Description of Rapid COVID-19 MS-based diagnostic test method

Summary

The aim of this study is to optimize and validate a rapid COVID-19 mass spectrometry (MS)-based diagnostic test that can be implemented at Schiphol airport or other places of need.

The overall method comprises two steps: in a first step a metabolic profile will be measured directly from the dry nasopharyngeal swabs using Desorption ElectroSpray Ionization (DESI)- high resolution (HR)MS, and in a second step two proteins specific for SARS-CoV-2 (NCAP and SPIKE) will be measured using a targeted MS/MS method. The dry swabs that we have successfully used are knitted polyester swabs on a polypropylene shaft.

The two methods are complementary and therefore together allow to minimize both false positive and false negative results. The integrated method is much faster and significantly lower in costs compared to current PCR tests.

While we think that this integrated method is very promising, we have to critically evaluate it and optimize it, and finally validate the optimized method for its suitability for rapid COVID-19 diagnostic testing versus PCR results as the current golden standard.

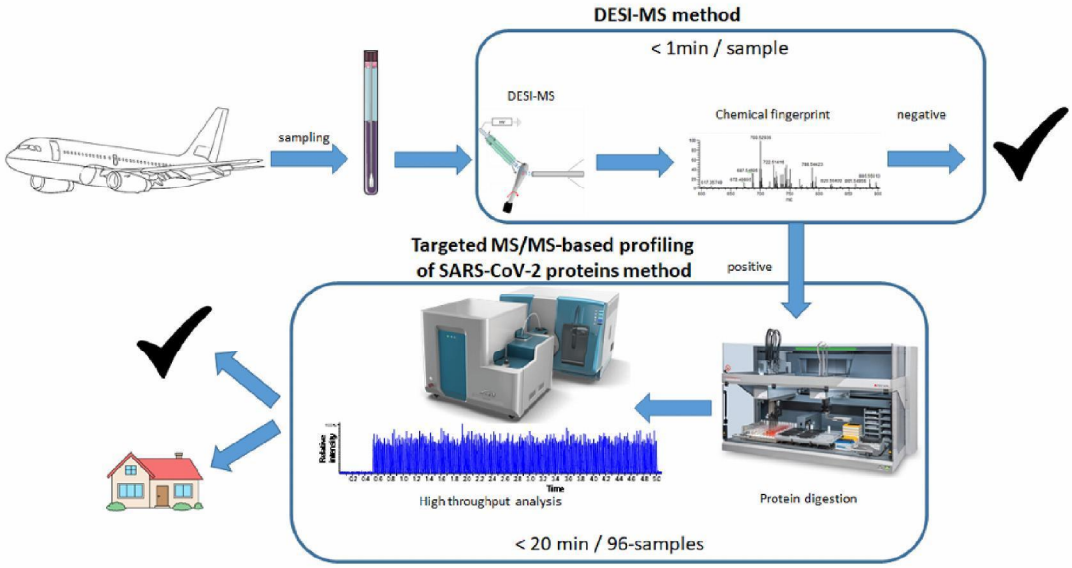

Figure 1. Scheme of integrated COVID-19 MS based diagnostic test comprising two analytical steps.

Description of DESI-MS method

Method description

Desorption electrospray ionization (DESI)-MS is an ambient sample introduction MS method, requiring only a few seconds per swab analysis (Figure 2). DESI is implemented by directing a pneumatically assisted electrospray onto the sample surface (Figure 3). The multiply charged solvent

droplets impacting the sample surface dissolve analyte molecules present on the surface. The secondary charged droplets produced by the impact event carry these species to the mass spectrometer. The DESI approach is able to collect a chemical fingerprint of any surface, including swabs, and has been applied for example to mucosal and vaginal swab samples [1].

