DECODING MPOX

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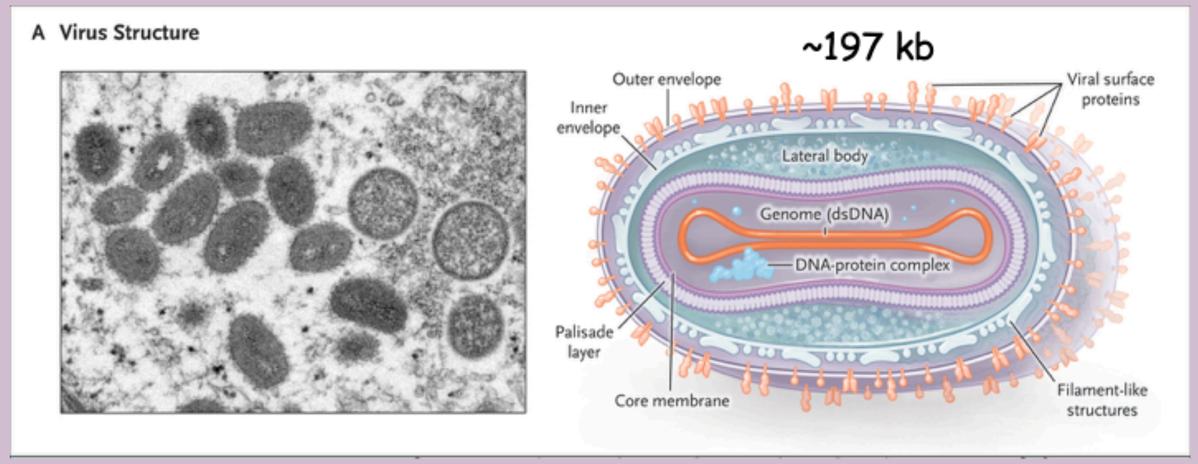
INTRODUCTION

- Mpox- Initial discovery in monkeys in a Danish laboratory in 1958
- First human case- 9-year-old boy in the democratic republic of the Congo in 1970
- Clade I : Congo Basin
  - subclades Ia and Ib
  - More deadly and infectious; Primarily through household contact frequently infects children
- Clade II : West African clade
  - subclades IIa and IIb
  - spread mainly through sexual contact



https://www.nejm.org/doi/full/10.1056/NEJMra2208860

- Enveloped double-stranded DNA virus
- Family: poxviridae
- Genus: orthopoxvirus

INTRODUCTION (CONC.)

- Pox viruses are grouped into eight genera
- Only four genera cause human infections

ORTHOPOXVIRUS	Variola
	Vaccinia
	Monkeypox
	Cowpox
	Buffalopox
	Cantagalo and Aracatuba
PARAPOXVIRUS	Orf
	Pseudocowpox (Paravaccinia)
	Bovine papular stomatitis
	Deerpox
	Sealpox
MOLLUSCIPOXVIRUS	Molluscum contagiosum
YATAPOXVIRUS	Tanapox
	Yabapox

MODE OF TRANSMISSION

Virus enters body through broken skin, respiratory tract, or mucous membranes [eye, nose, mouth]

ANIMAL-HUMAN TRANSMISSION:

- Direct contact or meat consumption : Bites, Scratches, Bush meat preparation

HUMAN-HUMAN TRANSMISSION:

- Direct contact : close contact with lesions, body fluids, Respiratory droplet
- Indirect contact : contaminated materials such as bedding

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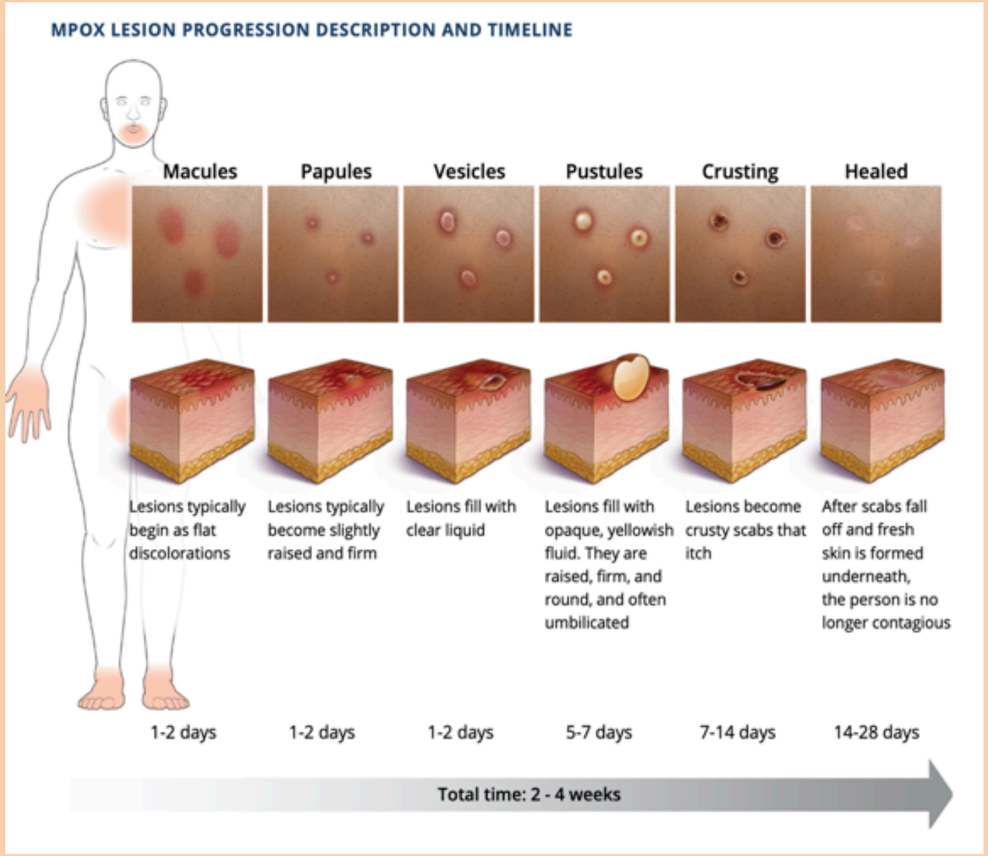


https://www.std.uw.edu/page/clinical-guides/guides#mpox

INCUBATION PERIOD:

Usually from 6 to 13 days but can range from 5 to 21 days

PERIOD OF COMMUNICABILITY:

1-2 days before the rash to until all the scabs fall off/get subsided

PRODROME (0-5 DAYS):

- Fever
- Lymphadenopathy (Occurs with fever onset, periauricular/axillary/cervical/inguinal, Unilateral or bilateral)
- Headache, muscle ache & exhaustion
- Chills and/or sweats
- Sore throat and cough

SKIN INVOLVEMENT (RASH):

- Usually begins within 1-3 days of fever onset
- Centrifugal spread
- Lasting for 2-4 weeks
- Deep seated, well circumscribed and often develop umbilication
- Lesions are painful until healing phase occurs
- Classical lesion is vesico-pustular
- Predilection for palm and soles is characteristi

STAGES OF RASH:

First lesion	Tongue and mouth
Within 24 hours	Macules
By 3rd days	Progression to Papules
By 4th-5th days	Lesion become vesicles (Raised and fluid filled)
By 6th-7th days	Pustular, Sharply raised, filled with opaque fluid
By the end of second week	Drying up and crust
Scabs remains for a week before falling off	

COMPLICATIONS:

- Secondary bacterial infections
- Dehydration
- Skin infection
- Pneumonia
- Encephalitis
- Corneal involvement (May lead to loss of vision)

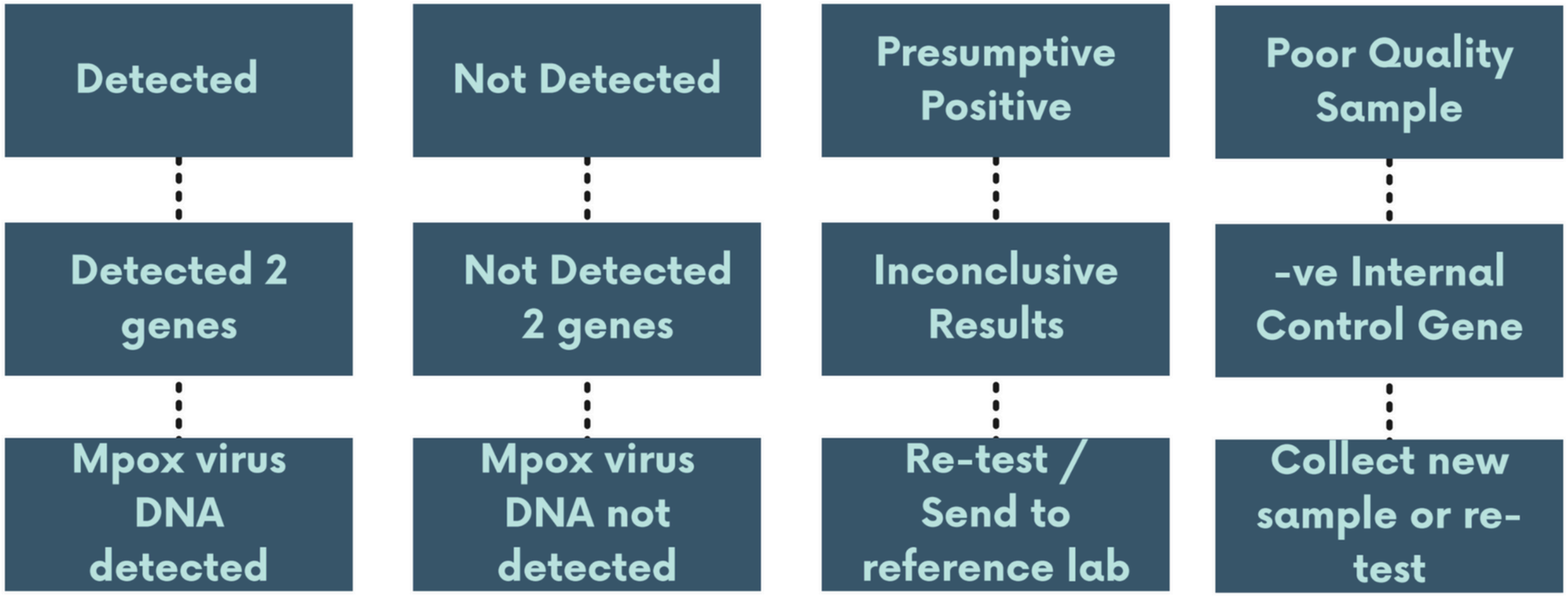
CLOSE DIFFERENTIAL DIAGNOSIS

- Varicella (Chicken pox)
- Disseminated herpes zoster
- Disseminated herpes simplex
- HFMD
- Measles
- Chancroid
- Secondary syphilis
- Infectious mononucleosis
- Other pox viruses causing disease

LABORATORY DIAGNOSIS

- Confirmatory test: Polymerase chain reaction (PCR) is the preferred laboratory test
- Nucleic acid amplification testing (NAAT), using real-time or conventional polymerase chain reaction (PCR), for detection of unique sequences of viral DNA
- PCR can be used alone, or in combination with sequencing.
- PCR protocols for the detection of OPXV and more specifically MPXV-With distinction of Congo basin and west African clades
- Some protocols involve two steps, in which the first PCR reaction detects
- OPXV, but does not identify which species.
- This can then be followed by a second step, which can be PCR-based or utilize sequencing, to specifically detect MPXV

GUIDELINE : MPOX VIRUS DNA DETECTION IN THAILAND

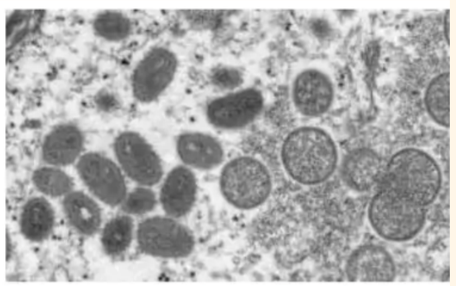


REAL-TIME PCR FOR MPOX DNA (AT LEAST 2 TARGET GENES)

