Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Consolidating the potency of matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) in viral diagnosis: Extrapolating its applicability for COVID diagnosis?

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ABSTRACT

MALDI-TOF-MS has essentially delivered more than expected with respect to clinical pathogens. Viruses are the most versatile entities of clinical pathogens that have challenged well-established microbiological methodologies. This review evaluates the existing scenario with respect to MALDI TOF-MS analytical technique in the successful analysis of viral pathogens. The milestones achieved with respect to detection and identification of COVID-19 has been presented. The fact that only a handful of scattered applications for COVID-19 exist has been pointed out in the review. Further, the lapses in the utilization of the available state-of-the art MALDI-TOF-MS variants/benchmark sophistications for COVID-19 analysis, are highlighted. When the world is seeking for rapid solutions for early, sensitive, rapid COVID-19 diagnosis, maybe MALDI-TOF-MS, may be the actual 'gold standard'. Reverting to the title, this review emphasizes that there is a need for extrapolating MALDI-TOF-MS for COVID-19 analysis and this calls for urgent scientific attention.

1. Introduction

The name virus is no longer technical, the years 2019–2021 ensured that every layman to every child became sensitized and familiar with the term 'virus'. In the past, viruses were microbiologist's agenda, but the pandemic made it a global crisis and concern. Pathogenic viruses are numerous and the adverse effects of these viruses have perpetually posed a huge challenge to humanity. In the last ten years, the class of respiratory viruses have been major cause of infections, natural outbreaks and epidemics. Yearly, more than 200 million cases of viral community-acquired pneumonia, 100 million in children and 100 million in adults have been reported [1]. The recent decades have witnessed the virulence of a wide range of respiratory viruses, including current circulating subtypes of influenza A virus (H1N1and H3N2), influenza B virus, respiratory syncytial virus (RSV), parainfluenza viruses, adenovirus and rhinovirus. New viruses have emerged as severe acute respiratory syndrome (SARS) virus, Middle East respiratory syndrome (MERS), avian influenza A (H5N1) virus, human meta pneumovirus (hMPV), corona viruses NL63 and HKU1 and human bocavirus. Rapid identification of respiratory viruses is of the utmost importance towards combating outbreaks and epidemics as well as initiation of timely therapy and prophylaxis. Currently, cell culture, serology and real-time reverse transcription-polymerase chain reaction (rRT-PCR) are those that are employed for virological diagnostics. However, there are a few drawbacks with these techniques: (i) serology and rRT-PCR-based assays are target based and miss non-selected or emerging pathogenic viruses [2,3]; (ii) culture based methods are time consuming; (iii) serology-based assays are not applicable in the acute phase and possess low sensitivity. Therefore, multiple tests are required in order to detect and subtype mixed infections [4].

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is a generic technique that has been

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proven for its efficacy in identifying microorganisms [5,6]. Bacterial analysis using mass spectrometry (MS) has made active progress over the last two decades and has been employed in many hospitals as a rapid and reliable alternative to provide precise, quick, easy and inexpensive comprehensive information on microorganisms [7].

There are various methods for identifying different cells; cell proteins are the most popularly used diagnostic methods [8,9]. MALDI-TOF MS technique is a successful method for detecting microorganisms that have been widely used in microbiological laboratories in recent years [10]. Essential applications of MALDI-TOF MS in virology include, identifying various mutations in viruses and identifying different strains, which might help the rapid and accurate diagnosis of viruses [11–13].

This review presents a consolidated report on the MALDI-MS applications and inputs towards viral detection and identification and diagnosis. The methodologies used for viral analysis using MALDI-MS by various researchers are comprehensively summarized. The specific inputs from MALDI-MS towards COVID-19, has been presented and the need to transfer the available state-of the art MALDI MS techniques demonstrated with respect to other viruses, for COVID diagnosis is discussed. The implementation of nano MALDI MS for COVID analysis is speculated under future perspectives.

2. MALDI-MS as a viral diagnostic tool

Viruses are traditionally detected via cell culture techniques, which in spite of being the gold standard, often took days or even weeks before any results were available. This was then replaced/complemented by lesser sensitive immunological methods, based on antibody analysis using immunoassays or immunofluorescence. The other more sensitive alternative was PCR and dot blot hybridization molecular methods. The use of MALDI-TOF MS as a viral diagnostic tool in clinical samples like influenza viruses, enteroviruses, human papilloma viruses (HPVs), herpes virus, hepatitis virus is sparingly reported [11–15].

The use of MALDI-TOF MS in virology is significantly lagging behind in its application, owing to the relatively low protein content of viruses [16], higher molecular weight of viral proteins (>20,000 Da) and reminiscent cell debris from the culture base in which the viruses are cultured *in vitro*. MALDI-TOF MS has proved its efficacy in detecting drug resistance to ganciclovir in cytomegaloviruses which frequently infect transplant recipients [17]. Usually, the viral genetic material needs to be amplified using PCR and the amplicons analysed/identified by MALDI. There are however, a few reports on the use of MALDI-TOF MS for viral diagnosis. We briefly present a snap shot of these in the following section.

