LAMP, COVID19, POC

LAMP - Loop-mediated isothermal amplification

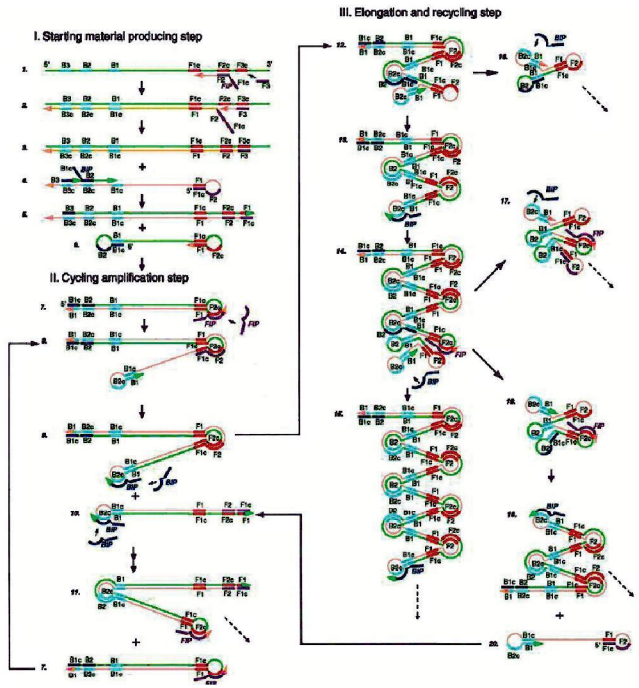
- Single-tube technique for the amplification of DNA
- Alternative for (RT-)qPCR detection
- Reverse Transcription LAMP (RT-LAMP) for RNA detection

(RT)-LAMP

- **Why ->** Proposal ZonMW based on LAMP
- **What is it** -> Technique, pros and cons
- **Where is it used** -> Applications, including SARSCOV2 detection
- **Proposal ->** objectives, role RIVM
- **Possibilities** -> Future applications POC

LAMP - technique

- Amplification at a constant temperature of 60–65 °C
- 10^9 copies DNA/hour, amount produced much higher than in PCR
- Polymerase with high strand displacement replication activity
- Typically, 4 different primers to amplify 6 distinct regions on the target gene (increases specificity)
- Detection of turbidity (pyrophosphate, amplification byproduct, precipitation) or fluorescence (intercalating dyes). Endpoint or real-time, which allows quantification.

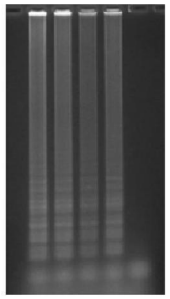


1. Forward Inner Primer (FIP):
2. Forward Outer Primer (FOP):
3. Backward Inner Primer (BIP):
4. Backward Outer Primer (BOP):

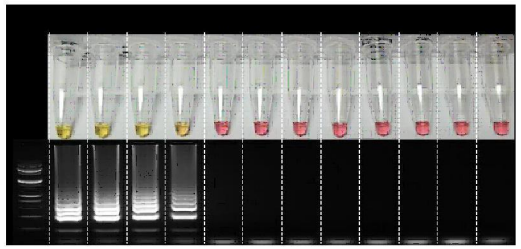
uses 4-6 primers recognizing 6-8 distinct regions of target DNA. A strand-displacing DNA polymerase initiates synthesis and 2 of the primers form loop structures to facilitate subsequent rounds of amplification.

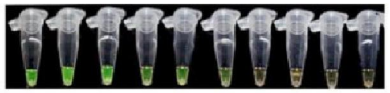
LAMP - technique

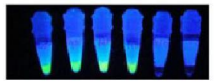
- Detection



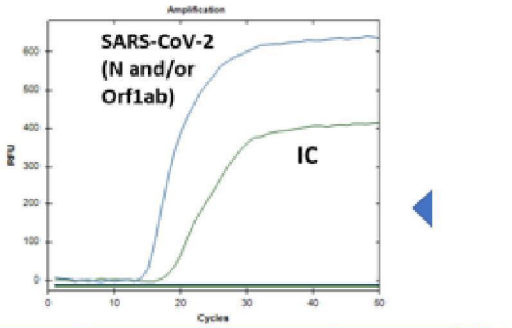
Gel electrophores is with typical ladder like pattern (positive indicated by 1-4, negative indicated 5)







Naked eye observation using DNA fluorescence SYBR Green I dye.