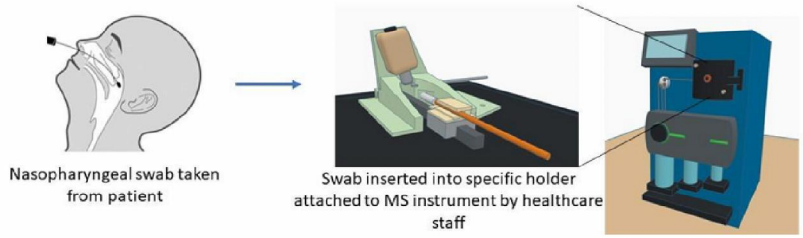

Figure 2. Workflow for the first step of the integrated method: DESI-MS of nasopharyngeal swabs.

Viral hijacking and metabolites/lipids

Pathogens (including the coronavirus) reprogram cellular metabolism to maximize the efficiency of virus particle production. It has been observed that TCA cycle-related pathways as well as amino acid biosynthesis is downregulated, while pathways contributing to nucleic acid biosynthesis are upregulated and the observed effect shows good correlation with the severity of symptoms. Significant alterations in lipid metabolism have also been observed, especially in case of free fatty acid and diglyceride levels indicating the upregulated utilization of adipose triglycerides. The detected shifts for specific amino are also predicted by reconstructed metabolic networks of SARS-CoV-2 infected cells (personal communication, Ines Thiele).

Although this approach will allow to detect significant differences in the pattern of a sick person, the lack of specificity of those markers may not fully allow to differentiate between different viral infections, leading to possible false positives; that is why we will use a confirmatory MS analysis targeting SARS-CoV-2 specific proteins.

First results

The current instrumentation includes a DESI-RDa swab analyser device introducing a nasal swab into the device automatically, analysing it.

The method has been validated for COVID-19 diagnostics for a limited set of 265 COVID-negative samples and 105 COVID-positive samples as analyzed in the lab by prof. Zoltan Takats (Imperial) and using standard PCR diagnostics as ground truth, with 92% agreement (Figure 3). This value can be expected for a first feasibility study, and this value will be significantly improved with the current optimization and validation. The method could detect a SARS-CoV-2 metabolic signature in a swab with a Ct value determined as 38 with PCR.

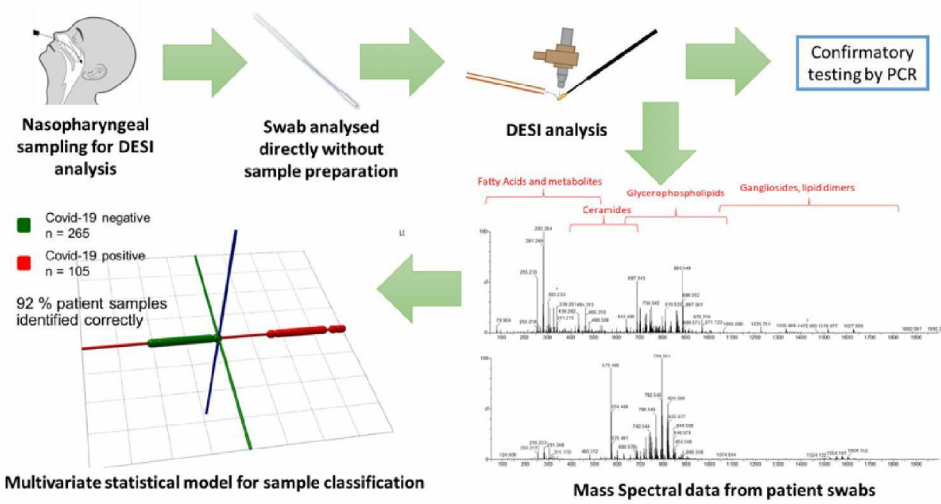

Figure 3. Nasopharyngeal testing and COVID-19 diagnostics by DESI-MS analysis of mucosal swabs.

In the planned integrated method, the positive swabs will be separated from the negative ones, the heads of the positive swabs will be cut off and placed into vials. The capped vials will be prepared for confirmatory analysis with the targeted MS/MS method of SARS-CoV-2 proteins (see below).