2.1. MALDI MS for influenza virus

The emergence and rapid transmittance of the antigenically novel virus, to which the population shows little or no immunity, results in the influenza pandemic [18]. The 20th century saw three influenza pandemics owing to the new viral subtype (H1N1, H2N2, H3N2) that consumed numerous lives [19]. In 2009, H1N1influenza virus led to the first declared pandemic of the 21st century that claimed 18,000 confirmed deaths [20]. Type A H1N1 influenza viruses have been circulating eversince the so-called 'Spanish flu' pandemic of 1918, followed by H2N2 outbreak of 1957. H1N1 re-emerged two decades later from an unknown reservoir [21] in 1977, causing much havoc [18].

Generally, influenza viruses are characterized using reverse transcriptase polymerase chain reaction (RT-PCR) [22]. Chou et al. [14] used MALDI-TOF MS in combination with antibody-magnetic nanoparticles for detection and rapid screening of influenza virus subtypes. Downard [15] described a method for detection of strains of influenza viruses using whole virus protein digests. This 'proteotyping approach' enabled typing, subtyping and tracing the lineage of human influenza viruses and in that of parainfluenza (another respiratory infectious agent). The use of MALDI MS greatly eased the otherwise long and cumbersome process.

MALDI-TOF MS was applied to the identification of influenza A and B viruses, adenovirus C species, parainfluenza virus types 1, 2 and 3, respiratory syncytial virus, echovirus, cytomegalovirus and meta pneumovirus. MALDI TOF MS led to the identification of cultivable respiratory viruses using viral protein enrichment method for the proteome profiling of virus infected and uninfected cell cultures. The reference virus strains and 58 viruses identified from respiratory samples of subjects with respiratory diseases were used for the *in vitro* infection of suitable cell cultures. The isolated viral particles, concentrated by ultracentrifugation, were used for subsequent protein extraction followed by MALDI-TOF MS analysis. A new library unique for virus profiles was created, allowed us to discriminate between uninfected and respiratory virus infected cell cultures [23].

Authors have devised a rapid, generic and robust sample preparation method for MALDI-TOF MS and LC–MS/MS, leading to rapid identification of respiratory viruses. Tenfold serial dilutions of influenza A virus, RSV and hMPV were prepared in CyMol medium (a collection, transport, and preservation medium for cells, viruses, and nucleic acids) [24]. Suspending viruses in CyMol significantly (approximately 100-fold) improved the identification limit compared to the other (ammonium bicarbonate, NTE buffer (100 mM NaCl, 10 mM Tris–HCl [pH 7.5], 1 mM EDTA), PBS (phosphate buffered saline)) tested solutions [4]. 200 μl aliquots were blended with 25 μl of RapiGest and incubated for 7 min at 95°C, after which DL-dithiothreitol and acetonitrile were added. Reduction and precipitation of the proteins was carried out successfully using controlled microwave radiation in a Rapid Enzymatic Digestion System (REDS). Digestion was performed in REDS at 37°C and 400 W and was terminated by the addition of trifluoroacetic acid (TFA) and centrifuged and filtered. Subsequently, obtained peptides were subjected to MALDI-TOF MS and LC–MS/MS for analyses [4].

According to a recent survey [25–28], the commercially available influenza diagnostic kits based on rRT-PCR were used to detect H1N1 virus with a limit of detection of 104.5–105.5 TCID50 (50% tissue culture infective dose) per mL. However, a negative result does not rule out possible infection with influenza virus due to the overall low sensitivity (40–69%) of the diagnostic kits [27]. With the power of peptide sequencing and database searches, mass spectrometry in combination with immunoassays utilizing monoclonal antibodies, can become useful for the identification of influenza virus proteins. Moreover, MS can confirm virus subtype and identify its antigenic determinants [28].

2.2. MALDI MS for hepatitis virus

Chronic hepatitis B (CHB) was treated using lamivudine (3 TC), this was limited by drug-resistant mutants emerging at the YMDD motif. Luan et al. validated the use of MALDI TOF MS to detect YMDD mutants and quantify viral subpopulations. A total of 21 Chinese patients with severe acute exacerbation of CHB treated with 3 TC were studied. Serum samples were tested for wild-type and YMDD mutants using MALDI TOF MS and compared with the conventional INNO-LiPA assay. 11 patients developed YMDD mutants (six had YIDD, four had YVDD and one had YV/IDD). The study proved that the MALDI TOF MS results were concordant with INNO-LiPA in all patients except one, where INNO-LiPA detected coexistence of YIDD and YVDD but MS and direct sequencing detected

YVDD only. MALDI OF MS was also able to detect a minor hepatitis B virus (HBV) subtype at 5% or above. This report established the fact that MALDI TOF MS is an accurate and cheap method for the detection of YMDD mutants [29] with minimal sample preparation.

Apart from viral identification, MALDI-TOF MS has also been successfully applied for genotyping hepatitis B and hepatitis C viruses [30,31] and for detection of mutations in hepatitis B viruses. Determination of the hepatitis C virus (HCV) genotype has become accepted as the "gold standard" for disease prognosis and assists in establishing the appropriate duration of treatment. More than 10 types and 70 subtypes of HCV have been described. Ilina et al., have determined the HCV genotype by mini sequencing followed by MALDI-TOF MS. Fragments of the HCV genome were amplified and three oligonucleotide primers were designed to detect genotype-specific single nucleotide polymorphisms. The primer extension reaction was performed and the reaction products were analysed with MALDI-TOF MS. The method was used to genotype HCV from HCV-positive blood sera/plasma. The 1a, 1b, 2a, 3a, and 4 genotype HCVs were detected in the clinical samples and the MS data confirmed by direct sequencing [30].

