Monkeypox Virus: What the Lab Needs to Know

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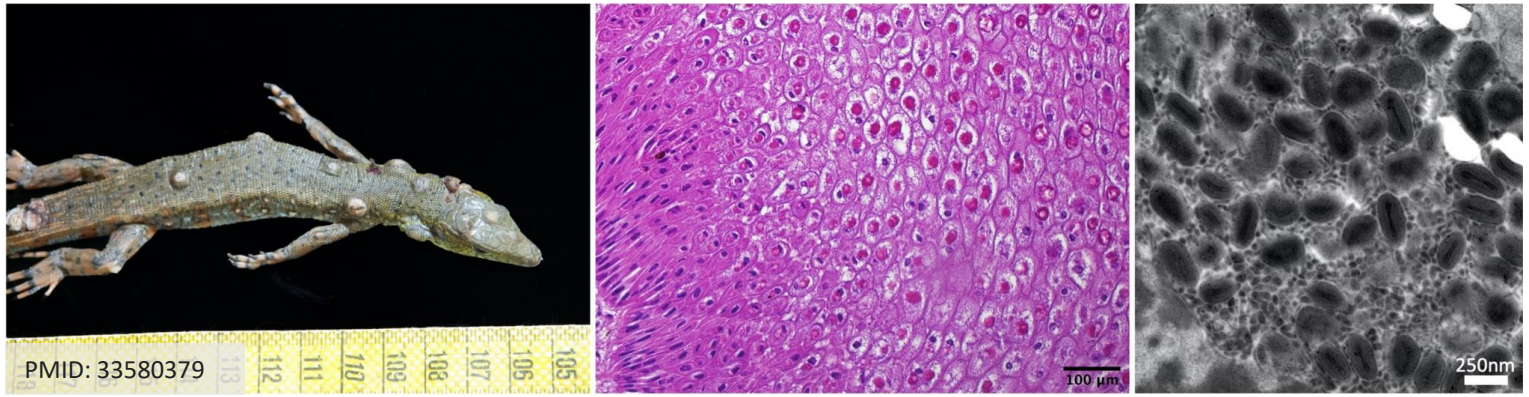
Objectives

- Describe basic virology and strain differences of monkeypox virus
- Appreciate the historical origins of monkeypox virus and the 2022 global outbreak
- Summarize how lab testing processes, including the pre- and post-analytic phases, affect results for monkeypox diagnosis

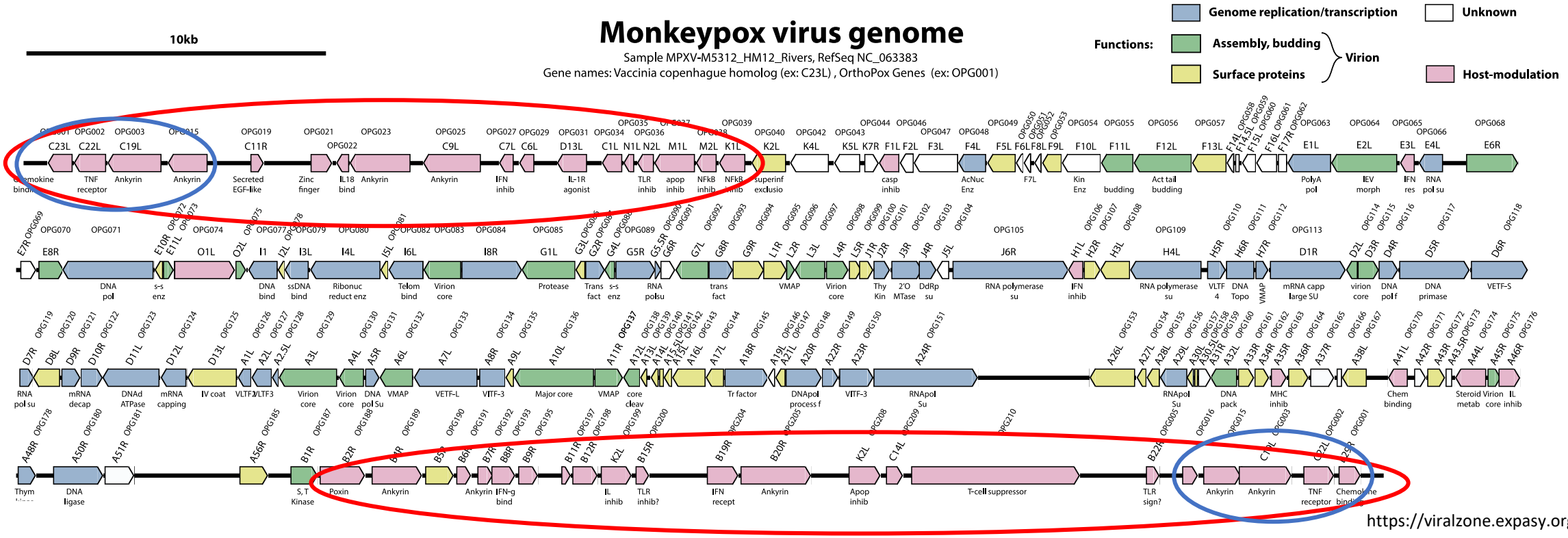
Virology of monkeypox virus

- Member of the Poxviridae family of which 4 genera are associated with human disease
  - Orthopoxvirus (variola, vaccinia, monkeypox, camelpox)
  - Parapoxvirus (Orf virus), Molluscipoxvirus (Molluscum), and Yatapoxvirus (Yaba monkey tumor virus)
- Poxviridae have an incredibly wide host range
  - Variola (smallpox) and Molluscum are human-only pathogens
  - Capable of infecting vertebrates (18 genera) and insects



Genetic structure of poxviruses

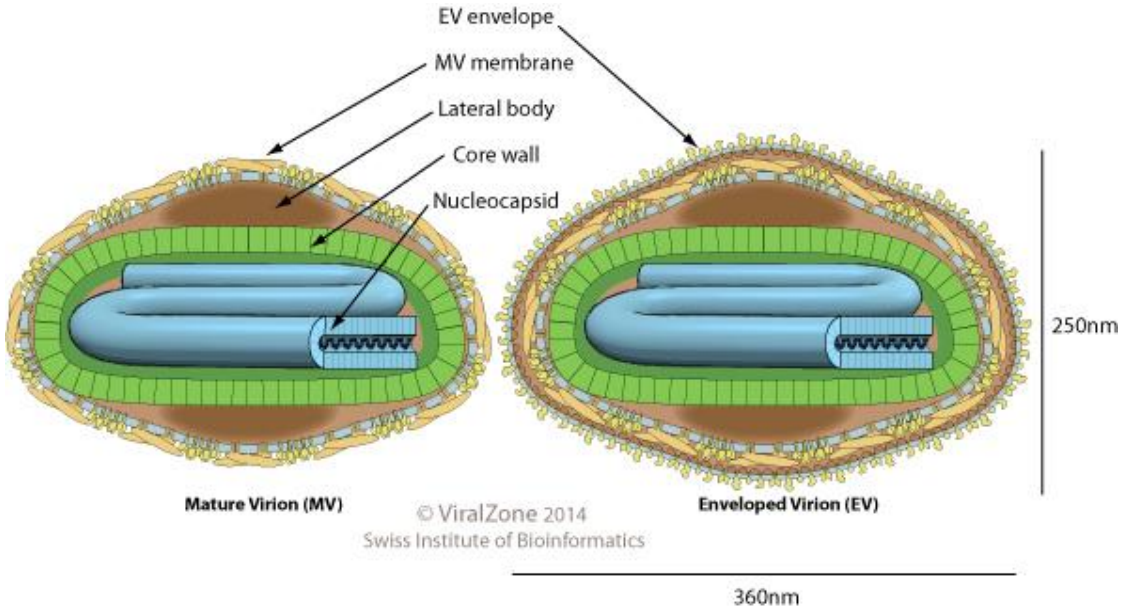
- Linear, double-stranded DNA genome, approx. 200kb in size
  - Genome consists of central core of essential proteins and flanking regions that affect the host response

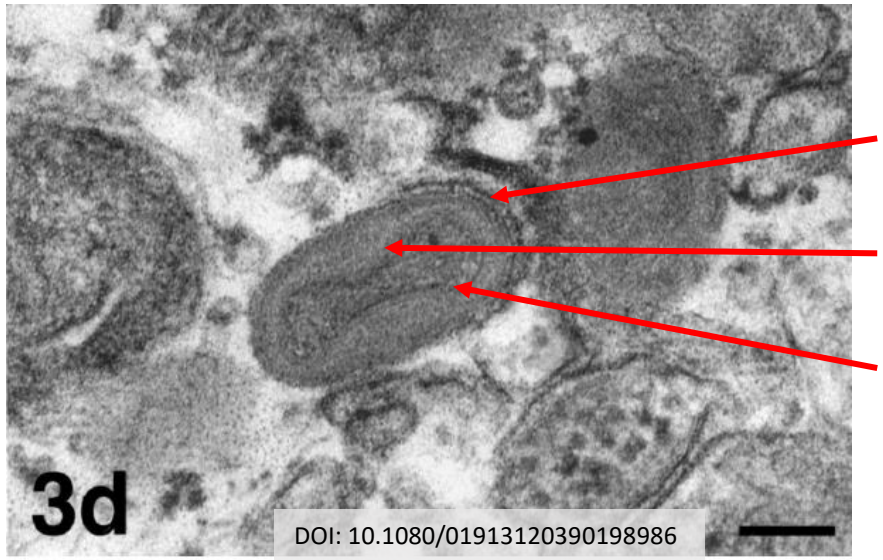


https://viralzone.expasy.org/9959

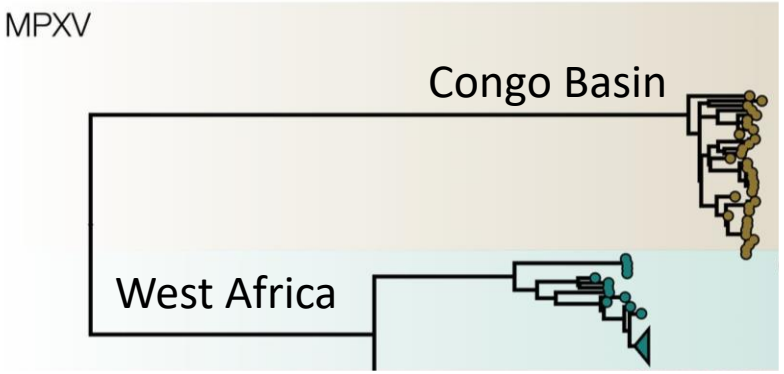
Structure of the poxvirus virion

- Poxviruses have a characteristic appearance
  - May exist in one of two forms: mature virus or enveloped virus
  - On EM, virions appear brick-shaped and are approx. 250nm x 200nm





Monkeypox virus clades



- Historically, MPXV was divided into two clades
  - Congo Basin – DRC (Zaire), Gabon, Cameroon
  - West African – Nigeria, Sierra Leone, Liberia
- Now: Clade I, IIa, IIb
  - Done to help decrease stigma caused by associating disease with a location
  - Proposed name for Clade IIB is *human monkeypoxvirus 1* (hMPXV1)
  - Further division into lineages is possible, but mostly academic at this point

