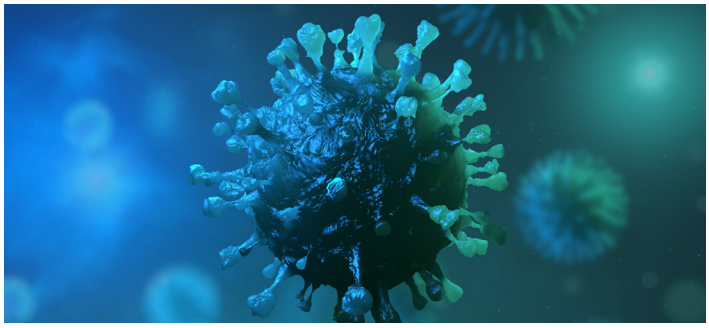
EXECUTIVE SUMMARY 000075

Reducing SARS-CoV-2 false positives with LC-MS for viral and host protein detection

The likelihood of developing severe COVID-19 symptoms is influenced by factors such as sex, age and the presence of co-morbidities. To improve diagnosis and treatment, clinical research is aiming to provide streamlined ways to detect SARS-CoV-2 in patient samples and identify biomarkers linked to specific symptom manifestations. Reverse transcription polymerase chain reaction (RT-PCR) testing for SARS-CoV-2 virus genetic material from nasopharyngeal swab samples is the gold standard for clinical detection and diagnosis of COVID-19. However, this approach is subject to false positives due to RT-PCR cross-contamination and amplification errors.

New clinical research shows that analysis of protein expression through surrogate peptides in patient samples with liquid chromatography-mass spectrometry (LC-MS) methods could greatly enhance COVID-19 diagnosis and treatment when used alongside RT-PCR. LC-MS offers specific and accurate ways of measuring peptides that are unique to a number of SARS-CoV-2 viral proteins to improve the selectivity and sensitivity of the measurements. Furthermore, it does not require an amplification step, thereby, minimizing the false negative and positive tests associated with amplification errors in RT-PCR. Systems like the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer and Thermo Scientific™ TSQ Altis™ triple quadrupole mass spectrometer are being used in clinical research to provide a comprehensive workflow for the analysis of COVID-19 samples from discovery to quantitation.



This executive summary provides an overview of a recent Thermo Fisher Scientific webinar presented by Dr. Sanjeeva Srivastava on Reducing SARS-CoV-2 false positives with LC-MS for viral and host protein detection. Dr. Srivastava discussed how LC-MS can identify proteins from the SARS-CoV-2 virus and the patient host response from a single nasopharyngeal swab or plasma sample. The workflow utilized the initial bottom-up characterization and label free quantitation (LFQ) to determine the set of surrogate peptides transitioned to routine, high-throughput screening using LC-MS/MS analysis on the TSQ Altis triple quadrupole mass spectrometer. Analysis of patient samples with LC-MS/MS offered a highly sensitive way to detect SARS-CoV-2 proteins, even in asymptomatic patients after negative RT-PCR results. Moreover, the workflow further developed a targeted peptide analysis based on depleted plasma profiling to predict disease severity and its progression in COVID-19 patients. With these high-throughput proteomics methods, Dr. Srivastava showed highly relevant diagnostic and prognostic information about COVID-19 patients, and highlighted several possible therapeutic targets for treatment of severe cases of COVID-19.

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Needles in a haystack: LS-MS identifies trace amounts of SARS-CoV-2 peptides in nasopharyngeal samples

The researchers began by working with patient samples from a COVID hot spot in Mumbai. These samples were known to be COVID-positive, as confirmed by routine RT-PCR testing, and were analyzed with LC-MS to test whether the method could detect unique peptides from SARS-CoV-2 proteins. Nasopharyngeal swabs were stored in a viral transport medium (VTM), and protein was precipitated in organic solvents (ethanol, IPA, acetone) (Figure 1). Protein pellets were then processed for mass spectrometry, and peptides resulting from digested proteins were analyzed with the Orbitrap Fusion Lumos Tribrid mass spectrometer with a Thermo Scientific™ Easy nano-LC™ 1200 system using data-dependent acquisition mode to obtain comprehensive proteome profiles.