LAMP - advantages

- Simplicity in application, ruggedness, and low cost (particularly wrt equipment: isothermal)
- Potential as a simple screening assay in the field or at the point of care (POC). Infectious disease diagnosis in low and middle income countries.
- Less sensitive (more resistant) than PCR to inhibitors in complex samples. Successful detection of pathogens from minimally processed samples such as heat-treated blood or in presence of clinical sample matrices.
- Uses different enzymes than PCR, e.g. DNA polymerase typically *Bst* instead of *Taq. Relevant for shortages such as now for SARSCOV2 detection*

LAMP - limitations

- Useful primarily as detection technique.

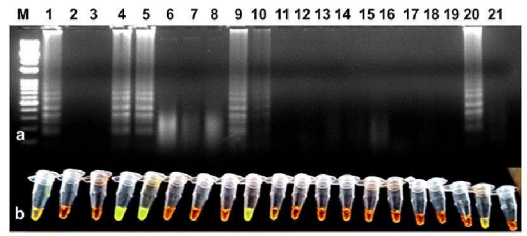
  Less versatile than PCR and not useful for other molecular biology applications such as cloning.

- Primer design difficult. Less freedom to choose the target site than with PCR
  - 4 (or 6) primers targeting 6 (or 8) regions within a fairly small segment of the genome, and primer design is subject to numerous constraints
  - choosing appropriate target can be difficult (e.g., a conserved site in a highly variable viral genome, or common genes in different bacterial strains). Multiple degenerated sequences may be required to cover the different variants, and such a cocktail of primers can produce non-specific amplification in the late amplification.

LAMP - limitations

- Multiplexing difficult. The larger number of primers per target increases the likelihood of primer-primer interactions for multiplexed target sets.
  - The LAMP product is a characteristic "ladder" on a gel, not a single PCR band. Therefore identity of a target cannot be confirmed by size of a band. Real-time multiplexing approaches based on fluorescence are possible yet complex

- False-positives may result from
  - carryover contamination due to the extremely high efficiency of the reactions.
  - primer-dimer amplification, which cannot be checked by performing melt curve analysis (if endpoint detection is used; may be distinguished In real-time detection)



LAMP applications

Succcessful detection of infectious diseases such as tuberculosis, malaria, and sleeping sickness.

Examples of its application:

- Mycobacterium tuberculosis - (Mitarai et al., 2011).
- Food-borne Staphylococcus strains
- Aquaculture pathogens
- Influenza virus - clinical results similar to real-time RTPCR (Nakauchi et al., 2011).
- Zika virus
- SARSCOV-2
  - Bhadra, S. et al (2020). High-surety isothermal amplification and detection of SARS-CoV-2, including with crude enzymes. BioRxiv,
  - Lamb, L. E., et al. (2020). Rapid Detection of Novel Coronavirus (COVID19) by Reverse Transcription-Loop-Mediated Isothermal Amplification. SSRN Electronic Journal.
  - Schmid-burgk, et al. (2020). LAMP-Seq : Population-Scale COVID-19 Diagnostics Using a Compressed Barcode Space.
  - Yu, L. et al. (2020). Rapid colorimetric detection of COVID-19 coronavirus using a reverse tran-scriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic plat-form: iLACO. MedRxiv, 2020.02.20.20025874.
  - Zhang, Y. (2020). Rapid Molecular Detection of SARS-CoV-2 (COVID-19) Virus RNA Using Colorimetric LAMP. MedRxiv, 2, 2020.02.26.20028373.
  - And more

LAMP applications: SARS-COV-2

Features

- Many of the kits contain three assays, targeting the **Orf1** gene (human RNA polymerase protein), the **N**-gene (the nucleocapsid protein) and the **E**-gene (envelope protein).
- Multiple targets decrease false-negatives due to virus mutations
- An increasing number of the products have received Emergency Use Authorisation (EUA) from the FDA

