Onderzoeks- en implementatieplan, Peptide-MS platform voor SARS-CoV-2 analyse

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Hoofdonderzoeker:

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Dit project wordt uitgevoerd in samenwerking met:

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Inhoudsopgave

Samenvatting

What?

Vanaf juli 2020 is het LACDR actief met de ontwikkeling van een 'dubbele' assay voor SARS-CoV-2-analyse op basis van massaspectrometrie (MS), een Direct MS-analyse gevolgd door een Peptide-MS analyse. Gezien de tijdsdruk is het onderzoek nu gefocust op het tweede 'gevoelige en specifieke' deel van de assay, op basis van Peptide-MS selectief SARS-CoV-2 peptiden te meten. Het onderzoek heeft tot de volgende resultaten geleid:

- Onderzoeksopzet is positief ontvangen in de VWS werkgroep 'Innovatieve testen'.
- ZonMw heeft op basis van het validatierapport het advies 'voldoende' afgegeven.
- Het OMT heeft aangegeven dat de methode potentie heeft maar heeft behoefte aan een omvangrijkere studie met meer (SARS-CoV-2 positieve) samples.

So What?

MS-Covid analyse kan voor een deel voorzien in de huidige acute behoefte aan 'sensitieve' sneltesten. Testen op basis van massaspectrometrie biedt:

- Een sneltestmethode op basis van 'nieuwe' technologie die een goede aanvulling is op bestaande testmethoden.
- De technologie biedt:
    - Hoge throughput,
    - Goedkope verbruiksartikelen en lage personele bezetting in lab,
    - Schaalbaar,
    - Ongevoelig voor virale mutaties,
    - De mogelijkheid mutaties te signaleren,
    - Een 'self-learning' model,
    - Flexibel; kan aangepast worden bij het LACDR om b.v. bepaalde mutaties te meten of andere virussen te detecteren.
- Uitbreiding van huidige sneltestcapaciteit, minder afhankelijk van bestaande sneltestcapaciteit.
- Potentie om eerste assay verder te ontwikkelen met 'direct MS' (analysetijd 10 seconden per sample).
- Voordelig pakket, goed verkrijgbare verbruiksartikelen.
- Hardware goed verkrijgbaar.
- Kan voorzien in de behoefte naar extra sensitieve sneltestcapaciteit t.b.v. spoor 2a.

De huidige assay dient verder onderzocht en ontwikkeld te worden op gebied van:

- Uitbreiding klinische validatie:
  De onderzoeksvraag en -opzet hiervoor is beschreven in hoofdstuk 1.
- Opschaling:
  Bouwen en automatiseren van onderzoeksfaciliteit op LACDR en tegelijkertijd een productiefaciliteit in Amsterdam. Benodigde set-up van hardware en ontwerpen zijn gereed. Automatiseringsplan is gemaakt (zie hoofdstuk 2).
- Zo spoedig mogelijke uitbreiding naar andere monsters zoals speeksel, mondspoeling of mid-turbinate neusuitstrijkjes.

Now What?

Gezien de acute behoefte wordt voorgesteld simultaan een onderzoekslocatie bij het LACDR in te richten en parallel te starten met de bouw van een productiefaciliteit op locatie. De onderzoeksfaciliteit op het LACDR en de productiefaciliteit zullen nauw verbonden zijn. Uitkomsten

van onderzoek naar implementatie worden direct geïmplementeerd in de facilitaire productiefaciliteit.

Verwacht wordt dat extra onderzoeksresultaten het OMT zullen overtuigen, waarna de eerste productiefaciliteit in gebruik kan worden genomen.

Om bovenstaande plannen te kunnen verwezenlijken is financiering benodigd.

