Apr 23, 2020 Version 3

A protocol for rapid detection of the 2019 novel coronavirus SARS-CoV-2 using CRISPR diagnostics: SARS-CoV-2 DETECTR V.3

DOI
dx.doi.org/10.17504/protocols.io.bfhcjj2w

Mammoth Biosciences[1], James P. Broughton[2], Wayne Deng[3,4], Clare L. Fasching[2], Jasmeet Singh[2], Charles Y. Chiu[3,4,5], Janice S. Chen[2]

[1][Mammoth Biosciences, Inc., South San Francisco, California, USA];
[2]Mammoth Biosciences, Inc., South San Francisco, California, USA;
[3]Department of Laboratory Medicine, University of California, San Francisco, California, USA;
[4]UCSF-Abbott Viral Diagnostics and Discovery Center, San Francisco, California, USA;
[5]Department of Medicine, Division of Infectious Diseases, University of California, San Francisco, California, USA

Coronavirus Method De...

Mammoth Biosciences

DOI: https://dx.doi.org/10.17504/protocols.io.bfhcjj2w

External link: https://mammoth.bio/2020/02/15/white-paper-a-protocol-for-rapid-detection-of-sars-cov-2-using-crispr-sars-cov-2-detectr/

Protocol Citation: Mammoth Biosciences, James P. Broughton, Wayne Deng, Clare L. Fasching, Jasmeet Singh, Charles Y. Chiu, Janice S. Chen 2020. A protocol for rapid detection of the 2019 novel coronavirus SARS-CoV-2 using CRISPR diagnostics: SARS-CoV-2 DETECTR. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bfhcjj2w

Protocol status: Working
We use this protocol and it's working

Created: April 23, 2020

Last Modified: April 23, 2020

Protocol Integer ID: 36100

Keywords: coronavirus, SARS, SARS-CoC-2, CRISPR, SARS-CoV-2 DETECTR, using crispr diagnostic, novel coronavirus sar, crispr diagnostic, fidelity crispr detection enzyme, coronavirus strain detection, novel coronavirus, coronavirus, cdc sar, crispr, differentiate sar, rapid detection, sar, detectr assay, schematic of detectr assay, transmitting sar, outbreak, available in cdc, pcr diagnostic panel, us centers for disease control,

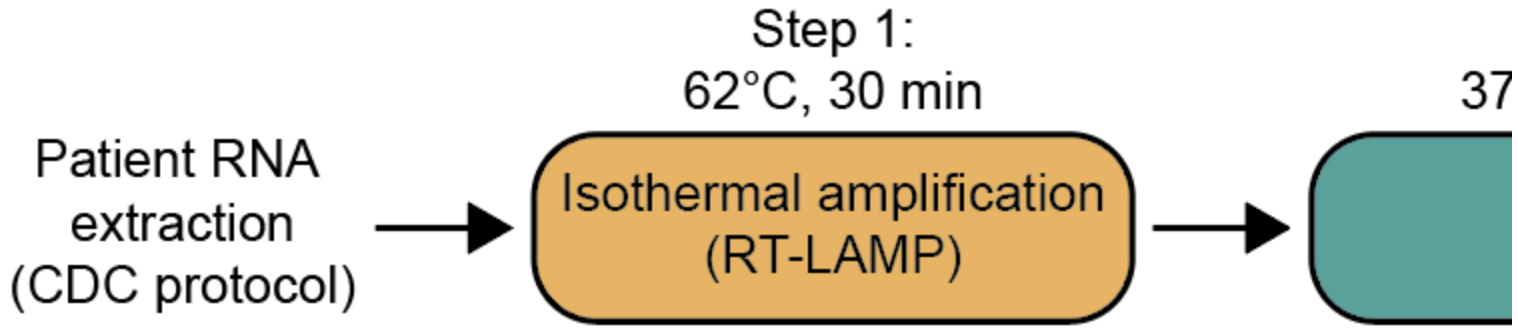
Abstract

DISCLAIMER: This protocol has not been approved by the FDA and should not be used as a clinical diagnostic