Upside of DESI-MS

DESI-MS does not only allow to detect a viral infection, but can in principle also differentiate between viral and bacterial infections. In addition, the metabolic profile can in principle also predict disease progression. Actually, several metabolite levels (e.g. glycerol-1-phosphate) were found to carry prognostic information and could be used for the identification of patients developing severe symptoms at early stage of the disease. However, the prognostic potential of the test will be only explored at a later stage than phase 1 or 2 of the project. In this project we will focus only on the detection of SARS-CoV-2 infection in persons using nasal swabs.

Dry swabs

The dry swabs that we have successfully used for DESI-MS are knitted polyester swabs on a polypropylene shaft that are available for medical use (https://harmonycr.com/swabs-applicators/puritan-cylindrical-tip-knitted-polyester-swab-6-polypropylene-shaft/3605). These dry swabs are also suited for the targeted MS/MS analysis of SARS-CoV-2 proteins. We are currently in discussion with DSM to produce swabs that are suitable for this MS-based COVID-19 test.

Description of targeted MS/MS analysis of SARS-CoV-2 proteins

Method description

During the first months of the COVID-19 crisis, several groups started working on the development of diagnostic tests for COVID-19 using LC-MS. All reported LC-MS methods target highly specific peptides obtained from SARS-COV-2 proteins after digestion. Earlier this year, Sinz *et al.* were able to detect few specific peptides of SARS-COV-2 S protein from gargle samples [2]. Armengaud *et al.* were able to accurately predict the contamination of a patient having a very low viral load (down to Ct 28-30) [3]. Bezstarosti *et al.* developed at Erasmus MC a method able to quantitatively detect in principle approximatively 10 000 SARS-COV-2 particles[3] (see Figure 4). Selective and sensitive detection of the peptides can be obtained using high resolution MS detection, MS/MS using high resolution detection or MS/MS using a QqQ detector[4]. Selectivity and sensitivity of these approaches can differ somewhat depending on the characteristics and performance of the MS system, but the sensitivity is rather comparable between high end Q-Orbitrap systems and high end QqQ systems such as the Sciex 6500 and the recent Sciex 7500 model.

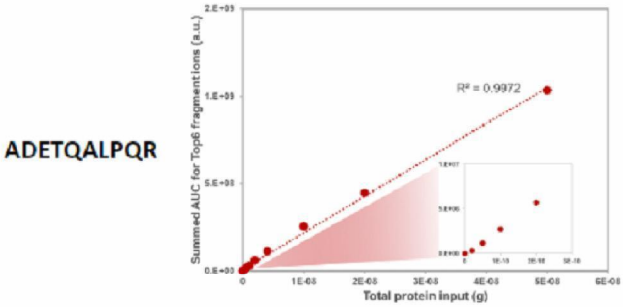

Figure 4. Calibration curve of selective peptide as obtained with LC-Orbitrap MS in SARS-CoV-2 infected cells as obtained by Bezstarosti et al[3] .

5.1.2e et al. developed a fast LC-MS/MS method for the quantification of the proteins NCAP and SPIKE down to the femtomolar level using a selective QqQ MS detector (Sciex 5500). The results of their method on real swab samples showed an excellent correlation to the PCR results (figure below), and they reported a limit of detection of a 1-4 fmol injected on-column for NCAP specific peptides (DGIIWVATEGALNTPK, RGPEQTQGNFGDQELIR, KQQTVTLLPAADLDDFSK), and slightly higher for NCAP specific peptide ADETQALPQR, and 0.6-6 fmol for SPIKE specific peptides (GWIFGTTLDSK, RSFIEDLLFNK, SFIEDLLFNK) using a Sciex 5500 detector[4].