The results showed LC-MS could detect the presence of 11 specific peptides from SARS-CoV-2 proteins: four from the spike protein (e.g., SARS-CoV-2 Spike glycoprotein P0DTC2); three from a nucleoprotein (SARS-CoV-2 nucleoprotein P0DTC9) and four peptides selected from replicase polyprotein 1ab (P0DTD1). Performing label free quantitation (LFQ) experiments on limited samples further indicated the potential of the identified SARS-CoV-2 peptide set for differentiating true positive samples from negative and were then transitioned to the high-throughput SRM methods. Incorporating the 11 targeted peptides from the three proteins in the SRM analysis improved the selectivity and sensitivity of the analysis for true positive responses.

LC-MS/MS proved to be highly sensitive at detecting SARS-CoV-2 and was able to detect viral particles in asymptomatic patients with very low viral load, and in patients who were recovering from COVID-19 and demonstrated negative test results with RT-PCR. These findings demonstrate how LC-MS/MS can screen for SARS-CoV-2 viral peptides with a high degree of sensitivity and selectivity, which minimizes false positive and negative tests associated with RT-PCR. The ability to cross-check multiple viral peptides also avoids misdiagnosis associated with analyzing peptides from only one viral protein.

Dr. Srivastava highlighted how the sample collection method for LC-MS could be particularly advantageous for testing people in remote locations, as the precipitation method offers a stable and rapid way for health professionals to collect and transport samples to the laboratory for testing. This precipitation method overcomes issues seen with RNA preparation of samples for PCR testing.

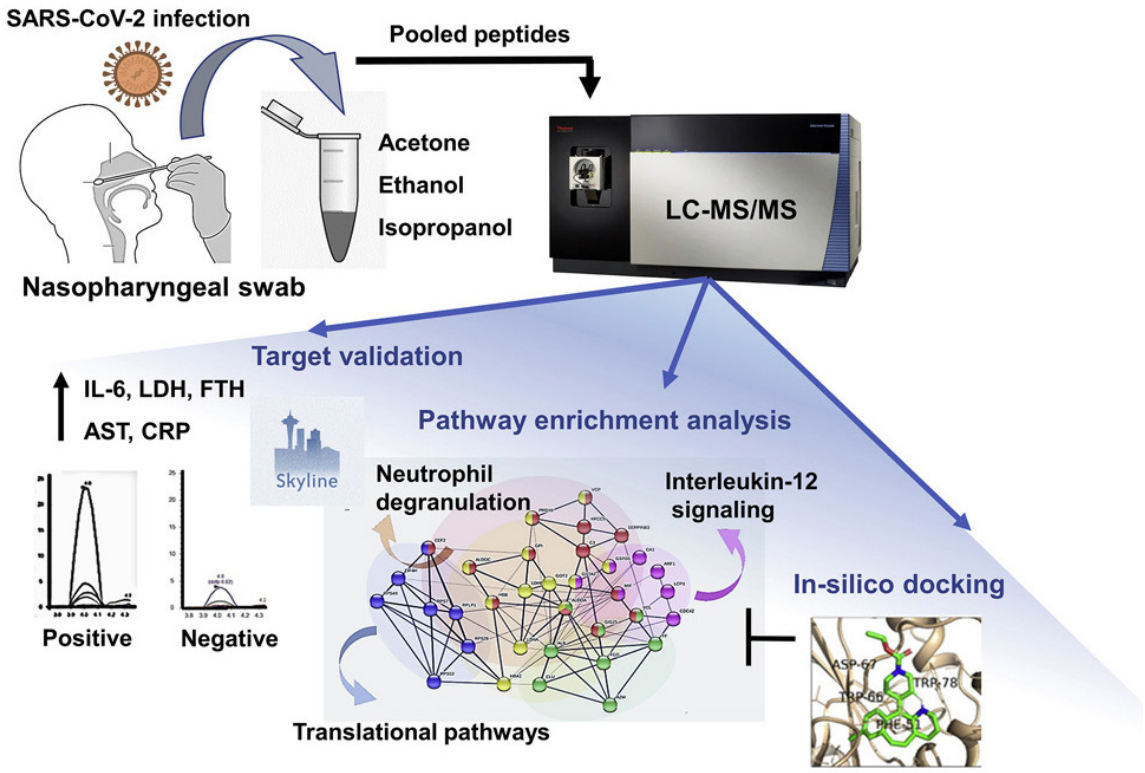

Figure 1. Schematic representation for mass spectrometry-based detection of nasopharyngeal swab samples collected from patients.

