

To: 5.1.2.e @rivm.nl]; 5.1.2.e @rivm.nl]; 5.1.2.e @rivm.nl];
Cc: 5.1.2.e @rivm.nl]; 5.1.2.e @rivm.nl];
From: 5.1.2.e
Sent: Mon 5/23/2022 5:20:45 PM
Subject: FW: MPXV EVD-LabNet update 2
Received: Mon 5/23/2022 5:20:46 PM
[WHO-MPX-Laboratory-2022.1-eng.pdf](#)
[Monkeypox-multi-country-outbreak.pdf](#)
[Orthopox-parapox PCR and sequencing protocols Viroscience ErasmusMC.pdf](#)

From: 5.1.2.e @rivm.nl>
Sent: maandag 23 mei 2022 19:18
Cc: 5.1.2.e @ecdc.europa.eu>
Subject: MPXV EVD-LabNet update 2

Dear EVD-LabNet colleagues,

A second mail with information regarding the MPXV outbreak.

WHO has just published an interim guidance for labs. Please find it attached.

ECDC has just published its first RRA. Please find it attached. Please take note of the case definitions.

Please refer to last week's e-mail for protocols and to the UPDATED EVD-LabNet directory at [EVD-LabNet directory \(europa.eu\)](#). Search for MPXV or orthopoxvirus generic under virus species to find member laboratories that have diagnostic capacity. ErasmusMC protocol that was send last week contained errors in primers (reverse primers were mistakenly the same as forward primers), Please find the corrected protocol attached, with apologies.

If you are in need for assistance please contact me or 5.1.2.e at ECDC (in cc). Either for implementation of protocols, primary/confirmatory testing.

Best wishes 5.1.2.e

5.1.2.e

5.1.2.e

5.1.2.e

([Emerging Viral Diseases-Expert Laboratory Network \(EVD-LabNet\) \(europa.eu\)](#))

Netherlands Centre for Infectious Disease Control,
 National Institute for Public Health and the Environment, Bilthoven



National Institute for Public Health
 and the Environment
 Ministry of Health, Welfare and Sport

Contact:
 P.O. Box 1
 3720BA Bilthoven, the Netherlands

Tel: 00-31 5.1.2.e
 e-mail 5.1.2.e @rivm.nl

To: 5.1.2.e @rivm.nl]; 5.1.2.e @rivm.nl]; 5.1.2.e
5.1.2.e @rivm.nl]
From: IDS-ddmicrobioloog
Sent: Fri 5/20/2022 5:55:42 AM
Subject: FW: EU lab response monkeypox virus detection (EVD-LabNet)
Received: Fri 5/20/2022 5:55:44 AM
[tropicalmed-01-00008.pdf](#)
[EQA PXV EVD-LabNet draft to share preliminary and confidentially..pdf](#)
[1-s2.0-S0891552019300170-main.pdf](#)
[UKHSA SPATH058 V7 FINAL Poxvirus Detection by Real-Time PCR MPX Edit external.pdf](#)
[RIVM Orthopox PCR 2.pdf](#)
[Orthopox-parapox PCR and sequencing protocols Viroscience ErasmusMC.pdf](#)

From: 5.1.2.e
Sent: Friday, 20 May 2022 07:55:38 (UTC+01:00) Amsterdam, Berlin, Bern, Rome, Stockholm, Vienna
To: 5.1.2.e IDS-ddmicrobioloog; 5.1.2.e LCI Voorwacht; 5.1.2.e
5.1.2.e
Cc: 5.1.2.e
Subject: FYI: EU lab response monkeypox virus detection (EVD-LabNet)

Beste allen,

Onderstaande e-mail is uitgegaan binnen EVD-LabNet en ook gedeeld met WHO-INT (ook daar begint lab response op gang te komen).

