID-19 test are referenced on pages 20 and 21 in our online Client Services Manual and include properly collected specimens using Nasopharyngeal Swabs, Nasopharyngeal Aspirates/Washes, Throat Swabs, Tracheal Aspirates, and Sputum Swabs. Specific instructions on specimen collections for these sources and those for other Eurofins Diatherix tests can be seen in the following link:

http://eurofins-diatherix.com/wp-content/uploads/2019/12/Client_Services_Manual_web_121119.pdf

It is important to note that the Centers for Disease Control recommends specimen collections that include swabs of the nasopharyngeal recess. If nasopharyngeal specimens cannot be obtained, swabs of the posterior pharynx can be combined with nasal or mid-turbinate specimens to optimize the collection. Properly collected lower respiratory secretions should also be collected in patients with a clinical diagnosis of pneumonia.¹¹

On April 22, 2020, the Food and Drug Administration (FDA) granted Emergency Use Authorization (EUA) for the Eurofins Diatherix SARS-CoV-2 assay.

Diatherix Eurofins SARS-CoV-2 virus has not been FDA cleared or approved. This test has been authorized by FDA under an EUA for use by authorized laboratories, and has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

References:

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