Mobile Diagnostics for Emerging Diseases moving to the point of need.

9th Infectious Diseases and Clinical Microbiology Speciality Society of Turkey
International Scientific Platform
May 20-23rd 2021

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https://mweidmann.com/

- Mobile real time PCR
- Mobile RPA
- Development of the lab disc (POC)
- RPA home test (PON)

Real-Time PCR for EID

Bunyaviridae

Orthobunyavirus
- Germiston
- CaliforniaEncephalitis
- Jamestown Canyon
- LaCrosse
- SnowshoeHare
- Guaroa
- Tahyna
- Inkoo
- Oropouche
- Batai

Nairovirus
- Crimean-Congo
- Erve

Phlebovirus
- Rift Valley Fever
- Toscana
- Sandfly Fever Sicillian
- Sandfly Fever Naples
- Sandfly Fever Turkey
- Bahnja
- Palma

Hantavirus
- Dobrava
- Hantaan
- Seoul
- Puumala
- Khabarowsk
- SinNombre
- Andes
- Cano Delgadito

Flaviviridae

- Dengue1-4
- Yellow Fever
- West Nile
- Japanese Encephalitis
- Tick-borne Encephalitis
- Murray Valley Encephalitis
- St. Louis Encephalitis
- Zika

Filoviridae

- Zaire Ebola
- Sudan Ebola
- Bundibugiyo
- Marburg

Togaviridae

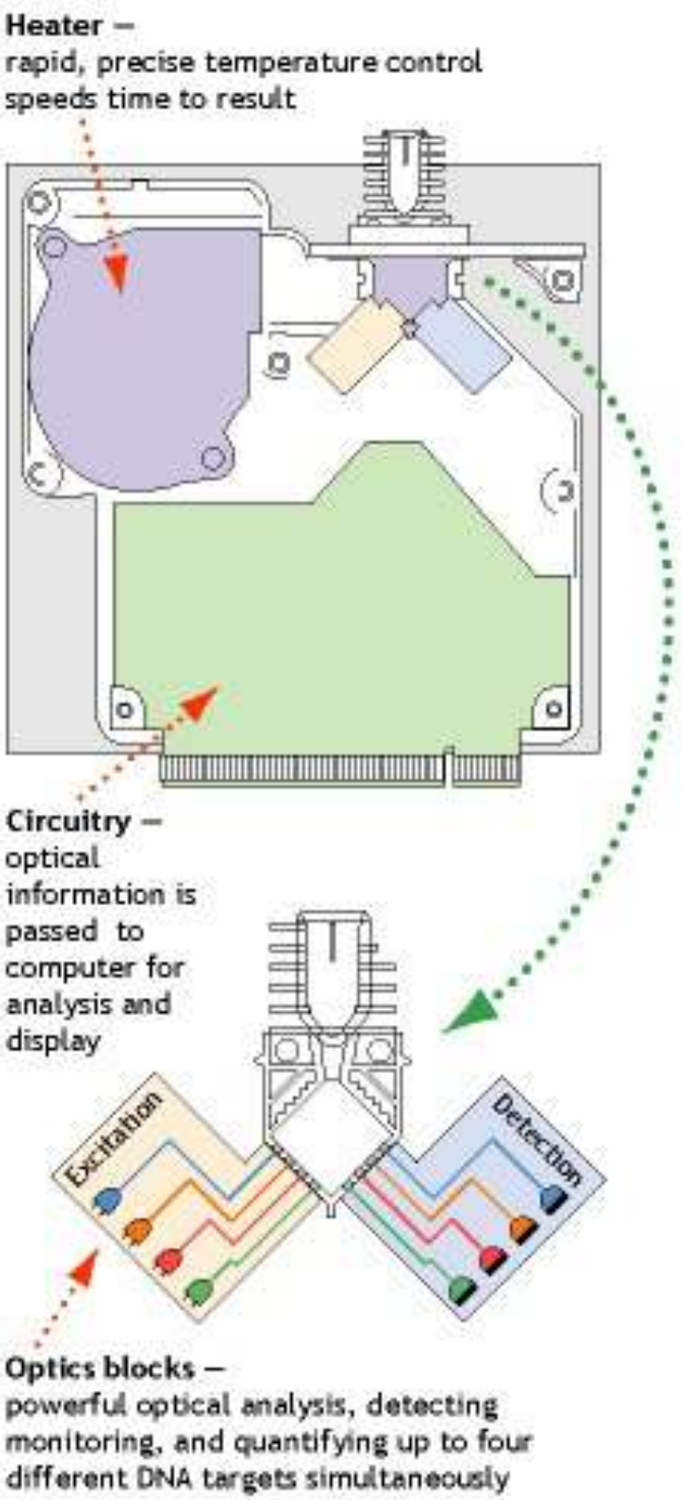
- Chikungunya
- Venzuelan Equine Encephalitis
- Western Equine Encephalitis
- Onnyongyong
- Semliki Forest Virus

Reoviridae

- Eyach
- Tripec

Mobile RT-PCR

Smart Cycler System



Dye Channel Characterization

Channel	1	2	3	4
Excitation (nm)	450–495 nm	495–527 nm	527–555 nm	555–593 nm
Emission (nm)	505–537 nm	537–565 nm	565–605 nm	605–800 nm
Simplex Dyes	FAM, SYBR Green	TET, JOE	TAM, CY3, Alexa, EtBr	ROX, Texas Red
Multiplex Dyes	FAM	TET	TAM	ROX