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  - West African
  - Congo Basin
- Now: Clade I, IIa, IIb
  - Proposed name for Clade IIB is *human monkeypoxvirus 1* (hMPXV1)
  - Further division into lineages is possible, but mostly academic at this point
  - Clinical features vary between clades
    - IIb has lower mortality and hospitalizations
    - Fewer lesions but increased human-to-human transmission

Historical name	Congo Basin	West Africa	
Clade	I*	IIa*	IIb (hMPXV1)^
Mortality rate	~10%	~1.0%	<0.03%
Hospitalizations	57.5%	35.5%	8.6%
>100 Lesions	73.7%	13.0%	<10%
Human-to-human transmission	31.5%	<1.0%	>99.9%

*Data from Likos 2004 and Jezek 1988

^Data from Patauner 2022

Important: Clade 1 (Congo Basin) is considered a select agent

Brief history of monkeypox virus outbreaks

1958 – Monkey poxvirus first isolated

1970 – First human cases identified

2003 – First outbreak in non-endemic areas

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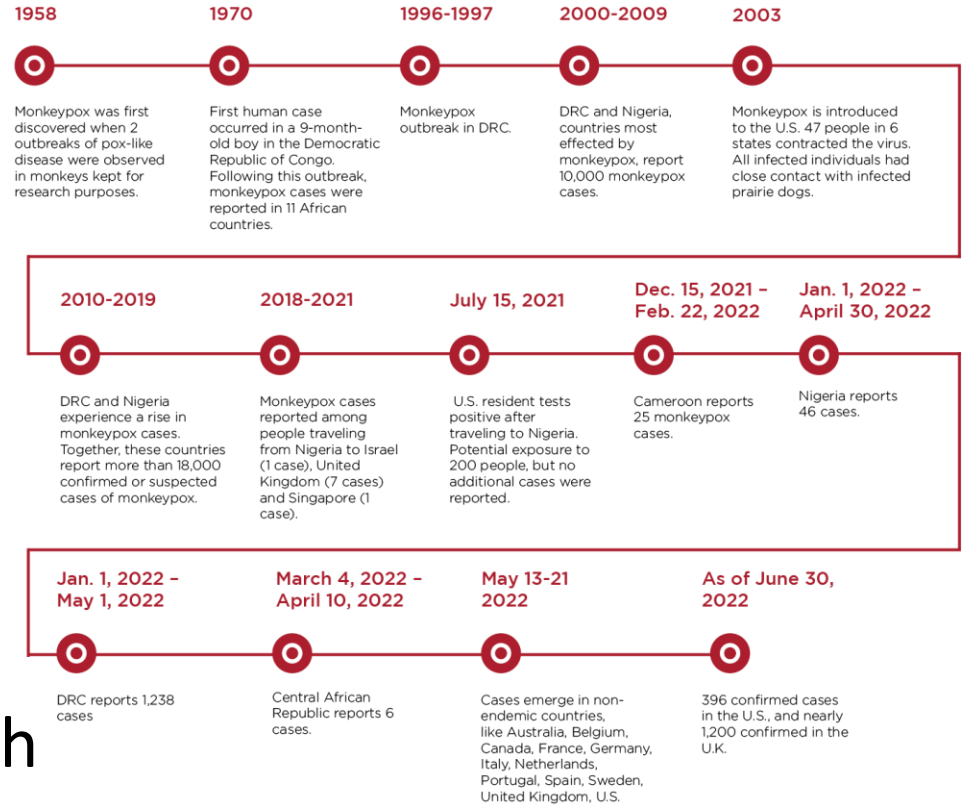
2003 – First outbreak in non-endemic areas

2003 – 2022 “Blips” begin increasing

- Travel-associated cases
- Local outbreaks in DRC and surrounding countries
- 2017 – Atypical outbreak in Nigeria

May 2022 - WHO declares MPXV “Public Health Emergency of International Concern”



https://asm.org/Resource-Pages/Monkeypox

2017 Nigerian Outbreak

- Dr. Ogoina observed a 9-year-old child with chickenpox-type rash
  - However, child previously had chickenpox disease…
- The last MPXV outbreak in Nigeria happened 25 years ago
  - Over 80% of cases were children in rural areas
  - Often small outbreaks associated with animal exposure
- However, this time it was different
  - Over 200 cases were identified, mostly in men from the city
  - Clinical history revealed a majority of these were MSM and had genital lesions

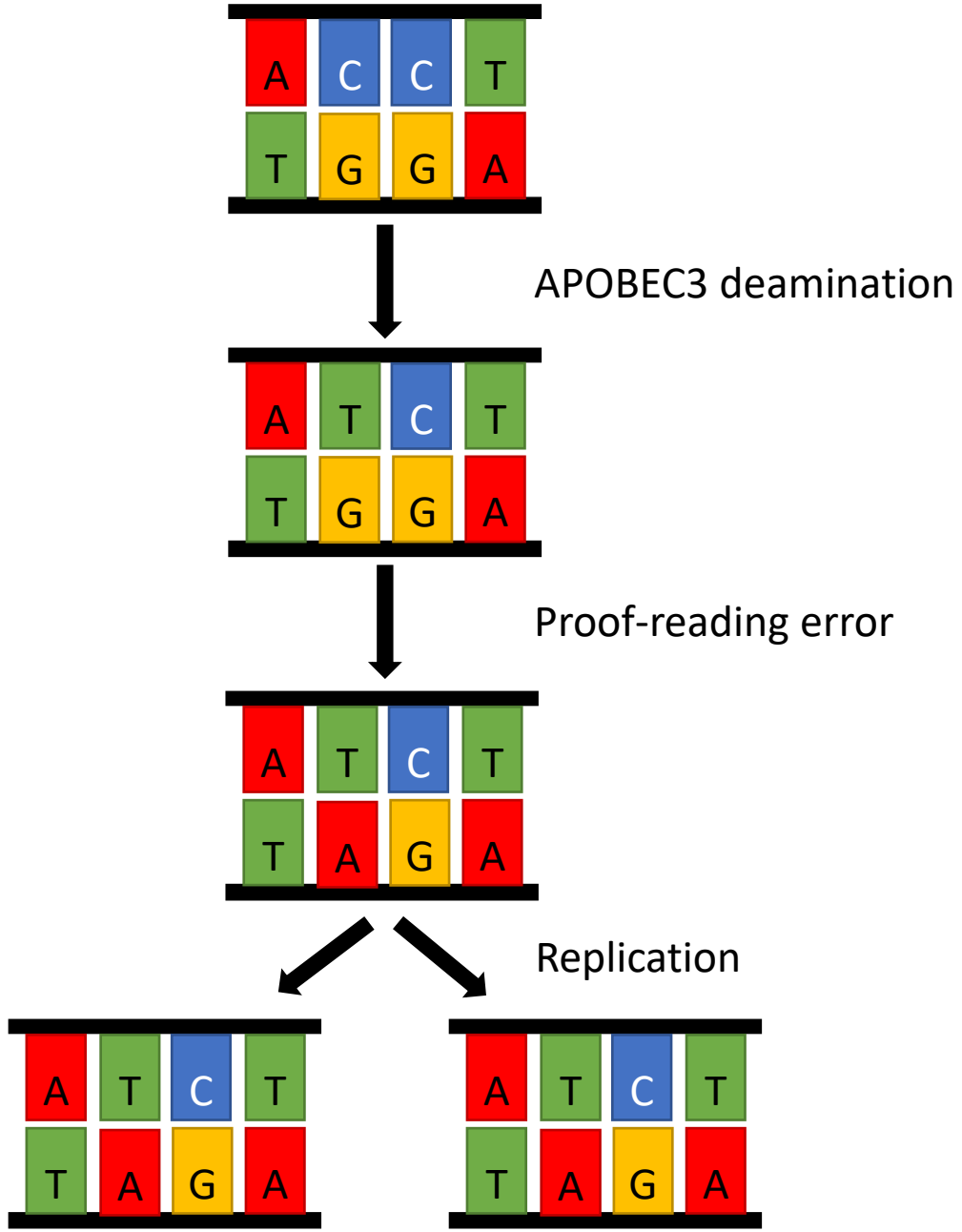
clinical findings in our study are like other studies [2,13,14], it is noteworthy that a substantial number of our cases who were young adults in their reproductive age presenting with genital ulcers, as well as concomitant syphilis and HIV infection. Although the role of sexual transmission of human monkeypox is not established, sexual transmission is plausible in some of these patients through close skin to skin contact during sexual intercourse or by transmission via

Did the outbreak really die out?

https://www.npr.org/sections/goatsandsoda/2022/07/28/1114183886/a-doctor-in-nigeria-tried-to-warn-the-world-that-monkeypox-had-become-a-global-t

Digression: APOBEC3

- APOBEC3 is a human enzyme that is critical in the defense against DNA viruses that infect our cells
- APOBEC3 functions as a “cytosine deaminase”
  - Essentially it changes an C -> T and disrupts viral replication
- Enzyme has well characterized mutation rate
  - Estimated ~9 APOBEC3 mutations per year for MPXV in humans



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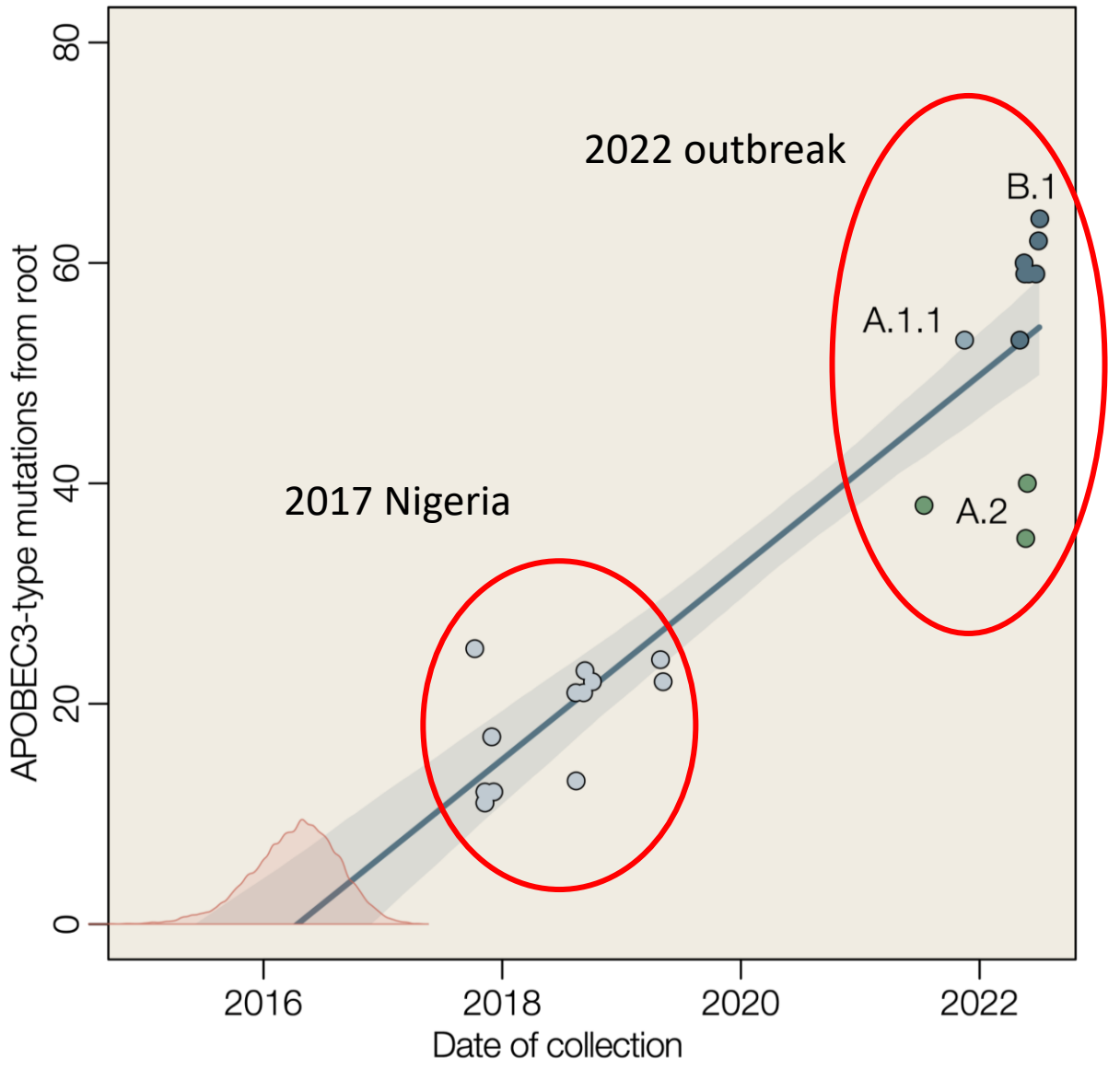
- APOBEC3 functions as a “cytidine deaminase”
- Enzyme has well characterized mutation rate
- April 5th 2016 - Estimated date of MPXV emergence in humans