Introduction

Given the global health emergency, rapid transmission, and severe respiratory disease associated with the outbreak of the 2019 novel coronavirus (SARS-CoV-2), Mammoth Biosciences has reconfigured our DETECTR platform to rapidly and accurately detect SARS-CoV-2 using a visual lateral flow strip format within 30 minutes from sample to result. To ensure specificity of detection, we selected a high-fidelity CRISPR detection enzyme and designed sets of gRNAs that can either 1) differentiate SARS-CoV-2 or 2) provide multi-coronavirus strain detection. SARS-CoV-2 DETECTR couples CRISPR detection with isothermal pre-amplification using primers based on protocols validated by the US Centers for Disease Control and Prevention (CDC) and World Health Organization (WHO). Currently in the United States, the CDC SARS-CoV-2 real-time RT-PCR diagnostic panel has a laboratory turnaround time of approximately 4-6 hours, with results that can be delayed for >24 hours after sample collection due to shipping requirements. In addition, these tests are only available in CDC-designated public health laboratories certified to perform high-complexity testing.

Mammoth is working to enable point of care testing (POCT) solutions that can be deployed in areas at greatest risk of transmitting SARS-CoV-2 infection, including airports, emergency departments, and local community hospitals, particularly in low-resource countries. Leveraging an “off-the-shelf” strategy to enable practical solutions within a short time frame, we describe here a protocol that is fast (<30 min), practical (available immediately from international suppliers), and validated using contrived samples.



	Specifications	
	Targets	• N-gene (SARS-CoV

		-2 specific) ● E-gene (SARS-CoV, bat-SARS-like-CoV, and SARS-CoV-2 coronaviruses) ● RNase P (human sample control)
	Limit of detection	10 copies/µl input

Table 1: SARS-CoV-2 DETECTR assay workflow and specifications.