Mobile qRT-PCR for Haemorrhagic Fever viruses

Virus	Target Gene	Sensitivity
CCHFV	M-fragment	10^2
RVFV	S-fragnment	10^2
YFV	5`- UTR-C	10
DENV	3`- UTR	10^2
ZEBOV	Nucleoprotein	10
SEBOV	Nucleoprotein	10
MARV	Nucleoprotein	10
LASV	-	-

Mobile qRT-PCR in Kedougou Senegal 2011

1. Route from Dakar to field trip site at Kedougou (700km)

2. Kedougou field station

3. Laboratory at Kedougou field station on arrival

4. Dry PCR mixes: 4x screening mixes, 10x patient mixes, RNA positive controls

5. PCR flow line up : Extraction, PCR 1-3, cyclers at far end.

6. Extraction site

7. PCR 1 (mastermix) site

8. PCR 2 (samples meet mix) site

9. PCR 3 (positive controls) site

10. Mobile PCR-cyclers

11. Bandafasi health post

12. Bandafasi laboratory

13. Electricity source from vehicle provided through laboratory window

14. PCR flow in Bandafasi laboratory

15. Mobile PCR-cyclers in Bandafasi laboratory

Weidmann M, et al. (2018) ***The Journal of Infectious Diseases*** **218(10): 1622-1630**

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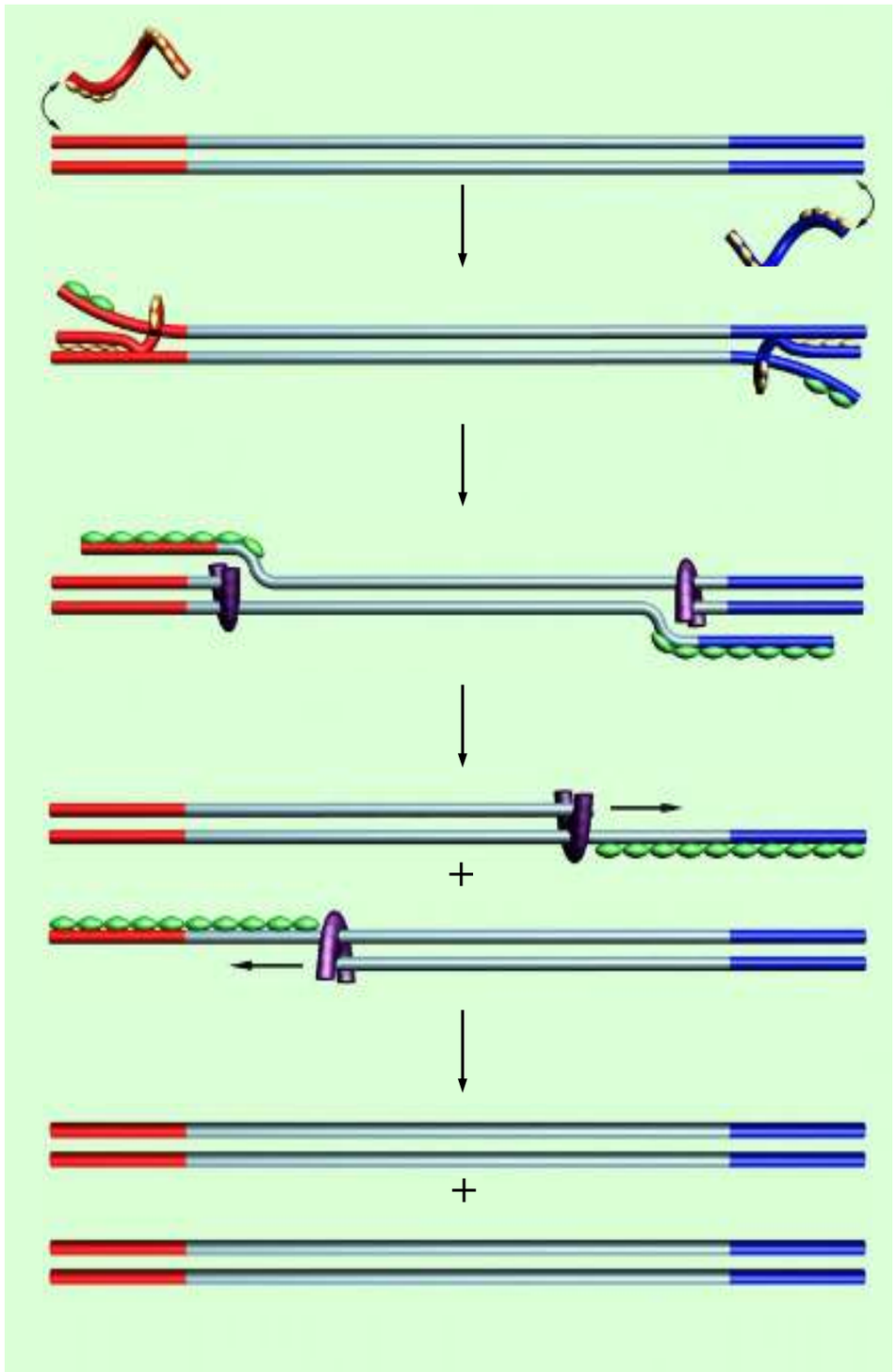
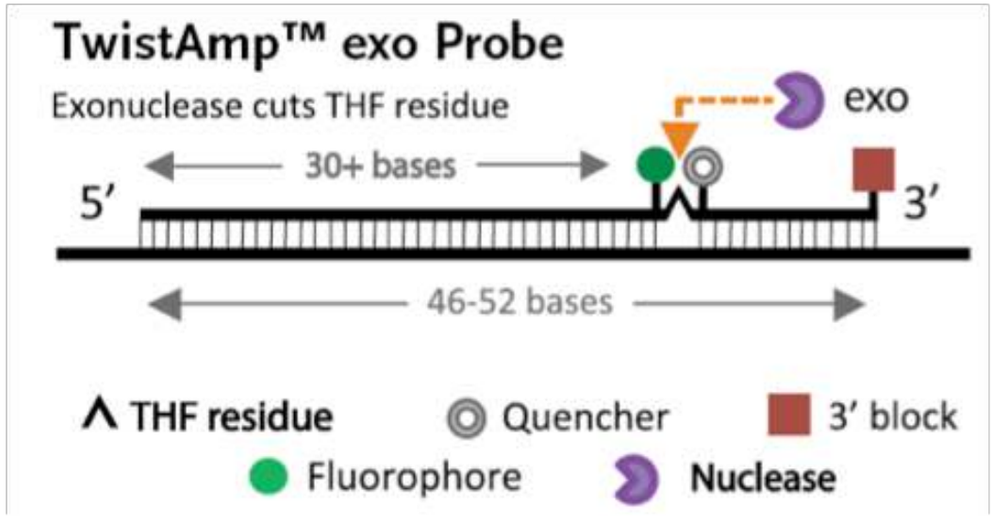
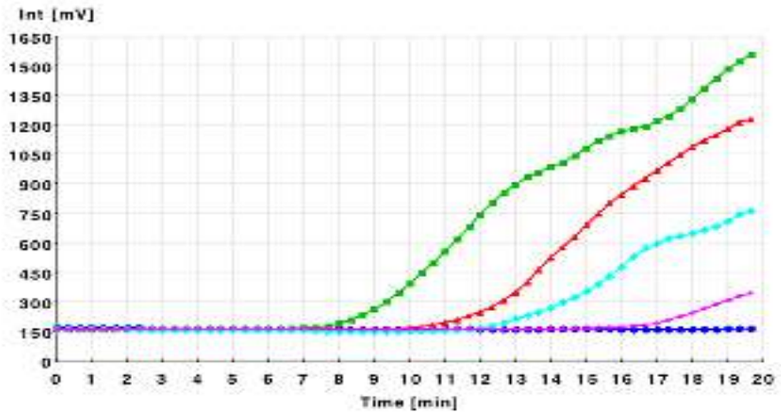
Recombinase Polymerase Amplification (RPA)