Financieringsbehoefte (x EUR 1.000):

Ontwikkeling automatisering en software	5.1.2b
Validatiestudie	
Inrichten en implementatie LACDR	
Facilitaire productielocatie (incl. 6mnd huur)	
Totaal financieringsbehoefte	

Vermelde bedragen zijn exclusief BTW

Kosten en tijdslijn:

- Kosten extra productielocaties
- Verwachte kosten per analyse
- Tijdslijn

Kosten opvolgende productielocatie (300 analyses per uur)

Facilitair lab 6mnd	5.1.2b
Hardware assay	
Totaal verwacht per opvolgende locatie	

Vermelde bedragen zijn exclusief BTW

Kosten per analyse (verbruiksartikelen en hardware)

Afnamebuizen met stok	5.1.2b
96-well 5.1.2b - p/s	
Ezymen (Trypsin)	
SPE Column	
Solvent & tips	
UPLC Column 5.1.2b	
Afschrijving hardware en huur productielocatie (6mnd, 2 shifts, 7 dagen, rest 25%)	
Kosten per analyse	

Vermelde bedragen zijn exclusief BTW

Tijdslijn

Onderzoeksfaciliteit LACDR gereed	10 maart 2021
Productiefaciliteit gereed	20 maart 2021
Oordeel OMT	30 maart 2021
Eerste fase automatisering gereed	4 april 2021
Tweede fase automatisering gereed	2 mei 2021
Start volle productie	6 mei 2021

Appendix 1 -Field evaluation of Peptide-MS method for SARS-CoV-2 testing

Aim: The aim is to evaluate the performance of the Peptide-MS rapid test in a large number (>2500 individuals, at least 200 positive tested cases) of symptomatic and asymptomatic individuals with RT-qPCR test as the reference.

Primary objective:

- Determine the sensitivity, specificity and number of predicted positive and negative samples of the Peptide-MS test (as index test) using PCR as reference standard.

Secondary objective

- To determine diagnostic characteristics (sensitivity, specificity) in individuals with and without COVID-19 like symptoms at time of testing.
- To correlate Peptide-MS result with amount of virus (viral load as determined by RNA), and if possible, infectivity (ability to grow in culture medium).
- To determine the number of participants who initially tested negative (RT-PCR) but who were tested positive for SARS-CoV-2 within 10 days of the initial test (if possible). For this follow up calls by GGD Amsterdam will be conducted. Alternatively, an app can be used for this if available.
- Assess critical operational parameters of this test before further implementation.

Study Design: Cross-sectional cohort study to compare the index test (Peptide-MS) with the reference test (RT-qPCR).

Study population: It is proposed to use a cross-sectional study with measurement by the index test (Peptide-MS) and the reference test (PCR). This cohort can comprise individuals at age of 18 and older who (i) enter a test street, or (ii) are requested to be tested by test and trace program ('GGD bron- en contactonderzoek'). Individuals participating will be asked to report symptoms at the time of entering the test street. If participants agree they will be called by GGD to determine whether individuals developed the disease and which symptoms.

Main study endpoints: The sensitivity, specificity and of the Peptide-MS test (as index test) will be determined using PCR as reference standard. The number of positive and negative predicted cases will be reported.

PCR reference standard test: The reference standard test can be RT-PCR or LAMP test. In case that the LAMP test is used, positive samples will be analyzed by RT-qPCR to obtain a quantitative measure. To determine viral load, the lab measuring RT-qPCR at a pre-defined platform (e.g. InBiome) will be requested to provide a calibration curve to allow to convert Ct-values into viral loads (measured by RNA) that can be compared between laboratories. If possible, some positive samples will be grown in culture medium to determine infectivity.

Evaluation criteria: We have defined the evaluation criteria as follows:

1. A sensitivity higher than 95% for Ct values up to 30
2. A specificity of at least 98%
3. A lead time of maximum 60 min (meaning the time from the moment the sample reaches the lab towards a validated result)
4. A scalable test, meaning it can be easily scaled to perform thousands of tests per day.

Evaluation of additional samples

We will include the evaluation of the use of the Peptide-MS method for other sample types such saliva, mouth flush or mid-turbinate nasal swab sampling if available.