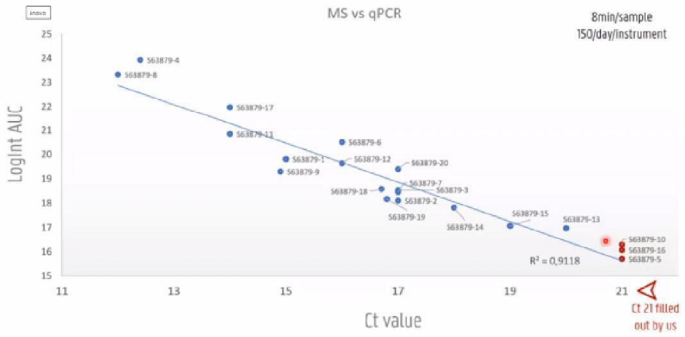

Figure 5. Comparison of signal of TIC (sum) of a 3 peptide specific for NCAP as measured by LC-MS/MS by Dhaenens group.

While the LC-MS methods described above are rather slow (from 7 min to 30 min per samples), other mass spectrometry approaches can overcome this limitation. Building on our expertise in high throughput mass spectrometry approaches for biomarker screening in the food industry (where we realized the quantitative analysis of biomolecules in 2000 samples per hour using an Echo-MS) we are convinced that it is possible to scale up these methods and have started to profile SARS-CoV-2 specific peptides with Echo-MS (Figure 6). Still with a high throughput of samples, it should be noted that the time from sampling to obtain a result in such high throughput MS/MS approaches of highly selective methods based on SARS-COV-2 protein analysis are limited by the amount of time required for the digestion of proteins (from about 10 min to several hours, although there are also methods reporting digestion of proteins in only a few minutes).

First results

We have been working on reproducing the LC-MS/MS results using a QqQ MS detector in collaboration with Maarten Dhaenens, using targeted MS/MS profiling of SARS-CoV-2 selective peptides. We mainly focussed on evaluating to which degree we can increase the throughput. Therefore our strategy was to apply solvent precipitation and extraction, trypsin-digestion at accelerated conditions for 10 min, and subsequently, SPE clean-up and direct analysis with electrospray-MS/MS analysed using a Sciex qTRAP 6500. Peptides of NCAP and SPIKE protein are monitored, and a human protein included for quality control purposes.

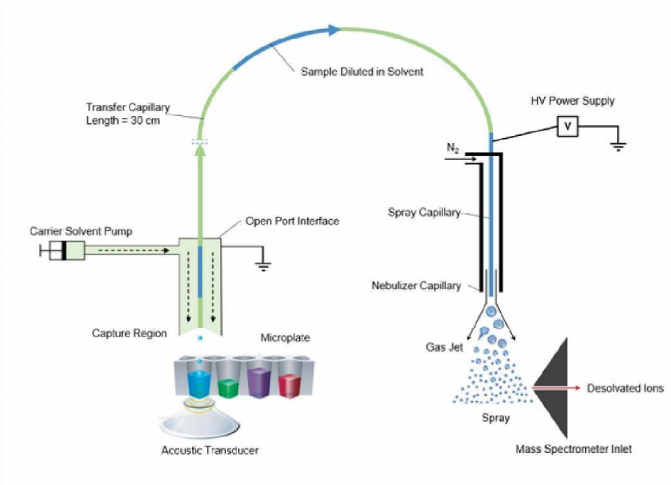

Figure 6. Echo-MS/MS system using acoustic injection into an open port interface and electrospray ionization.

Preliminary results with Echo-MS

We have an ECHO-MS platform (Sciex) in house which we have had available for nearly a year to support the evaluation and development of applications for high-throughput mass spectrometry applications. We have therefore evaluated the capability of the ECHO-MS platform for peptide quantification using bovine serum albumin (BSA) as reference. From our preliminary tests, we find that the intensity and sensitivity of the ECHO-MS/MS was similar to conventional LC-MS/MS

analysis for the analysis of a BSA digest, with LOD/LOQ at a concentration level down to few fmol/microlitre at a throughput of 3 seconds per sample (Figure 7).