Why does this matter?



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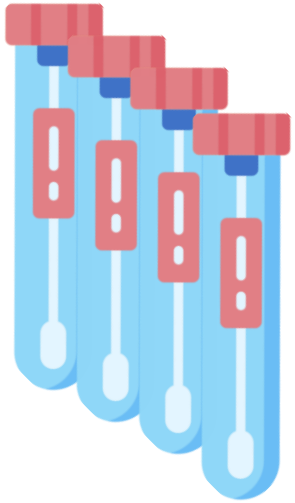
What the Lab Needs to Know about MPXV

Pre-analytical



- Specimen sources
- Disease onset
- Number of specimens
- Collection media

Analytical

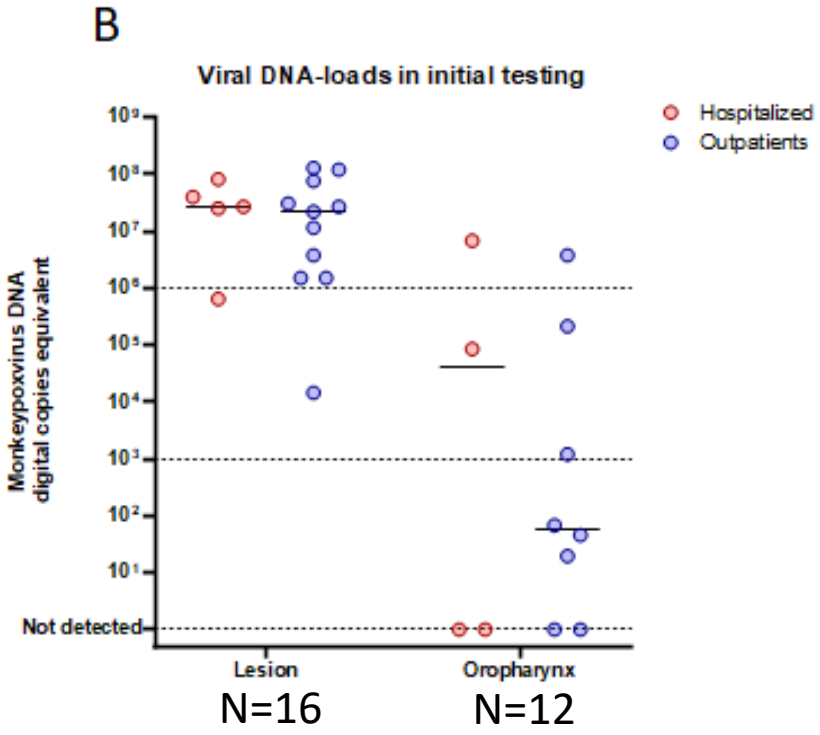
Post-analytical

https://diagnostics.roche.com/us/en/products/instruments/cobas-6800-ins-2693.html
https://www.flaticon.com/free-icon/chart_8960963
https://www.flaticon.com/free-icon/test-tube_3159741

Short communication

Clinical characteristics and comparison of longitudinal qPCR results from different specimen types in a cohort of ambulatory and hospitalized patients infected with monkeypox virus.

Dominik Nörz [a], Thomas Theo Brehm [b,g], Hui Ting Tang [a], Ilka Grewe [b], Lennart Hermanussen [b], Hanna Matthews [c], Julia Pestel [c], Olaf Degen [c], Thomas Günther [d], Adam Grundhoff [d], Nicole Fischer [a], Marylyn M. Addo [b,e,f,g], Sabine Jordan [b], Sandra Hertling [h], Stephan Unger [i], Guido Schäfer [j], Knud Schewe [j], Christian Hoffmann [j], Martin Aepfelbacher [a], Susanne Pfefferle [a,k], Julian Schulze zur Wiesch [b,g,1], Stefan Schmiedel [b,1], Marc Lütgehetmann [a,*,1]



Pre-analytic Questions:

1. What specimens are best to collect?
2. When should specimens be collected?
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Method:

- Compared viral load of lesion swabs to OP swabs for MPXV patients

Results:

- Lesion samples
  - 16/16 with MPXV detected
  - 14/16 >10^6 copies/mL
- OP samples
  - 8/12 with MPXV detected
  - 2/12 samples with >10^6 copies/mL

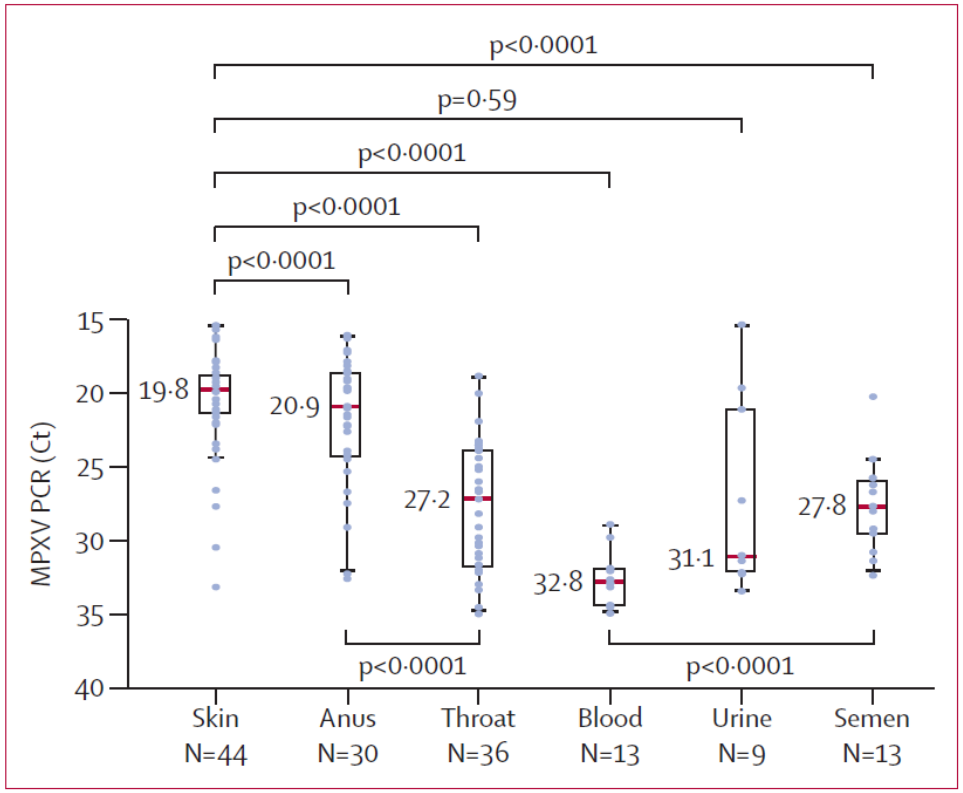
Limitation:

- Specimen type or Specimen site?

Viral loads in clinical samples of men with monkeypox virus infection: a French case series

Romain Palich, Sonia Burrel, Gentiane Monsel, Agathe Nouchi, Alexandre Bleibtreu, Sophie Seang, Vincent Bérot, Cécile Brin, Ariane Gavaud, Yara Wakim, Nagisa Godefroy, Antoine Fayçal, Yanis Tamzali, Thomas Grunemwald, Michel Ohayon, Eve Todesco, Valentin Leducq, Stéphane Marot, Vincent Calvez, Anne-Geneviève Marcelin, Valérie Pourcher

Retrospective study of 356 specimens collected from 50 individuals with sampling at multiple anatomic sites



Pre-analytic Questions:

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Results:

- Skin was the most sensitive site with earliest Ct values
- Despite the presence of skin lesions in all individuals, MPXV DNA was not detected in 12% (6/50)
- Urine was the least sensitive with 22% (9/41) positive

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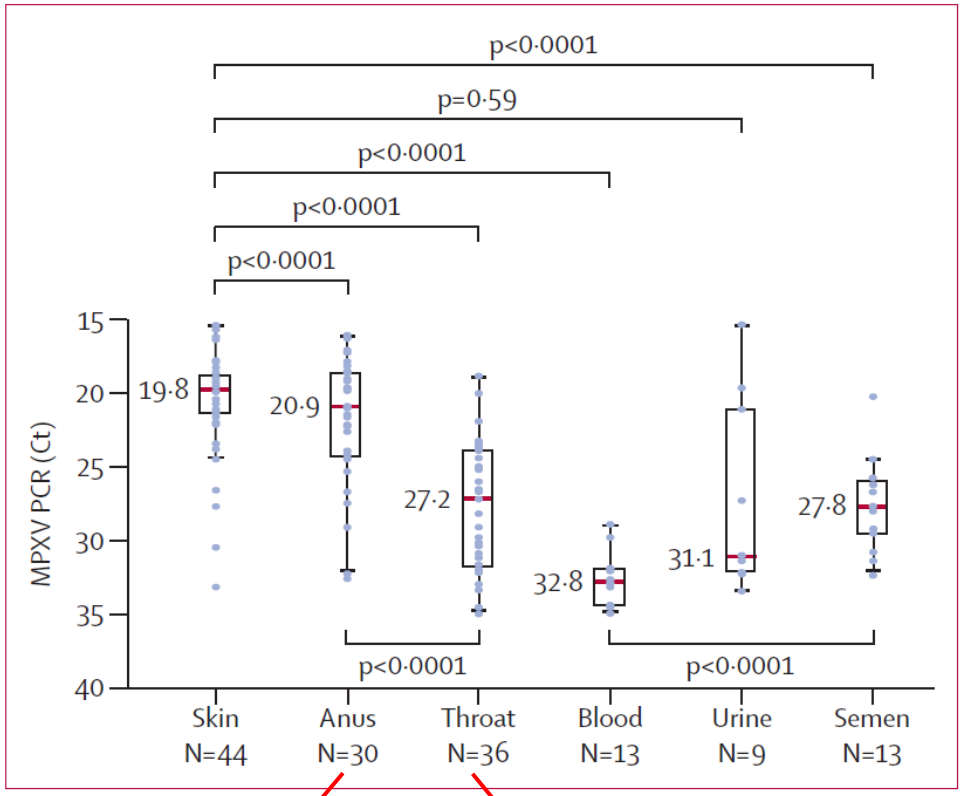

ulcer +: 19.6
ulcer - : 25.3

pharyngitis +: 23.5
pharyngitis -: 29.0

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Results:

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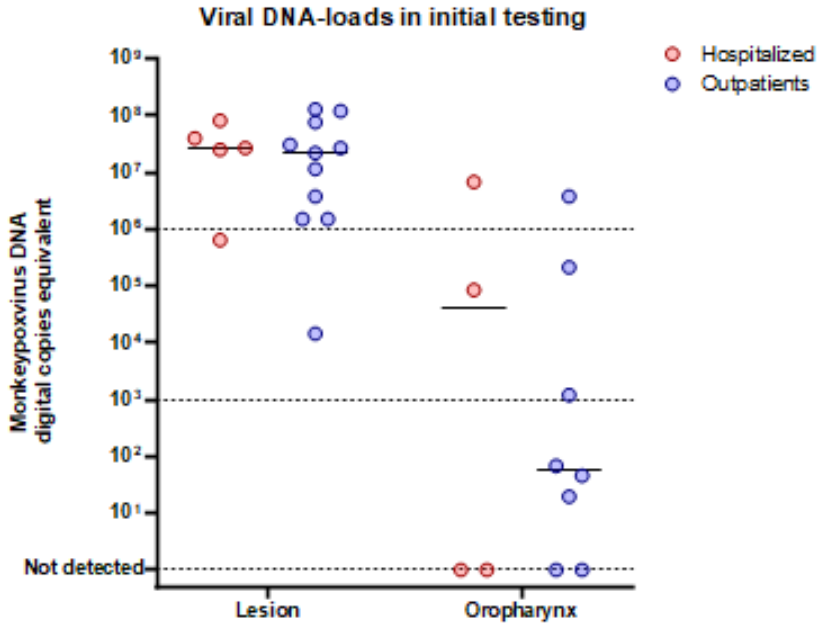
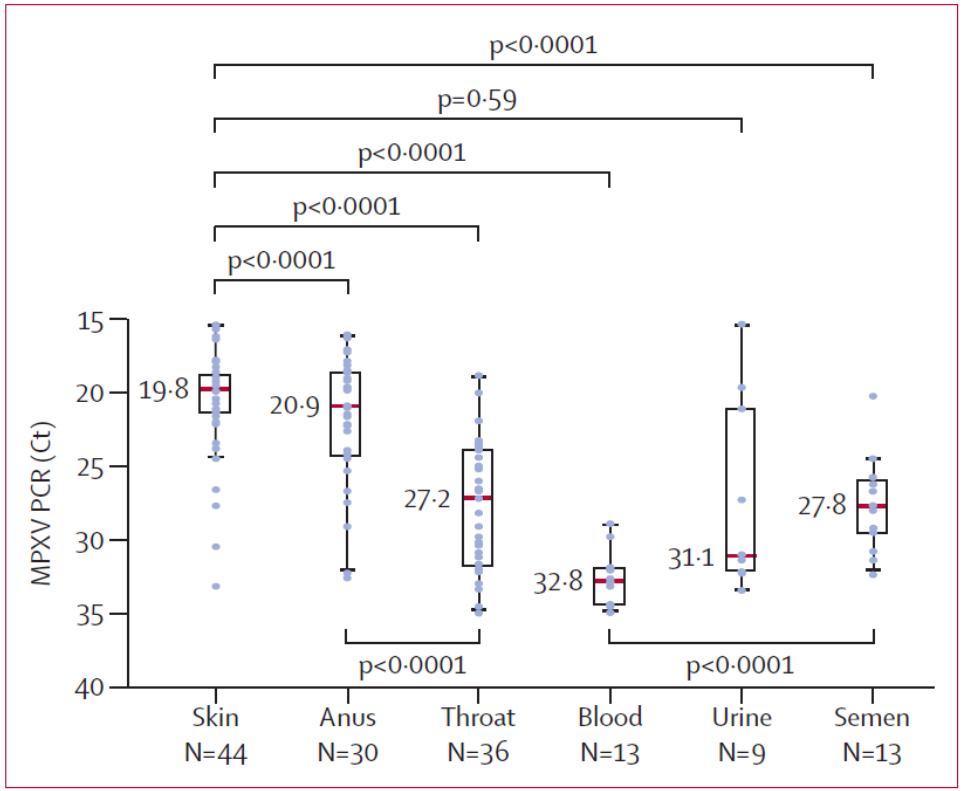
- Despite the presence of skin lesions in all individuals, MPXV DNA was not detected in 12% (6/50)
- Urine was the least sensitive with 22% (9/41) positive
- **For anal and throat samples, earlier Cts are associated with tissue injury**





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Conclusions:

- Specimen site and type matter
- Skin is the most sensitive site for MPXV detection
- Presence of injury (inflammation, ulceration, etc.) associated with higher viral load and likely better analytic sensitivity

Short communication

Clinical characteristics and comparison of longitudinal qPCR results from different specimen types in a cohort of ambulatory and hospitalized patients infected with monkeypox virus.

- Longitudinal measurement of viral load in 5 individuals up to 21 days following symptom onset
- VL measured from lesion swabs, OP, and blood

Pre-analytic Questions:

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		Median Viral Load (copies/mL)		
Source	**Initial positive specimens**	Week 1	Week 2	Week 3
Lesion	5/5	3.31×10^7	3.04×10^6	8.55×10^3
OP	3/5	8.44×10^4	4.04×10^3	0
Blood	4/5	585	7.8	23.7

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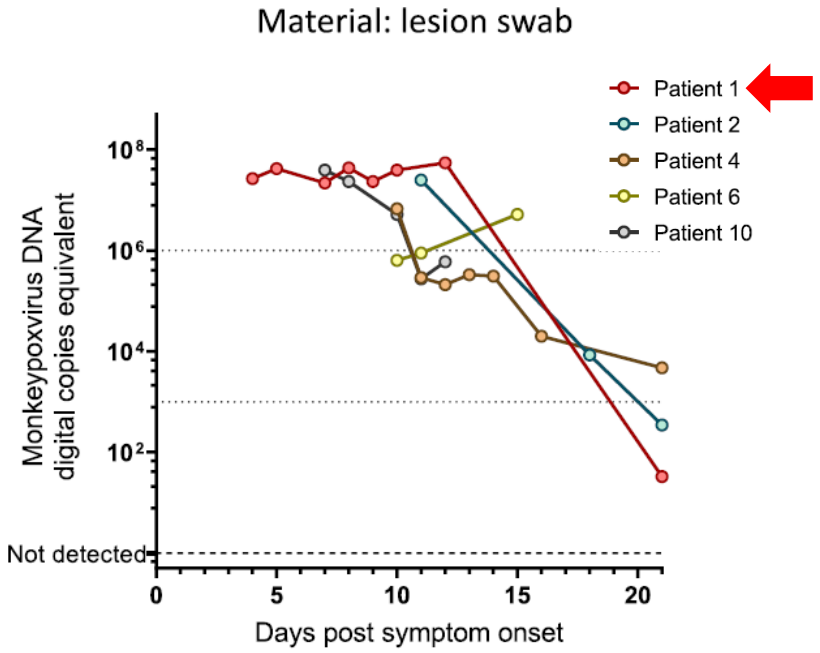

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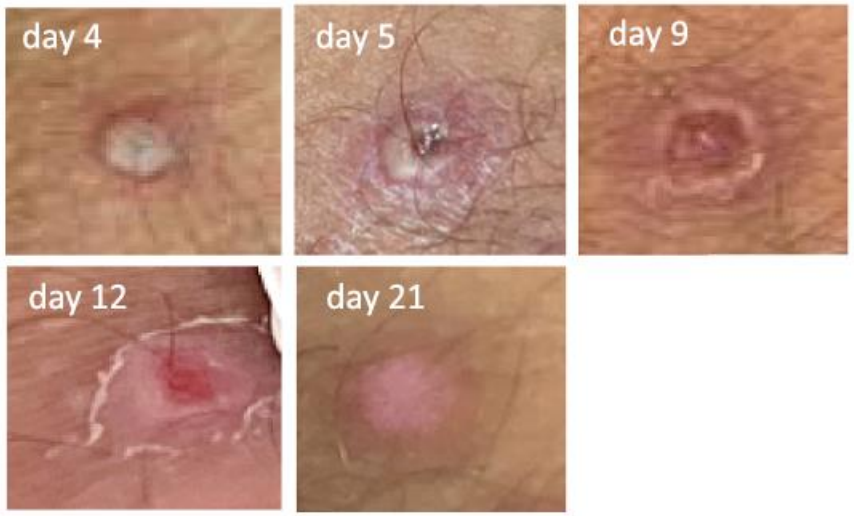
Lesion timeline for Patient 1:



Conclusions:

- VL is highest at initial diagnosis
- Skin lesions remain positive for the longest duration
- Resolving lesions contain MPXV DNA

5/31/22 Lab Alert

- Collect at least two dry swabs from the same lesion
- Place swabs in individual sterile containers
- No mention of number of sites to collect

6/24/22 Update

How to collect lesion specimens

1. Wear appropriate personal protective equipment (PPE).
2. Collect two swabs from each lesion, preferably from different locations on the body or from lesions which differ in appearance. (2-3 lesions)
   - For further characterization of a specimen at CDC, three types of specimens are accepted.
     - » Dry swabs of lesion material
     - » Swabs of lesion material in viral transport media (VTM)

Pre-analytic Questions:

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IMPORTANT NOTES

When collecting multiple swabs from the same lesion, place two swabs in a single container and submit both under the same order. When collecting from different body sites or lesions, place in separate containers and submit one order per body site or lesion.

