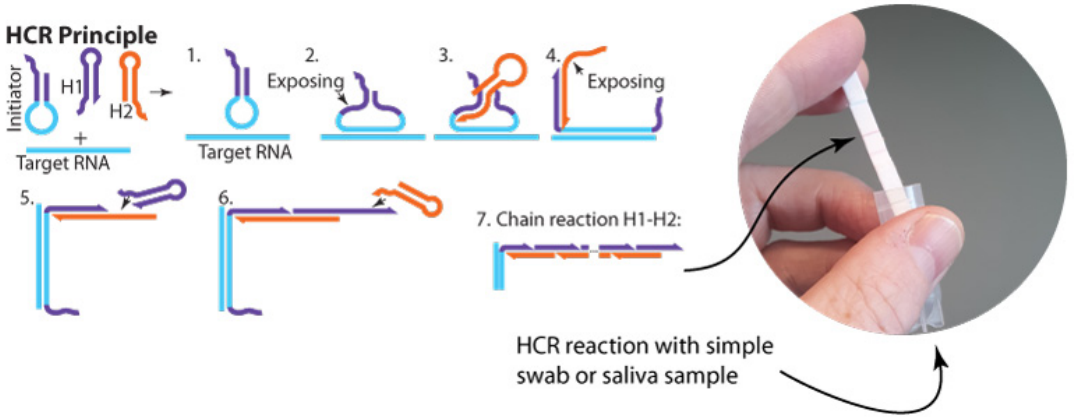
Simple and cheap remote test for infection based on HCR

SciLifeLab

Principal Investigators: Björn Högberg, Björn Reinius (Karolinska Institutet)
Program research area: Diagnostics for virus
Collaborators:

May 2020

- The aim of the project is to develop a simple method for self-testing with a sensitivity high enough for simple self-isolation decisions.
- We can now detect viral RNA using this approach in the lab. Currently we are collecting patient samples and working on the home-test setting.
- The method uses HCR (Hybridization Chain Reaction) to create long DNA polymers only in the presence of the target DNA. These long DNA can then be detected using an LFA (Lateral Flow Assay)-strip similar to a pregnancy test.



RCA-based COVID-19 diagnostics (RCA-COVID-DIA)

SciLifeLab

Principal Investigators: Prof. Mats Nilsson (Stockholm University)
Program research area: COVID-19 diagnostics
Participants: Felix Neumann; Hao Zhe Lee; Erik Samuelsson; Ruben R. G. Soares; Nicola Crosetto; Mats Nilsson

May 2020

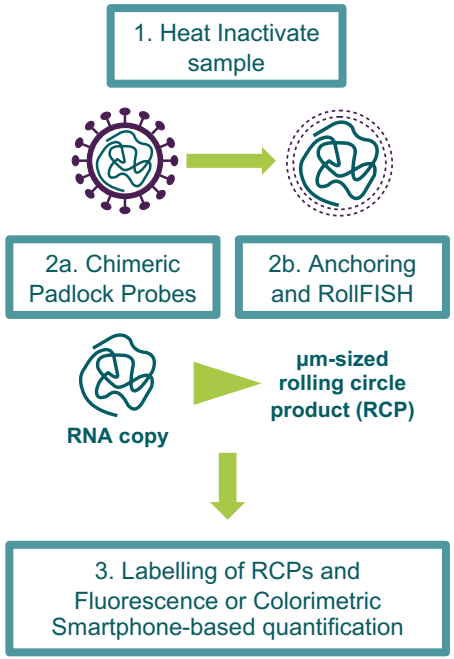
Project Goal

Development of a novel COVID-19 diagnostic method based on rolling circle amplification (RCA) to detect SARS-CoV-2 RNA **directly from a heat inactivated biological sample** without the need of RNA extraction and reverse transcription, and read out by the naked eye, mobile-phone, or simple microscope