Piepenburg et.al. **(2006)** PLoS Biology 4(7): e204 (courtesy ASM Scientific)





TubeScanner

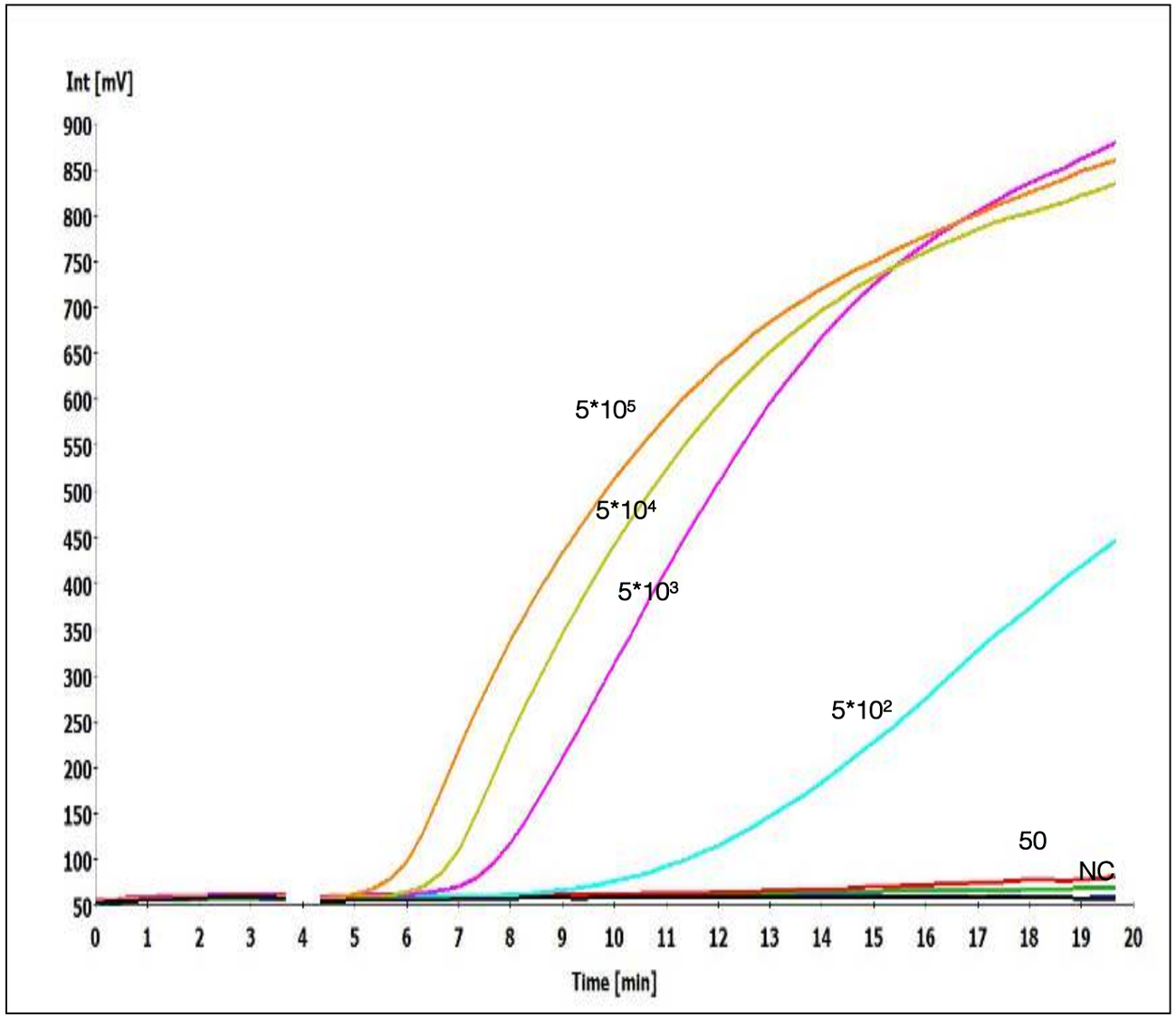
RPA-assay performance

Agent	Target gene	Probit sensitivity (95%) 8 runs	Run time (min)
Escherichia coli	*ssrA*	18	10
Clostridium difficlle	*tcdB*	22	10
Bacillus anthracis	pagA	16	8
Bacillus anthracis	*capC*	778	7
Francisella tularensis	tul4	19	10
Yersinia pestis	*pla*	16	8
Rift Valley fever virus	N	21	7
Sudan virus	NP	21	8
Ebola virus	NP	17	8
Variola virus	HA	16	10
Marburg virus	NP	16	4
Influenza A Virus (M)	M	218	8
Influenza B Virus (HA)	HA	131	8
Human Adenovirus-1	*hexon gene*	12	13
Human Adenovirus-4	*hexon gene*	131	14
Human Adenovirus-7	*hexon gene*	16	14
Parvovirus B19	VP1	15	7
Parinfluenza virus 3	HN	912	10
Herpes virus 1	gD	100	8
Varizella Zoster virus	*polymerase*	10	12
Foot and Mouth Disease virus	polymerase	102	9
Yellow fever virus	*E gene*	153	8

Mobile RT-RPA in Kedougou Senegal 2013

Abd El Wahed A, et al.(2015) *Journal of Clinical Virology* 69:16-21

Point-of-care diagnostic testing for Ebola virus disease in Ebola treatment centers