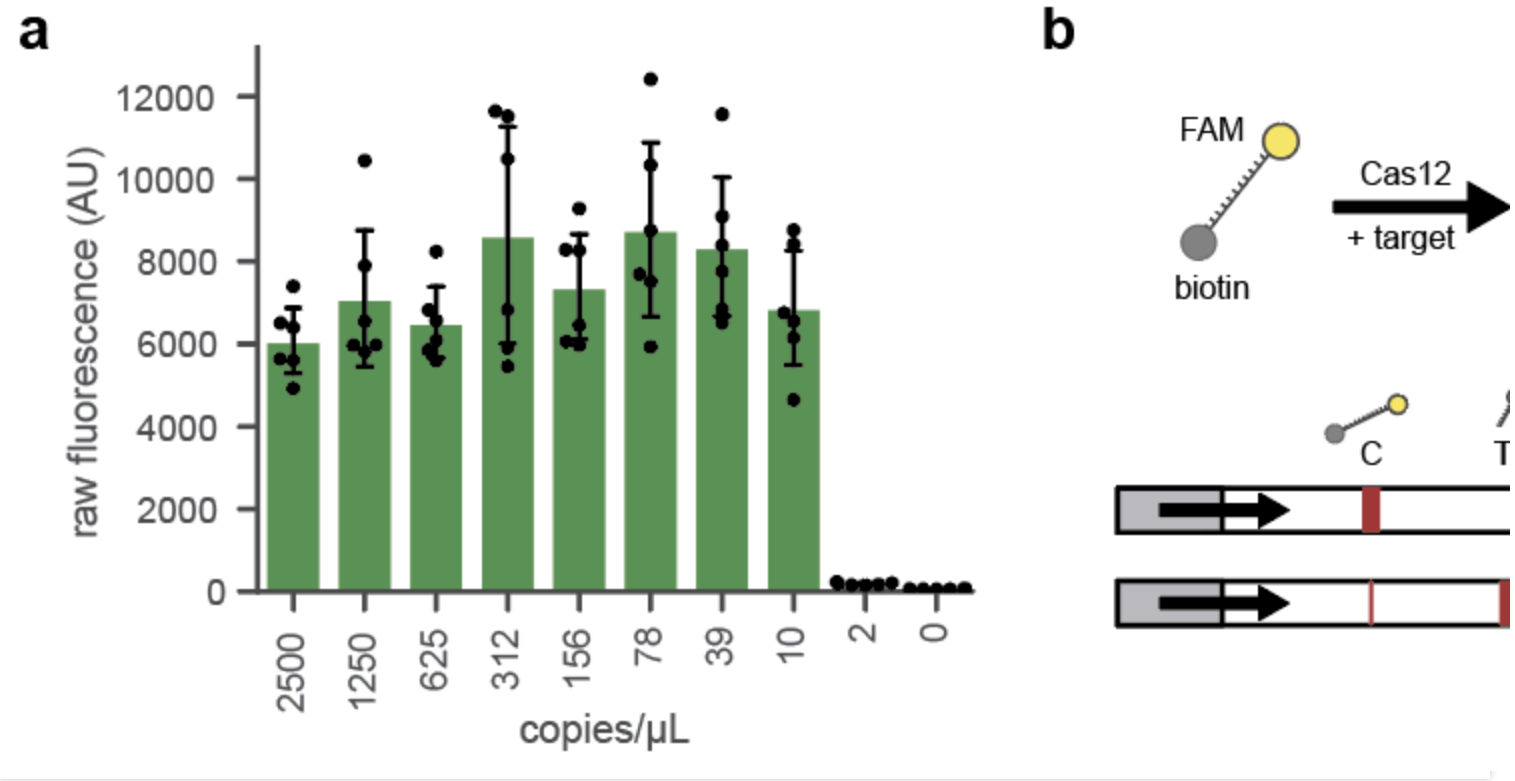


Figure 1 | a) SARS-CoV-2 DETECTR has a limit of detection (n=6) of 10 copies per µl input. **b)** Schematic of DETECTR assay coupled to lateral flow strip. **c)** Representative lateral flow results for the assay shown for 0 copies per µl and 10 copies per µl

Acknowledgements: We thank Vikram Joshi, Nefeli Tsaloglou and Xin Miao for advice and helpful discussions in the preparation of this whitepaper.

Conflicts of Interest: JPB, CLF, JS and JSC are employees of Mammoth Biosciences, CYC is on the Scientific Advisory Board of Mammoth Biosciences, and JSC is a co-founder of Mammoth Biosciences. JPB, CLF, JS, CYC and JSC are co-inventors on CRISPR-related technologies.

Attachments

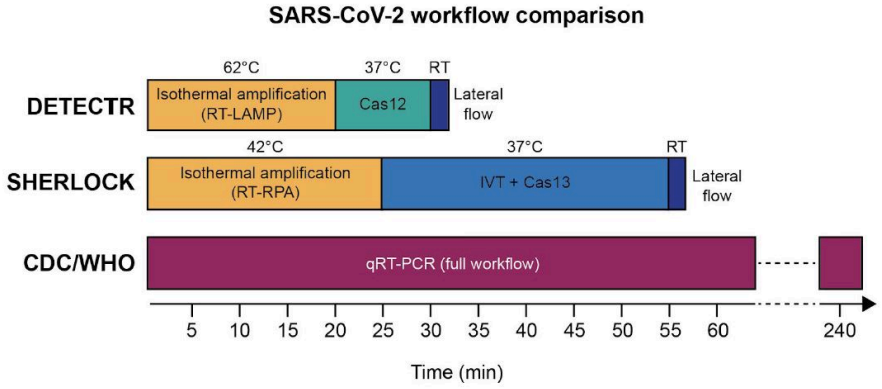
SARS-CoV-2.pdf 603KB

A protocol for rapid... 793KB

Guidelines

Appendix

While we were preparing this whitepaper, another **protocol for SARS-CoV-2 detection using CRISPR diagnostics (SHERLOCK, v.20200214)** was published. We compare the assay workflows and specifications between CRISPR diagnostics and established CDC/WHO protocols below. (Note: as of this publication, CRISPR diagnostics workflows have not yet been approved by the FDA)



Appendix Figure 1: Comparison of SARS-CoV-2 assay workflows for DETECTR, SHERLOCK, and CDC/WHO

		SARS-CoV-2 DETECTR	SARS-CoV-2 SHERLOCK	CDC SARS-CoV2 qRT-PCR
	Target	N gene & E gene (N gene gRNA compatible with CDC N2 amplicon, E gene compatible with WHO protocol)	S gene & Orf1ab gene	N-gene (3 amplicons)
	Sample control	RNase P	None	RNase P
	Limit of Detection	70-300 copies/µl input	10-100 copies/µl input	1 copy/µL input
	Assay reaction time	~30 min	~60 min	~45-60 minutes

	Assay components	RT-LAMP (62 °C, 290 min), Cas12 (37 °C, 10 min), Lateral flow (RT, 2 min)	RT-RPA (42 °C, 25 min), IVT + Cas13 (37 °C, 30 min), Lateral flow (RT, 2 min)	UDG digestion (25 °C, 2 min), reverse transcription (50 °C, 15 min), denature (95 °C, 2 min), amplification (95 °C, 3 min; 55 °C 30 sec; 45 cycles)
	Heavy instrumentation required	No	No	Yes
	FDA EUA approval	No	No	Yes

Appendix Tavle 1: Comparison of SARS-CoV-2 specifications for CRISPR diagnostic protocols to the current CDC assay.

