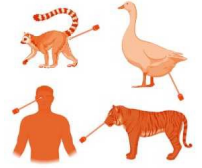


Jun 29, 2023

TELEVir Field Protocol

DOI

dx.doi.org/10.17504/protocols.io.n2bvj694xlk5/v1



Anna S Fomsgaard^{1,2}

¹Statens Serum Institut; ²University of Copenhagen



Anna S Fomsgaard

OPEN ACCESS



DOI: dx.doi.org/10.17504/protocols.io.n2bvj694xlk5/v1

Protocol Citation: Anna S Fomsgaard 2023. TELEVir Field Protocol . protocols.io <https://dx.doi.org/10.17504/protocols.io.n2bvj694xlk5/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 06, 2022

Last Modified: June 29, 2023

Protocol Integer ID: 60387

Funders Acknowledgement:

Horizon 2020

Grant ID: 773830

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 emerging virus threats. The protocol was used to detect 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)

Protocol materials

- MagNA Pure LC Total Nucleic Acid Isolation Kit **Roche Catalog #03038505001** In [2 steps](#)
- REPLI-g Cell WGA & WTA **Qiagen Catalog #150052** Step 30
- Rapid Sequencing Kit **Oxford Nanopore Technologies Catalog #SQK-RAD004** Step 37
- DNase Set I **Zymo Research Catalog #E1010** Step 1

Pretreatment

15m

1

15m

 DNase Set I Imerco 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-%2C%2B5m%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 **Imerco Catalog #03038505001**

Inactivation

20m

- 8 Incubate
🕒 00:20:00 at room temperature for viral inactivation

20m

CITATION

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

CITATION

Rosenstjerne 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 **Imerco 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


Rosenstjerne 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 μ l 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

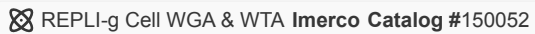
<https://www.minipcr.com/>

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_oxoxo_products#color=White | LINK |
|  | |


Kit used for WTA and WGA



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**

Add  to PCR tube with 10 µl eluted sample material

  on MiniPCR

10m

33 **Repair (WGA) and Reverse Transcription (WTA)**

33.1 For **WGA**, mix following and add  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

Rosenstjerne 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

01:00:00 42 °C On MiniPCR

Afterwards immediatly

00:03:00 95 °C on MiniPCR

1h 3m

34 **Ligation**

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**

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 |

2h 5m

| Reagents | Volume (µL) per sample |
|----------|------------------------|
| 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 **Imerco** **Catalog** #SQK-RAD004

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

Citations

Step 33.1

Rosenstierne MW, McLoughlin KS, Olesen ML, Papa A, Gardner SN, Engler O, Plumet S, Mirazimi A, Weidmann M, Niedrig M, Fomsgaard A, Erlandsson L. The microbial detection array for detection of emerging viruses in clinical samples—a useful panmicrobial diagnostic tool.

<https://doi.org/10.1371/journal.pone.0100813>

Step 8

Vinner L, Fomsgaard A. Inactivation of orthopoxvirus for diagnostic PCR analysis.

Step 8

Rosenstierne MW, Jensen CE, Fomsgaard A. Rapid, Safe, and Simple Manual Bedside Nucleic Acid Extraction for the Detection of Virus in Whole Blood Samples.

<https://doi.org/10.3791/58001>

Step 9

Rosenstierne MW, Jensen CE, Fomsgaard A. Rapid, Safe, and Simple Manual Bedside Nucleic Acid Extraction for the Detection of Virus in Whole Blood Samples.

<https://doi.org/10.3791/58001>