2.3. MALDI MS for enteric virus

Hand, foot, and mouth disease (HFMD) is caused by acute enterovirus infections and is common among children, in Asian-Pacific regions [32–35]. Human enteroviruses (HEV) originally consisted of polioviruses, coxsackie viruses group A and B (CVA and CVB), echoviruses and the numbered enteroviruses. The major causative agents of HFMD are EV71 and CVA16, both classified under HEV-A [36,37]. Although HFMD infections caused by these enteroviruses are clinically indistinguishable, an infection with EV71 is often accompanied by severe complications. Fatalities due to EV71 infection have occurred in the Asian-Pacific region since 1997 [38]. CVA16 has only been associated with uncomplicated HFMD. HEVs are currently identified by neutralization with type-specific antisera or reverse-transcription polymerase chain reaction (RT-PCR) followed by sequencing. The limitation of this test makes identification of multiple genotypes difficult, possibly leading to misdiagnosis and delayed treatment [39,40].

Simultaneous detection of enteric viruses that cause similar symptoms, is key to the prevention of outbreaks and control of infections. A novel PCR-Mass assay combining multiplex polymerase chain reaction (PCR) with MALDI-TOF MS was developed and applied for simultaneous detection of eight distinct human enteric viruses. Enteric viral isolates and standard viral RNAs were examined to determine the sensitivity and specificity of the PCR-Mass assay on 101 clinical specimens from patients suspected of hand, foot and mouth disease (HFMD). The results were compared with that obtained from real-time RT-PCR. These experiments confirmed the performance of the PCR-Mass technique over standard methods. Notably, the PCR-MS technique was able to disclose the presence of multiplex pathogens in a single specimen.

Multiplex MALDI-TOF MS is a powerful tool for the detection and confirmation of microbes [41–43]. Peng et al., developed a sensitive and easy assay of 18 HEV serotypes associated with HFMD using the Sequenom MassARRAY MALDI-TOF MS system [40]. A total of 241 clinical specimens collected from HFMD patients in the Chinese 210 outbreak, were tested by DNA sequencing and MassARRAY analysis. In 14 (5.8%) samples, the MassARRAY method detected multiple types, whereas the DNA sequencing method detected a single type. This resulted in rapid reduction in detection time. In another 2 (0.8%) samples, the MassARRAY method detected single types, whereas the DNA sequencing method detected no HEV. The MassARRAY assay is therefore proven to be a highly sensitive and accurate method for the type-specific detection of 18 HEVs in HFMD and is a powerful complement to current detection methods.

Peng et al. (2013) proved the utility of multiplexed MALDI-TOF for type-specific detection of human enteroviruses associated with hand, foot and mouth diseases [40]. Piao et al. [13] combined multiplex PCR with MALDI-TOF MS and developed a PCR-Mass assay which simultaneously detected eight distinct enteric viruses in humans. Calderaro et al. [44] used MALDI TOF MS as an effective, rapid and inexpensive tool to identify various poliovirus serotypes from different clinical samples. Through MALDI-TOF MS, specific viral biomarkers were detected which were helpful in differentiating virus-infected cells from healthy cells.

Apart from viral identification, MALDI-TOF MS has also been used in virology for genotyping of JC polyomaviruses [45]. Currently, the detection of point mutations and identification of JCV genotypes is via population-based Sanger di-deoxy sequencing (SS) procedures and alignment tools which lack sensitivity and can only identify low levels of viral expression. MALDI TOF MS comparative sequencing was compared to SS, in urine samples taken from a small group of patients. Twelve samples were successfully identified using MALDI TOF MS, while eight were identified via SS. Of those samples which were successfully identified, the majority were subtypes of types 1 and 2. This is in keeping with a previous study of JCV genotyping in 105 urine samples taken from an Australian population which suggested that subtypes of JCV types 1 and 2 accounted for over 85% of samples [46]. The MALDI TOF MS assay subjects PCR amplified sequences to *in vitro* transcription and base-specific RNA cleavage, generating mass signal peaks which are specific to each viral subtype. These peak patterns were compared to reference sequences, taken from the NCBI website, using Sequenom's iSEQTM software platform and matched according to a confidence score and sequence variation probabilities. The results confirmed that MALDI TOF MS assay was twice as sensitive as traditional SS. A basic cost analysis of the consumables required to amplify, transcribe and cleave samples via MALDI TOF MS totalled $9.70 to sequence all four cleavage reactions for one sample, while that from SS totalled over $11 per sample, making MALDI TOF MS a viable, economical alternative to SS for samples with low JCV load [45].

Amexis et al. [47] demonstrated the monitoring of oral poliovirus vaccine with the use of mutant analysis by PCR and restriction enzyme cleavage (MAPREC). They studied the genetic variation in live attenuated mumps virus vaccine using both MAPREC and a DNA MassArray platform based on MALDI-TOF MS. The results obtained from MAPREC and MALDI-TOF methods showed excellent correlation. This suggests the potential utility of MALDI-TOF for routine quality control of live viral vaccines and for assessment of genetic stability and quantitative monitoring of genetic changes in other RNA viruses of clinical interest.

Few studies have so far been conducted on identifying and detecting viruses partly due to the high molecular weight of viral proteins (>20 KDa) and because viral identification still relies strongly on cell culture and antigen or nucleic acid detection. In addition, extraction protocols for viral proteins frequently require *in vitro* cell cultures, which could alter the proteins and take several days. A recent 2014 study from Calderaro et al. discriminated among three different serotypes of Sabin poliovirus, tuning the range of MALDI–TOF to identify differential PMF peaks for the VP4 capsid [44].