Materials

STEP MATERIALS

WarmStart LAMP Kit (DNA and RNA) - 100 rxns **New England Biolabs Catalog #**E1700S

-

SARS-CoV-2 DETECTR Reagents

Step 1: Isothermal amplification (62°C, 20 min)

- RT-LAMP Master Mix (Supplier: NEB)

WarmStart LAMP Kit (DNA and RNA) - 100 rxns **New England Biolabs Catalog #**E1700S

- **DNA oligos (Supplier: IDT)**

- Primer sequences:

	Name	**Sequence (5′ → 3′)**
	N-gene F3	AACACAAGCTTTCGGCAG
	N-gene B3	GAAATTTGGATCTTTGTCATCC
	N-gene FIP	TGCGGCCAATGTTTGTAATCAGCCAAGGAAATTTTGGGGAC

	N-gene BIP	CGCATTGGCATGGAAGTCACTTTGATGGCACCTGTGTAG
	N-gene LF	TTCCTTGTCTGATTAGTTC
	N-gene LB	ACCTTCGGGAACGTGGTT
	E-gene F3	CCGACGACGACTACTAGC
	E-gene B3	AGAGTAAACGTAAAAGAAGGTT
	E-gene FIP	ACCTGTCTCTTCCGAAACGAATTTGTAAGCACAAGCTGATG
	E-gen	CTAGCC

	e BIP	ATC CTT ACT GCG CTA CTC ACG TTA ACA ATA TTG CA
	E-gene LF	TCG ATT GTG TGC GTA CTG C
	E-gene LB	TGA GTA CAT AAG TTC GTA C
	RNaseP POP7 F3*	TTG ATG AGC TGG AGC CA
	RNaseP POP7 B3*	CAC CCT CAA TGC AGA GTC
	RNaseP POP7 FIP*	GTG TGA CCC TGA AGA CTC GGT TTT AGC CAC TGA CTC GGA TC

	RNaseP POP7 BIP*	CCT CCG TGA TAT GGC TCT TCG TTT TTT TCT TAC ATG GCT CTG GTC
	RNaseP POP7 LF*	ATG TGG ATG GCT GAG TTG TT
	RNaseP POP7 LB*	CAT GCT GAG TAC TGG ACC TC

* RNaseP POP7 primers published in Curtis et al. , (2018) .

Citation

Curtis KA, Morrison D, Rudolph DL, Shankar A, Bloomfield LSP, Switzer WM, Owen SM (2018) . A multiplexed RT-LAMP assay for detection of group M HIV-1 in plasma or whole blood.. Journal of virological methods.

https://doi.org/10.1016/j.jviromet.2018.02.012 LINK

Step 2: Cas12 detection (37°C, 10 min)

- LbCas12a (Supplier: NEB)

EnGen Lba Cas12a (Cpf1) - 70 pmol **New England Biolabs Catalog #**M0653S

- **crRNA (Supplier: Synthego)**

-
- Reporter (Supplier: IDT)

	Name	Sequence (5′ → 3′)
	N gene gRNA (SARS-CoV-2 specific)	UAAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUCAGCGUUC
	E gene gRNA (pan-coronavirus)	UAAUUUCUACUAAGUGUAGAUGUGGUAUUCUUGCUAGUUAC
	RNase P gRNA (Sample control)	UAAUUUCUACUAAGUGUAGAUGACCUGCGAGCGGGUUCUGA

	Reporter	/56-FAM/TTATTATT/3Bio/

Step 3: Lateral flow (RT, 2 min)