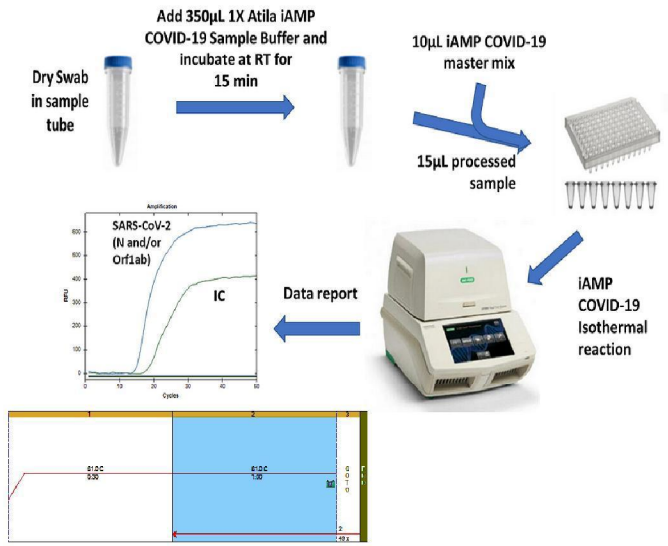
LAMP applications: SARS-COV-2

Features

- Nasal, nasopharyngeal (NP), and oropharyngeal (OP) swabs
- Detection SARS-CoV-2 RNA directly from samples without prior RNA extraction (lysisbuffer at RT for 15')
- Sample–to–result ~90 minutes
- Multiplex by using fluorescence resonance energy transfer (FRET) probes
  - FAM detection of 2 SARSCOV2 genes (N and orf1ab),
  - HEX detection of human gene (Gapdh)
- FDA approved

iAMP® COVID-19 Detection Kit (Atila Biosystems)

workflow



LAMP applications: SARS-COV-2

Validation

➢LOD 4000 cps/ml

RTqPCR:

Abbott ~100, Biofire ~330, CDCassay 300-1000

BUT in the LAMP protocol, the sample preparation "front end" processes a much larger quantity of sample and therefore delivers more viral RNA to the LAMP reaction.

SARS-CoV-2 - Tentative LoD: SARS-CoV-2 RNA spiked into negative oropharyngeal swab

Target Level	Valid results	SARS-CoV-2 (N/ORF) Positive		SARS-CoV-2 (N/ORF) Detection Rate	Internal Control Positive		Internal control Detection Rate
		n	Mean Ct		n	Mean Ct	
0.4 cp/µL	5	4	18.55	80%	5	24.73	100%
4 cp/ µL	5	5	16.18	100%	1	35.31	20%
40 cp/ µL	5	5	13.87	100%	0	NA	0%
2 cp/ µL	5	4	20.35	80%	5	25.4	100%
4 cp/ µL	5	5	19.84	100%	3	32.6	60%
6 cp/ µL	5	5	17.14	100%	0	NA	0%
Negative	10	0		0%	10	23.5	100%

Tentative LoD: 4 cp/µL [lowest target level demonstrating >95% detection rate of SARS-COV-2]

SARS-CoV-2 - Confirmatory LoD: Pseudovirus spiked into negative oropharyngeal swab specimen

Target Level	Valid results	SARS-CoV-2 (N/ORF) Positive		SARS-CoV-2 (N/ORF) Detection Rate	Internal Control Positive		Internal control Detection Rate
		n	Mean Ct		n	Mean Ct	
4 cp/µL	20	20	16.87	100%	14	25.77	70%

LAMP applications: SARS-COV-2

Validation

➢Inclusivity

primer-annealing regions Orf1ab and N 100% match to all SARS-CoV-2 genomes (n=154 as of March 21, 2020).

➢Cross-Reactivity

In silico: no oligos with homology ≥ 80% with the organisms listed

In vitro: purified DNA/RNA spiked into negative oropharyngeal swabs at 10^5 genome copies/µL

in silico
Human coronavirus 229E
Human coronavirus OC43
Human coronavirus HKU1
Human coronavirus NL63
SARS-coronavirus B093
MERS-coronavirus NL140422
Human Metapneumovirus (hMPV) isolate 00-1
Parainfluenza virus 1 isolate NM001
Parainfluenza virus 2 isolate VIROAF10
Parainfluenza virus 3 strain HPIV3/AUS/3/2007
Parainfluenza virus 4a isolate HPIV4_DK(459)
Parainfluenza virus 4b strain 04-13
Influenza B B/Illinois/13/2005 (segment 7)
Enterovirus 68 isolate NZ-2010-541
Respiratory syncytial virus
Human Rhinovirus B3 strain SC2606
Chlamydia pneumoniae TW-183
Bordetella pertussis strain B3921
Pseudomonas aeruginosa UCBPP-PA14
Streptococcus salivarius CCHSS3

Organisms wet-tested in swab matrix	SARS-CoV-2 (N/ORF) Detection Rate
Adenovirus (ATCC VR-1516)	0% (0/3)
Influenza A (H1N1)	0% (0/3)
Haemophilus influenzae	0% (0/3)
Legionella pneumophila	0% (0/3)
Mycobacterium tuberculosis	0% (0/3)
Streptococcus pneumoniae	0% (0/3)
Streptococcus pyogenes	0% (0/3)
Mycoplasma pneumoniae	0% (0/3)
Pneumocystis jirovecii (PJP)	0% (0/3)
Candida albicans	0% (0/3)

LAMP applications: SARS-COV-2

Validation

- Endogenous Interference Substances Studies

SARS-CoV-2 RNA spiked into negative oropharyngeal swabs at 2.5X LOD (10 copies/µL sample).