If a longitudinal cohort of students or 16+ children at school or other individuals is available, we will include samples of this study. The goal is to determine the correlation between RNA level and protein level for the testing.

Appendix 2 - Plan for implementation of Peptide-MS for large-scale testing in the Netherlands

We will describe here how we will implement the Peptide-MS method at the GGD Amsterdam for the evaluation study and make it ready for large-scale testing in the Netherlands in the near future. For further implementation, automation of the workflow is critical. We have already made important steps towards a fully automated workflow and have made a plan with PAA for the automation for the workflow. LACDR has worked with PAA (Peak Analysis & Automation (www.paa-automation.com) before.

Currently, the transfer of samples and some sample processing steps are carried out by lab technicians and/or researchers. We propose to optimize and integrate the workflow (see Figure 1) at (1) the lab at LACDR/Leiden University and (2) a lab established at the GGD Amsterdam test street (location RAI). In parallel, PAA will work on the automation of all steps, and will transfer parts ready for implementation first to Leiden to validate its performance. Upon good performance the automation will be integrated at the lab in the test street at the GGD Amsterdam. We will integrate and automate in steps: one part will be already automated and carried out by a robot, the steps not automated yet will be carried out by a technician. In this manner we expect that 3-4 weeks after approval of this proposal we can start to set up a first workflow at the GGD test street at the RAI, and that this workflow will be fully operational 6 weeks after approval of this proposal. This will allow a full roll-out of the Peptide-MS test 8 weeks after approval of this proposal.

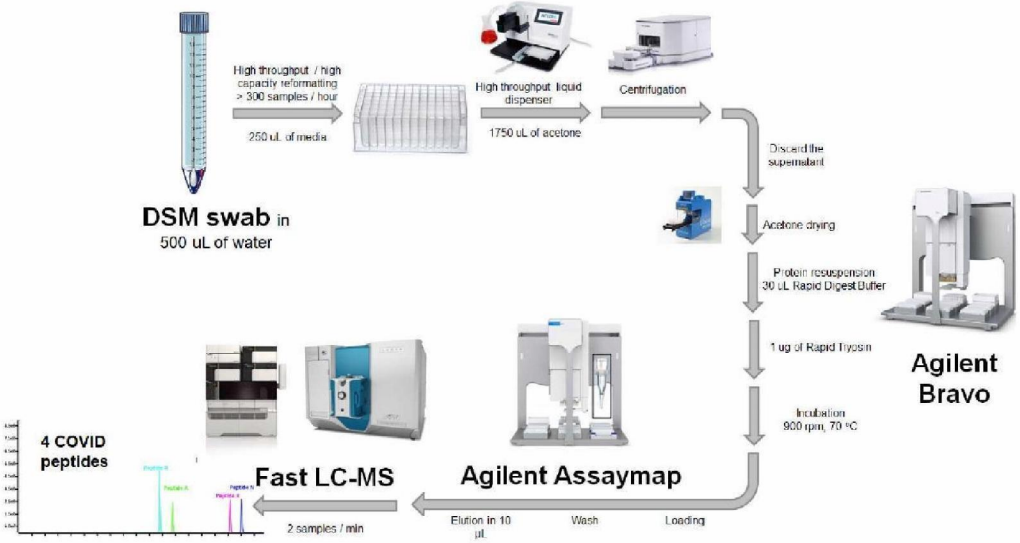

Figure 1. Workflow of Peptide-MS method

Initially it is necessary to transfer the swab manually into a compatible well plate, all other liquid handling steps will be automated to a large extent using an Agilent Bravo robot.