Test Kits Available in Thailand	Clade (s) detected
Uni_medica Multiplex real_time PCR Kit	MPXV/ MPXV Clade I/ MPXV Clade II
QIAstat-Dx Viral Vesicular Panel (Pan OPXV is being added by QIAGEN to cover clade Ib)	OPXV/ MPXV Clade I/ Clade II/ others
BioPerfectus Monkeypox Virus Real_time PCR Kit, Life Sciences	OPXV/ MPXV MPXV Clade I/ MPXV Clade II
Luna Universal Probe One-Step RT-qPCR Kit	MPXV/ MPXV Clade I/ MPXV Clade II
Certest Biotec	MPXV
VIASURE	MPXV
VIRCELL	OPXV/ MPXV

OTHER METHODS

- Electron microscopy: Electron microscopy can be used this method is not routinely used for the diagnosis of poxviruses.
- Viral culture: Virus isolation is not recommended as a routine diagnostic procedure
- Antigen and antibody detection methods do not provide monkeypox_specific confirmation due to serological cross-reactivity
- Not recommended for diagnosis



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GLOBAL HISTORICAL & CURRENT SCENARIO

1958 — Mpox first discovered in colonies of monkeys

1970 — First human case reported in DRC (Democratic Republic of Congo)

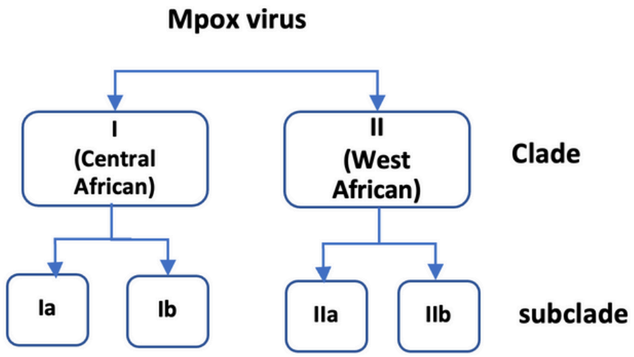
2003 — First Mpox outbreak outside Africa was reported in USA

2017 — Mpox reemerged in Nigeria

May 2022 — Global outbreak

สถานการณ์เชื้อไวรัส Mpox ทั่วโลก

- ปัจจุบันได้จัดจำแนกไวรัสฝีดาษวานรออกเป็น clade I Central African และ clade II West african

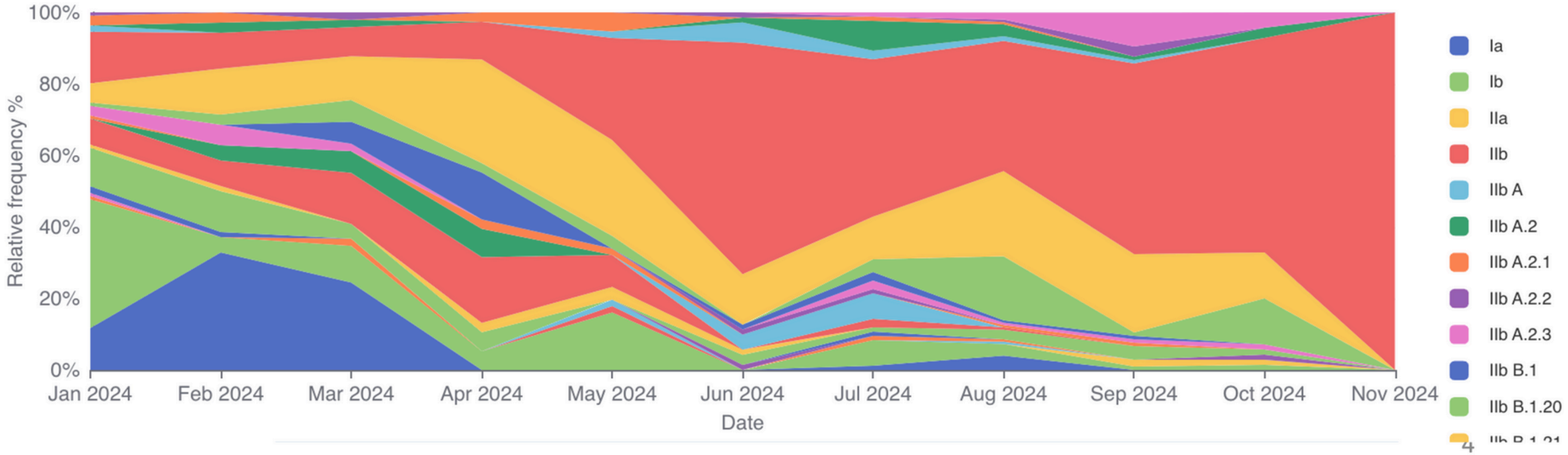


Data as of: 22-Nov-24

Confirmed* mpox cases since January 1, 2024**

21,401 Total clade I and clade II mpox	**11,911** Cases in locations*** with only clade I mpox
5,813 Cases in locations with only clade II mpox	**3,673** Cases in locations with both clade I and clade II mpox

- Clade IIb ซึ่ง clade IIb เป็นสายพันธุ์ที่มีการระบาดมากที่สุดในปัจจุบันจัดแบ่งได้เป็น 34 สาย พันธุ์ย่อย (lineage) สถานการณ์ติดเชื้อจากทั่วโลกตั้งแต่วันที่ 1 มกราคม พ.ศ. 2567 ถึงปัจจุบัน พบผู้ติดเชื้อทั้งสิ้น 21,401 ราย