Milenia HybriDetect 1 lateral flow strips (Supplier: TwistDx

Minimum sample equipment


(2)
Benchmark
37.0°C OK
SET
myBLOCK
Mini Dry Bath
(1)
Nalgene

Sample Equipment

- Pipette tips
- 37 °C heat block
- 62°C heat block
- Microcentrifuge
- Eppendorf tubes
- Pipettes
- Lateral flow strips
- Sample collection device (nasopharyngeal swab)
- Timer

Troubleshooting

Safety warnings

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Before start

Amplicon Contamindation Risk Mitigation

Three approaches of decreasing the risk of amplicon contamination include physical (using hoods), chemical (using DNAzap) and procedural (workflow, PPE, GLP) strategies. We recommend three levels of control: 1. Separate locations for pre- and post-amplification activities, 2. Separate environmental control between the rooms and 3. Separate equipment and personnel. This standard operating procedure (SOP) will provide detailed guidelines that will minimize the risk of amplicon contamination.

Directional workflow (Personnel, reagents, equipment)

The ideal workflow moves from the pre-amp to the post-amp area.

Personnel:

1. One-way directional workflow is critical to minimize carrying amplicons to less contaminated and amplicon-free (pre-amp room) zones.
2. If possible, operators should exclusively work in the pre-amp or post-amp room on any given shift.
3. If only one operator is available, it is recommended that the operator do all amplification reactions first, then proceed to the DETECTR reactions.
4. Proper PPE should be worn to minimize amplicon movement on clothing.
5. Immediately don a disposable lab coat when entering the pre-amp room or prior to working in the post-amp area and dispose of the lab coat prior to leaving the pre-amp room.
6. Immediately don a disposable lab coat when entering the post-amp room or prior to working in the post-amp area and dispose of the lab coat prior to leaving the post-amp room.

Reagents:

1. Ensure your reagents also have a one-way directional flow from receiving/main lab to the pre-amp room.
2. Reconstitute all oligos in the pre-amp room.
3. Make aliquots of primers and reagents to keep in the pre-amp freezer.
4. Amplification reagents may be stored in the pre-amp room in the 4oC fridge or the -20oC freezer.

Equipment:

1. If possible use dead air boxes or hoods for pre-amp and post-amp processes.
2. All equipment found in the pre-amp stays in the pre-amp area.
3. All equipment found in the post-amp stays in the post-amp area.

Prepare nucleic acid sample and CRISPR reagents

1 Extract patient RNA following **CDC recommendations**.

2 Prepare *LbCas12a RNP complexes* for the samples to be tested. **One** complex for N-gene, E-gene, and RNase P gRNAs is needed **for each sample**.

	Reagent	*Volume*	*Final Concentration*
	Nuclease-free water	15.75 µl	
	10X NEB uffer 2.1	2 µl	1X
	1 µM LbCas12a	1 µl	50 nM
	1 µM gRNA	1.25 µl	62.5 nM
	TOTAL VOLUME	**20 µl**	

3 Incubate LbCas12a with gRNA to generate RNP complexes for 00:30:00 at 37 °C .

4 Add *reporter substrate* to final concentration of [M] 500 nanomolar (nM) .

5 Place reactions On ice until ready to proceed.

Note

Complexes are stable at 4°C for at least 24 hours.

Run DETECTR reaction

6 On ice , prepare **three** *RT-LAMP reactions*, **one each** for N-gene, E-gene, and RNase P primer sets:

	Reagent	*Volume*	*Final Concentration*
	10X Isothermal Amplification Buffer (NEB)	2.5 µl	
	100 mM MgSO 4 (NEB)	1.13 µl	6.5 mM (4.5 mM added, 2 mM in 1X IsoAmp Buffer)
	10 mM dNTPs (NEB)	3.5 µl	1.4 mM
	10X Primer Mix	2.5 µl	0.2 µM F3 / 0.2 µM B3 /

			1.6 µM FIP / 1.6 µM BIP / 0.8 µM LF / 0.8 µM LB
	Bst 2.0 polymerase (NEB)	1 µl	8 units / rxn
	Warmstart RTx (NEB)	0.5 µl	7.5 units / rxn
	Nuclease-free water	3.87 µl	
	Nucleic acid sample	5 µl	
	TOTAL VOLUME	**25 µl**	

7 Incubate at 62 °C for 00:30:00 .

Note

Note: Use precaution when opening amplification tubes to prevent amplicon contamination.