For the peptide digestion procedure, an Agilent Bravo robot will be used to process plates of 96 samples. The Bravo will be able to do liquid transfers, heating and shaking and will be coupled to several external accessories including an evaporation unit and a centrifuge via an external robot arm as part of the Peptide-MS platform. In previous projects, we have shown that the wash station on the Bravo allowed the tips to be reused many times to keep costs and wastes low. Using a second Agilent Bravo robot including the AssayMap will allow sample enrichment using SPE in a parallel manner. This

approach is able to purify the target peptides in parallel in a 96 well plate format. SPE columns are reusable so do not increase the costs significantly. In the first phase, transfer of 96 well plates between the Bravo and the autosampler on the LC-MS will be manual. The throughput of the LC-MS will be increased through method optimization and hardware improvements. The time needed to detect peptides using the LC-MS method will be reduced to half a minute by using a two-column system that can wash and re-equilibrate one column while using the other for measurement. With the set-up currently planned one sample preparation platform can process about 300 samples/hour. Subsequent MS analysis will be about 100 samples per hour per mass spectrometer; for a throughput of 300 samples/hour three mass spectrometers are required. In our preliminary experiments, no loss of sensitivity was seen when using the faster LC-MS method of 30 sec.

Another approach to increase the throughput of Peptide-MS is to use acoustic injection of the peptides after SPE using the Agilent Bravo robotic sample preparation platform. Acoustic injection coupled to mass spectrometry is able to reduce sample measurement time to less than 6 seconds per sample with no carryover between samples. When coupling the very selective MS protocol with the SPE enrichment of target peptides, we anticipate to measure 8,000-12,000 samples/day by one acoustic injection MS instrument (Echo-MS). We will test this method, and if it proves reliable, we will use acoustic injection rather than LC as introduction system into the MS system.

After testing the high throughput method in Leiden, three weeks after approval of this proposal the workflow will be set-up as prototype at the lab at the GGD Amsterdam, for further validation and implementation, and evaluation of its robustness. The Peptide-MS sample handling platform will include one Bravo liquid handling robot, one Bravo robot including an SPE unit (AssayMap) and several instrumentations of the automated workflow. The platform handles the samples to a HT LC or a Sciex ECHO and a Sciex 7500 Qtrap mass spectrometer (Figure 2). This workflow will not be fully automated at the beginning (as discussed above), and a technician will be responsible for the transfer of well plates from one unit to the next.

Parallel to the development of the workflow for the GGD and transferring it to the lab at the GGD, the high throughput method will be further optimized and automated at the LACDR at Leiden University. Issues encountered at the GGD will be addressed using the prototype workflow at LACDR.

During this period, the automation of the initial sample reformatting steps will be finalized, and the transfer of well plates will be automated. Proper sample tracking software that could interface with the GGD tracking system will be developed. Improvements will be transferred to the prototype at the GGD lab. After six weeks we expect a partly (70%) automated workflow, and after 8 weeks or at the latest after 10 weeks a nearly fully automated workflow. This workflow can be then copied to other labs. The final automated Peptide-MS workflow will be enclosed in a biological safety cabinet (Fig. 2)

Figure 2. Scheme of automated HT peptide-MS workflow. The full enclosure in a biological safety cabinet (S-CEL4000 with HEPA filter) is shown in insert.

Appendix 3 - Required investment for described implementation

To integrate and automate the workflow, we plan to have one automated workflow being built at PAA, one prototype at LACDR to optimize and automate the Peptide-MS method, and one prototype lab at a teststreet to run samples collected at the GGD or somewhere else. For this we propose the following investments:

1. Automation of the workflow by PAA as integrator including (ca. 5.1.2b):
   a. Design of rapid opening of vials, extraction and transfer to 96 well plate using one robot.
   b. Transport of well plates from and towards one unit to another unit (see workflow Figure 1); for this at least two robot arms will be used for loading the centrifuge, Bravo robot, and LC-MS or Echo MS.
2. Development of software for integration of whole workflow and analysis of data (ca. 5.1.2b)
3. Set up workflow at LACDR/Leiden University for optimization and further development of platform:
   4. Robot, centrifuge, evaporation unit, and other parts for sample preparation (ca. 5.1.2b)
   5. HT LC system (Shimadzu or Agilent; 5.1.2b) and 7500 Qtrap MS (Sciex) (5.1.2b) or Echo MS system (ca. 5.1.2b)
6. Set up prototype at GGD comprising:
   7. Sample preparation workflow comprising robot, centrifuge, evaporation unit (ca. 5.1.2b)
   8. HT LC system (Shimadzu; 5.1.2b) and 7500 Qtrap MS (Sciex) (ca. 5.1.2b or Echo MS system (ca. 5.1.2b)