ข้อมูล ณ วันที่ 26 พฤศจิกายน 2567

แหล่งอ้างอิง : https://gisaid.org/mpox-variants-dashboard/

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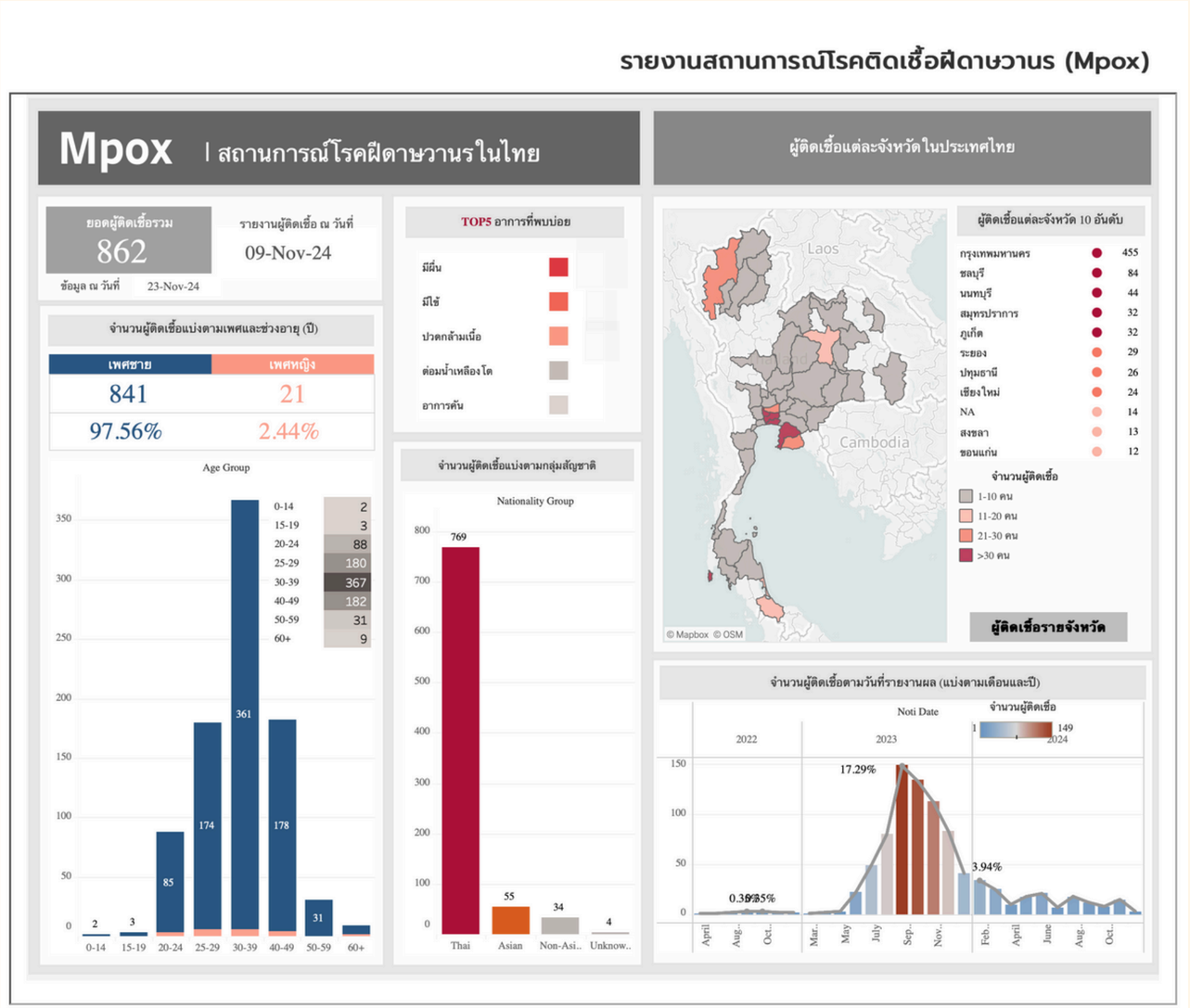
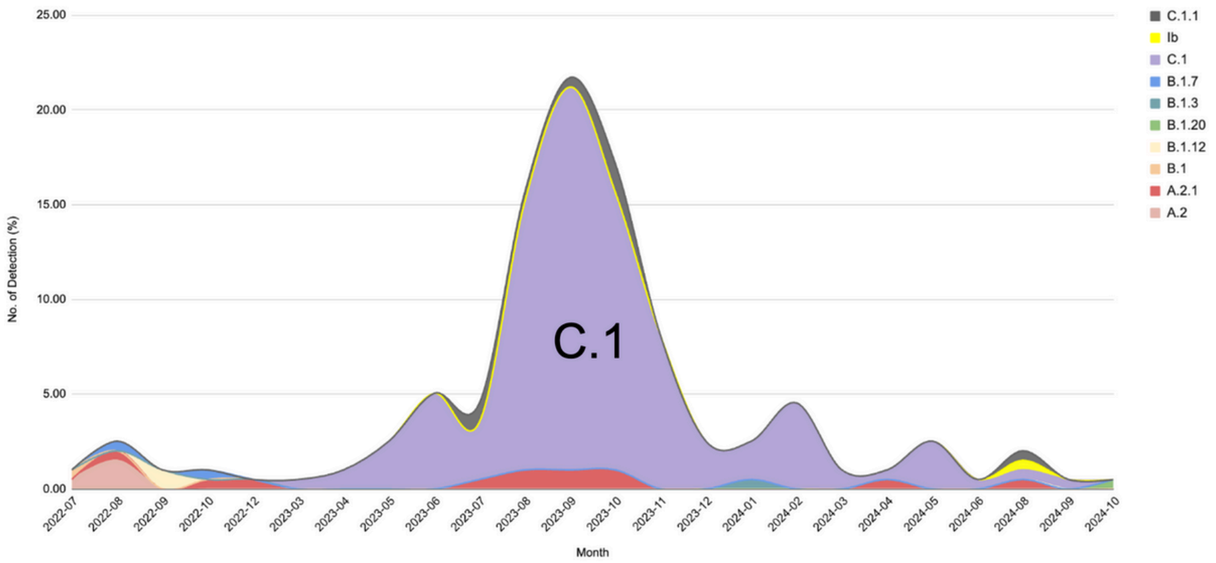
- **17 July 2022** - First case in Thailand
- **23 July 2022** - WHO declared Mpox as PHEIC (Clade IIb)
- **August 2022** - Mpox outbreak peak