De lab response is voor de huidige situatie adequaat. In hoeverre dit blijft gaat denk ik ook samenhangen met hoeveel sub-klinische/a-specifieke symptomatiek er plaatsvindt en daarmee hoe groot dit ongemerkt kan worden. Gezien de huidige situatie, met waarschijnlijke ongemerkte community transmissie, is het einde denk ik nog niet in zicht. IDS_RIVM is beschikbaar voor verzoeken van ECDC en WHO voor lab (nat en droog) ondersteuning, na overleg mbt capaciteit in samenhang met aantallen aanvragen op dat moment.

Hartelijke groet 5.1.2.e mede namens 5.1.2.e

From: 5.1.2.e
Sent: vrijdag 20 mei 2022 07:39
Subject: First information on monkeypox virus detection (EVD-LabNet)

Dear EVD-LabNet members,

As promised some first round of information on monkeypox virus situation and laboratory detection in Europe. Currently an increasing number of countries report confirmed MPXV cases, including UK, Belgium, Sweden, Italy, Portugal, USA and suspected cases in Spain and Canada. ECDC is preparing a rapid risk assessment that will be published early next week.

Information on MPXV can be found here:

ECDC:

[Monkeypox cases reported in UK and Portugal \(europa.eu\)](#)

[Factsheet for health professionals on monkeypox \(europa.eu\)](#)

[Rapid risk assessment: Monkeypox cases in the UK imported by travellers returning from Nigeria, 2018 \(europa.eu\)](#)

WHO on-line course on MPXV:

<https://openwho.org/courses/monkeypox-intermediate>

A few years ago EVD-LabNet organised an EQA for molecular detection of poxviruses including MPXV. Due to the pandemic the manuscript on this EQA has not been submitted for publication yet. For your information I have added the draft manuscript as attachment to this e-mail. *Please treat this manuscript with the confidentiality needed so EVD-LabNet can still officially publish the results.*

Positive control materials to set-up your own diagnostics are available through the EU-funded European Virus Archive (EVAg). Please use this link to search for the needed materials: [Monkeypox virus | EVAg \(european-virus-archive.com\)](#). It is expected that in the course of next week more materials will become available through the catalogue.

When in need for support for primary/confirmatory testing: please refer to the EVD-LabNet directory at [EVD-LabNet directory \(europa.eu\)](https://europe.ecdc.europa.eu/en/evd-labnet). Please select "Monkeypox virus" under virus species and a list of labs and the techniques they use will appear. Alternatively select "orthopox virus generic". Please contact [redacted] 5.1.2.e @rivm.nl) or [redacted] 5.1.2.e @ecdc.europa.eu) if financial support for shipment and testing is needed. We will discuss putative funding options with you.

Finally, some first protocols to share kindly provided by UKHSA (PHE), ErasmusMC and the WHO CC for smallpox vaccines at RIVM. Please note that MPXV is a RG3 pathogen.

Contact information in case of questions to the protocols provided now:

1. UKHSA; [redacted] 5.1.2.e @phe.gov.uk
2. ErasmusMC; [redacted] 5.1.2.e @erasmusmc.nl
3. RIVM; [redacted] 5.1.2.e @rivm.nl

1. UKHSA detection of cases in the UK is based on: Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. Journal of virological methods. 2010;169(1):223-7. See attachment for detailed protocol. Further notes from Dan: "We've modified reagents slightly from original paper to match our workflows. Likewise we would recommend you run the assay with an multiplexed internal control, but have removed that in edited version as laboratories may have their own local version they want to employ. Original paper also has clade differentiation assays which we employ as confirmatory if required in new cases, but see comment below. Extraction process we typically use MagNpure 96 with its external lysis total nucleic acid protocol, using external lysis 100ul sample (swab or plasma) + 250 µl MP96 lysis buffer (10mins) to make safe the sample and inactivate before removing from containment. For the differentials; parapox/molluscum and orthopox we use assays based on: Schroeder K, Nitsche A. Multicolour, multiplex real-time PCR assay for the detection of human-pathogenic poxviruses. Mol Cell Probes. 2010;24(2):110-3.

