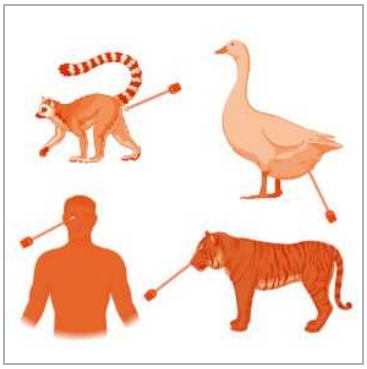

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Protocol status: Working
We use this protocol and it's working

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TELEvir Field Protocol

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DISCLAIMER

This protocol was prepared for a workshop held the 23-24 June 2022 at Statens Serum Institut, Denmark. Here 25 TELEVIR partners from 10 European countries participated to train in the usage of a field-deployable point-of-incidence toolbox to identify emgerging virus threats. The protocol was used to detec SARS-CoV-2 and Human Papillomavirus a RNA and DNA virus detection test-of-concept.

ABSTRACT

The TELE-Vir project key aim is to develop a fast point-of-incidence (poi) toolbox for identification and characterisation of emerging virus threats for humans and/or domestic and wildlife animals.
Presented here is a protocol including sample pretreatment, NA extraction and random amplification for metagenomic virus detection using the MinION device (Oxford Nanopore Technologies)

Pretreatment

15m

1

15m

DNase Set I Zymo Research Catalog #E1010

Equipment

BD Disposable Syringe with Luer-Lok Tip (5ml)	NAME
Syringe	TYPE
Becton Dickinson	BRAND
309649	SKU

Equipment

Syringe Filter pore size 0.2 µm	NAME
Filter	TYPE
Minisart	BRAND
16532	SKU
https://www.sartorius.com/shop/ww/en/usd/applications-laboratory-filtration-sterile-filtration/minisart-syringe-filter%2C-polyethersulfone-%28pes%29%2C-pore-size-0-22-%C2%B5m%2C-ethylene-oxide%2C-female-luer-lock%2C-male-luer-lock%2C-pack-size-50/p/16532-	LINK
Polyethersulfone (PES), Pore Size 0.22 µm, Ethylene Oxide, Female Luer Lock, Male Luer Lock, Pack Size 50	SPECIFICATIONS

400 µL Sample

50 µL DNase I

50 µL DNase Digestion buffer

00:15:00 at room temperature

Note

If 400 µL sample is not available, downscale the reagents to fit the lower sample volume.

2 Add 500 µL PBS (or equivalent to 1 ml sample material in total)

3 Poor the diluted sample into the lid of the Syringe Filter (Minsart). The filter can be placed on a sterile surface meanwhile e.g. the Syringe Filter paper lid (inner side).

4 Suck in air corresponding to app 1 ml in a 5 ml syringe

5 Extract the 1 ml sample material from the petri dish to the 5 ml syringe

6 Attach the 0,22 µM Minisart syringe filter to the 5 ml syringe with Luer-Lok

7 Filter the sample and air directly into a 4.5 ml cryotube containing 1 ml MPLB-buffer. Put lid on, mix by turning tube.

MagNA Pure LC Total Nucleic Acid Isolation Kit **Roche Catalog #03038505001**

Inactivation

20m

8 Incubate 20m

00:20:00 at room temperature for viral inactivation

CITATION

Vinner L, Fomsgaard A (2007). Inactivation of orthopoxvirus for diagnostic PCR analysis.. Journal of virological methods.

CITATION

Rosenstierne MW, Jensen CE, Fomsgaard A (2018). Rapid, Safe, and Simple Manual Bedside Nucleic Acid Extraction for the Detection of Virus in Whole Blood Samples.. Journal of visualized experiments : JoVE.

LINK

https://doi.org/10.3791/58001

Extraction

10m

9 Kit used for hand held NA extraction:

MagNA Pure LC Total Nucleic Acid Isolation Kit **Roche Catalog #03038505001**

Equipment

4.5 ml cryotube	NAME
tube	TYPE
Thermo Fisher Scientific	BRAND
363452	SKU
https://www.thermofisher.com/order/catalog/product/363452	LINK

Equipment

3.6 ml cryotube	NAME
Tube	TYPE
Thermo Fisher Scientific	BRAND
379189	SKU
https://www.thermofisher.com/order/catalog/product/363452	LINK

Cylinder magnet
A rubber band to attach the magnet to a tube.
A 50 ml Nunc tube or similar to pour excess solutions into for disposing.

Following the protocol by:

CITATION

Rosenstierne MW, Jensen CE, Fomsgaard A (2018). Rapid, Safe, and Simple Manual Bedside Nucleic Acid Extraction for the Detection of Virus in Whole Blood Samples.. Journal of visualized experiments : JoVE.
LINK
https://doi.org/10.3791/58001

10 Per *X* number of samples, aliquot in cryotubes or Eppendorf tubes 10m
960 µL Magnetic Glass Particles (MGPs) in an Eppendorf tube
4 mL Wash buffer I in a 4.5 ml cryotube
1.5 mL Wash buffer II in an Eppendorf tube
3 mL Wash buffer III in a 3.6 ml cryotube
100 µL Elution buffer in an Eppendorf tube
Prepare one sample at the time.
Approximate time 00:10:00 per sample