https://ddc.moph.go.th/monkeypox/dashboard.php

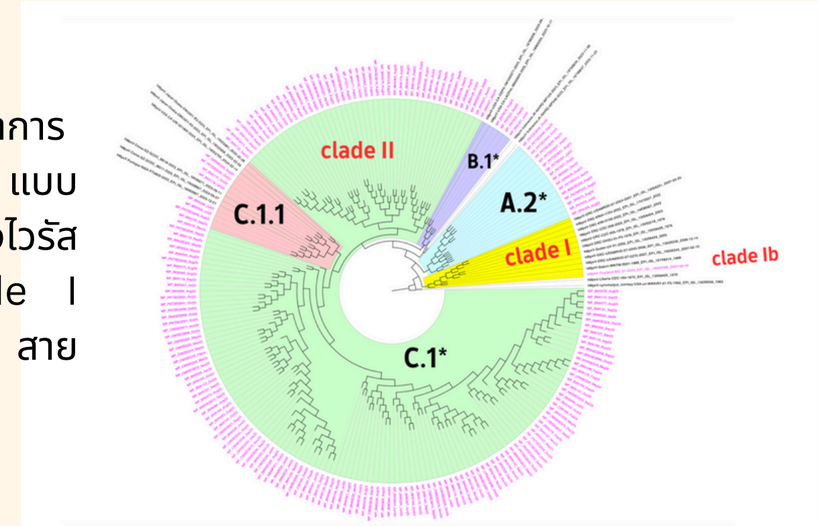
•สถานการณ์การระบาดของโรคฝีดาษวานรในประเทศไทยมีการเพิ่มขึ้นอย่างต่อเนื่อง นับตั้งแต่พบผู้ติดเชื้อรายแรกเมื่อวันที่ 17 กรกฎาคม พ.ศ. 2565 ซึ่งเป็นชาวต่างชาติที่อาศัยอยู่ในพื้นที่จังหวัดภูเก็ต
•ปัจจุบัน (ข้อมูล ณ วันที่ 26 พฤศจิกายน 2567) พบผู้ป่วยยืนยันสะสมทั้งสิ้น862 ราย โดยส่วนใหญ่เป็นเพศชาย อายุระหว่าง 30-39 ปี และมีผู้เสียชีวิตสะสม 13 ราย

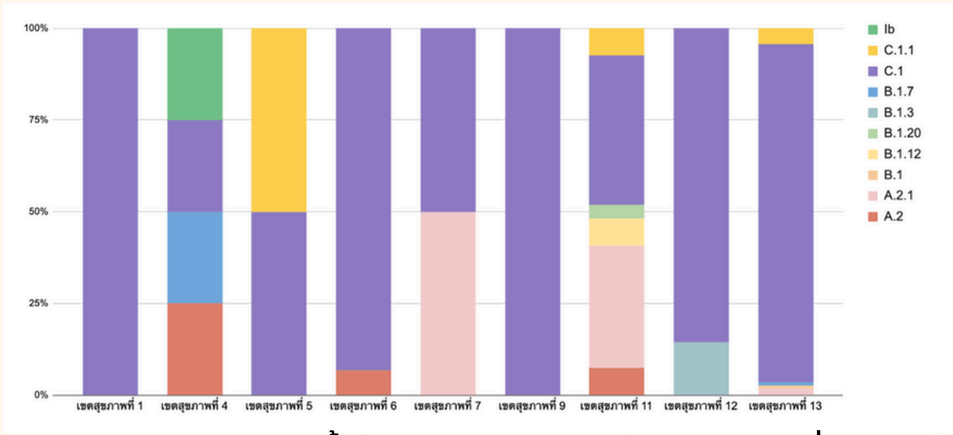
สถานการณ์เชื้อไวรัส Mpox ในประเทศไทย



- จากการเฝ้าระวังการเปลี่ยนแปลงสายพันธุ์ด้วยการถอดร[illegible]ม ทั้งจีโนม (whole genome sequencing) จำนวน 198 ราย ผลการวิเคราะห์พบ 9 สายพันธุ์ย่อย (lineage) ได้แก่ A.2, A.2.1, B.1, B.1.12, B.1.3, B.1.7, B.1.20, C.1 และ C.1.1 โดยพบสายพันธุ์ย่อย C.1 มากที่สุดคิดเป็นร้อยละ 83.84 รองลงมาคือสายพันธุ์ย่อย A.2.1 (ร้อยละ 6.06), C.1.1 (ร้อยละ 4.04) และ A.2 (ร้อยละ 2.02) ตามลำดับ ทั้งนี้พบว่ามีการระบาดสูงสุดในช่วงเดือนกันยายน 2566 คิดเป็นร้อยละ 21.72 ของจำนวนผู้ป่วยทั้งหมด

ภาพแสดงความสัมพันธ์เชิงวิวัฒนาการด้วย phylogenetic tree แบบ Maximum Likelihood ของไวรัส Mpox โดยแยกออกเป็น Clade I และClade II โดยพบ Clade II สายพันธุ์ย่อย C.1* มากที่สุด





•พบการระบาดของเชื้อไวรัส Mpox มากสุดในเขตสุขภาพที่ 13 คิดเป็นสัดส่วน 59.09% รองลงมาคือเขตสุขภาพที่ 11 คิดเป็นสัดส่วน 13.64%

GLOBAL HISTORICAL & CURRENT THAILAND SCENARIO

- September 2023 - New Clade Ib strain first detected in sex work in Eastern Congo
- Since early 2024 - Upsurge in number of cases in African region
- 14th August 2024 - WHO declared Mpox as PHEIC for second time (Clade Ib)
- August 2024 - New Clade Ib strain detected in a 66 year old man who travel from Africa to Thailand

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LABORATORY ALGORITHM USED FOR CONFIRMATION OF THE FIRST MPOX CLADE IB CASE IN THAILAND

Suspect case
Collection date
: 16 Aug 2024
Receiving date
: 19 Aug 2024

swab sample of the skin lesion

Real-time PCR

OPXV (+)
MPXV generic (+)
MPXV Clade I (-)
MPXV Clade II (-)

Metagenome analysis

Case Confirmation
22 Aug 2024

- ไม่มีโรคประจำตัวและไม่เคยได้รับวัคซีนฝีดาษมาก่อน
- มีประวัติเดินทางไปทำธุรกิจที่เมือง **BUKAVU**แคว้น **SOUTH KIVU** สาธารณรัฐประชาธิปไตยคองโก ในช่วงต้นเดือนสิงหาคม **2567**
- หลังจากกลับจากเดินทางในช่วงต้นเดือนสิงหาคม **2567** เริ่มมีอาการคันบริเวณอวัยวะเพศ และเมื่อเดินทางกลับมาถึงประเทศไทยพบว่ามีอาการไข้ ปวดกล้ามเนื้อ เจ็บคอ อ่อนเพลีย พร้อมกับมีผื่น ตุ่มหนอง และตุ่มน้ำ กระจายตามร่างกาย