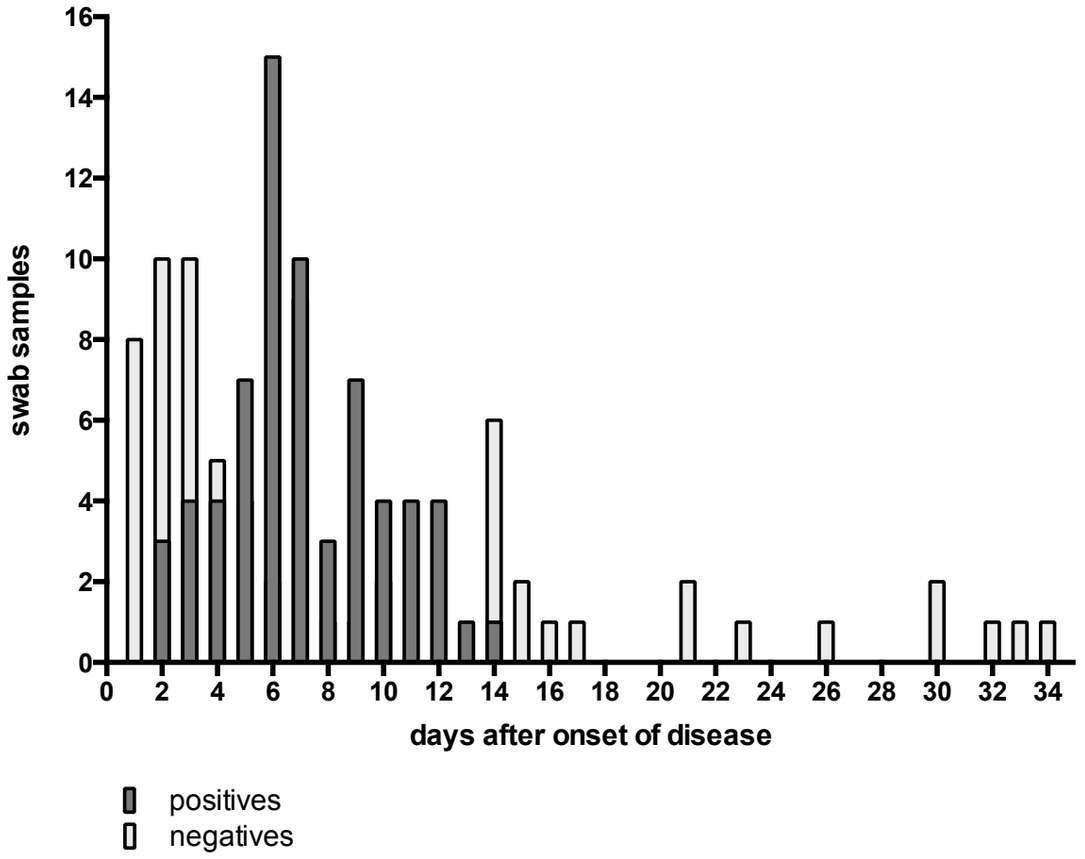


Mobile suitcase laboratory

Mobile EBOZV-RT-RPA clinical evaluation





n = 928

Verstorbene mit Symptomen: 138

Positiv: 67

Faye O et al. (2015) *Eurosurveillance* 20(44) Article 5

RPA assays developed so far…..

Emerging viral Diseases

Ebola virus
Sudan virus
Marburg virus
Bundibugiyo virus
Monkeypox virus

Arboviruses

Yellow Fever virus
Zika virus
Japanese Encephalitis virus
Rift Valley Fever virus
Dengue virus
Chikungunya virus

Neglected Diseaes

Rabies virus
Mycobacterium ulcerans
Treponema pallidum
Leishmania donovani
Mycobacterium leprae

Respiratory viral Diseases

Influenza virus A
Influenza virus B