Dry swabs discouraged

Clinical Performance and Trends During the First Two Months of Monkeypox Virus PCR Testing at Two United States Reference Labs

Nicole A. P. Lieberman, Patrick C. Mathias, Benjamin T. Bradley, Alexander L. Greninger

doi: https://doi.org/10.1101/2022.09.20.22280169

Pre-analytic Questions:

1. What specimens are best to collect?
2. When should specimens be collected?
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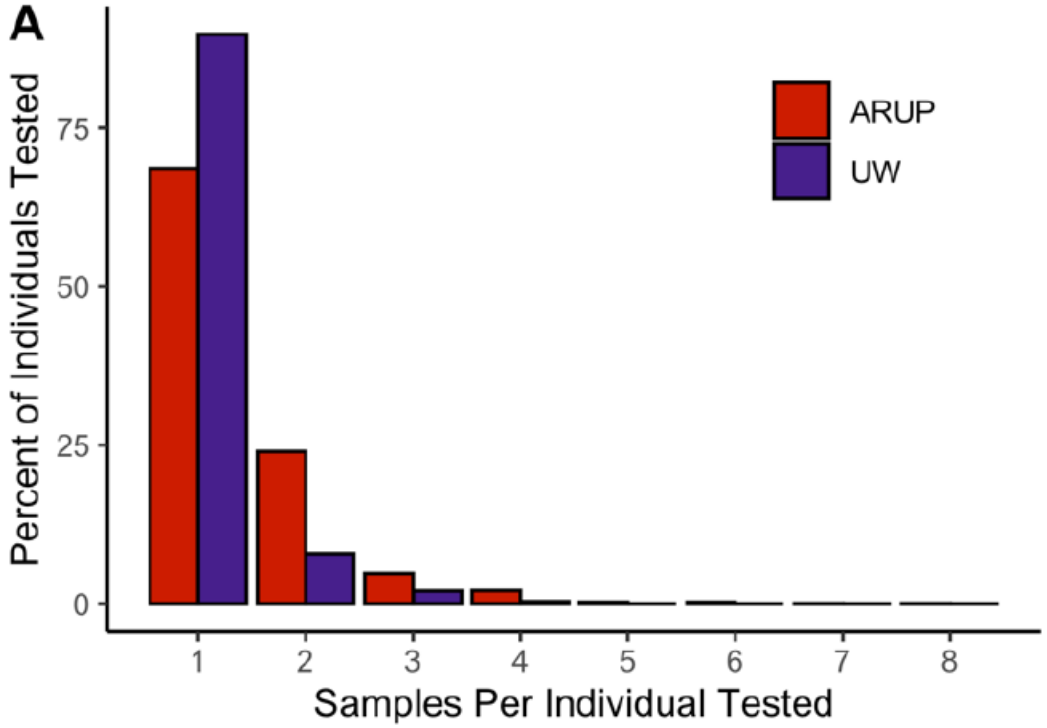
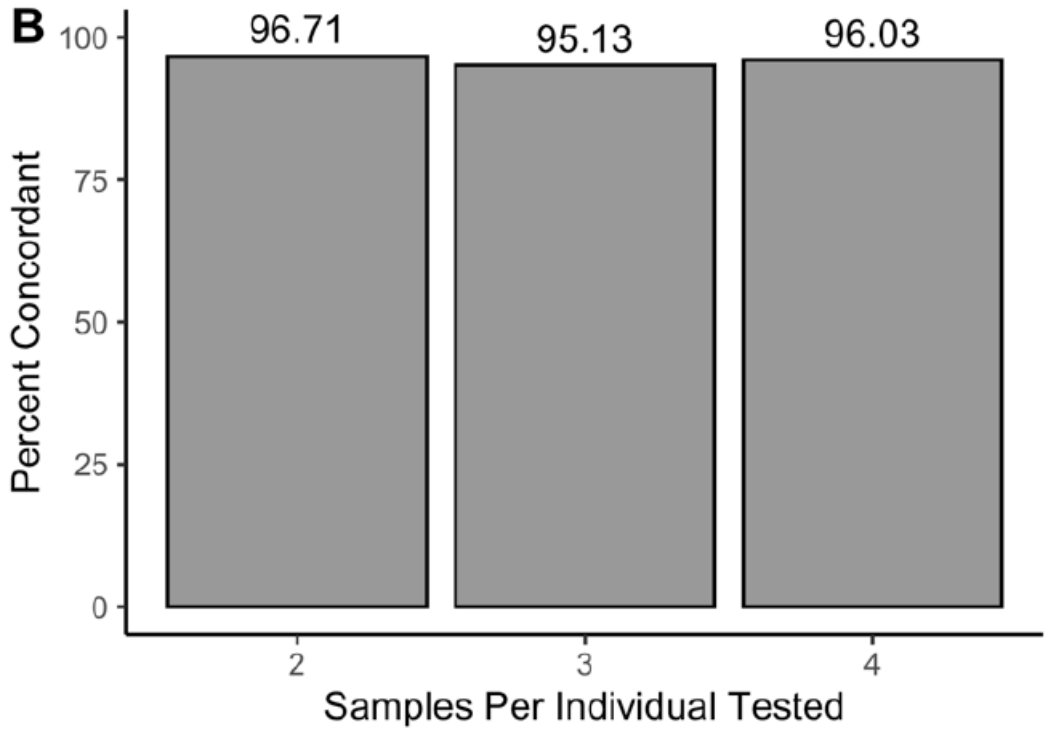
Over 10,000 samples collected between mid-July to early Sept.

- 31% of ARUP specimens and 10% of UW specimens had multiple samples collected





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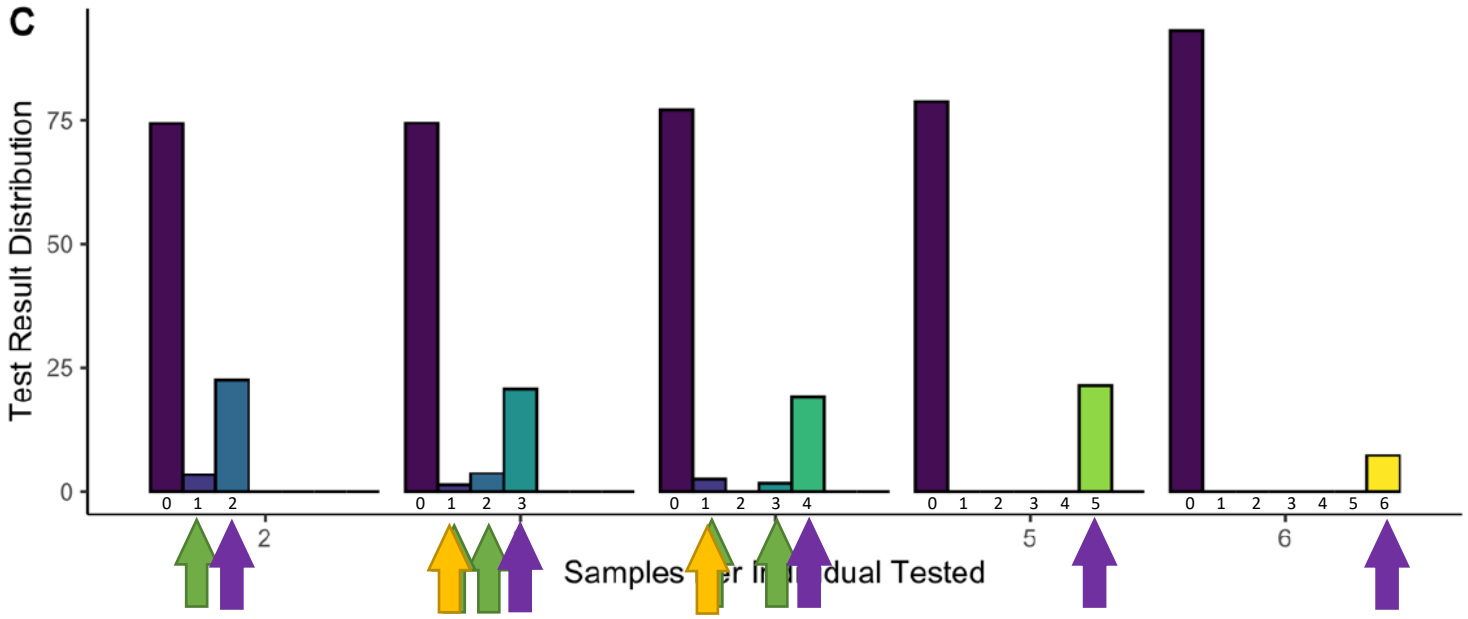

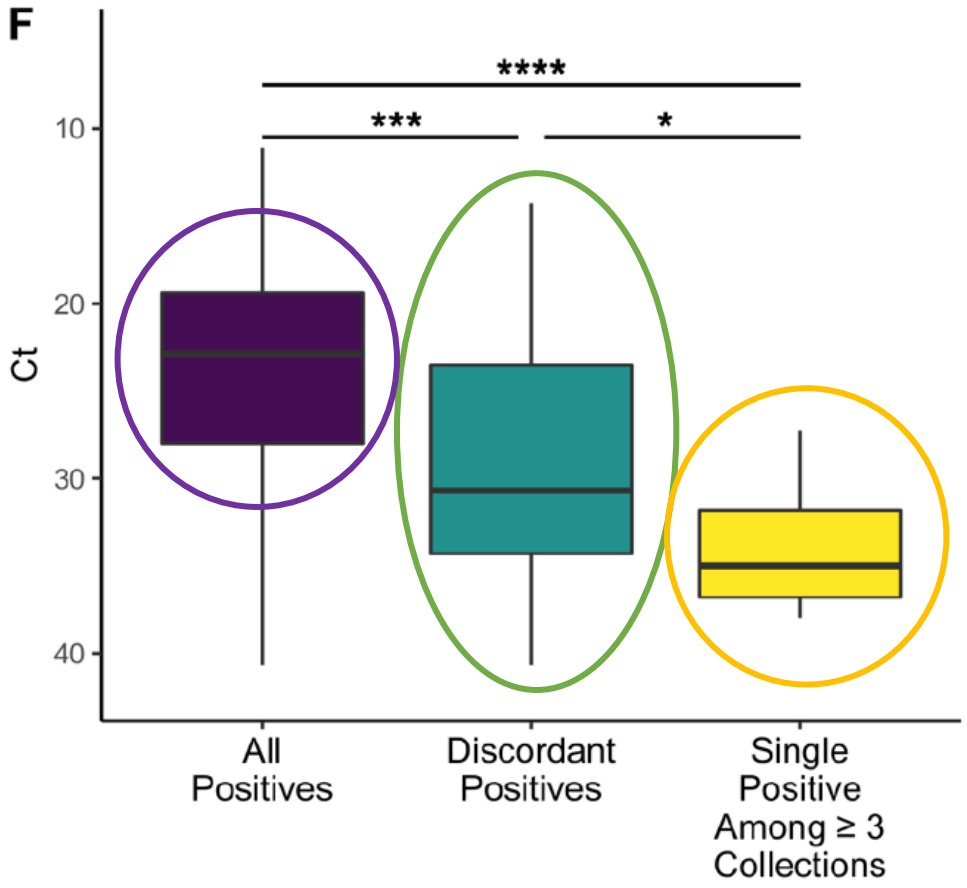

Conclusions:

- Two specimens appear sufficient for diagnosis
- Late CTs in discrepant samples may represent contamination or resolving disease