Virus detail

Virus name:	hMpxV/Thailand/NIC-31/2024
Accession ID:	EPI_ISL_19350788.1
Clade / Lineage	Ib
AA Substitutions	A5L L270I, A5L T63V, A5L T122A, A8L F594S, A9R E226D, A10L V90E, A11L A229T, A11L D267N, A11L F283Y, A11L M740I, A11L N98D, A14L T17A, A15L P39H, A18L T184I, A19R A348P, A19R I249V, A19R I264T, A19R K62E, A19R K435E, A19R P76Q, A19R Q243R, A19R Q463R, A22R I313S, A24R D32N, A24R D256E, A24R L307S, A24R N100D, A25R D6V, A25R N282D, A25R R273Q, A26L K52R, A26L N71D, A26L R23C, A26L V36A, A27L A580D, A27L N443S, A27L S36T, A27L T494I, A28L I355T, A28L I370del, A28L I371del, A28L M14T, A28L Q493P, A28L Y358H, A29L H107R, A29L R74H, A31L T222A, A31L Y42S, A33R L36S, A33R N120del, A33R N121del, A33R T2A, A33R Y119del, A34L P2S, A35R K67E, A35R V88A, A37R V46A, A38R I111V, A38R ins212stop, A38R Y217H, A39R D106N, A39R S161L, A40L I112V, A40L Y107S, A41L G31D, A41L M111I, A41L V96I, A43R A125V, A43R M145T, A43R Y160D, A44R N21S, A45L R315C, A47R D24N, A47R L227S, A47R Y221H, A49R I139V, A51R L85M, A51R S127Y, B1R F106V, B1R L94V, B1R V66G, B2R D177E, B2R I205T, B2R T167A, B2R T239I, B3R K300stop, B4R S227F, B4R S419R, B4R V265I, B5R C506H, B5R K185R, B7R F80C, B7R F166Y, B7R I167L, B7R P50Q, B8R G100E, B9R F263L, B9R I108R, B10.5R M1I, B10.5R S18T, B10R C120S, B11R I100V, B11R N71D, B11R Q133R, B12R K230E, B13R E134G, B14R E157D, B14R K140E, B14R L158I, B14R T156A, B14R Y99C, B15L I5M, B15L Y6H, B16R N178K, B17R C349Y, B17R G354S, B17R R733Q, B17R V621A, B17R V690A, B17R W169R, B18R A58P, B18R D57M, B18R I51L, B18R I62del, B18R I79V, B18R N61del, B18R N68S, B18R Q64del, B18R R69T, B18R S39P, B18R V26A, B18R V59S, B18R V63del, B18R Y65I, B19R A34V, B19R I142T, B19R T300A, B19R V311A, B20R I11V, B20R L21F, B21R A98D, B21R A475V, B21R A1305T, B21R D379G, B21R D1717G, B21R E1057D, B21R F3L, B21R H363R, B21R I1741M, B21R K331N, B21R L734del, B21R L1704R, B21R N209D, B21R N334I, B21R S722P, B21R S781P, B21R T928A, B21R V173I, B21R V515A, B21R V788A, C1L C279Y, C2L M160I, C2L P254A, C2L Y374S, C3L F36S, C4L T159I, C5L N68S, C5L T3A, C6R M148L, C6R T109A, C6R V63A, C7L A185T, C7L H17del, C7L V16del, C8L S95N, C9L C48R, C9L I232K, C11L E17V, C11L M37V, C11L R314del, C11L T263M, C15L S78P, C16L R438G, C18L C622Y, C18L K125E, C18L V323A, C19L K353E, C20.5L

Sample information

Collection date:	2024-08-16
Location:	Asia / Thailand / Bangkok
Host:	Human
Additional location information:	Resident of Bukavu, City in eastern Democratic Republic of the Congo (DRC)
Gender:	Male
Patient age:	66
Patient status:	Hospitalized
Specimen source:	Lesion swab from the tip of the penis
Additional host information:	Travel history from Democratic Republic of the Congo (DRC)
Sampling strategy:	
Outbreak:	
Last vaccinated:	
Treatment:	Tazocin, Doxycycline Tecovirimat
Sequencing technology:	Illumina MiSeq
Assembly method:	bwa-0.7.18, samtools-1.20 and ivar1.4.3
Coverage:	12.43x
Comment:	Stretches of NNNs (3.40% of overall sequence). F4.5R_I41stop results in 69.9% truncation of the protein sequence. F4.5R_M22stop results in 84.2% truncation of the protein sequence. F4.5R_V38stop results in 72.2% truncation of the protein sequence. F4.5R_G64stop results in 52.6% truncation of the protein sequence. F4.5R_M28stop results in 79.7% truncation of the protein sequence. F4.5R_A48stop results in 64.7% truncation of the protein sequence. A38R_ins212stop results in 7.5% truncation of the protein sequence. B3R_K300stop results in 1.3% truncation of the protein sequence. Gap of 3 nucleotide(s) found at refpos 2254 (FRAMESHIFT). Insertion of 1 nucleotide(s) found at refpos 4614 (FRAMESHIFT). Insertion of 1 nucleotide(s) found at refpos 7000 (FRAMESHIFT). Gap of 6 nucleotide(s) found at refpos 7033 (FRAMESHIFT). Gap of 4 nucleotide(s) found at refpos 7159 (FRAMESHIFT). Gap of 1 nucleotide(s) found at refpos 9009 (FRAMESHIFT). Gap of 6 nucleotide(s) found at refpos 16625 (FRAMESHIFT). Gap of 3 nucleotide(s)