Our clinical director also provides this advice on sampling; *Use Viral Transport fluid, and just swab the vesicle. Sampling the fluid is no longer necessary and in a good case we would a CT of 14 or less anyway. If the patient is unwell, an EDTA, urine and throat swab are useful for both diagnoses and monitoring progress and recovery.* It is important to consider the differential diagnoses such as VZV, HSV, molluscipox and syphilis. In sexually transmitted diseases context it is essential to exclude the other causes as well as to look for monkeypox. With the increasing case no's we are beginning to take the stance that if its orthopox positive and clinical picture/transmission fits then it is MPX"

2. ErasmusMC detection of cases is based on: Schroeder K, Nitsche A. Multicolour, multiplex real-time PCR assay for the detection of human-pathogenic poxviruses. Mol Cell Probes. 2010 Apr;24(2):110-3. Please find more details in the attachment to this e-mail.

3. RIVM detection of orthopox cases by PCR/SEQ: see attachment.

As soon as more information on successful assays is available we will share this information. Please share if you have additional protocols. Please note that early next week the ECDC RRA will provide further information on case definitions including the laboratory component of the definition.

Further reading: see two overview manuscripts attached.

Best wishes [redacted] 5.1.2.e

[redacted] 5.1.2.e

[redacted] 5.1.2.e

[redacted] 5.1.2.e

(Emerging Viral Diseases-Expert Laboratory Network (EVD-LabNet) (europa.eu))

Netherlands Centre for Infectious Disease Control,
National Institute for Public Health and the Environment, Bilthoven



**National Institute for Public Health
and the Environment**
Ministry of Health, Welfare and Sport

Contact:
P.O. Box 1
3720BA Bilthoven, the Netherlands

Tel: 00-31 5.1.2.e
e-mail: 5.1.2.e [@rivm.nl](mailto:5.1.2.e@rivm.nl)

To: 5.1.2.e @rivm.nl]; 5.1.2.e @rivm.nl]; 5.1.2.e @rivm.nl];
 5.1.2.e @rivm.nl]
Cc: 5.1.2.e @rivm.nl]; 5.1.2.e @rivm.nl]; 5.1.2.e
 5.1.2.e @rivm.nl]
From: 5.1.2.e
Sent: Tue 5/24/2022 7:34:42 AM
Subject: Werkzaamheden met Monkeypox
Received: Tue 5/24/2022 7:34:43 AM
[Pokken LCI richtlijnen.pdf](#)
[Pathogen Safety Data Sheets Infectious Substances Monkeypox virus - Canada.ca.pdf](#)

Beste 5.1.2.e

Graag wil ik jullie informeren dat er vandaag gestart wordt met Monkeypox werkzaamheden in het BSL3 virologie lab. Voor deze week zal er enkel een kweek worden ingezet, zodat er materiaal voor diagnostiek klaar ligt mocht dat noodzakelijk zijn. Voor zover ik weet zijn er verder nog geen werkzaamheden gepland

Op dit moment zijn we hard bezig om de risicobeoordeling rond te krijgen. Volgens SOP 5.2 introductie nieuw agens zou er nog het een en ander met jullie afgestemd moeten worden.

Voor de arts-microbiologen en bedrijfsarts

- Controleren de factsheet. De factsheet van de LCI en Canadian-public health gaan we voor nou gebruiken, zijn er nog aanvullende aandachtspunten waar we rekening mee moeten houden die niet opgenomen zijn in deze factsheets? Anders graag akkoord hierop
- UMCU protocol - er zal over nagedacht moeten worden of dit door gegeven moet worden aan UMCU – mogelijk afhankelijk aan wat er nog gaat volgen aan werkzaamheden
- Vaccinaties – werkzaamheden gaan uitgevoerd worden door pokken gevaccineerde werknemers, overige V331 geautoriseerd personeel is niet gevaccineerd tegen pokken. Deze personen zijn niet aanwezig in het lab op het moment dat er werkzaamheden met monkeypox worden uitgevoerd. Vaccinaties zal nog verder besproken moeten worden – wel of niet vaccineren van overig geautoriseerd V331 personeel

Voorstel is om op korte termijn (deze week/volgende week) een overleg in te plannen om UMCU-protocol en Vaccinaties te bespreken.