8 Combine 2 µL of the *RT-LAMP reaction* with 18 µL of the *LbCas12a RNP complex* with the appropriate gRNA.

9 Add 80 µL 1X NEBuffer 2.1 .

10 Incubate at 37 °C for 00:10:00 .

11 Insert *Milenia HybriDetect 1 (TwistDx) lateral flow strip* directly into reaction.

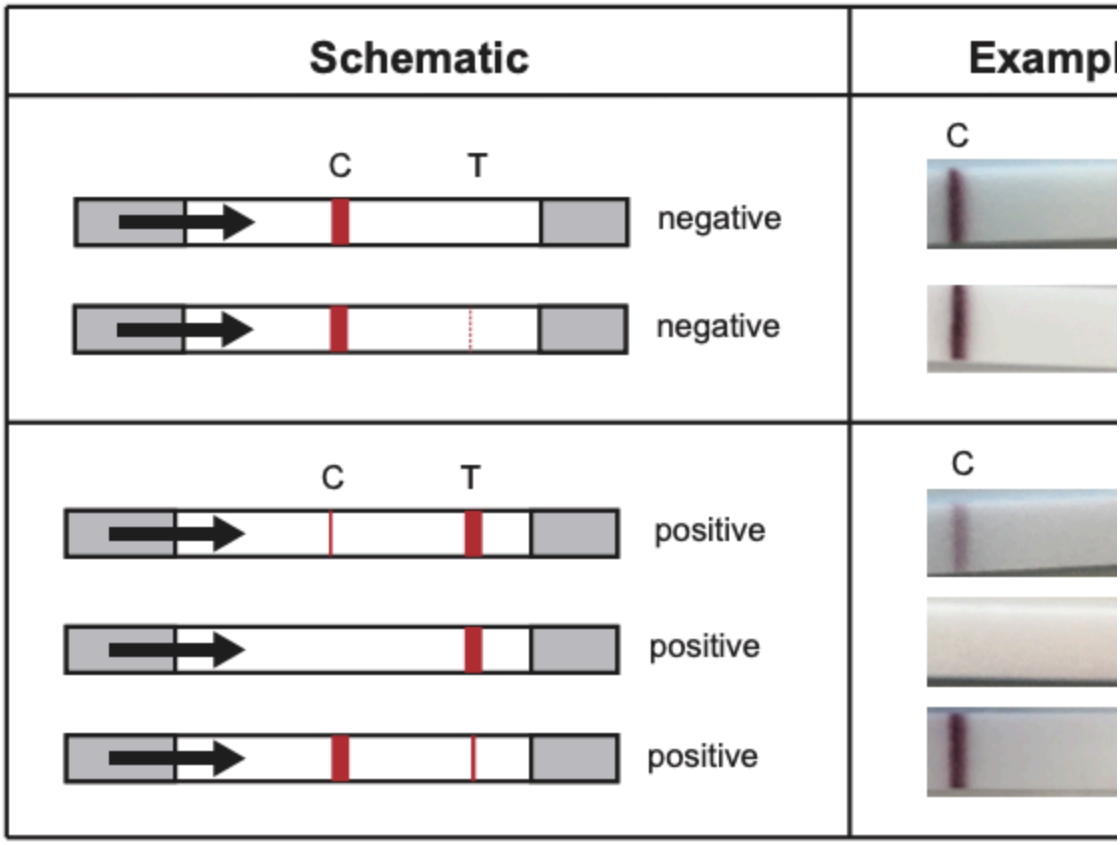
12 Allow the lateral flow strip to run for 00:02:00 at Room temperature and observe
the result.

Test interpretation

13

Note

Note: The *line closest to the sample pad* is the *control line* and the line that appears farthest from the sample pad is the *test line* (see Figure 1). A sample with complete cleavage of the reporter molecule may appear to have no signal at the control line.



	N-gene	E-gene	RNase P	Interpretation
	+	+	+/-	SARS-CoV-2 positive
	+	-	+/-	Indeterminate
	-	+	+/-	Indeterminate

	-	-	+	SARS-CoV-2 negative
	-	-	-	QC failure

Citations

Curtis KA, Morrison D, Rudolph DL, Shankar A, Bloomfield LSP, Switzer WM, Owen SM. A multiplexed RT-LAMP assay for detection of group M HIV-1 in plasma or whole blood.
https://doi.org/10.1016/j.jviromet.2018.02.012