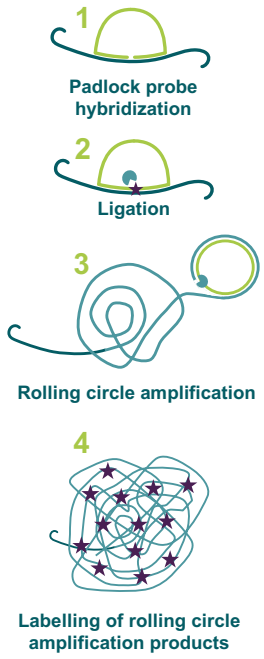
Impact

Provide a rapid COVID-19 viral load test which is **simple**, **scalable** and **amenable to miniaturization in a point-of-care test**, allowing an effective response to **present and future pandemic situations**

Our strategy

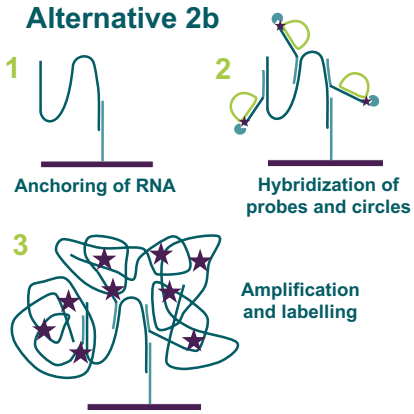


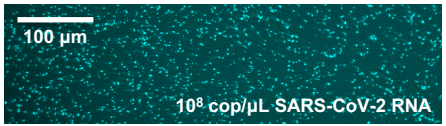
Alternative 2a



Methods and Results

Alternative 2b





Detection of amplification products using, e.g., a mobile phone.

iLACO-Sweden: RT-LAMP based SARS-CoV-2 detection.

Principal Investigators: Vicent Pelechano (SciLifeLab – Karolinska Institutet)
Program research area: Diagnostic of virus.
Collaborators: Rebecca Howard (SU), Tomas Nyman(KI), Gustaf Sandg(KH), Xiushan Yin(Shenyang), Simon Elässer(KI), Björn Reinius(KI), Björn Högberg(KI)

May 2020

Aim of project and impact on society

Achieved:

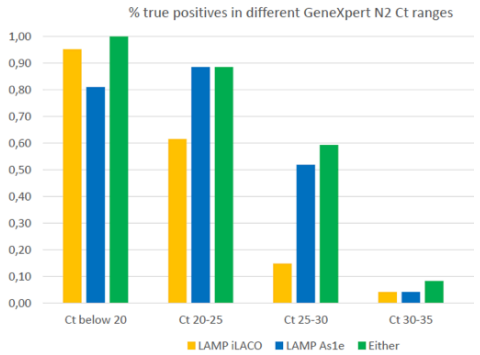
- ✓ Develop and optimize in-house reagents to be independent of supply chain. (<10SEK/sample)
- ✓ Optimize detection conditions and benchmarking
- ✓ Improve direct test of patient samples.

Next steps:

- Further improve sensitivity
- Expand to direct saliva tests

Results and conclusions

- We have produced an optimized reagents for 100K+ test (RT-MashUP + v5.9/v716)
- Benchmark with RT-qPCR in 200+ patients
- Purified RNA: very good
- Raw sample: good for medium/high viral load.

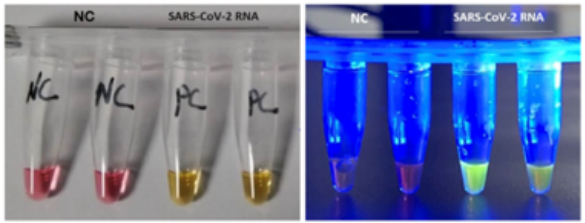


Method

Isothermal amplification (RT-LAMP)