Respiratory Syncytial virus
Adenovirus 1 / 4 / 7
Parainfluenzavirus 3
MERS CoV
SARS-CoV-1/2

Other human infections

Parvovirus B19
HSV1
VZV
Fransciella tularensis
Bacillus anthracis pacA/capC
Clostridum difficile tcdB/tcdA
Pan-Ricketsia
Salmonella typhi / paratyphi

Livestock infections

Foot and Mouth Disease Virus
Lumpy Skin Disease Virus
Bovine Coronavirus
Influenza virus H5N1
Influenza virus H7N9
Influenza virus H9N2
Mycobacterium avium subs paratuberculosis
Franscicella noutaensis orientalis

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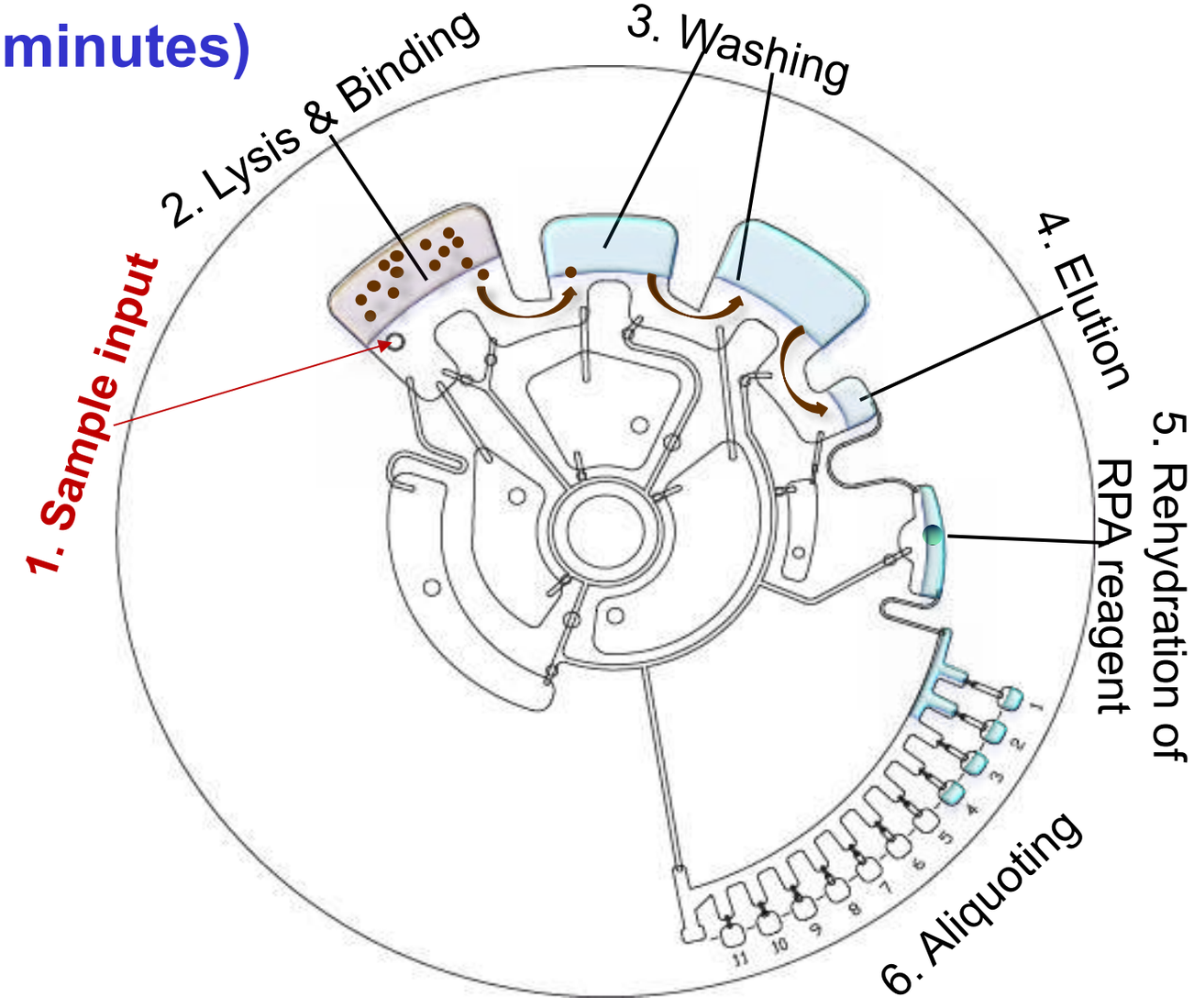
Microfluidic unit - integration of NA extraction and RPA