2.4. MALDI MS for human papilloma virus

Human papillomavirus (HPV) DNA testing was shown to have a higher sensitivity and negative predictive value for the detection of clinically relevant preinvasive disease than the conventional

Papanicolaou smear and liquid-based cytology methods [48,49]. Currently, there are 3 HPV assay platforms approved for clinical use by the US Food and Drug Administration. The non−PCR-based assays tend to have slow throughput and high cost per case and provide no or limited genotyping information. The new Cobas HPV assay is PCR-based but with characteristics like the other 2 assays. Misdiagnosis might be frequent in clinical cases.

The Mass ARRAY (Sequenom, San Diego, CA) technique based on the MALDI-TOF-MS platform allows detection and genotyping of all 14 HPV types simultaneously in 1 reaction with high throughput. This method has the power to analyse 3000 samples in 2 working days with an automatic PCR working station, and a 24-h laboratory could provide a throughput of 4500 samples per day with the current configuration [50] and is cost effective too.

Yi et al. [12] reported the use of a PCR-based MS method for detection of high-risk HPVs, a prime cause of human cervical cancer. They claimed that the high-throughput and cost effectiveness make it ideal for routine clinical diagnosis and for epidemiological studies. The MALDI-TOF MS system is a high-throughput technology with multiplex capacity. It has already been used for genotyping [5,51].

2.5. MALDI MS for herpes simplex virus

The herpes viruses infecting humans include: herpes simplex virus (HSV) types 1 and 2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV) types A and B, cytomegalovirus (CMV), human herpesvirus 6 (HHV6) types A and B, HHV7, and HHV8 (Kaposi's sarcoma-associated herpesvirus). Common laboratory techniques for herpes virus detection include antibody detection such as enzyme-linked immunosorbent assay [52], PCR [53−55], and dot blot hybridization. PCR-based methods for the multiplex detection of all known human herpes viruses were developed on the MALDI-TOF MS system. A variety of 882 archival samples, including bronchoalveolar lavage, conjunctival fluid, sore secretion, blister material, plasma, serum, and urine, analysed for herpesviruses using PCR-based reference methods, were used to validate the MALDI-TOF MS method. The overall concordance rate between the MALDI-TOF MS method and the reference methods was 95.6%. In summary, the MALDI-TOF MS method is well suited for large-scale detection of human herpes viruses in a wide variety of archival biological specimens [11]. Table 1 summarizes the milestones achieved using MALDI-TOF MS for viral diagnosis.

3. State of the art diagnosis/detection technology for COVID

The novel coronavirus disease 2019, known as COVID-19, is caused by the SARS-CoV-2 virus and has been declared a pandemic by the World Health Organization on March 12th following its emergence in Wuhan, China [56]. There were more than 1.2 million confirmed cases of COVID-19 in 175 countries, with more than 65,000 deaths (COVID-19 Map-Johns Hopkins Coronavirus Resource Center, 2020). SARS-CoV-2 is one of the four new pathogenic viruses which have jumped from animal hosts to humans in the past 20 years and the current pandemic alerts the entire scientific community about the need for research in diverse fields to help contain this crisis. Corona Virus SARS-CoV-2 (Covid-19) primarily affects the respiratory system by attaching to the ACE2 Receptor lining the epithelial cells of the respiratory tract. Many other extra pulmonary organs and tissues also express the ACE2 receptor

Table 1
MALDI TOF MS applications in virology.

Mass technique employed	Identified strain(s) of virus	Methodology used
MALDI TOF MS protein profiling	58 respiratory tract viruses	viral proteins enrichment method to the proteome profiling of virus infected and uninfected cell cultures.
MALDI-TOF MS or MALDI Fourier Transform Ion Cyclotron Resonance MS (MALDI-FT-ICR MS)	Purified Influenza viruses	virus concentration and/or purification with either ultracentrifugation, differential centrifugation, precipitation, filtration, isolation of viral particles or protein(s) with an affinity capture immunoassay or gel electrophoresis
MALDI TOF MS	Respiratory viruses - Influenza A H3N2, Influenza A H1N1	Inhouse Rapid, generic and robust sample pretreatment and preconcentration method
MALDI TOF MS	influenza A and B; adenovirus C; parainfluenza types 1, 2, and 3; respiratory syncytial virus (RSV); echovirus; CMV; andhuman metapneumovirus	mass spectra of infected samples(differential peaks) that had previously been compared with uninfected cells
MALDI TOF MS	JC polyomaviruses, hepatitis B and hepatitis C viruses and for detection of mutations in hepatitis B viruses	viral genotyping for identification
MALDI TOF MS	Hepatitis C virus	genotype by minisequencing followed by MALDI-TOFMS
DNA massArray −MALDI TOF MS	Hepatitis B virus, mumps virus	PCR and restriction enzyme cleavage (MAPREC)
Multiplex matrix-assisted laser desorption/ ionization time-of flight mass spectrometry	Human enteric viruses	Sequenom MassARRAY MALDI-TOF MS system
multiplex PCR with MALDI-TOF MS	Detection of 8 enteric viruses	PCR-Mass assay
MALDI-TOF	type-specific detection of human enteroviruses	multiplexed MALDI-TOF
MALDI TOF MS	JC polyomavirus	genotyping JCV DNA in urine samp
MALDI TOF MS Sequenom's iSEQTM	JCV	genotype in 105 urine samples taken from an Australian population
MALDI TOF MS	Human papillomavirus	The Mass ARRAY (Sequenom, San Diego, CA) technique based on the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS
MALDI TOF MS	Human papillomavirus	PCR-based MS method
PCR-based MALDI TOF MS	Herpesviruses	882 archival samples, including bronchoalveolar lavage, conjunctival fluid, sore secretion, blister material, plasma, serum, and urine, analysed for herpesviruses
MALDI TOF MS	Herpesviruses	Multiplex MALDI TOF MS
MALDI TOF MS	Sabin poliovirus	Tuning range of MALDI TOF MS