Predicting symptom severity from the patient proteomic response

Analyzing patient nasopharyngeal swab samples with LC-MS was highly sensitive at detecting viral particles. The researchers next tested whether the technique could identify host proteome changes after COVID-19 infection, specifically asking whether LC-MS could be used to find peptide or protein biomarkers of COVID-19 infection that correlate with symptom severity.

The researchers analyzed data from three patient groups: COVID-positive, COVID-negative and COVID-recovered patients. Both nasopharyngeal and plasma samples were used in the analysis. Nasopharyngeal tests were used to measure the initial host response when patients came to the clinic, and testing plasma samples taken at regular intervals in hospitalized patients allowed longitudinal tracking of patient responses. The same methods of protein precipitation and processing were used as before, and samples were analyzed with a LC-MS/MS system using selected reaction monitoring (SRM), in which a TSQ Altis Mass Spectrometer was coupled to a Thermo Scientific™ Vanquish™ Horizon Binary UHPLC system for higher throughput peptide detection and quantitation.

The results identified distinct protein expression profiles in each patient group. These profiles had notable similarities and important differences between COVID-positive and COVID-recovered patients, while COVID-negative patients had a distinct profile (Figure 2), showing that each patient group could be clustered according to their responsive proteome.

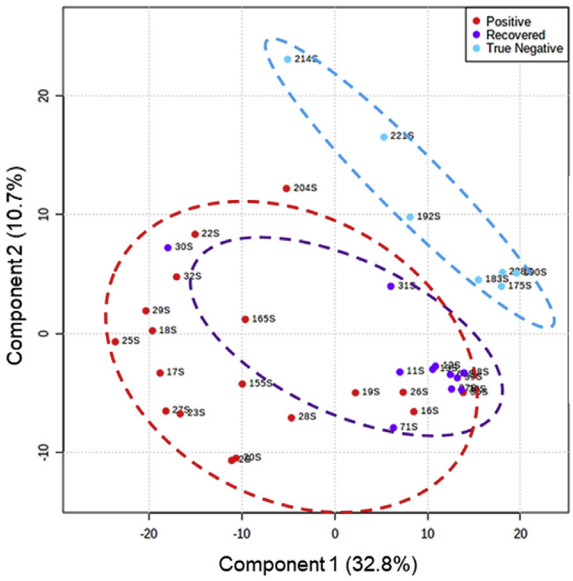

Figure 2. Map of the protein expression segregation clusters from COVID-negative (blue), COVID-recovered (purple) and COVID-positive (red) patients.

To further analyze and learn if there were differences in proteomic profiles based on symptom severity, two types of COVID-positive patients were compared: those with mild symptoms and those with severe symptoms. Analysis of nasopharyngeal swabs and plasma samples showed different protein patterns in the two groups, which were associated with a variety of host response pathways. Around 3,600 host proteins were detected, but this list was narrowed down to a set of previously known clinical biomarkers of COVID-19.

When analyzing this refined list, the results showed that patients with severe symptoms had high expression of distinct sets of these known COVID-19 biomarkers compared to non-severe symptomatic patients. For example, nasopharynx samples included high levels of LDHA, LDHB, STAT1 and hemoglobin subtype proteins. Blood serum showed upregulation of FGG, SERPIN-like proteins (Figure 3), D-Dimer and APOB, a plasma indicator of significant cardiovascular symptoms.

Using LC-MS/MS to measure protein expression response profiles in patients allowed the researchers to find protein response patterns associated with COVID-19 symptom severity in both nasopharyngeal and plasma samples. Importantly, many of these proteins were known COVID-19 biomarkers, suggesting that LC-MS can be used to identify key proteins for diagnostic testing to predict symptom severity.

