Background Characteristics

The DiaSorin Molecular Simplexa™ COVID-19 Direct real-time RT-PCR assay is intended for use on the LIAISON® MDX instrument for the in vitro qualitative detection of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in nasopharyngeal swabs (NPS), nasal swabs (NS), nasal wash/aspirate (NW), or bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. The Simplexa™ COVID-19 Direct assay is an aid in the diagnosis of SARS-CoV-2 infection.

Individuals suspected of COVID-19 are defined by the CDC as having:

- The presence of signs and symptoms
- Living, or have traveled, in a place where transmission of COVID-19 is known to occur
- Have been in close contact with an individual suspected or confirmed to have COVID-19


The solution includes:

- the Simplexa™ COVID-19 Direct kit, the LIAISON® MDX instrument (with LIAISON® MDX Studio Software), the Direct Amplification Disc and the associated accessories.

LIAISON® MDX: The intuitive LIAISON® MDX studio software integrates total workflow control with data QC and final result interpretation. Experience running individual assay targets or the flexibility of multiple assays in one run using the multiple assay suite featured in software 2.2 and higher.

Direct Amplification Disc: An 8-well disc that enables the direct amplification of a sample without the need for DNA/RNA extraction.

Simplexa™ COVID-19 Direct kit: The kit contains the reaction mix in 24 single use vials which are ready to use. For each sample to be analyzed, the entire content of a single vial is transferred into one wedge of the disc, followed by the pipetting of the primary sample (NPS in viral transport media, NS in viral transport media, nasal wash/aspirate or BAL). The need for traditional extraction is bypassed with the benefit of a rapid sample-to-answer turnaround time (90 minutes). It also reduces the risk of error with a streamlined workflow, single-use reagents, and extraction-free chemistry ultimately enabling proper diagnostic and treatment decision making.

The quality of treatment starts with diagnosis.
Both of the regions chosen for the test design are specific for the SARS-CoV-2. The S gene encodes the spike glycoprotein of the SARS-CoV-2 (COVID-19 virus), while the ORF1ab region encodes well-conserved non-structural proteins and therefore is less susceptible to recombination. An RNA internal control is used to detect RT-PCR failure and/or inhibition.

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The Simplexa™ COVID-19 Direct assay is validated for:
- Nasopharyngeal swabs (NPS) or nasal swabs (NS) in approximately 3 mL of Universal Transport Media (UTM, Copan), Universal Viral Transport (UVT, BD), Remel M5, Remel M6, Copan ESwab™ (Liquid Amies), Copan MSwab, Mwe Sigma Transwab®, Mwe Sigma Virocult®, Puritan UniTrans-RT® Transport System or 0.9% Sodium Chloride.
- Bronchoalveolar lavage (BAL) undiluted or diluted 1:1 (v/v) in a mucolytic such as Remel Sputasol.
- Nasal wash/aspirate undiluted.

**Clinical Performance**

The clinical performance of the Simplexa™ COVID-19 Direct assay was established in multi-site clinical evaluations. Fresh clinical NPS specimens were tested with the Simplexa™ COVID-19 Direct assay at three (3) different clinical sites from February 2020 to March 2020. For each of the sites, an established comparator was used. Sites 1 and 3 used the same comparator while Site 2 used a different comparator. Negative individually collected and positive contrived NS or NW specimens were tested internally with the Simplexa™ COVID-19 Direct assay in April or May 2020. Contrived samples were prepared by spiking heat-inactivated 2019-nCoV/USA-WA1/2020 strain (ATCC® VR-1986HK™) into individual negative NS or NW specimens. BAL specimens, diluted 1:1 with Sputasol, were tested with the Simplexa™ COVID-19 Direct assay at one clinical site using an established comparator.

**ANALYTICAL SENSITIVITY/LOD**

The Limit of Detection (LoD) for NPS was determined to be the lowest detectable concentration of quantitated extracted viral genomic RNA (copies/mL) at which ≥ 95% of all replicates test positive. Initially, the tentative LoD was identified with serial dilutions of the characterized SARS-CoV-2 viral genomic RNA in UTM with RNasin tested in five (5) replicates during design and development. The LoD was then confirmed by testing forty eight (48) replicates with concentrations at the tentative limit of detection. The final LoD was confirmed to be the lowest concentration resulting in positive detection with a minimum 95% positivity.