Groet 5.1.2.e

5.1.2.e

Rijksinstituut voor Volksgezondheid en Milieu
Stafeenhed Organisatie, Bedrijfsvoering en Personeel (OBP)
Afdeling Veiligheid en Accreditatie (V&A)
 Antonie van Leeuwenhoeklaan 9 | 3721 MA Bilthoven
 Postbus 1 | Postvak 73 | 3720 BA Bilthoven

5.1.2.e

T: +31 (0) 8 5.1.2.e
M: +31 (0) 6

Werkdagen: ma, di, wo en vr

Pan-Orthopox/Parapox duplex realtime PCR v2.0:

Based on: Schroeder K, Nitsche A. *Multicolour, multiplex real-time PCR assay for the detection of human-pathogenic poxviruses*. Mol Cell Probes. 2010 Apr;24(2):110-3.

This Realtime PCR detects and differentiates between orthopox and parapox. Orthopox is further confirmed by a second conventional PCR and Sanger sequencing. Note: MGB probes are used which are available from a limited number of suppliers.

DNA isolation

Roche MagNAPure96 using the total nucleic acid extraction kit and external lysis protocol. (400 ul original material + 600 ul MagNAPure lysisbuffer)

Primer/probe mix

Orthopox F primer:	5'- TAATACTTCGATTGCTCATCCAGG -3'	30 pmol/ul
Orthopox R primer:	5'- ACTTCTCACAAATGGATTGAAAATC -3'	30 pmol/ul
Orthopox Probe:	5'-FAM-TCCTTTACGTGATAAATCAT-NFQ-MGB- 3'	5 pmol/ul
Parapox F primer:	5'- TCGATGCGGTGCAACAC -3'	30 pmol/ul
Parapox R primer:	5'- GCGGCGTNTTCTTCTCGGAC -3'	30 pmol/ul
Parapox Probe:	5'-VIC-TGCGGTAGAAGCC -NFQ-MGB-3'	5 pmol/ul

Amplificationmix:

5.0 ul	4x polymerase mastermix (Taqman Fast Advanced MM, Appliedbiosystems)
0.4 ul	Primer/probe mix
6.6 ul	PCR-grade water
8.0 ul	DNA

Cycling conditions (Roche Lightcycler480-II):

5 min 50°C
 20 sec 95 °C
 3 sec 95°C, 30 sec 60°C (x45)

Orthopox positives (FAM channel) are further confirmed with conventional PCR and Sanger sequencing (see below).

Sanger sequencing Orthopox:

Based on: Espy MJ, Cockerill III FR, Meyer RF, Bowen MD, Poland GA, Hadfield TL, Smith TF. Detection of smallpox virus DNA by LightCycler PCR. J Clin Microbiol. 2002 Jun;40(6):1985-8

Primers

Forward primer:	5'- CTAATATCATTAGTATACGCTACAC -3'	20 pmol/ul
Reverse primer:	5'- GAGTCGTAAGATATTTTATCC -3'	20 pmol/ul

Amplificationmix:

5.0 ul	10x PCR buffer (Qiagen)
2.0 ul	MgCl ₂ (Qiagen)
1.0 ul	dNTP plus mix (Roche)
1.0 ul	Forward primer (20 pmol/ul)
1.0 ul	Reverse primer (20 pmol/ul)
0.5 ul	Hotstar polymerase (Qiagen)
29.5 ul	PCR-grade water
10.0 ul	DNA

Cycling conditions:

15 min 95°C
 1 min 95°C, 1 min 45°C, 1 min 72°C (40X)
 10 min 72°C
 hold at 4°C

This should give a 204 nt fragment that can be Sanger sequenced using the same primers with your favorite Sanger sequencing protocol. Sequence discriminates between Cowpox, Monkeypox, Vaccinia, and Smallpox.