In addition, costs for personnel are estimated to be 5.1.2b , for chemicals consumables and reagents 250 k€ and 250k€ for other lab facilities. The throughput of the GGD lab will be 300 samples/hour.

Table 1. Overview of estimated costs for implementation study (excl. VAT)

Partner	Item	Costs (k€)
PAA	Automation (personnel etc)	5.1.2b
PAA or 3rd party	Software	5.1.2b
LACDR/Leiden University	Robotic reformatting	5.1.2b
	Agilent Bravo	
	Agilent Bravo Assaymap	
	Tube/plate barcode reader/labeller	
	Robot framework (with table, robot arm, biosafety enclosure)	
	2 LC-MS[$] or Echo-MS	
	Personnel[#]	
	Chemicals & reagents	
GGD lab	Robot reformatting	
	Agilent Bravo	
	Agilent Bravo Assaymap	
	Tube/plate barcode reader/labeller	
	Robot framework (with table, robot arm, biosafety enclosure)	
	2 LC-MS or Echo-MS	
	2 Technicians for 6 months	
	Chemicals & reagents	
	Other lab facilities and rental	

[#] 2 LC-MS experts, 1 automation expert, 2 technician and IT/data expert for 6 months

[$] If LC-MS is used rather than ECHO-MS, one system can be transferred to RAI to increase throughput during implementation.

Appendix 4 – Timeline and deliverables

Below the timeline of the activities at the LACDR and at the lab at the GGD Amsterdam (location RAI is planned) are described, as well as the deliverables of this project and their timeline.

Timeline of activities

Table 2. Planning of activities

Week	LACDR	GGD mobile lab	
1	Ordering all parts for Peptide-MS method at LACDR	Ordering all parts for Peptide-MS lab	
2	Set-up HT lab for LACDR workflow and assemble workflow for GGD prior to transfer to GGD	Start collecting samples at GGD (for validation at LACDR)	
3	40% of sample preparation workflow automated	Set-up lab at GGD	
4	Throughput of LC-MS close to 100 samples/min. Start of small validation with new workflow.	Set-up Peptide-MS method at GGD lab	
5	Implement parts for automation as delivered by PAA (automation provider)	Optimize and analytically validate Peptide-MS workflow at GGD lab	
6	70% of sample preparation workflow automated	Peptide-MS method is validated at RAI; start of validation study	
8	90 % of sample preparation workflow automated	Finish of validation study (if enough samples are available). Implementation of automation at set-up at RAI; ready for roll out	
10	100% of sample preparation workflow automated, problems encountered addressed	Fully automated method can be enrolled, also last manual steps have been eliminated	

Deliverables:

1. Report of intermediate validation at LACDR (week 5)
2. Report of full validation (week 8)
3. Report on expected costs per sample for large-scale implementation in the Netherlands (week 7)
4. Report of other sample types (saliva, mouth flush, mid-turbinate nasal swabs if samples are available; week 8)

Appendix 5 - Costs per sample

Supplies and instrumentation

The supplies and instrumentation are described here below:

- Peptide-MS uses widely available devices. No devices or consumables used in the Peptide-MS assay are used for PCR or LAMP.
- Peptide-MS is expected to run flawlessly on a Sciex Qtrap MS, which is readily available.
- We expect that the lab analysis can be fully automated, further increasing speed and further reducing the need for specialised personnel.
- The tables below list required reagents, consumables and instrumentation and some key suppliers.