like vascular endothelial cells, lungs, heart, brain, kidneys, intestine, liver, pharynx, and are targets for the virus attack. Host cell entry requires priming of the spike protein of the corona virus by the cellular serine protease, TMPRSS2 and ACE 2 receptors followed by replication within the host cell [57].

Key mechanisms by which the Covid-19 virus induces cell damage in various organs are by the following 4 mechanisms. The virus can directly cause toxic damage to the cells or injury to the endothelial cells of the vasculature supplying the organs causing thromboinflammation. Other proposed mechanisms are disruption of the RAAS (renin-angiotensin-aldosterone system) and dysregulation of the immune response to the virus and exaggerated proinflammatory cytokine response (a cytokine storm) that induces widespread inflammation and damage to multiple organs [58]. Pathologically, the infected lungs have patchy consolidation (pneumonia) along with pleural fibrinous exudate and/or fibrosis, sometimes with purulent inflammation due to secondary bacterial infection. As the disease progresses there is diffuse alveolar damage with transparent hyaline membrane formation (ARDS) and severe pulmonary edema [59]. Fig. 1 gives an overview of the typical impacts of COVID 19 on the human body.

Treatment depends on the duration of onset of first symptom of the patient, diagnostic tests like RT PCR, assessment of the extent of involvement of lungs by Chest CT scan and other tests like complete blood count (CBC), LFT, RFT, ECG, ECHO, D-Dimer to assess function of liver, kidney, heart and coagulation profile as needed. The vital signs especially respiratory rate and SpO_2 (Oxygen saturation) is monitored periodically to assess the severity and progression of the disease. In early stages of the disease the patient can be quarantined at home with antipyretics, nasal decongestants, vitamins C, D, Zinc. If the SpO_2 drops to 90–93%, then patient is admitted and administered oxygen, started on prophylactic dexamethasone and anticoagulants. If patient is on O_2 requiring more than 5L/min by NIV, HFNC or ventilator support and if SpO_2 drops below 90, patient might require admission in ICU for further meticulous management with continuous monitoring and evaluation.

Rocca et al. have recently reported their attempts to assess the potential of MALDI-TOF MS technology to create mass spectra directly from nasopharyngeal swabs. Using machine learning algorithms, specific discriminatory peaks were identified and applied to differentiate COVID-19 positive samples from COVID-19 negative samples. This study used MALDI-TOF MS for COVID-19 identification, and for the detection of specific biomarkers, differentiating between virus-infected and uninfected patients. The preliminary results confirmed that MALDI-TOF MS coupled with ClinPro Tools software are an interesting alternative for diagnosis of SARS-CoV-2 virus. The research group is working on enhancing the values of performance obtained in this first approach. To achieve that, the same group is increasing the numbers of samples, evaluating different extraction methods and making improvements of the machine learning algorithms. Nevertheless, this study launches forth the use of MALDI-TOF MS combined with machine learning algorithms as a revolutionary screening assay that deserves further development to braise itself to become an alternative approach for the currently available methods [60].

Several research groups have proposed strategies based on targeted proteomics to detect SARS-CoV-2 in clinical specimens [61–64]. However, lengthy and laborious manual sample preparation procedures [61–63] and long analysis times for liquid chromatography separation and MS detection [62,63] would limit their application in large-scale testing. Besides, their analytical performance was not fully demonstrated since they relied on preliminary validations with a limited number of clinical samples [61–64]. Carr et al., developed a rapid, specific and robust method to enable high-throughput screening to support large-scale SARSCoV-2 clinical diagnostics. For this purpose, two different assays were validated based on the parameters for MS-based targeted proteomic assays: qualitative (Tier 3) and quantitative (Tier 1) [65]. Clinical respiratory tract samples were first analysed using a bottom up proteomics workflow, resulting in a spectral library that generated a list of targeted peptides. The peptide selection was further refined using parallel reaction monitoring (PRM) on a microflow chromatography–high-resolution MS platform. The selected SARS-CoV-2 peptides were used to develop a high throughput targeted proteomics assay based on turbulent flow chromatography coupled to tandem mass spectrometry (TFCMS/MS). When applied to several hundred clinical samples, the assay detects up to 84% of the SARS-CoV-2 positive cases identified by an in-house real-time PCR