Rijksinstituut voor Volksgezondheid
en Milieu
Ministerie van Volksgezondheid,
Welzijn en Sport

Operating Procedure Orthopox PCR

5.1.2.e

A. van Leeuwenhoeklaan 9
3721 MA Bilthoven
Postbus 1
3720 BA Bilthoven
www.rivm.nl

KvK Utrecht 30276683

T +31 88 689 8989
info@rivm.nl

Datum
19 mei 2022

Ons kenmerk

Mastermix Worksheet Orthopox FastVirus

PCR-mix:

4x Taqman Fast Virus Mastermix	5.0	µl
Orthopox primer/probe mix	3.0	µl
PCR Grade Water	7.0	µl
TOTAL	15.0	µl

4x Taqman Fast Virus Mastermix	5.0	µl
EAV primer/probe mix	3.0	µl
PCR Grade Water	7.0	µl
TOTAL	15.0	µl

PCR-program LightCycler480:

Detection Format:	Multi Color Hydrolysis Probe					
PCR Program	Segment number	Temp (°C)	Hold Time (sec.)	Slope (°C/sec.)	Acquisition mode	
Denaturation	1	95	60	4.4	None	LC 480
Amplification	1	95	10	4.4	None	
(cycles:50)	2	60	30	2.2	Single	
Cooling	1	40	30	4.4	None	

- 1 Aliquot 15 µl portions of PCR-mix in 96-wellsplate
- 2 Add 5 µl RNA/DNA to 15 µl PCR-mix
- 3 Seal the 96-well plate
- 4 Centrifuge briefly
- 5 Incubate directly 15 minutes at 50°C (heating block)
- 6 Incubate directly 2 minutes at 95°C (heating block)
- 7 Centrifuge briefly
- 8 Keep plate at 4°C if you can't run directly
- 9 Run PCR program on LightCycler 480

Controls

Positive control
Internal control

Synthetic PC Blaasjes 10-5
EAV culture 10-3

Datum

19 mei 2022

Ons kenmerk**Primers & Probes****Orthopox Primer/Probe mix:**

POXRPF1	(10 pmol)	CTATACACCCGATCAACTAAAGGG
POXRPR	(10 pmol)	CTAGGAATAGAAATATTCTGCATCCA
POXRPP2	(5 pmol)	CTCTAATTCATCATGTGCC (FAM-BHQ1)

EAV Primer/Probe mix:

EAV-2043F	(15 pmol)	CTGTCGCTTGTGCTCAATTTAC
EAV-2193R	(15 pmol)	AGCGTCCGAAGCATCTC
EAV 2102P-2	(5 pmol)	TGCAGCTTATGTTCTTGCACTGTGTTT (TXR-BHQ2)

To: [redacted] 5.1.2.e [redacted]@rivm.nl]
From: [redacted] 5.1.2.e [redacted]
Sent: Mon 5/23/2022 3:30:37 PM
Subject: FW: Questions on the monkeypox outbreak - input for tomorrow's HSC meeting
Received: Mon 5/23/2022 3:30:37 PM

Hoi [redacted] 5.1.2.e

Ik zit morgen samen met [redacted] 5.1.2.e van VWS bij een meeting van HSC.

Zou iemand van de LCI onderstaande vragen kunnen beantwoorden of eventueel kunnen bespreken om 10.15 met mij en [redacted] 5.1.2.e?

Alvast bedankt voor je reactie.