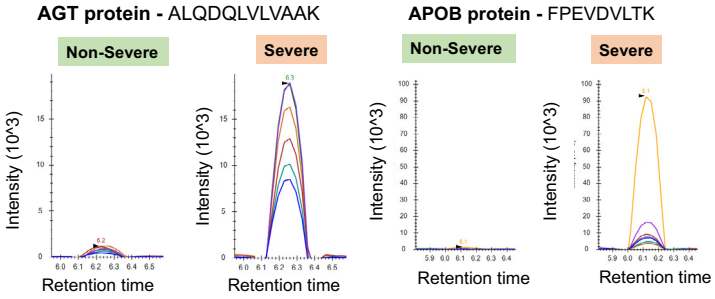

Figure 3. Expression of known COVID-19 peptide biomarkers were higher in the plasma of severe-COVID patients than non-severe-COVID patients.

Identifying therapeutic targets based on host response biomarkers

The depth of the proteomic datasets allowed the researchers to explore the mechanistic aspect of COVID-19 and understand which protein pathways were altered in patients with more severe symptoms. In the nasopharynx, this included the neutrophil degranulation pathway, interleukin signaling pathways and mRNA translation of proteins. In the plasma, major altered pathways included platelet activation and peptidase activity, complement activation pathways, and leukocyte activation pathways involved in an immune response.

The researchers used these altered pathway maps to design an in silico approach for the identification of potential drug candidates and therapeutic targets for COVID-19 treatment. Computationally simulated molecular docking experiments were performed with the identified proteins and a selection of 29 FDA approved drugs. The results showed a high binding affinity of several drugs to proteins that appeared in the nasopharyngeal swab and plasma sample datasets in severe COVID-19 patients. For example, datasets from nasopharyngeal swab samples in COVID-severe patients detected high expression of interleukin-12 signaling pathway proteins ADP-ribosylation factor 1, Carbonic anhydrase 1 and MIF. FDA-approved drugs Loratadine and UCPH-101 were found to have predicted binding sites in these three proteins (Figure 4). Similarly, Selinexor was predicted to target six proteins upregulated in plasma samples from COVID-19-positive patients, including S100A9 and SERPIN proteins. While these findings suggest how drugs already in circulation could be applied to treat COVID-19 patients, further preclinical work needs to assess efficacy and safety.

Improving COVID-19 diagnosis and prognosis with mass spectrometry

From identifying 11 SARS-CoV-2 virus peptides in patient samples to identifying novel biomarkers and therapeutic targets of severe COVID-19 symptoms, the use of LC-MS in this clinical research project demonstrated the potential of this approach for COVID-19 patient testing.

Throughout the study, Dr. Srivastava and colleagues utilized Thermo Scientific mass spectrometry instruments. These instruments allow users of all expertise levels to acquire high-quality results with increased confidence, making them ideal for rapid implementation in clinical research laboratories testing COVID-19 patients. The systems come with streamlined data processing software, algorithms and libraries to address routine applications for COVID-19 assays.