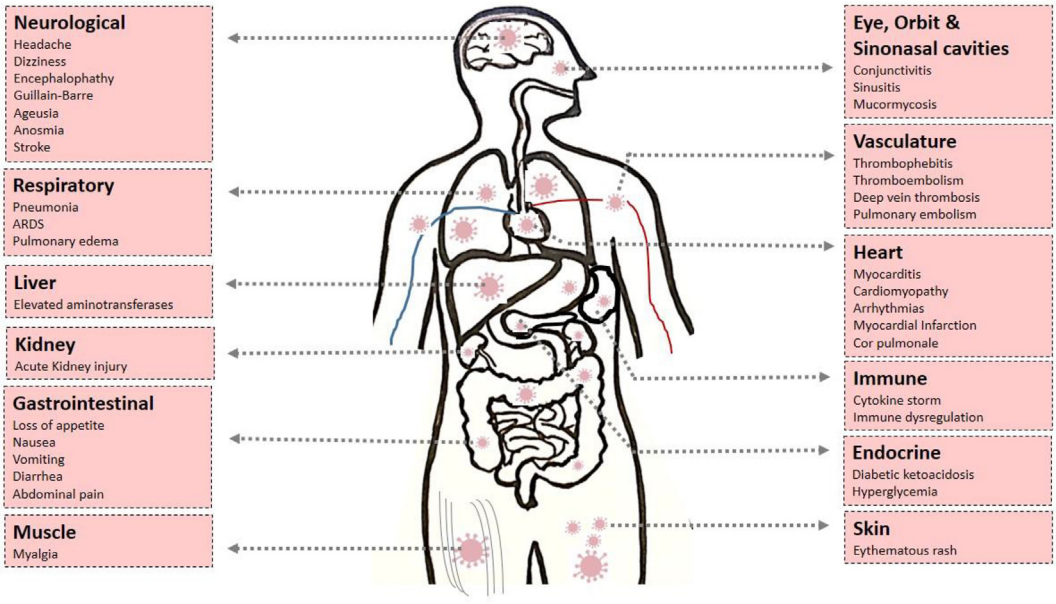

Fig. 1. Overview of the adverse effects of corona virus on humans.

method, demonstrating the utility of this MS-based approach for high-volume SARS-CoV-2 testing. Currently, RT-PCR testing is the gold standard for SARS-CoV-2 RNA. During the acute phase of infection, SARS-CoV-2RNA is generally detectable in upper respiratory swabs and bronchoalveolar lavage (BAL) samples. Positive results merely indicate the presence of SARS-CoV-2 RNA; while clinical plasma biomarkers such as D-dimer, CRP, IL-6 levels with patient history are necessary to determine the patient's clinical COVID-19 status and level of therapeutic intervention. The 2020 global pandemic failed with respect to timely testings, this was the key factor that curtailed COVID-19 spread in even the wealthiest countries. The development of MALDI-TOF MS diagnostics for SARS-CoV-2 detection is driven by the need for greater diagnostic capacity and alternative applications to complement standard PCR and antibody-based diagnostics.

Validation studies against saliva/gargle spiked with cultured virus are on-going and the current study indicate a close to 100% sensitivity, if measuring S1 peaks alone as the indicator of corona virus infection. Gargle/saliva samples are collected and subjected to PCR testing as well as MALDI TOF MS diagnosis simultaneously. Other markers measured in this technique may give further valuable clinical information such as other viral infections and magnitude of the mucosal humeral immune response [66].

To date, there is still an urgent need for accurate and high-throughput detection of COVID-19 for large population screening. In a recent study, serum samples of 146 COVID-19 patients, including mild, typical, severe and critical classifications, and 152 control individuals, including non-COVID-19 patients with fever/cough symptoms, TB patients, and healthy controls, were analysed using MALDI-TOF MS. 25 MS peaks statistically significant within patients and control individuals were identified. Using various machine learning methods, a model constructed with the 25 peaks was established, showing 99% accuracy for the identification of COVID-19 patients with a sensitivity of 98% and specificity of 100% on a test cohort (100 samples). This accurate and rapid method provides a powerful tool for high throughput screening and surveillance of COVID-19 [67].

Illes RK et al. [66] achieved a multifaceted clinical MALDI−TOF MS screening test, not limited to SARS-CoV-2 by detecting viral envelope glycoproteins, including peaks of spike protein fragments S1, S2b and S2a. The method offers ease of sampling, speed of analysis and a much lower cost of testing. Combining MS-based methods with machine learning (ML) and artificial intelligence (AI) [68], demonstrated reliable detection of SARS-CoV-2 in swab samples. When the cost of RT PCR based COVID detection is set at 10-15USD, the MALDI-TOF MS COVID detection is reported to be less than 1 USD [66]. Tran et al. [69] evaluated an automated ML platform, Machine Intelligence Learning Optimizer (MILO), combined with MALDI−TOF MS for rapid high-throughput screening of COVID-19 and showing promising accuracy (96.6−98.3%), sensitivity (positive percent agreement of 98.5−100%), and specificity (negative percent agreement of 94−96%) [69]. Delofeu et al. analysed 236 nasopharyngeal swab samples, and the subsequent mass spectra data was used to build different ML models, showing a performance of >90% accuracy, sensitivity, and specificity. They compared extreme gradient boosting trees and support vector machines (SVMs) and the best results were obtained from an SVM [70].