NA extraction

(12 minutes)



Sample in result out system !

Isothermal real-time recombinase polymerase amplification (RPA) (15 minutes)

Strohmeier O., et.al (2015) RSC Advances 5: 32144

DiscoGnosis
Disc-shaped point-of-care platform for infectious disease diagnosis

Differential diagnosis of acute fever in developing countries

Disease panel (all have same clinical sympomts but different treatment needs)

(1) Malaria (*P. falciparum, P. ovale, P. vivax, P. malariae*)

(2) *Salmonella typhi / paratyphi*

(3) *Streptococcus pneumoniae*

(4) Dengue virus

(5) Chikungunya virus

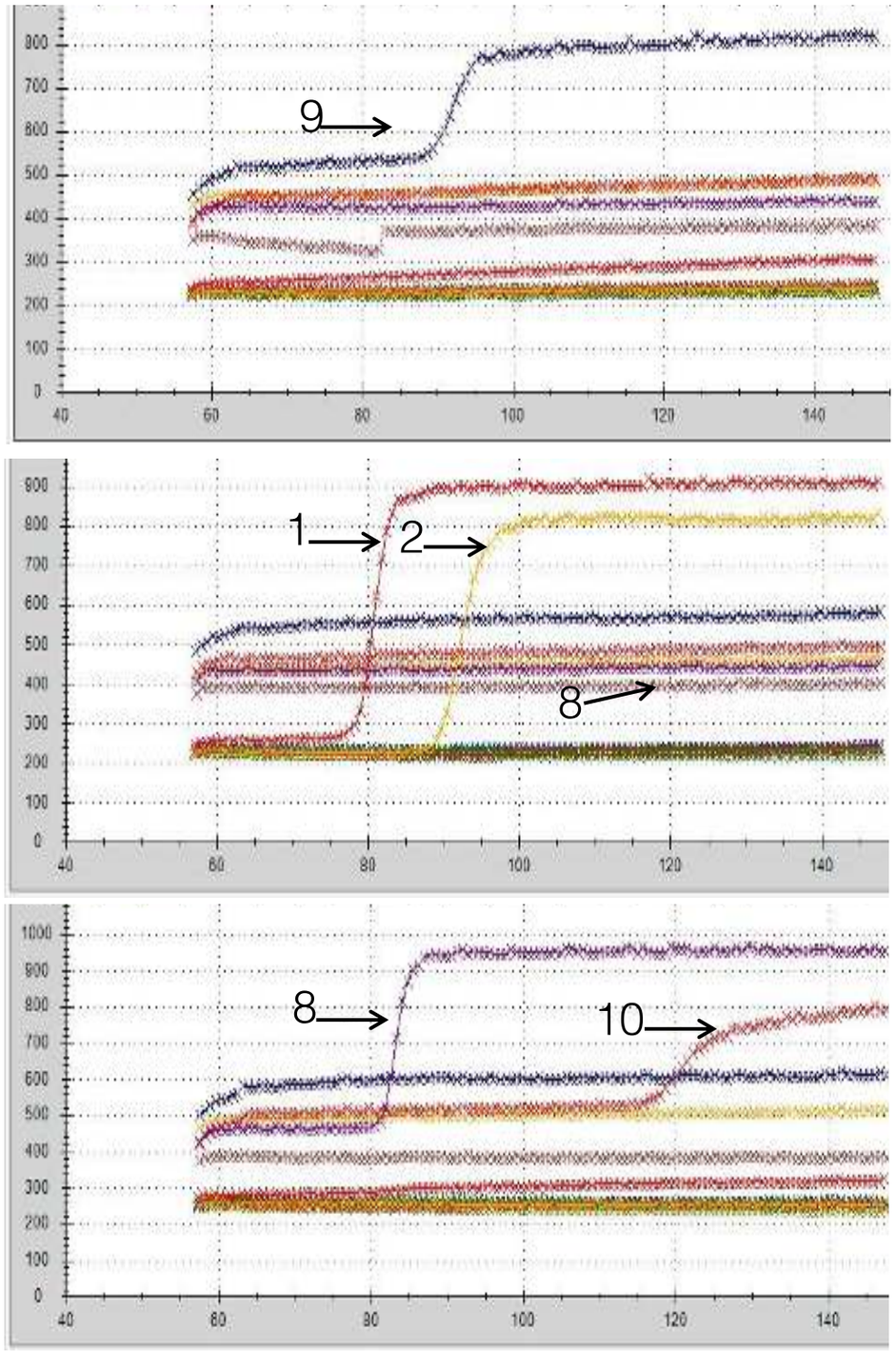
- Full automation (from 200µL whole blood)
- LAMP isothermal amplification
- All reagents pre-stored.
- Amplification reagents lyophilised (incl. RT)
- cold chain independent storage
- Sample-to-answer in 70-120 min (including the extraction/purification steps)