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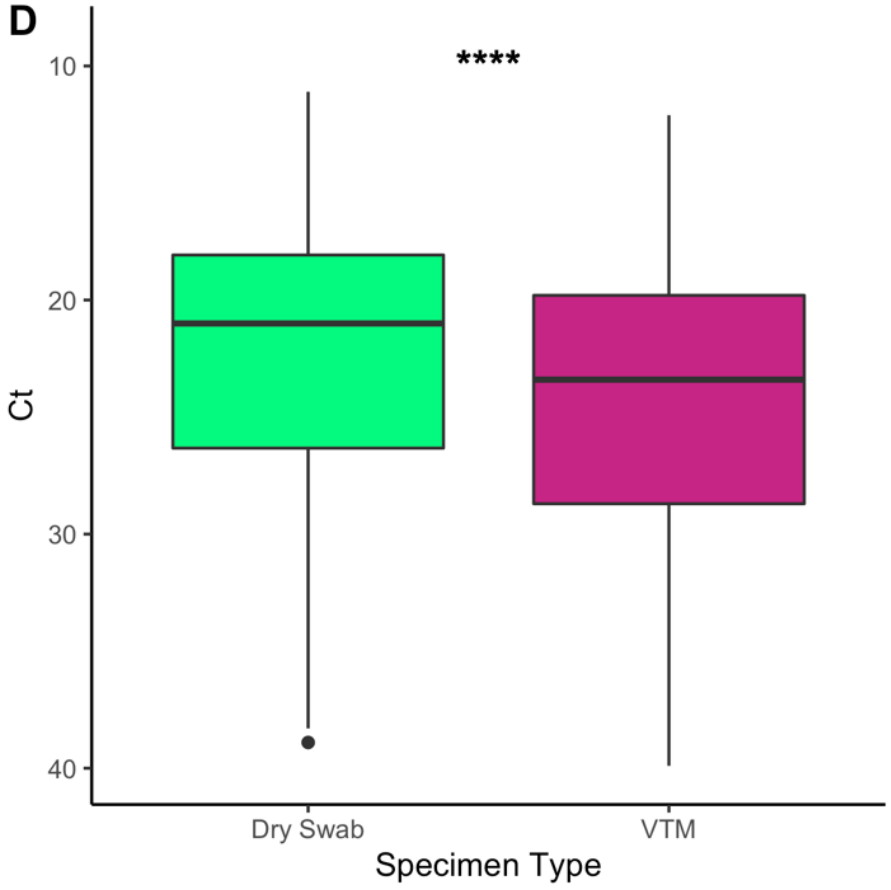


Results:

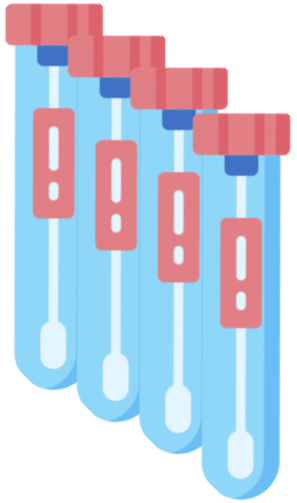
- Cts were similar despite statistical significance
- Dry swab: 22.8; VTM: 24.4

Conclusions:

- Analytically, dry swabs and VTM have similar performance
- However, impacts on processing and TAT make VTM a more favorable option

What the Lab Needs to Know about MPXV

Pre-analytical



Analytical

- Molecular targets
- Association of CTs to infectivity
- Detection in non-routine assays

Post-analytical

Targets for MPXV detection

Analytic Questions:

1. How do different PCR targets compare?
2. Does a positive PCR result reflect infectivity?
3. Detection in non-standard assays

Non-variola, orthopox

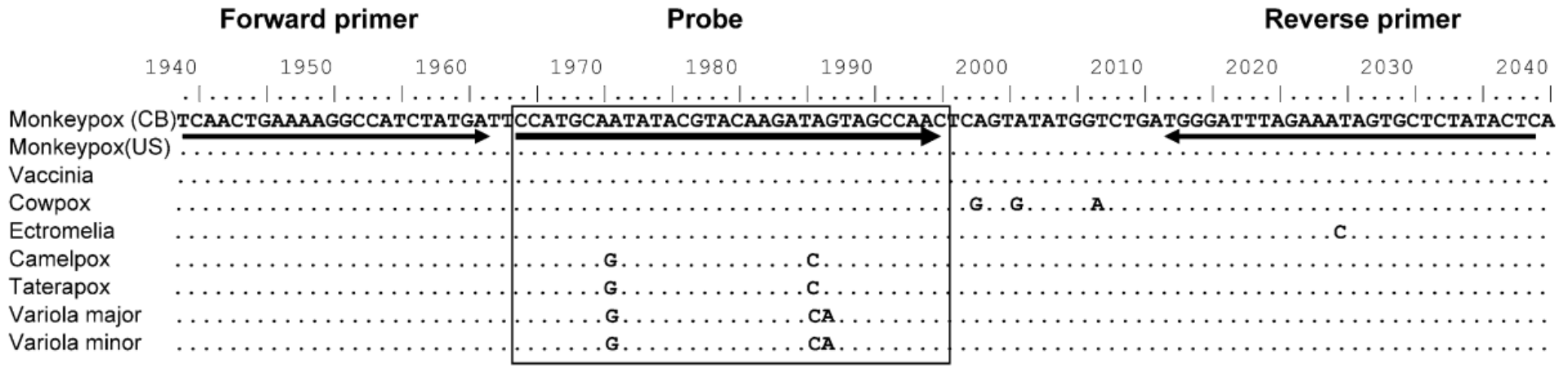
Advantages

- Can detect other orthopox infections (vaccinia, cowpox)
- Does not detect variola

Disadvantages

- Dx relies on epidemiological data

Monkeypox, Clade II specific



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Disadvantages

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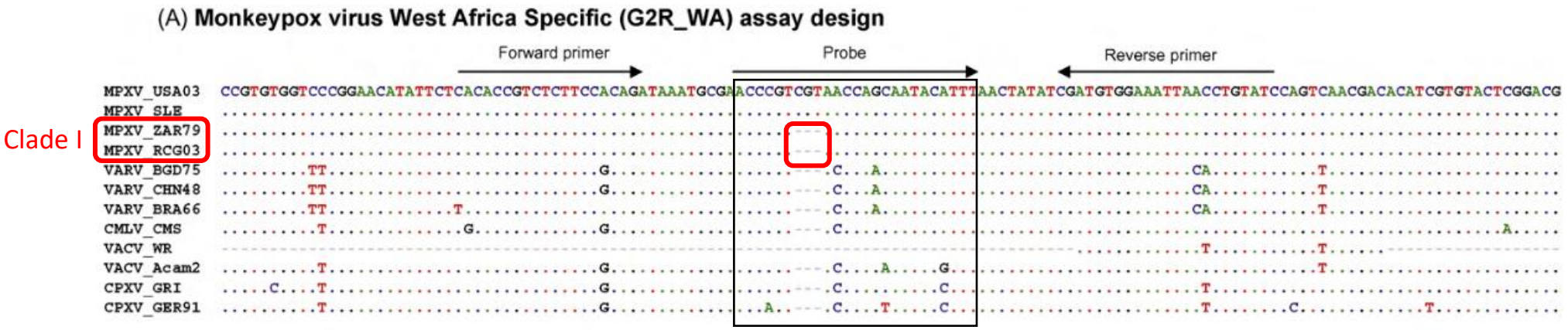
Monkeypox, Clade II specific

Advantages:

- Definitively identifies monkeypox virus
- Target has two copies per genome

Disadvantages

- Challenging to find ideal target, Clade I and Clade II share >99% sequence identity
- Reports of false negative results with this assay



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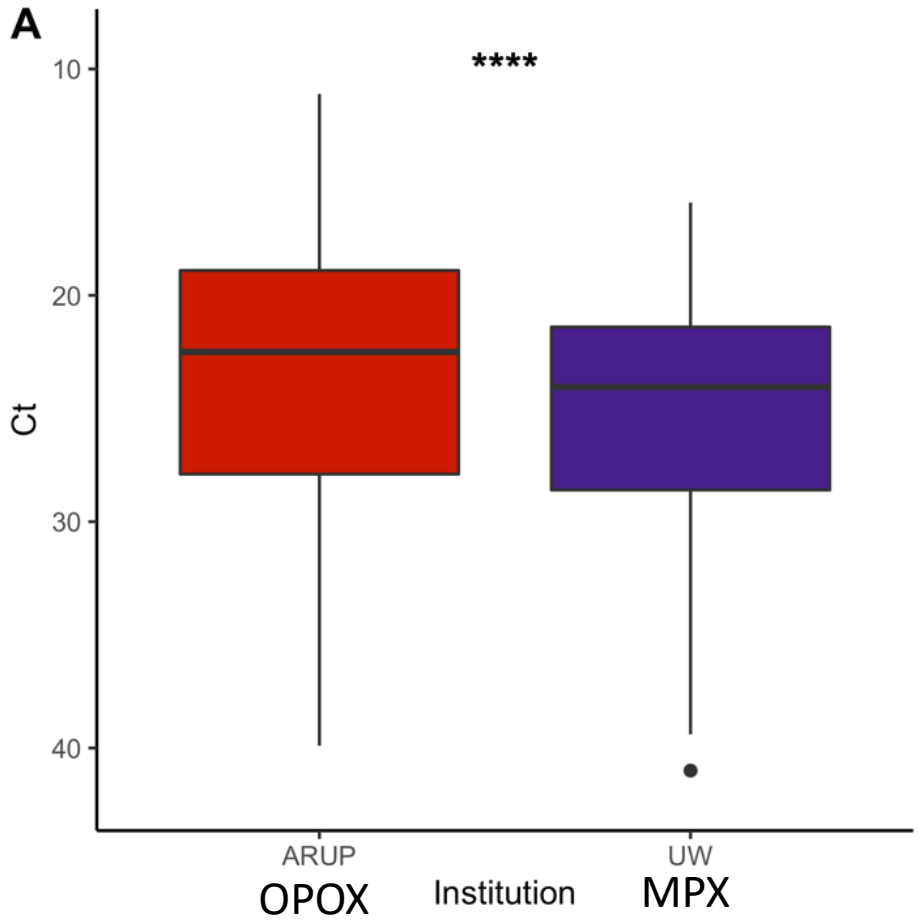


Results:

- Orthopox: Median CT- 23.9
- Monkeypox: Median CT- 25.4

Conclusions:

- Targets demonstrate similar cycle thresholds and likely similar analytic performance
- However, each target has potential shortcomings