Nachtigall et al. [71] described a method to detect SARS-CoV-2 in nasal swabs using MALDI-MS and machine learning analysis. This approach uses equipment and expertise commonly found in clinical laboratories in developing countries. Mass spectra from a total of 362 samples (211SARS-CoV-2-positive and 151 negative by RT−PCR) without prior sample preparation from three different laboratories were subjected to testing. Two feature selection methods and six machine learning approaches were deployed for SARS-CoV-2 detection. The support vector machine model provided the highest accuracy (93.9%), with 7% false positives and 5% false negatives. These results suggest that MALDI-MS and machine learning analysis can be used to reliably detect SARS-CoV-2 in nasal swab samples. These results prove that the six different ML models demonstrated high accuracy and reliability with the highest accuracy (93.9%). Preiano et al. [72], have very recently published a report reviewing the analysis of SARS-CoV-2 class of viruses. Table 2 consolidates the updated list of MALDI TOF MS based reports on COVID. These are few of the handful reports available for the applicability of MALDI-TOF MS for detection of COVID 19. Fig. 2 gives the work flow of MALDI TOF MS based analysis of COVID.

4. Proposed improvisations and practical implementations

MALDI MS and its accomplishments in the area of microbiology, especially in facets of identification and diagnosis/detection is immense. The most prominent MALDI MS inputs are into bacteria, followed by fungi and viruses. MALDI MS applications into viral diagnosis and detection and treatment, is backed up by a decent number of publication reports. Also, the review revealed the fact that MALDI MS has been used for virology, about two decades back, yet no surge or significant progress is evident. The reason behind MALDI MS lagging behind the rest although it is proven as a viral diagnosis tool, is another area worth probing. A plausible reason could be the very fact that, this application combines two diverse research disciplines, namely, mass spectrometry and virology. Bacteria and fungi do not require technical expertise, while culturing of viruses very much do. Viruses are rather sensitive and their culturing process cumbersome, handling delicate and this may pose a challenge for a basic microbiologist let alone an analytical chemist. To overcome this barrier, cross disciplinary collaborations will greatly help.

This review highlights the lack of technology transfer on the use of MALDI MS and its associated techniques into the much needed area of COVID 19 detection. When there is a considerable amount of data on the use of various MALDI MS combinatorial techniques proven for detection of influenza, herpes, human papilloma virus, hepatitis B/C virus, HIV and enteric viruses, it is strange that when the world was seeking for rapid detection tools, nothing prominent from MALDI MS was offered. With MALDI TOF MS proven for biomarker detection, it is strange that nothing has been probed in the direction of viral biomarkers. This is an avenue worth investing some research attention into.

When more than a dozen researchers, who had worked on respiratory viruses, that were close allies with COVID 19, were still at large, except for a handful of scattered reports on the use of MALDI MS for COVID 19, no progress has been made. With early detection being the need of the hour, with MALDI MS ability to enable detection within minutes, still nothing authoritative established is some valid lacunae. This review, insists that the methods reported and used successfully on other viruses, should be tried out on COVID 19.

Next-generation sequencing (NGS) is a high-throughput, impartial technology with numerous attractive features compared to established diagnostic methods for virus detection. NGS-based studies have improved our understanding of viral diversity. There is considerable interest within virology to explore the use of metagenomics techniques, specifically in the detection of viruses that cannot be cultured. Ilya et al. [73], recently based on the existing SARSCoV2 pandemic situation (April 2020) tested SARS-CoV2 positive Illumina libraries with Lazypipe and confirmed that the pipeline detected SARS-CoV in 9 out of 10 libraries with default settings and without SARS-CoV2 reference genome. The utility of

Table 2
MALDI TOF MS applications for COVID 19.

MALDI TOF MS technique	Sample	Identification Method	COVID Application	Cost	References
MALDI-TOF MS technology using machine learning algorithms/ClinPro Tools	nasopharyngeal swab	Biomolecular host profiling	create mass spectra directly from sin order to find specific discriminatory peaks by and whether those peaks were able to differentiate COVID-19 positive samples from COVID-19 negative samples.	Unspecified	Rocca et al. [60],
MALDI-TOF MS- Machine learning methods	serum samples	Biomolecular host profiling	146 COVID-19 patients, including mild, typical, severe, and critical classifications, and 152 control individuals, including non-COVID-19 patients with fever/cough symptoms, TB patients, and healthy controls were analysed	Unspecified	Yan et al. [67],
MALDI-TOF MS- Machine learning methods	nasal swab samples	Biomolecular host profiling	mass spectra from a total of 362 samples (211 SARS-CoV-2-positive and 151 negative by RT−PCR) without prior sample preparation from three different laboratories were tested for their accuracy of SARS-CoV-2 detection. The support vector machine model provided the highest accuracy (93.9%), with 7% false positives and 5% false negatives.	Unspecified	Nachtigall et al. [71]
MALDI TOF MS	Saliva/gargle samples	Biotyping and Biomolecular Host Profiling	Host/viral proteins analysed for 45 min for sample preparation, 3 min per sample for MALDI-TOF analysis, a few seconds for data results analysis	<1 USD	Illes t al. [66],
MALDI TOF MS	nasopharyngeal swab	Biomolecular host profiling	Host proteins analysed by machine learning algorithms Sensitivity of 100%, Specificity of 92%, Accuracy of 97%	Unspecified	Deulofeu et al. [70]
MALDI-TOF MS	Nasal samples	Biomolecular Host Profiling Host proteins	- 107 COVID-19 positive samples (28 asymptomatic and 79 symptomatic) - 92 COVID-19 Machine learning algorithms Total turnaround time <1 h negative samples	Unspecified	Tran et al. [69],
MALDI-TOF MS	Gargle samples	Biotyping and Biomolecular Host Profiling	30 COVID-19 positive samples (89% asymptomatic) and 30 COVID-19 negative samples tested Host proteins and viral proteins analysed using online database Searching. Sensitivity of 93.33−100%: Specificity of 90−93.33%	Unspecified	Chivte et al. [74]
RT-PCR/MALDI-TOF MS	Saliva samples	Genotyping	- 34 COVID-19 positive samples - 26 COVID-19 negative samples	Unspecified	Hernandez et al. [75]
RT-PCR/MALDI-TOF MS	Oral or nasopharyngeal samples	Genotyping	Viral genes: N1, N2; N3; ORF1; ORF1ab analysed in 168 suspected COVID-19 samples using Online database searching. Time: 8 h for the entire process	~10 EUR	Rybicka et al. [76],
RT-PCR/MALDI-TOF MS	Oral or nasopharyngeal samples	Genotyping	Viral genes: N1; N2; N3; ORF1; ORF1ab. 22 COVID-19 positive samples and 22 COVID-19 negative samples were tested Time: 8 h for the entire process -	Unspecified	Wandernoth et al. [77]