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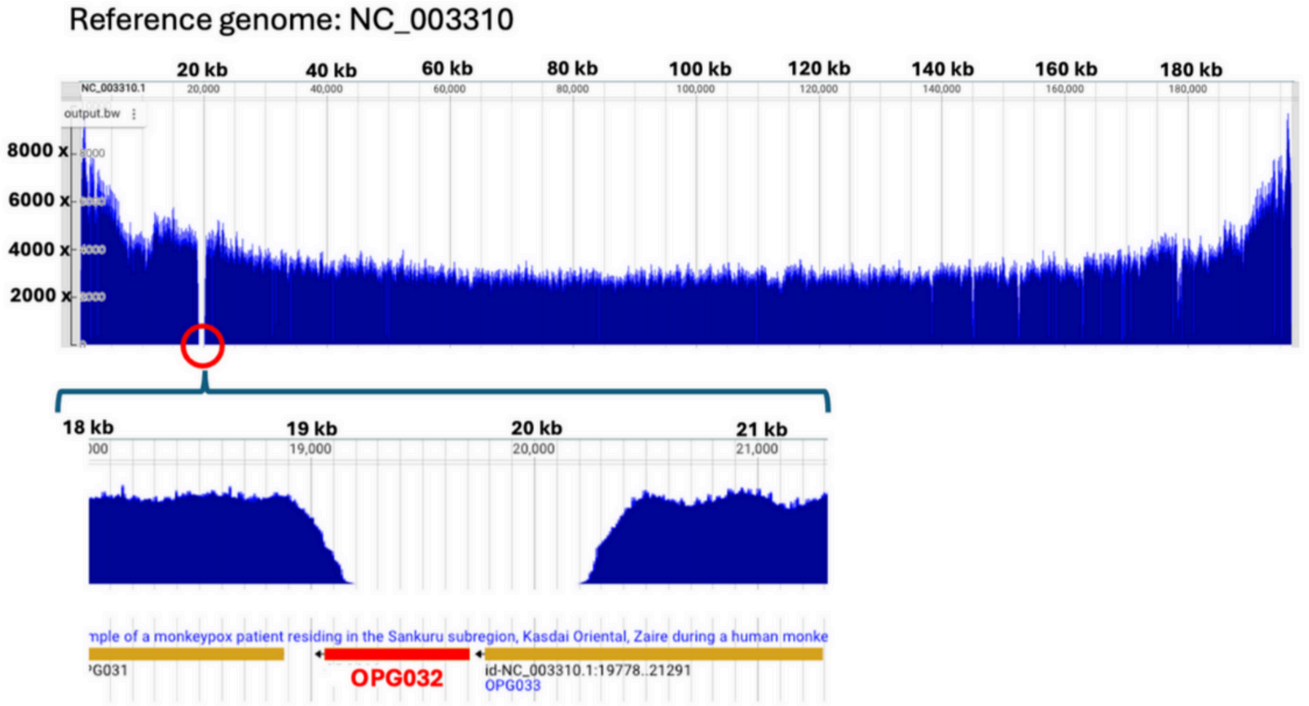
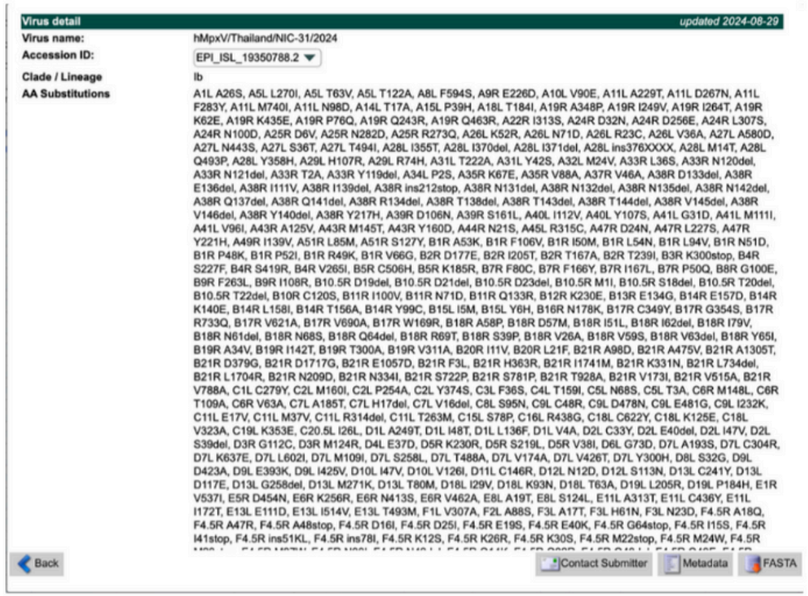
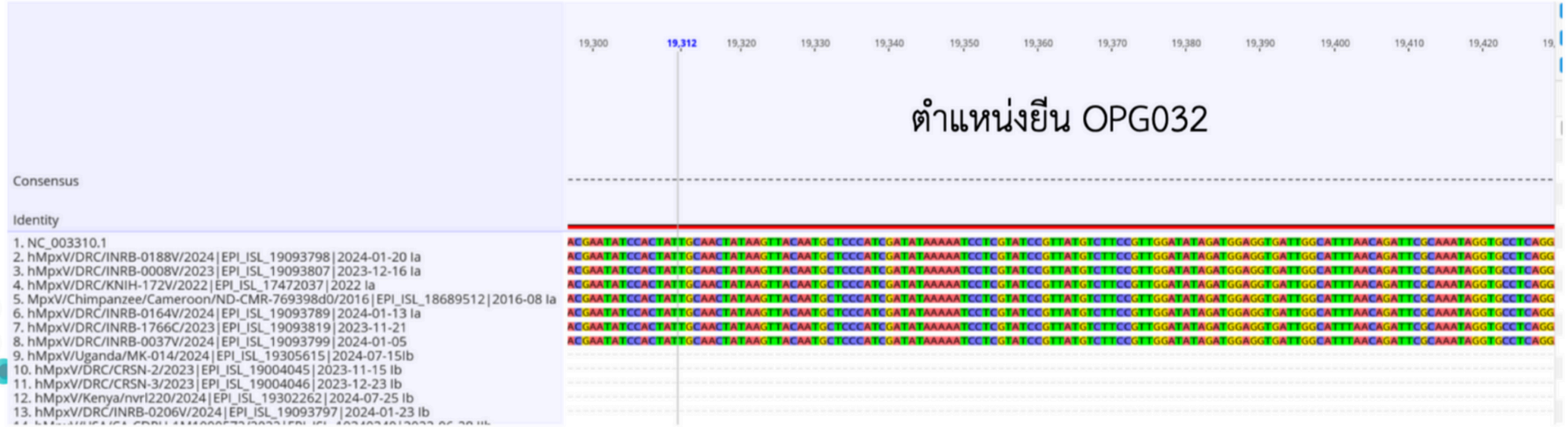
COMPLETE GENOME OF THE FIRST MPOX CLADE IB CASE IN THAILAND