RAPID COMMUNICATION

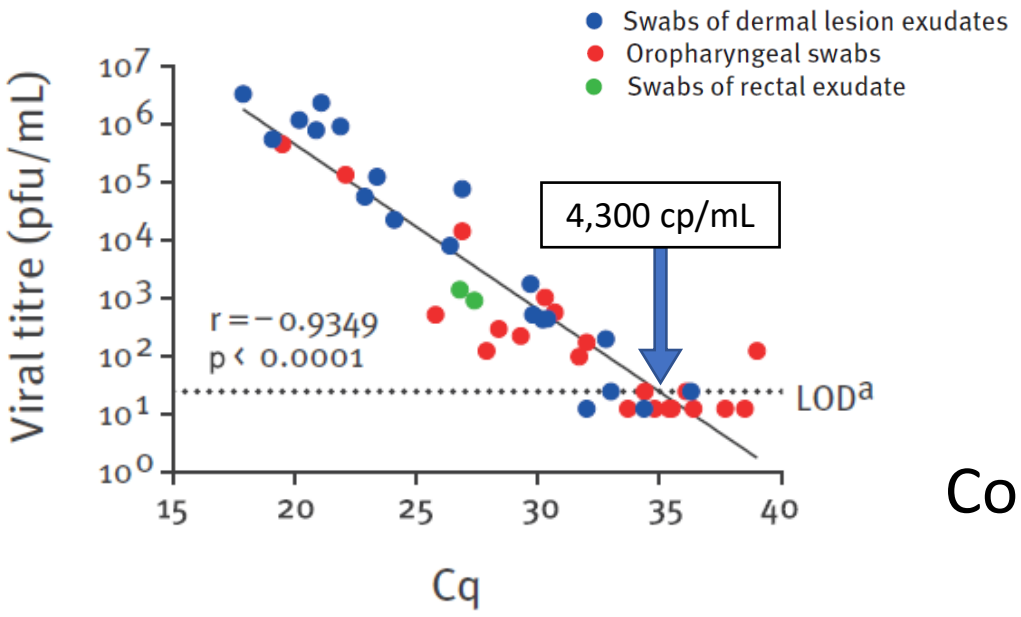
Monkeypox DNA levels correlate with virus infectivity in clinical samples, Israel, 2022

Nir Paran[1,*], Yfat Yahalom-Ronen[1,*], Ohad Shifman[2], Shirley Lazar[2], Ronen Ben-Ami[3,10], Michal Yakubovsky[3], Itzchak Levy[4,10], Anat Wieder-Feinsod[4,10], Sharon Amit[5], Michal Katzir[6,10], Noga Carmi-Oren[7], Ariela Levcovich[7], Mirit Hershman-Sarafov[8,11], Alona Paz[8,11], Rebecca Thomas[9], Hadas Tamir[1], Lilach Cherry-Mimran[1], Noam Erez[1], Sharon Melamed[1], Moria Barlev-Gross[1], Shay Karmi[1], Boaz Politi[1], Hagit Achdout[1], Shay Weiss[1], Haim Levy[1], Ofir Schuster[1], Adi Beth-Din[2], Tomer Israely[1]

43 specimens from 32 patients

21 OP, 20 skin lesion, and 2 rectal swabs

Plaque assay using BSC-1 cells, fixed at 72hrs



Analytic Questions:

1. How do different PCR targets compare?
2. Does a positive PCR result reflect infectivity?
3. Detection in non-standard assays

Monkeypox virus isolation from a semen sample collected in the early phase of infection in a patient with prolonged seminal viral shedding

https://doi.org/10.1016/ S1473-3099(22)00513-8

	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 13	Day 14	Day 15	Day 16	Day 17	Day 19
Plasma	NA	NA	NA	Positive (34·5)	NA	Negative	NA	Negative	NA	Negative	Negative	Negative	Negative
Urine	NA	NA	Negative	NA	Negative	NA	Negative	Negative	NA	Negative	NA	NA	Negative
Semen	Positive (28·0)	Positive (29·3)	Positive (27·8)	NA	NA	NA	NA	NA	Positive (34·3)	Positive (35·6)	NA	Positive (38·7)	Positive (40·6)
Rash or skin lesion	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Negative

Quantification cycle values are indicated in brackets after positive results. The cutoff cycle threshold is 45, thresholds of 42 or higher are retested for confirmation. Negative indicates no detection of monkeypox virus DNA or absence of rash or skin lesions. Positive indicates detection of monkeypox virus DNA or presence of rash or skin lesions. NA=not available.

Table: Timeline of monkeypox virus DNA detection in plasma, urine, and semen samples with increasing days from symptom onset

Culture positive

Conclusions:

- Culturable virus can be obtained from clinical sources including non-lesional specimens (e.g. OP and semen)
- Culturable virus is observed at viral loads >4,300 cp/ml
- However, infectious virus in cell culture =/= transmissibility

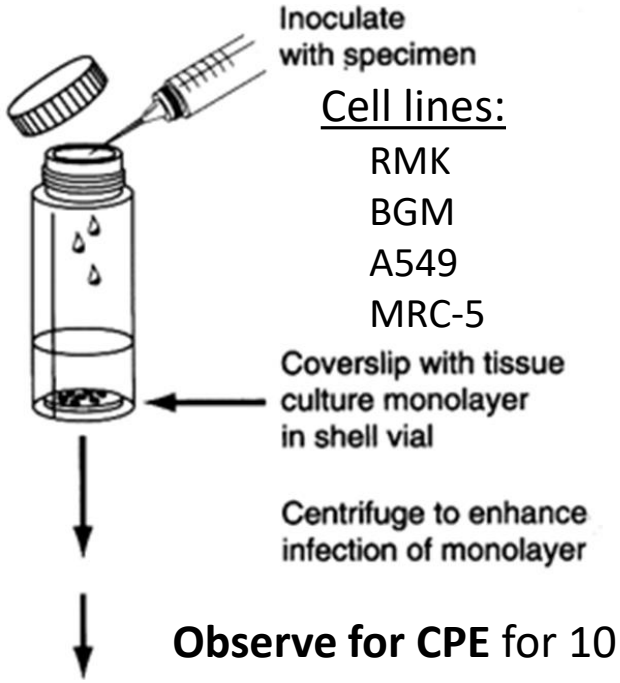
Characterization of the cytopathic effect of monkeypox virus isolated from clinical specimens and differentiation from common viral exanthems

Angela Ma, Janine Langer, Kimberly E. Hanson, Benjamin T. Bradley

doi: https://doi.org/10.1101/2022.09.13.507875

3,468 specimens collected between July and August
- 14 MPXV specimens were identified

Growth and CPE of MPXV was compared to HSV and VZV



Cell lines:
- RMK
- BGM
- A549
- MRC-5

Observe for CPE for 10 days

Analytic Questions:

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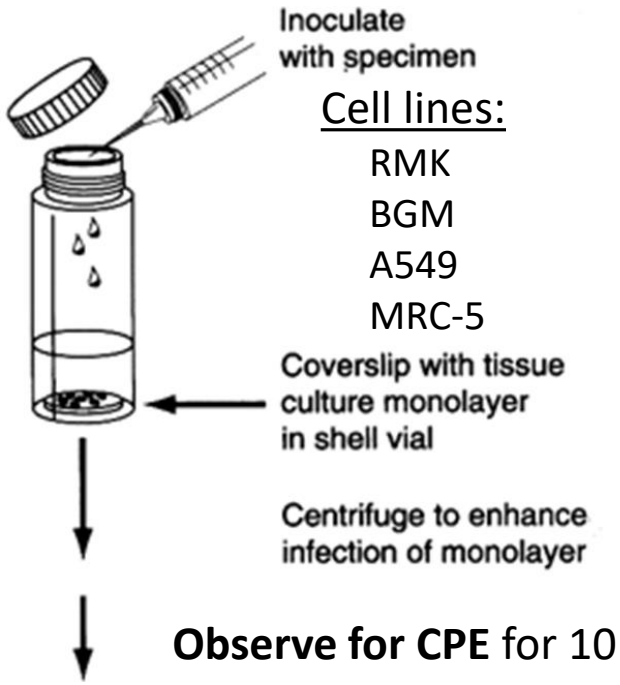


Cell lines:

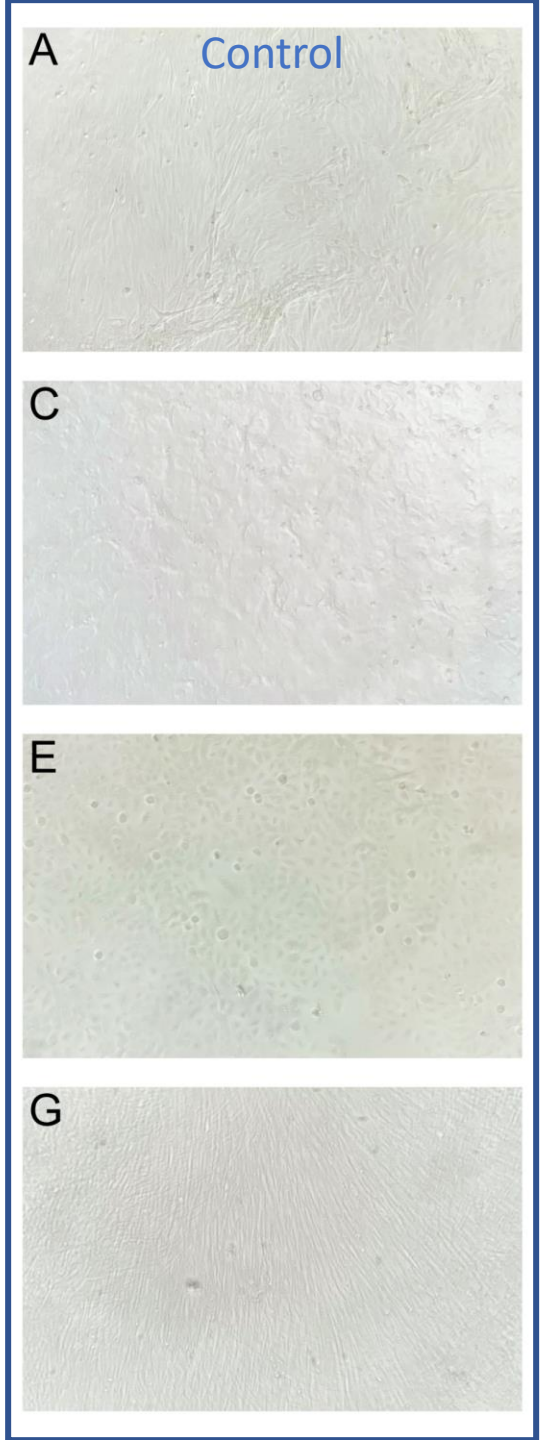
RMK
BGM
A549
MRC-5

Observe for CPE for 10 days

BGM

A549

MRC-5



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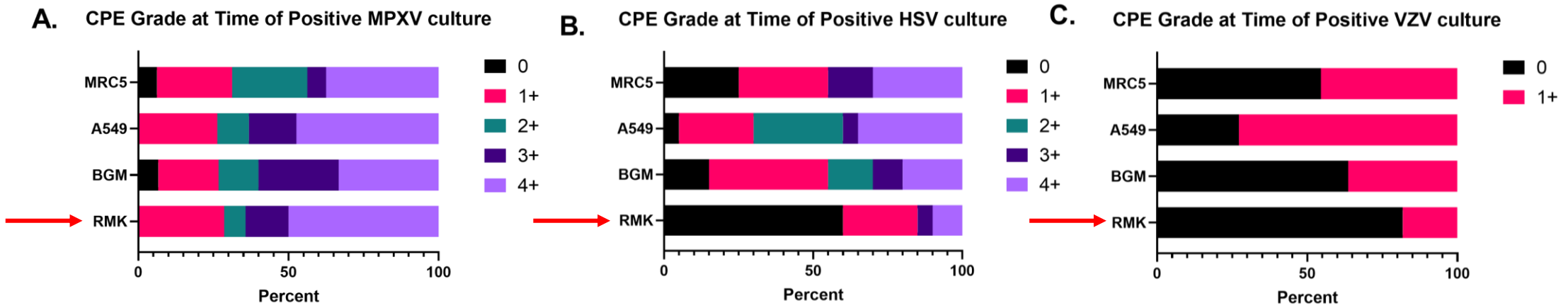