LabDisc Validation results

In two DENV samples detection of DENV1

In a CHIKV negative sample additional detection of malaria

In CHIKV positive sample additional detection of DENV2



LAMP assays

Primers: *Uni Stirling, Mast Diagnostica GmbH*
Lyopellets: *Mast Grp Ltd*
Extraction buffers: *Mangamedics Diagnostics BV*
Magn. beads: *Analytik Jena*

Lopez-Jimena B et al. (2018) PLOS Neglected Tropical Diseases 12(5): e0006381
Lopez-Jimena B et al. (2018) PLOS Neglected Tropical Diseases 12(5): e0006448
Hin S. et al. (2021) *PLOS Negl Trop D*is 15(2): e0009177

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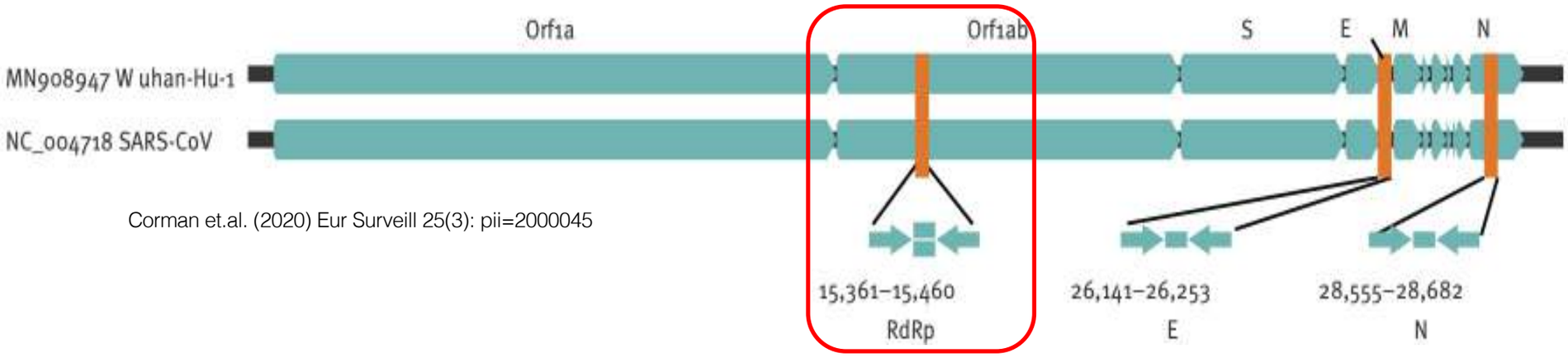
RdRp gene RPA for detection of SARS-CoV-2



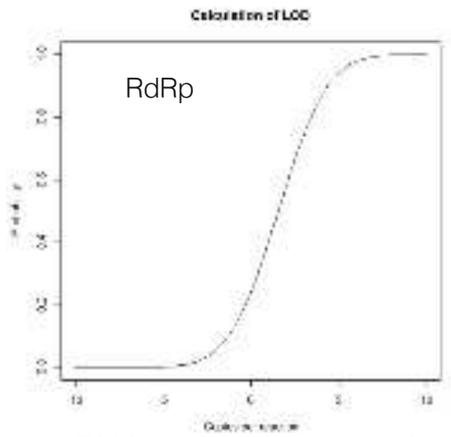
Corman et.al. (2020) Eur Surveill 25(3): pii=2000045

Cross Reactivity

Viral nucleic acid	RdRP
SARS-COV-2	+
SARS-COV-1	+
Coronavirus 229E, NL63, and OC43	-
MERS-Coronavirus	-
Influenza A (H1N1 pdm09)	-
Influenza A (H3N2)	-
Influenza A (H5N1)	-
Influenza A (H1N1 H275Y)	-
Influenza B (Victoria)	-
Influenza B (Yamagata)	-
Parainfluenza virus 1-4(patient isolate)	-
Respiratory syncytial virus A and B	-
Human rhinovirus A 16	-
Human rhinovirus B 5	-
Human metapneumovirus A1 and B2	-
Adenovirus type 1, 4, and 34	-
A/Anhui/1/13 (H7N9)	-
A/ Chicken /Germany/79 "Taucha" (H7N7)	-
A/Chicken/Brescia/19/02 (H7N7)	-
A/Cygnus olor/Germany/R1377/07 (H5N1)	-
Newcastle disease virus clone 30	-
Infectious laryngotracheitis virus U76	-
Infectious bronchitis M41	-