- 5 min heat inactivation (95°C)
- 20-40 min RT-LAMP reaction

Colorimetric or fluorescent detection



Yu *et al.* Clinical Chemistry 2020

Alekseenko*, Barret*, Pareja-Sanchez* *et al.* in preparation

Extraction-free high-sensitive RT assay for SARS-CoV-2 RNA detection

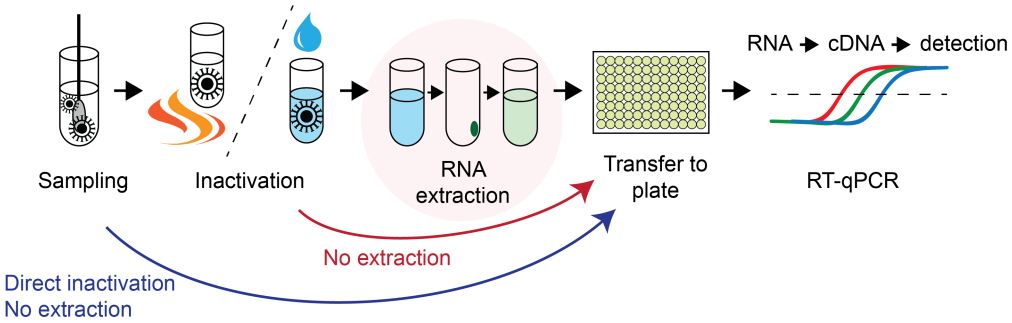
SciLifeLab

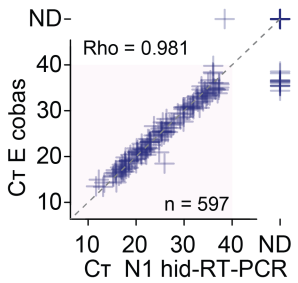
Principal Investigators: Björn Reinius (Karolinska Institutet, MBB) www.reiniuslab.com
Program research area: Diagnostics for virus
Collaborators: Björn Högberg (KI, MBB), Jan Albert (KI, MTC), Antonio Gigliotti Rothfuchs (KI, MTC)

June 2020

- **Aim of project and impact on society:** RNA extraction is a major bottleneck in current COVID-19 testing in terms of turn-around, logistics, component availability and cost, which delays or completely precludes COVID-19 diagnostics in many settings. Efforts to simplify the current methods are critical, as increased diagnostic availability and efficiency would benefit patient care and infection control. We aimed to develop **a rapid, sensitive and affordable RNA-extraction-free RT-PCR based method for COVID-19 testing**, which could aid the expansion of testing.

- **Results and conclusions:** Through a joint collaborative effort across academic and clinical departments **we developed the SARS-CoV-2 heat-inactivated direct RT-PCR protocol**. The method has been cross-validated using ~600 clinically diagnosed patient samples and standardized diagnostics. The method is ready for use and is already employed at various sites around the world. The protocol is publicly available as a preprint on **medRxiv.org** and the scientific manuscript is in end-phase of external review.
- **Article: Massive and rapid COVID-19 testing is feasible by extraction-free SARS-CoV-2 RT-PCR, Smyrlaki et al. 2020.**





Alternative test design for high-throughput SAR-CoV-19 screening

SciLifeLab

Principal Investigators: Prof. Masood Kamali-Moghaddam, Uppsala University
Program research area: Diagnostics for virus
Collaborators: Dr. L. Löf, UU; Dr. P. Kharaziha, KI; Dr. B. Torabi, Sepanteck AB; Dr. M. Hammond, SciLifeLab PLA & Single Cell Proteomics Facility

- The aim of this project is to establish a platform for detection of SAR-CoV-19 in different body fluids using advanced molecular tools based on padlock technology.
- A successful outcome will allow detection of the virus with high sensitivity and accuracy in thousands of samples per day, with a great potential impact on healthcare, to remove the current pressure.
- Two different test designs, a) targeting directly the viral RNA and b) targeting the cDNA from the virus, with a set of padlocks are currently tested on synthetic and purified SAR-CoV-19 RNA. The best performing design(s) will subsequently be evaluated in patient samples.