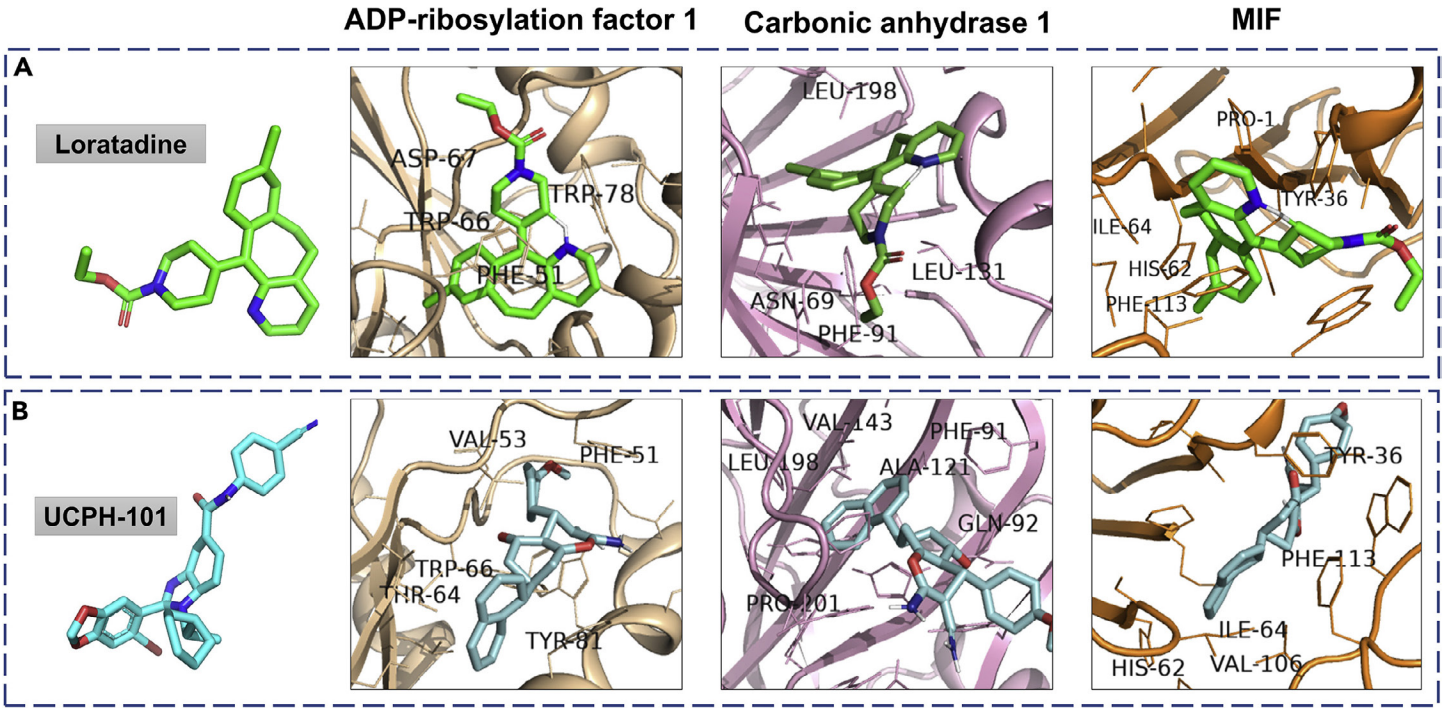

Figure 4. Molecular docking studies of drugs binding to host proteins involved in interleukin-12 signaling pathways in COVID-19.

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To detect SARS-CoV-2 virus peptides in patient nasopharyngeal and plasma digests, the Orbitrap Fusion Lumos Tribrid mass spectrometer with EASY-nLC 1200 system was used to obtain deep proteome profiles. This mass spectrometer is well-suited to perform proteome analysis and identify COVID-19 proteins from ultra-low-level samples, e.g., low-quality nasopharyngeal samples. The EASY-nLC 1200 system provided stable chromatography for low-level sample amounts and was used for untargeted proteome profiling.

For measuring host proteome responses in patients with different COVID-19 symptom profiles, the researchers used SRM methodology with a TSQ Altis mass spectrometer coupled to a Vanquish Horizon Binary UHPLC system to obtain robust and reproducible screening and quantitation. When analyzing complex samples, the TSQ Altis mass spectrometer system can quantify peptides at extremely low concentrations in complex matrices using segmented quadrupole mass filters operated in tandem MS. The Vanquish Horizon Binary UHPLC system was ideal for routine sample analysis due to its reproducible retention time performance specialized for scheduled SRM acquisition.

A piece of the puzzle to better diagnose and treat COVID-19

Dr. Srivastava demonstrated that using LC-MS systems for analysis of COVID-19 patient nasopharyngeal and plasma samples was highly sensitive and selective in detecting SARS-CoV-2 peptides and patient host response peptides. The implementation of mass spectrometry systems in COVID-19 testing facilities could compliment routine RT-PCR testing to facilitate diagnosis and treatment of COVID-19 patients, and minimize false-negative testing associated with RT-PCR. High-throughput mass spectrometry systems allow profiling of patient proteome responses and prediction of patient symptom severity with high accuracy, instructing patient treatment programs and informing vaccination programs.

For additional reference, see Technical Note 65974: LC-MS for Detection of SARS-CoV-2 Viral and Host Proteins. https://assets.thermofisher.com/TFS-Assets/CMD/Technical-Notes/tn-65974-lc-ms-detection-sars-cov2-viral-host-proteins-tn65974-en.pdf

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