Analytical sensitivity

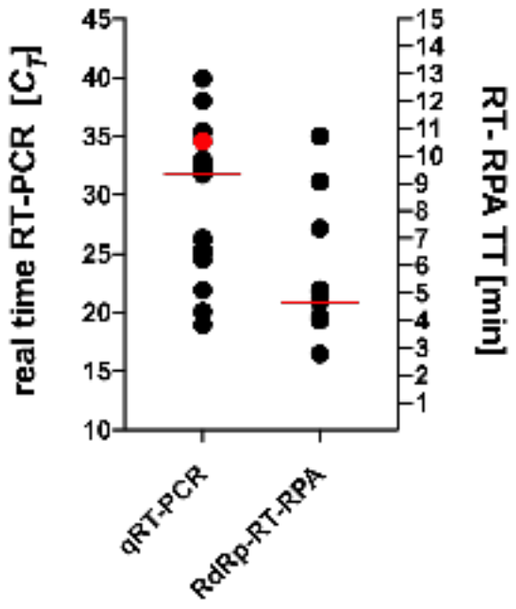
Probit analysis (n=5)



2 RNA molecules / reaction
(Probit 95%)

Preclinical evaluation

(n=36 (18 positive & 18 negative)



	RdRP
Sensitivity	0,94
Specificity	1,00
PPV	1,00
NPV	0,95

RdRp-RPA	E-PCR +	E-PCR -
RPA +	17	0
RPA -	1	18

RdRp gene testing shows exceptional SARS-CoV-2 performance with clinical samples.

First results from EDCTP project:

Senegal

	PCR [CT]		RPA	
Sample	E	RdRp	E	N
1.	19.1	+	+	+
2.	20.0	+	+	+
3.	22.9	+	+	+
4.	24.3	+	+	+
5.	25.7	+	+	+
6.	27.8	+	+	+
7.	28.4	+	-	+
8.	28.9	+	-	+
9.	30.8	+	-	-
10.	31.9	+	-	-
11.	32.8	+	-	+
12.	33.5	+	-	-
13.	34.8	+	-	+
14.	35.5	+	-	-
15.	37.2	+	-	-
16.	39.1	+	-	-

Methods

RNA was extracted from swab VTM by Qiagen kit

RT-RPA
RPA primers & probe
Thermofisher RevertAid
Twist DX exo kit
Axxin T8

RT-PCR
TIBMOLBIOL primers & probe
Luna® Universal One-Step RT-qPCR Kit
CFX96 Real-Time PCR System

Egypt

	PCR [CT]		RPA	
Sample	E	RdRP	E	N
1.	16.54	+	-	-
2.	16.67	+	+	+
3.	17.17	+	-	-
4.	18.05	+	-	-
5.	18.18	+	-	-
6.	18.79	+	+	+
7.	18.8	+	+	+
8.	18.84	+	-	-
9.	19.24	+	-	-
10.	19.26	+	-	-
11.	19.27	+	+	+
12.	19.32	+	-	-
13.	19.83	+	-	-
14.	19.97	+	+	+
15.	19.99	+	+	+
16.	20.10	+	-	-
17.	20.32	+	+	+
18.	20.41	+	+	+
19.	20.44	+	-	-
20.	21.22	+	+	+
21.	21.57	+	-	-
22.	21.91	+	+	+
23.	22.23	+	+	+
24.	22.50	+	+	+
25.	22.67	+	-	-
26.	22.70	+	-	-

RT-PCR
TIBMOLBIOL primers & probe
Superscript III Platinum One-Step qRT-PCR kit
Stratagene Mx 3000p Cyler

Abd El Wahed et al. (2021) *Analytical Chemistry* 93 (4): 2627–2634

The at home test solution is easy to use by untrained persons and developed for high volume manufacturing

Simple, affordable test device

Power supply for test by rechargeable battery

- Process control and readout wireless by smartphone
- Scalable manufacturing designed into device
- Pilot and volume manufacturing with renowned contract manufacturers (consumer electronics)

Smartphone enables additional functionality

- Optimized User interface and user guidance
- Verification of sample taking procedure
- Immediate display and transfer of result for processing by authorities via cloud backend

SARS CoV 2 RPA advanced test procedure
Time to result: 15 minutes

Take nasal or throat or swab

Wash the swab in the disposable for 20 sec

Place the disposable on the device and turn it

Readout the result 15 minutes later

MIDGE MEDICAL

- Ahmed Abd El-Wahed
- Frank Hufert

- Amadou Alpha Sall
- Oumar Faye
- Ousmane Faye

- Felix von Stetten
- Oliver Strohmeier
- Kostas Mitskakis

EDCTP

https://edctp-drc.stir.ac.uk/

https://www.vetmed.uni-leipzig.de/en/institut-fuer-tierhygiene-und-oeffentliches-veterinaerwesen/forschung/arbeitsgruppe-tierhygiene-und-tierseuchenbekaempfung/africa-suitcaselab/