The final LoD for NPS, according to the assay results interpretation, is 500 copies/mL.
The Limit of Detection (LoD) for NS was determined to be the lowest detectable concentration of inactivated titered COVID-19 viral particles, strain 2019-nCoV/USA-WA1/2020, at which ≥ 95% of all replicates tested positive according to the results interpretation algorithm in pooled negative nasal swab specimens in UTM. Initially, the tentative LoD was identified with serial dilutions of the viral particles tested in four (4) replicates. The lowest concentration at which all replicates were positive was interpreted as the tentative LoD. The LoD was then confirmed by testing twenty (20) replicates with concentrations at the tentative limit of detection. The final LoD was confirmed to be the lowest concentration resulting in positive detection with a minimum 95% positivity.

The final LoD for NS, according to the assay results interpretation, is 242 copies/mL.

The Limit of Detection (LoD) for NW was determined to be the lowest detectable concentration of inactivated titered COVID-19 viral particles, strain 2019-nCoV/USA-WA1/2020, at which ≥ 95% of all replicates tested positive in pooled negative NW specimen matrix. Initially, the tentative LoD was identified with serial dilutions of the viral particles tested in four (4) replicates. The lowest concentration at which all replicates were positive was interpreted as the tentative LoD. The LoD was then confirmed by testing twenty (20) replicates with concentrations at the tentative limit of detection. The final LoD was confirmed to be the lowest concentration resulting in positive detection with a minimum 95% positivity.

The final LoD for NW, according to the assay results interpretation, is 500 copies/mL.

The Limit of Detection (LoD) for BAL was determined to be the lowest detectable concentration of inactivated titered COVID-19 viral particles, strain 2019-nCoV/USA-WA1/2020, at which ≥ 95% of all replicates tested positive in pooled negative BAL matrix. Initially, the tentative LoD was identified with serial dilutions of the viral particles tested in four (4) replicates. The lowest concentration at which all replicates were positive was interpreted as the tentative LoD. The LoD was then confirmed by testing twenty (20) replicates with concentrations at the tentative limit of detection. The final LoD was confirmed to be the lowest concentration resulting in positive detection with a minimum 95% positivity.

The final LoD for BAL, according to the assay results interpretation, is 1208 copies/mL.

The FDA website reports a sensitivity for the Simplexa™ COVID-19 Direct assay of 6,000 NDU/mL.10

ANALYTICAL SENSITIVITY AND CLINICAL INTERPRETATION

Viral loads in COVID-19 patients have been studied and reported in the literature and show high values, between 10^3 and 10^11 copies/ml. The work led by the Yale School of Public Health and conducted at Yale New Haven Hospital on 44 patients hospitalized for and 98 health care workers, shows viral loads in NPS greater than 1000 copies/ml with peak in the course of the disease. A work by La Scola et al (Méditerranée Infection University Hospital Institute in Marseille) concludes that individuals with low viral loads (Ct> 34 with their LDT system) are not infectious. In this regard, CDC underlines the discontinuation of isolation based on symptoms and not on molecular positivity in the update of February 2021 (https://www.cdc.gov/coronavirus/2019-ncov/community/). Following recovery from clinical illness, many patient no longer have detectable viral RNA in upper respiratory specimens. Among those who continue to have detectable RNA, the concentrations are in the range at which replication competent virus has not been reliably isolated by the CDC.

REACTIVITY/INCLUSIVITY

An in silico inclusivity analysis of the Simplexa™ COVID-19 Direct primers and probes was performed. All primer sets designed for detection of the ORF1ab and S gene were tested against the complete available SARS-CoV-2 genome sequence (as of June 2020). The analysis demonstrated that the regions recognized by
the designed primers and probes have 100% homology with all available SARS-CoV-2 sequences from the National Center for Biotechnology Information (NCBI) and Global Initiative on Sharing Avian Influenza Data (GISAID) databases/databanks.

CROSS-REACTIVITY
Cross-reactivity of the Simplexa™ COVID-19 Direct assay was evaluated using both in silico analysis and by testing whole organisms or purified nucleic acid from other organisms. Test specimens for laboratory testing were prepared by spiking cultured isolates/ inactivated organisms/purified nucleic acids (whole genome) (i.e., a minimum of $10^6$ CFU/mL or higher for bacteria and $10^5$ TCID$_{50}$/mL or PFU/mL or higher for viruses) into negative matrix (NPS in UTM) and determining cross reactivity based on three replicates. RNasin® was added to UTM for specimens containing extracted RNA.

No cross-reactivity was observed for the 72 organisms tested including coronaviruses other than SARS-CoV-2.