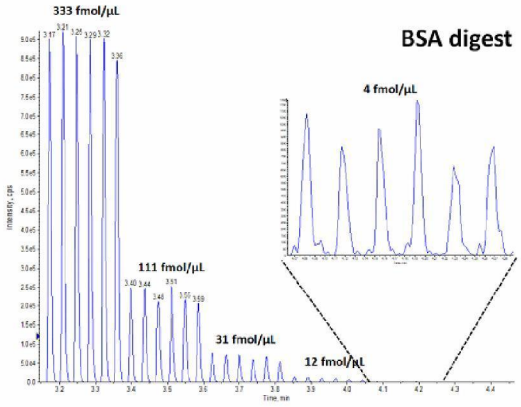

Figure 7. Echo-MS/MS of peptide of BSA digest. Each ' peak' represents one sample, and in this analysis one

The second step of our evaluation was to test recombinant protein of SARS-COV-2. After fast digestion (15 min) of the NCAP protein, we were able detect the NCAP protein. We roughly estimate the limit of detection of this protein equivalent to BSA (Figure 8).

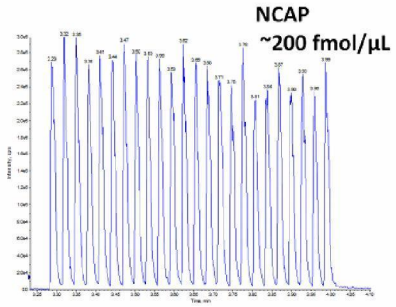

Figure 8. Echo-MS/MS of KQQTVT LLPAADLDDFSK peptide obtained from digestion of NCAP protein.

Currently we are optimizing the sample preparation and analysis workflow for ECHO-MS/MS and evaluate the novel 7500 Sciex detector to further increase the sensitivity. If the sensitivity is not enough, we will use rapid LC-MS/MS. We therefore expect to detect 10,000-100,000 virus particles at least in dry swabs.

Expected performance of integrated method comprising DESI-HRMS and MS/MS of peptides

The method will be validated with a second dry swab sampled at Amsterdam RAI as proposed in the protocol 'Rapid mass spectrometry-based COVID-19 viral test' submitted for METC approval at Erasmus MC.

Response time

The negative result in estimated 90% of the cases will be available in principle within a minute following the sampling procedure using DESI-HRMS only. For the other cases, the result will be available within 20-25 minutes.

Such a rapid test would allow to provide rapid feedback to the passengers, still before boarding, or leaving the airport and travelling home.

Diagnostic accuracy

The recall rate of first tier testing is expected to be 10% (i.e., requiring measurement with Instrument II as second tier) at <1% false negative rate. The diagnostic accuracy of the second tier testing is expected to be >95% using nasopharyngeal PCR as a reference method.

Expected throughput

The initial throughput of the integrated method is estimated to be at least 200 samples/hour. We expect to be able to increase the throughput to more than 1000 samples/hour within 2-4 weeks after installation, which may require more than one DESI-MS system in the set-up. The costs per sample are estimated to be 5-8 Euro/sample, and on the long-term the price per sample is expected to be lower.

References

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2. Ihling, C. *et al.* Mass Spectrometric Identification of SARS-CoV-2 Proteins from Gargle Solution Samples of COVID-19 Patients. *J. Proteome Res.* 0–3 (2020) doi:10.1021/acs.jproteome.0c00280.

3. Bezstarosti, K., Lamers, M. M., Haagmans, B. L. & Demmers, J. A. A. TARGETED PROTEOMICS FOR THE DETECTION OF SARS-COV-2 PROTEINS. *bioRxiv* (2020).

4. Lane, C. S., Puyvelde, B. Van, Uytfanghe, K. Van & Dhaenens, M. *Targeted assay for quantification of proteins from the SARS- CoV-2 coronavirus (Sciex application note).*