Table 3. Required supplies and instrumentation for large-scale implementation (excl. VAT)

Reagents and consumables	Suppliers	Typical costs
swabs	DSM	5.1.2b
96 well plates	Fisher Scientific AB-1127	5.1.2b for 96 samples
Trypsin	Promega Rapid digestion kit	5.1.2b each
SPE column	Agilent	5.1.2b each cartridge (may vary between 0.6-4)
Solvents & tips	Divers	5.1.2b per sample
UPLC column	Waters	5.1.2b for 4000 runs

For Peptide-MS, the consumable costs per sample are estimated at 5.1.2b per sample:

- The costs for enzymes and chemicals are circa 5.1.2b per sample.
- The SPE columns used by the Agilent Assaymap can be re-used. We do not yet know how often we can re-use them, which is one of the reasons that we cannot yet specify the exact costs per sample. Based on recent experience EUR 1.2- per run is reasonable.
- Other consumables as well plates, vials, tips and solvents are circa 5.1.2b per sample, depending on the conditions that can be agreed upon with suppliers.
- The chromatography supplies are circa € 5.1.2b per sample.

Promega is the chosen supplier of trypsin because they have heat-stable enzymes allowing for much faster digestion. Promega has confirmed they can provide the requested enzyme at scale and will custom pack it to fit our needs.

Table 4. Required instrumentation for 1 workflow allowing 300 samples/hour (excl. VAT)

Instrumentation	Suppliers	Typical costs
Liquid handler	Agilent Bravo	5.1.2b – 1 per workflow
Liquid handling including SPE unit	Agilent Bravo Assaymap	5.1.2b – 1 per workflow
Robotic reformatting	PAA	5.1.2b – 1 workflow
Centrifuge	Hettich	5.1.2b - 1 per workflow
Tube / plate Barcode reader / labeller	Agilent	5.1.2b 1 per workflow
HT LC system	Shimadzu	5.1.2b - 3 per workflow
Mass spectrometer	Sciex 7500 QTrap	5.1.2b – 3 per workflow
Robot framework (with table, robotic arms and biosafety enclosure)	PAA	5.1.2b – 1 workflow

As mentioned above, LACDR will test whether after sample preparation acoustic injection-MS is an option. In that case the total investment for Mass spectrometry is about 900kE and does not require an HT LC system.

Depreciation costs of production facilities are estimated to be 5.1.2b Euro/sample, assuming depreciation over 6 months, 2 shifts (15 hours), 7 days a week, estimated residual value 25% on hardware.

Appendix 6 – Outlook on implementation and up-scaling

The MS-based diagnostic testing platform is very cost-efficient, scalable, and complementary to existing testing platforms in the Netherlands. The method is insensitive for virus-mutations. LACDR will further improve the throughput and cost efficiency of the method.

Next to SARS-CoV-2 diagnostics the MS-based platform can also contribute to monitoring the efficiency and effect of the current COVID-19 vaccination program.

We will further develop the MS-based SARS-CoV-2 diagnostic testing platform in the next months:

- We will finetune Direct-MS to monitor for presence of viral infections. Direct-MS can identify the fingerprint of viruses by detecting metabolites and lipids that are the result of viral hijacking, immune responses to the virus, or from the viral particles, directly acquired from the nasopharyngeal swabs using e.g. Desorption ElectroSpray Ionization (DESI)- high resolution (HR)MS or direct infusion mass spectrometry after a straightforward extraction. The two methods are complementary and could also possibly be combined as Direct-MS is extremely fast (after optimisation down to 1-2 minutes) and cost-efficient while Peptide-MS is very specific and rapid but not as ultra-fast (after optimisation down to about 60 minutes from sample to result).
- We will explore how to include monitoring of relevant mutations using the Peptide-MS method.
- We will explore whether we can predict disease severity or infectivity (if proper metadata are available).

The MS-based platform can be fast adapted to include e.g. specific mutations or additional viruses.