POTENTIAL INTERFERING SUBSTANCES
Potential interfering substances from respiratory specimens were tested for ability to generate false negative results using samples containing the extracted viral RNA at 3x LoD in nuclease free water. Testing was performed with 3 replicates per substance.

No interference was observed with systemic antibacterial, Antibiotic nasal ointment, Nasal corticosteroids, Nasal gel, Homeopathic allergy relief medicine, Nasal spray or drops, Cold Eeze (Throat lozenges, Oral anesthetic and analgesic), Anti-viral drug, Mucin, and Whole blood.

SELECTED PUBLICATIONS
Results of selected publications are summarized here:

1. Comparison of four molecular in vitro diagnostic assays for the detection of Sars-CoV-2 in nasopharyngeal specimens - Zhen et al, Journal of Clinical Microbiology, April 2020 - Northwell Health New York, USA.
   - Samples involved in the study: 104 NPS from symptomatic patients.
   - Methods: Simplexa™, Genmark, CDC, Hologic.

   • Results:
     - Simplexa™: 100% sensitivity 100% specificity; CDC and Hologic: 100% sensitivity, 98% and 96% specificity respectively; Genmark: 93% sensitivity 100% specificity.
     - An analytical comparison was also conducted by testing serial dilutions of synthetic RNA with the 4 methods in parallel and Simplexa™ was found to be 1 more Log sensitive than CDC.

   - Samples: 278 NPS and Nasal swabs, 33 BAL, from symptomatic patients.
   - Methods: Simplexa™, WHO Corman.
   - Results: 100% sensitivity and 100% diagnostic specificity (WHO Corman missed 8 positives that were detected by Simplexa™).

   - Samples: 169 nasopharyngeal swabs.
   - Methods: Simplexa™, Cepheid, Hologic Panther, Roche Cobas all evaluated versus CDC based LDT.
   - Results:
     - 100% specificity for all assays.
     - CDC-based LDT versus Hologic Panther Fusion: Panther Fusion RUO and EUA assays were slightly less sensitive than the CDC-based LDT.
     - CDC-based LDT versus Simplexa™: 100% concordance, with lower Cts recovered by the Diasorin compared to the LDT on all specimens (average Ct difference -2.1 [IQR -2.3 – -1.7]).
     - CDC-based LDT versus Roche Cobas: One of the 20 positives was not detected by the Roche assay.

The quality of treatment starts with diagnosis.
CONCLUSIONS

The CE marked and FDA Emergency Use Authorized Simplexa COVID-19 Direct assay has demonstrated excellent analytical and clinical performance in both internal and peer-reviewed studies. This simple and reliable assay is an aid in detecting the presence of SARS-CoV-2 and supports the clinical need of timely diagnosis of COVID-19.


- Comparison 1 = 310 NPS from symptomatic patients tested in parallel with Simplexa™ and Abbott ID Now; 100% specificity for both, 100% sensitivity for Simplexa™ and 86% for Abbott.
- Comparison 2 = 184 NPS from symptomatic patients tested in parallel with Simplexa™, Abbott ID Now and Roche Cobas 6800; specificity 100% for both, sensitivity 100% for Simplexa and Roche, 91% for Abbott.
- An analytical comparison was also performed by testing serial dilutions of 1 positive sample: Simplexa™ and Cepheid are comparable, 1 Log less sensitive than Roche and 1 Log more sensitive than Abbott ID NOW.

5. Direct Comparison of SARS-CoV-2 Analytical Limits of Detection across Seven Molecular Assays - Fung et al. - University of California, San Francisco.

- Analytical limits of detection for seven SARS-CoV-2 assays using serial dilutions of pooled patient material quantified with droplet digital PCR were determined.
- While the high-throughput laboratory-based instruments (Roche Cobas, Abbott m2000, and Hologic Panther Fusion) achieved the best analytical sensitivity, other sample-to-answer methods (DiaSorin Molecular Simplexa™ and GenMark ePlex) were only approximately 2- to 4-fold less sensitive, indicating good performance with more rapid results. In this study, the Abbott ID NOW POC instrument achieved a sensitivity of approximately 511 copies/ml, 1 log lower than high-throughput laboratory instruments.
REFERENCES

1. Simplexa COVID-19 Direct kit IFU


USC19WP0321

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The Simplexa COVID-19 Direct test has not been FDA cleared or approved. The test has been authorized by FDA under an EUA for use by authorized laboratories. The test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens. The Simplexa COVID-19 Direct 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.

Product availability subject to required regulatory approvals.