Mass Spectrometry for COVID-19

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Word Count: 1145
Abstract: NA
Total Pages: 6

Key words: COVID-19, Coronavirus Disease 2019, SARS-CoV-2, MALDI, matrix-assisted laser desorption/ionization, mass spectrometry, COVIDWISE

In the United States, response to the COVID-19 coronavirus pandemic has been hampered by inadequate testing resources for the causative virus SARS-CoV-2. In the early part of the pandemic, United States laboratories were initially heavily regulated and slow to provide testing. As the pandemic has progressed, the supply chain for instruments and reagents has been inconsistent and has revealed weaknesses in traditional sophisticated infectious disease testing. Testing capabilities of clinical laboratories could be substantially improved by assays that are more simplified and do not require multiple consumable reagents for extraction, purification, amplification and detection. One such technology with the potential to require minimal reagents is matrix-assisted laser desorption/ionization combined with mass spectrometry (MALDI-MS). Recently, Nachtigall and colleagues reported the development of a MALDI-MS method for the diagnosis of SARS-CoV-2 infection (1).

In this study, nasal swab samples were directly applied to MALDI-MS and a spectral pattern was used to classify patients that were infected or non-infected. This report is an interesting application of mass spectrometry for the detection of viral infections. The protocol analyzes unpurified nasal swab specimens in transport media. The study claims high accuracy (93.9%) with 7% false positives and 5% false negatives. The assay protocol has multiple desirable features for a clinical test during the COVID-19 pandemic, including minimal specimen preparation, few reagents, flexible sample throughput, and rapid data acquisition. However, there are several important caveats in the study design: 1) Some test samples were used for both machine learning model selection and machine learning model validation; 2) Samples were characterized as either SARS-CoV-2 positive or negative by RT-PCR, however, samples with potentially cross-reactive viruses or bacteria were not included; 3) A limit of detection for viral load was not determined. In their sample set, the selected machine learning

model identified SARS-CoV-2 positive specimens with 92.6% specificity and 95% sensitivity. However, there was only one reproducible signal peak. The single reproducible peak had lower intensity in infected individuals; this suggests that the assay is identifying a spectral pattern from host response rather than the virus. Leveraging host-response to predict infection is an intriguing alternative to current testing for identification of SARS-CoV-2 infected individuals. However, the proposed MALDI-MS method and interpretative algorithm has not been thoroughly tested and its ability to differentiate types of infectious or non-infectious causes of host modulation remains unknown.

Over the past decade, MALDI-MS has become a routine laboratory technique for the identification of enriched cultures of bacterial and fungal organisms. This is a rapid (minutes) method that requires minimal consumables or reagents. Unlike traditional biochemical or molecular microbiology techniques with expensive consumables, the major expense for MALDI-MS is the instrument. Theoretically, MALDI-MS is appealing to laboratories in low resource settings or in distant geographic locations because supply chains for reagents do not need to be robust. For the analysis of purified bacterial cultures, a single MALDI-MS instrument can process hundreds of samples daily without substantial specialized training of technologists for either the performance or interpretation of assays. However, the upfront capital investment of MALDI-MS is currently cost prohibitive for many laboratories in the US and throughout the world.

An unusual aspect of this study is that non-purified viral samples were assessed. Nasal swabs were placed into viral transport media, and transport media was directly assessed. Within transport media, any virus would be markedly diluted. Non-purified viral samples have not been previously reported by mass spectrometry methods.

A separate study of purified viral culture identified a limit of detection by MALDI-MS of approximately 10^6 genomic copies (1μL assessed from a stock of 10^9 copies per mL) (2). In comparison, amplified nucleic acid techniques such as PCR have a limit of detection for viruses that is routinely 10 to 10^2 copies per reaction. The relatively higher viral load needed for detection by mass spectrometry is another indication that the assay reported by Nachtigall and colleagues is indirectly detecting a viral infection by assessing biomarkers from a host response.

In the context of sepsis not related to COVID-19, monitoring the host response to infection has been explored as a more sensitive method for disease detection compared to the direct detection of an organism (3). The most basic method for monitoring host response to infections is the use of elevated white blood cell counts in peripheral blood. Additional host biomarkers that are now routinely used for the diagnosis and monitoring of infection include C-reactive protein, procalcitonin and lactate. In addition, there are multi-marker approaches for diagnosing infections by profiling host immune gene expression (mRNA transcript profiling). An RNA transcriptome-based approach to examine host response to infection has shown promise in predicting 1) Whether an infection exists; 2) Viral versus bacterial etiology; 3) Risk for 30-day mortality (3). Similarly, the use of MALDI-MS has been investigated in peripheral blood mononuclear cells of patients with sepsis (4). This MS method showed promise in differentiating an immune response to bacterial versus viral infection.

A major challenge of the COVID-19 pandemic is difficulty in predicting who will have severe or mild symptoms; some patients can deteriorate very quickly. Monitoring host response could have a role in rapidly stratifying patients into high and low risk categories. This risk stratification could identify individuals who need higher intensity treatment. An unbiased system such as MS has the potential to detect host immune signatures which predict disease severity.

Despite the limitations of the study by Nachtigall et al, the proposed method is an interesting proof-of-concept for the indirect diagnosis of infections. Other modalities of indirect detection of infections within populations include non-laboratory techniques. For example, aggregate search histories such as *Google Flu Trends* have previously been shown to identify regional outbreaks of influenza 7 to 10 days prior to traditional surveillance methods (5). Indeed, there are multiple reports of the use of *Google Trends* for regional surveillance of COVID-19. Additionally, mobile phone apps have been developed that allow individuals to report their symptoms in real time. When used uniformly, this type of app could be monitored centrally for early warning of infection outbreak and allow pre-emptive measures to be taken. Other smart phone capabilities include contact tracing and prospectively determining the risk of infection by recording the duration of physical proximity to phones of individuals who are diagnosed with COVID-19; conversely, if an individual is infected, an alert notification is sent to all individuals in recent physical proximity. Such contact tracing apps have been implemented in Singapore and South Korea. In the United States, some regions have implemented the COVIDWISE smartphone contract tracing app, a collaboration between Apple and Google.

The COVID-19 outbreak response in the United States has revealed the weakness of relying on assays dependent on complex supply chains for consumables ranging from extraction reagents, primers, probes, tips, and cartridges. In addition, there are multiple challenges to manufacturing and maintaining analyzers. As part of future pandemic preparations, the use of alternative technologies, like MALDI-MS, with indirect detection of infections should be explored.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:*

Employment or Leadership: K. Patel, *Clinical Chemistry*, AACC; J.Y. Park, *Clinical Chemistry*, AACC.
Consultant or Advisory Role: J.Y. Park, Miraca Holdings; Baylor Genetics, Fujirebio, and SRL Labs are subsidiaries.
Stock Ownership: J.A. SoRelle, Myriad Genetics.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.
Patents: None declared.

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