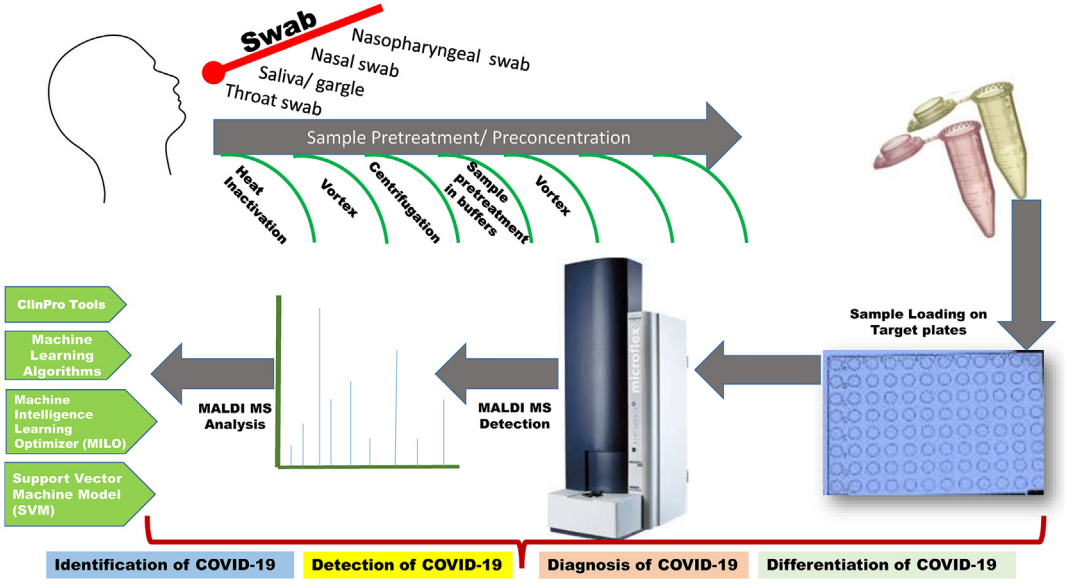

Fig. 2. Typical work flow of MALDI TOF MS analysis of COVID 19.

Lazypipe for swift detection by NGS sequencing from clinical samples has been demonstrated. Combining such bioinformatics approaches with MALDI-MS could escalate its efficacy for viral detection.

MALDI MS, is backed up by a large number of variants, SALDI, SELDI, Nanoparticle assisted MALDI MS, imaging MALDI (MSI), Silicon nanopost array (NAPA), Desorption/ionization on silicon (DIOS), this review highlights the fact that none of these state of the art sophistications of the MALDI MS technology have been attempted with respect to viruses and more so with COVID 19. Each of these LDI variants had demonstrated enhanced detection and breaking down limits of detection and yet, with their proven effectiveness, them not being applied to viral detection using MALDI MS, is abnormal. This is one of the times that one feels the pain when an available resource is not deployed, in spite of the rising global crisis. There is definitely a lot that the above variants can offer towards viral studies using MALDI MS. This review expects researchers' attention to be drawn towards this core area to enable progress in the right direction. Pandemics are a global threat and the need of the hour is that early detection can save a life and save the spread, MALDI MS does have the speed, the ease, the ability, fine tuning is all that is needed. When the world is struggling to sort out ways to detect COVID, the constantly emerging mutants and COVID variants will no doubt require upgraded MS techniques.

Testing of clinical COVID samples employing MALDI MS is also a less reported area, which needs to be worked on in order to hit at the limitations of the conventional MALDI MS system, so that enhanced variants can be sought after. There is definitely plenty of room unexplored, when it comes to COVID- MALDI MS. Expanding and exploring and extrapolating will lead to successful deliverables. This is what this review points at.

5. Conclusions

This review focused on consolidating the available reports on detection and diagnosis of viruses using MALDI MS. Compared to bacteria based MALDI MS reports, viral reports are very few and compared to the viral applications, COVID 19 applications of MALDI MS are reduced to a handful reports. This review points out to the lack of use of sophistication in terms of various state of the art MALDI MS variants, for COVID 19. Who knows what lies to be unravelled beyond these unprobed horizons, could hit the keys for viral and COVID diagnosis and subsequent treatment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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