Potentially interfering substances added at the indicated concentration to positive and negative swabs

Potential Interfering Substance	Conc.	Positive Samples	Negative Samples
Mucin: bovine submaxillary gland, type I-S	2.5 mg/ml	3/3	0/3
Blood (human)	2.5% v/v	3/3	0/3
Afrin Original nasal spray	15% v/v	3/3	0/3
Basic Care allergy relief nasal spray (Gluococorticoid)	5% v/v	3/3	0/3
NeilMed Nasal gel	1.25%	3/3	0/3
GoodSense All Day Allergy, Cetirizine HCl Tablets 10 mg	1mg/mL	3/3	0/3
Cepacol Sore Throat (benzocaine/menthol lozenges)	5 mg/mL	3/3	0/3
Zanamivir	3.3 mg/mL	3/3	0/3
Tamiflu	2.2 ìg/mL	3/3	0/3
Mupirocin ointment	5mg/mL	3/3	0/3
tobramycin	4ug/mL	3/3	0/3

ZonMW proposal

- uitvraag op verzoek van VWS.
- Partners: TNO, RIVM, DSM, LUMC/UMCG, Integrated DNA Technologies, Inc. (IDT)
- Duration 6 months

Initial plan (last week)

1. Protocol dry swabs – lysisbuffer - LAMP detection, validated for **safety** and performance
2. Implementation in clinical laboratory and clinical validation
3. Production LAMP enzymes (DSM)
4. **Exploration additional sample types** (saliva, stool, fomites,wastewater, air)
5. Exploration LAMP-Seq and **POC applications**
6. Plan for implementation

ZonMW proposal

OMT specification (Friday 8/5)

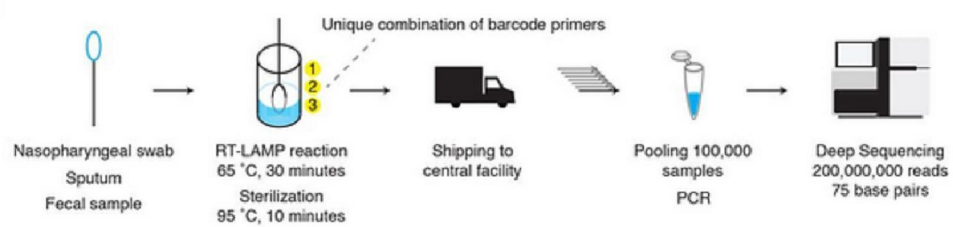
- Focus on additional value for goal up-scaling to 70,000 tests/day
- No POC (arguments: data integration, notifyable disease, safety)
- Interest in LAMP seq.

Final plan

1. Protocol swabs – lysisbuffer - LAMP detection, validated for **safety** and performance
2. Implementation in clinical laboratory
3. Production LAMP enzymes (DSM)
4. **Exploration additional sample types** (saliva, stool, fomites, wastewater, air)
5. Exploration LAMP-Seq
6. Plan for implementation

LAMP-Seq

Population-scale testing



LAMP-Seq: Population-Scale COVID-19 Diagnostics Using a Compressed Barcode Space. (10)(2e) L. Schmid-Burgk *et al.* 2020 bioRxiv

Single heating step for each individual sample, followed by pooled processing, parallelizable deep sequencing

Simple thermal protocol for individual samples and pooling many samples prior to resource-intensive steps -> , requirement for specialized reagents, equipment, and labor is greatly reduced as compared to established RT-qPCR protocols.

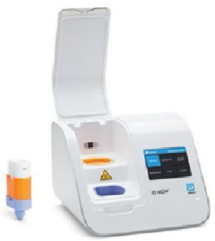
Estimated cost per sample would be < 7 USD, scalable to hundreds of thousands of samples per day per sequencing facility.

POC – Future possibilities

- PCR disadvantages: complex equipment, sample prep important due to sensitive to inhibitors in sample, slow

- LAMP may provide an inexpensive, robust and rapid alternative. Importantly, also for use in developing countries that do not have access to high tech laboratories
- Validation for different sample types important

example



ID NOW™ COVID-19

- Positive results may be detected in as little as 5 minutes. Negative results in 13 minutes
- RdRp gene