11 Add 960 µl MGPs to 2 ml solution of sample and MPLB-buffer

12 Put lid on and turn tube in hand gently until well mixed

13 Attach magnet and trap beads.

14 Pour solution into trash tube. The MGPs should stay behind trapped on the side of the tube.

15 Pour Wash buffer I in the sample tube

16 Put a lid on, remove magnet and turn the tube in hand gently until well mixed

17 Attach magnet and trap beads.

18 Pour solution into trash tube. The MGPs should stay behind trapped on the side of the tube.

19 Pour Wash buffer II in the sample tube

20 Put a lid on, remove magnet and turn the tube in hand gently until well mixed

21 Attach magnet and trap beads.

22 Pour solution into trash tube. The MGPs should stay behind trapped on the side of the tube.

23 Pour Wash buffer III in the sample tube

24 Put a lid on, remove magnet and turn the tube in hand gently until well mixed

25 Attach magnet and trap beads.

26 Pour solution into trash tube. The MGPs should stay behind trapped on the side of the tube.

27 Pour Elution buffer in the sample tube

28 Put a lid on, remove magnet and turn the gently until beads are eluted.

29 Transfer 10 µL Elution Buffer with MGPs to **2 x** 0.2 ml PCR tube

Whole Transcriptome and Genome Amplification

4h

30 Equipment to enable portability
- A MiniPCR to run isothermal incubations
- A powerbank that can provide 20V. Use one to power the miniPCR and another to power the MinION for approximately 5 hours. Use brand of choice.
- A salad swing as a hand driven centrifuge. Use brand of choice.
- A coolbox for reagents and cooling samples. Use brand of choice.

Equipment

All-in1 Laptop Powerbank 24000	NAME
Power Bank	TYPE
Sanberg	BRAND
420-57	SKU
https://sandberg.world/da-dk/product/all-in-1-laptop-powerbank-2400	LINK
Any powerbank that can produce 20V is acceptable. Have 2-3 to run the MiniPCR and the MinION	SPECIFICATIONS

Equipment

miniPCR® mini8 thermal cycler NAME

Thermal cycler TYPE

miniPCR® BRAND

mini8 SKU

https://www.minipcr.com/ LINK

Equipment

A Salad Spinner Centrifuge NAME

Centrifuge TYPE

undefined BRAND

undefined SKU

https://www.oxo.com/salad-spinner.html?queryID=975936c98686e2b0325b684dd613daff&objectID=2251&indexName=magento2_prod_oxooxo_products#color=White LINK

Kit used for WTA and WGA

REPLI-g Cell WGA & WTA **Qiagen Catalog #150052**

Quantiscript RT Enzyme Mix, Ligase Mix, and REPLI-g SensiPhi DNA Polymerase should be thawed on ice.

All other components can be thawed at room temperature (15–25°C)

31 The two aliquots of same sample enables uniform whole genome amplification (WGA) and whole transcriptome amplification (WTA) in parallel reactions.

32 Cleanup for WTA

10m

Add 2 µL gDNA Wipeout Buffer to PCR tube with 10 µl eluted sample material

00:10:00 42 °C on MiniPCR

33 Repair (WGA) and Reverse Transcription (WTA)

33.1

For **WGA**, mix following and add 10 µL Repair reagent mix to sample

	Reagents	Volume (µL) per sample
	RT/Polymerase Buffer	4
	gDNA Wipeout buffer	2
	H2O sc	1
	Random primer	1
	Random Hex-P primer (20µM)	1
	WGA Ready Enzym	1
	Total	10

This modified protocol uses 5'-phosphorylated random hexamers (P-N6) instead of kit provided oligo-dT primers according to Rosenstierne et al (2014).

CITATION

Rosenstierne MW, McLoughlin KS, Olesen ML, Papa A, Gardner SN, Engler O, Plumet S, Mirazimi A, Weidmann M, Niedrig M, Fomsgaard A, Erlandsson L (2014). The microbial detection array for detection of emerging viruses in clinical samples--a useful panmicrobial diagnostic tool.. PloS one.
LINK
https://doi.org/10.1371/journal.pone.0100813

33.2 For **WTA**, mix following and add 8 µL RT reagent mix to sample

	Reagents	Volume (µL) per sample
	RT/Polymerase Buffer	4
	H2O sc	1
	Random primer	1
	Random Hex-P primer (20µM)	1
	Quantiscript RT Enzym Mix	1
	Total	8

33.3 Incubate 1h 3m

01:00:00 42 °C On MiniPCR

Afterwards immediatly

00:03:00 95 °C on MiniPCR

34 Ligation 35m

Mix following and add 10 µL Ligation mix to each tube

	Reagents	Volume (µL) per sample
	Ligation buffer	8
	Ligase Mix	2
	Total	10

Incubate

00:30:00

24 °C This is the Repli-G recommended temperature, however, the miniPCR only goes to 25C. Instead of incubation in the MiniPCR, try table-top incubation.

00:05:00 95 °C

Hold at 4 °C

35 Amplification 2h 5m

Mix following and add 30 µL amplification mix to each tube

	Reagents	Volume (µL) per sample
	Repli-g reaction buffer	29
	Repli-g SensiPhi DNA polymerase	1
	Total	30

Incubate

02:00:00 30 °C

00:05:00 65 °C

Hold at 4 °C

36 For library preparation use amplified material **without** MGPs.

Library Preparation

37

Multiple rapid library preparation kits are available through **Oxford Nanopore Technology.** For Workshop we use.

Rapid Sequencing Kit **Oxford Nanopore Technologies Catalog #SQK-RAD004**

Equipment

MinION Mk1C	NAME
Sequencer	TYPE
Oxford Nanopore Technology	BRAND
MIN-101C	SKU
https://store.nanoporetech.com/minion-mk1c.html	LINK