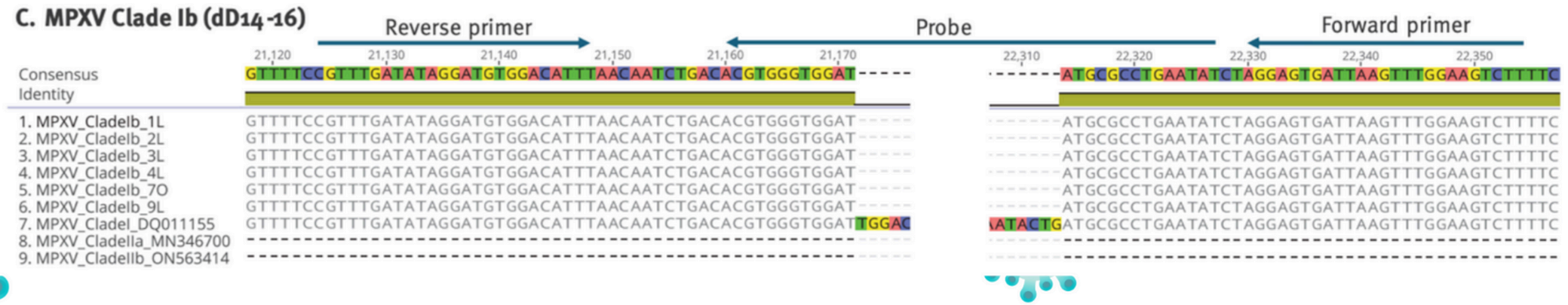
A large ~1 kbp deletion in the MPXV genome (Δ19,128–20,270 coordinates relative to the clade I reference genome, GenBank accession NC_003310) has been reported in genome sequences from Kamituga mpox outbreak. (Ref. E. H. Vakaniak, Nature Medicine, 2024)





Primers and Probes Design in Mpox Diagnostics

- Primers and probes are designed to target specific clades of the Mpox virus.
- Mutations or deletions in primer/probe binding regions can lead to false-negative results.
- Example: Detection of the Congo Basin Clade Ib strain using Real-Time PCR with Clade I-specific primers (Congo Basin (C3L)).
- **Limitation:** Deletion of ~1,000 base pairs in the OPG032 gene (Vakaniaki et al., 2024).
- Result: Primers fail to bind to Clade Ib target sequence.
- This can cause false-negative results, even when the patient is infected with Clade Ib.
- **Key takeaway:** Careful selection and design of primers and probes are critical to avoid false-negative results caused by mutations or deletions.



Real-Time PCR Specific Primer Used for Clade Ib Differentiation (L. Schuele et al., 2024)

Monkeypox Virus Clade Ib (dD14-16) Assay Design

- This diagram outlines the specific primer sequences used in the Real-Time PCR assay designed to differentiate between the Monkeypox virus Clade Ib
- The assay targets unique genetic markers present in the dD14-16 region of the viral genome, which helps in precise clade identification
- This method improves the diagnostic accuracy of Clade Ib infections, facilitating better outbreak monitoring and control.

HIGHLIGHTS ON SURVEILLANCE OF MPOX

- Even one case of Mpox is to be considered as an outbreak
- Key stake holders in surveillance are:
  - Points of entries (PoEs)
  - Antenatal clinics
  - Sexually Transmitted Diseases clinics
  - Designated Lab network

CONTACT TRACING

- A contact is defined as any person, who has one or more of the following exposures with a probable or confirmed case of Mpox during the period of communicability.
- Home quarantine and active follow up for contacts of confirmed cases
- Self monitoring for contacts of probable cases
- Monitor at least daily for 21 day

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THE ROLE OF LABORATORY NETWORKS IN MPOX OUTBREAKS

1. Early detection and rapid diagnosis
2. Data sharing
3. Coordination for outbreak control

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EXTERNAL QUALITY ASSURANCE (EQA) PROGRAM

1. DMSc plays a crucial role in ensuring laboratory accuracy nationwide
2. EQA ensures testing accuracy across labs
3. Regular assessments and global standards compliance

Annual Assessment
The EQA program is conducted once per year to ensure consistent quality

LABORATORY NETWORKS

- Department of Medical Sciences
  - National Institute of Health
  - 15 Regional Medical Sciences Centers
- Hospitals/ Research Institute/ Office of Disease Prevention and Control/ etc.

IMPORTANT POINTS TO REMEMBER REGARDING SAMPLE COLLECTION

- Best sample - Collected from lesion at any stage of the rash from multiple sites
- Lesion is swabbed vigorously, to ensure adequate viral DNA is collected. Both dry swabs and swabs placed in viral transport media (VTM) can be used.
- Two lesions of the same type should be collected in one single tube, preferably from different locations on the body and which differ in appearance
- In the absence of skin lesions - Prefer NPS/OPS/anal/rectal swab
- Blood sample is least preferable

- All specimens being transported should have triple packaging, labelling and documentation.
- Specimens should be refrigerated or frozen within an hour of collection and transported to the laboratory as soon as possible.
- If transport exceeds 7 days, specimens should be stored at -20°c or lower.
- Longer term specimen storage (>60 days from collection) is at -70°C.

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FUTURE DIRECTIONS AND CONCLUSION

1. Improve rapid testing and expand lab networks globally to stay ahead of diseases like Mpox
2. New technologies like Whole Genome Sequencing (WGS) will be crucial for real-time monitoring
3. Key to success: Early detection, accurate testing, and well-coordinated lab networks supported by EQA

Annual Assessment
The EQA program is conducted once per year to ensure consistent quality

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THANK YOU
FOR YOUR ATTENTION