Groeten,

[redacted] 5.1.2.e

Van: [redacted] 5.1.2.e [redacted]@minvws.nl>

Datum: 23 mei 2022 om 17:23:44 CEST

Aan: [redacted] 5.1.2.e [redacted]@rivm.nl>

CC: [redacted] 5.1.2.e [redacted]@minvws.nl>

Onderwerp: FW: Questions on the monkeypox outbreak - input for tomorrow's HSC meeting

Beste [redacted] 5.1.2.e

Zie hieronder de vragen voor de discussie morgen.

Groet,

[redacted] 5.1.2.e

International Policy

.....
 Ministry of Health, Welfare and Sports
 Directorate of Public Health | Crisismanagement and Infectious Diseases
 Parnassusplein 5 | 2511 VX The Hague
 P.O. Box 20350 | 2500 EJ The Hague
 The Netherlands

.....
 T +31.7 [redacted] 5.1.2.e
 M +31.6 [redacted] 5.1.2.e
 [redacted] 5.1.2.e @minvws.nl
www.rijksoverheid.nl

Van: [redacted] 5.1.2.e [redacted]@ec.europa.eu - [redacted] 5.1.2.e [redacted]@ec.europa.eu>

Verzonden: maandag 23 mei 2022 16:29

Aan: [redacted] 5.1.2.e [redacted]@ec.europa.eu

Onderwerp: Questions on the monkeypox outbreak - input for tomorrow's HSC meeting

Dear members of the HSC,

Dear colleagues,

In preparation of the urgent HSC meeting tomorrow on Monkeypox (and hepatitis), please find below a set of questions. We would like to ask each country to please provide us with your responses – **either in writing to the HSC mailbox or orally tomorrow at the meeting**. These questions will form the basis for the Monkeypox discussion point at tomorrow's meeting.

Note that the Commission is consulting the HSC ComNet with questions on risk communication and community engagement activities (with the MSM communities) for awareness raising.

Finally, please note that ECDC has published its rapid risk assessment on the Monkeypox outbreak, which is available online at:

<https://www.ecdc.europa.eu/en/publications-data/risk-assessment-monkeypox-multi-country-outbreak>.

Best regards,

HSC Secretariat

For the answers provided to the questions below, please indicate whether your country's guidance/recommendations are in line with ECDC's RRA.

Disease management of cases and contacts

1. Is your country **recommending to isolate confirmed cases and/or quarantine high risk contacts* (including pets)?**

2. Can your country accept the **recommendations on quarantine and isolation** made by the ECDC in their rapid risk assessment?
3. Has your country's **contact tracing mechanism been updated in response to the monkeypox outbreak**? For example, are you looking into imported cases (contacts abroad)?
4. Has your country **issued guidance for the management of monkeypox cases (and close contacts*)**? If so, please provide the link/indicate whether this can be shared with other countries. Also, is your guidance in accordance with the proposed ECDC risk assessment?

Laboratory diagnostics and sequencing

1. Has your country **sufficient diagnostic testing capacities and capabilities in place** for orthopoxviruses (in particular for the molecular diagnostic of monkeypox virus 2022 strain)? If not, what are your exact needs in diagnostic testing of monkeypox virus (MPXV)?
2. Does your country have procedures in place that allow you to **refer specimens for confirmatory testing** (e.g. in another EU/EEA country)? Do you require support for sample shipping?
3. Is there a need for **sequencing support** in your country?

Vaccination & Antivirals

1. Is your country **recommending to vaccinate at risk contacts*** with the smallpox vaccine for post exposure prophylaxis?
2. Is your country **recommending to vaccinate certain healthcare professionals** with the smallpox vaccine for pre or post- exposure prophylaxis? If so, please specify which healthcare professionals.
3. Is your country **recommending antiviral treatment of confirmed cases**? If so, please provide details on therapies available.

** Please see definitions of 'close contacts' as proposed by ECDC in its RRA published on 23 May 2022.*