Results:

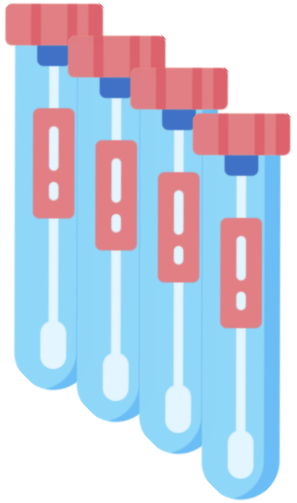
- MPXV demonstrates characteristic CPE in A549 and MRC-5 cells
- MPXV grows more quickly than VZV and demonstrates higher CPE in RMK cells

Conclusions:

- MPXV can be separated from HSV and VZV by CPE pattern and growth rate in cell lines
- Cell culture provides unique benefits not present with PCR

What the Lab Needs to Know about MPXV

Pre-analytical



Analytical

Post-analytical

- Discrepancy reporting
- Repeat algorithms

Morbidity and Mortality Weekly Report

Orthopoxvirus Testing Challenges for Persons in Populations at Low Risk or Without Known Epidemiologic Link to Monkeypox — United States, 2022

Faisal S. Minhaj, PharmD[1,2]; Julia K. Petras, MSPH[1,2]; Jennifer A. Brown, DVM[3]; Anil T. Mangla, PhD[4]; Kelly Russo, MD[5]; Christina Willut[4]; Michelle Lee, MPH[4]; Jason Beverley, MS[4]; Rachel Harold, MD[4]; Lauren Milroy, MPH[3]; Brian Pope[3]; Emily Gould, MD[6]; Cole Beeler, MD[6]; Jack Schneider, MD[6]; Heba H. Mostafa, MD, PhD[7]; Shana Godfred-Cato, DO[1]; Eleanor S. Click, MD[1]; Brian F. Borah, MD[1,2]; Romeo R. Galang, MD[1]; Shama Cash-Goldwasser, MD[1,2]; Joshua M. Wong, MD[1]; David W. McCormick, MD[1]; Patricia A. Yu, PharmD[1]; Victoria Shelus, PhD[1,2]; Ann Carpenter, DVM[1,2]; Sabrina Schatzman, PhD[1,8]; David Lowe, PhD[1]; Michael B. Townsend, PhD[1]; Whitni Davidson, MPH[1]; Nhien T. Wynn, MS[1]; Panayampalli S. Satheshkumar, PhD[1]; Siobhán M. O'Connor, MD[1]; Kevin O'Laughlin, MD[1]; Agam K. Rao, MD[1]; Andrea M. McCollum, PhD[1]; María E. Negrón, DVM, PhD[1]; Christina L. Hutson, PhD[1]; Johanna S. Salzer, DVM, PhD[1]; CDC Monkeypox Emergency Response Team

CDC report of three false positive MPXV results in low-risk patients

- All patients received treatment

Why do these errors occur?

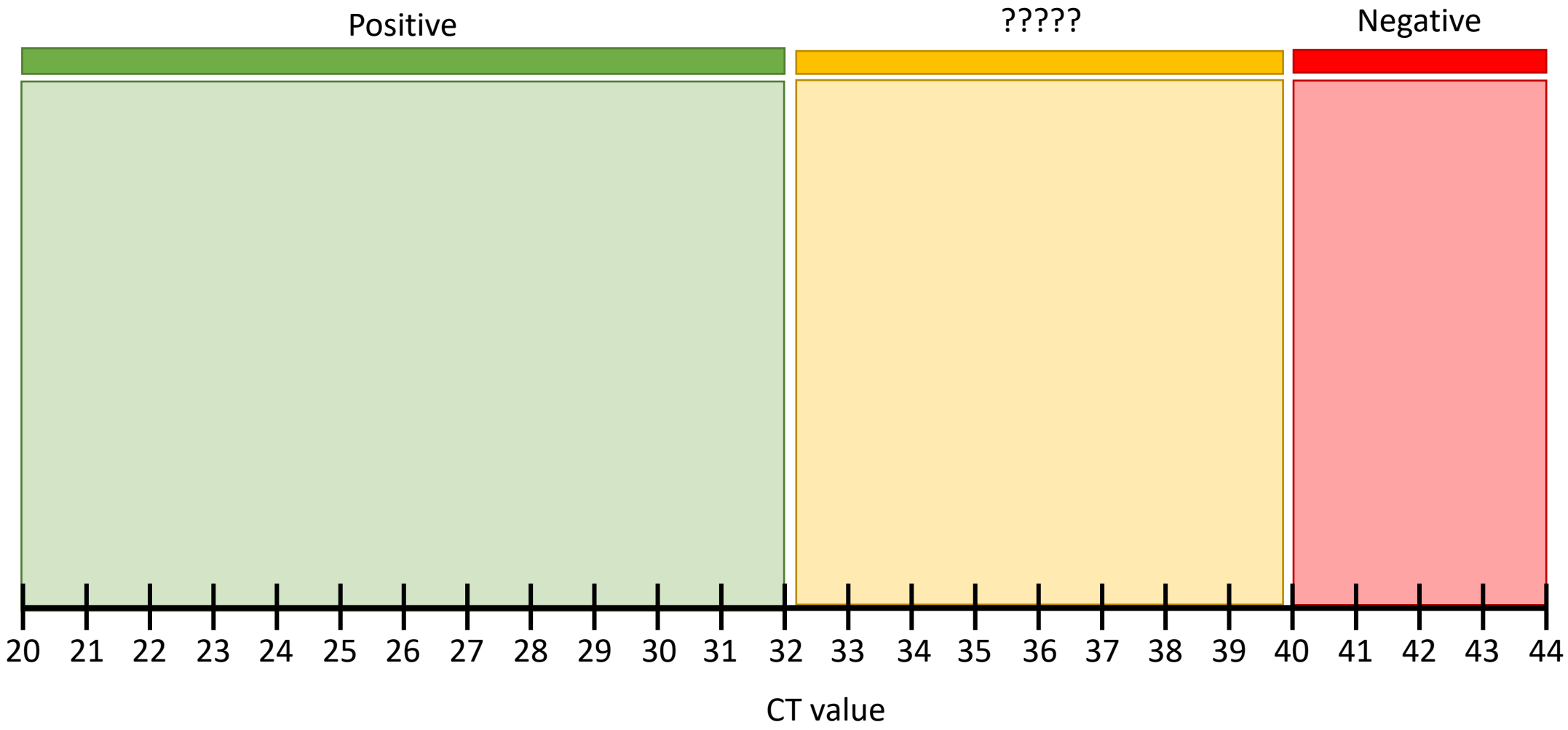
- Pipetting error
- Specimen mislabel
- Cross-contamination

Is repeating the best answer?

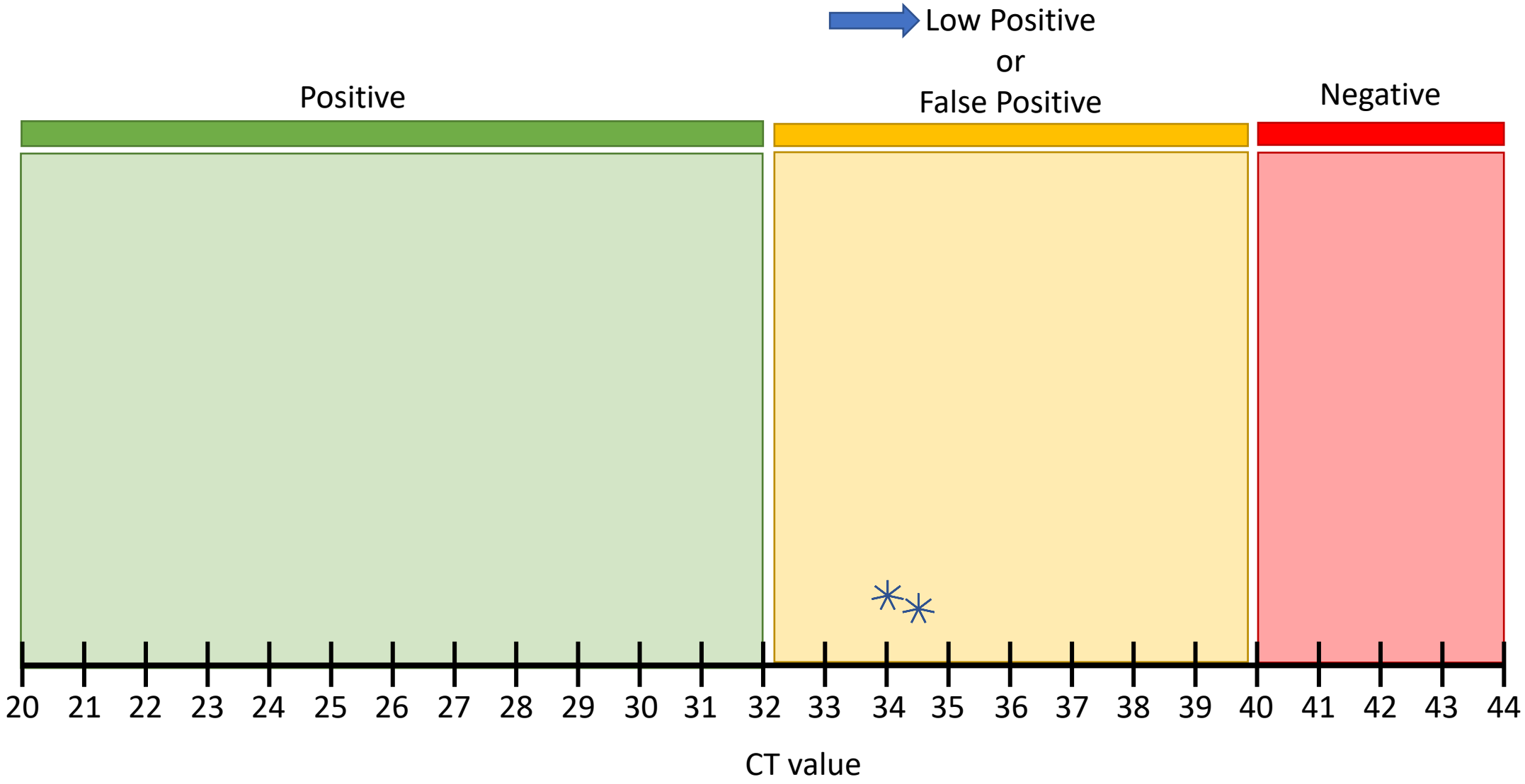
Patient	Patient characteristic	Symptoms	Initial real-time PCR test result*	Additional MPXV, NVO, or OPXV real-time PCR test result*	IgM†	Treatment administered
A	Pregnant woman, 37 weeks' gestation	Rash, pruritus	Pos NVO Ct: 34.30	MPXV: inconclusive¶	Neg	Tecovirimat to patient A, VIGIV to neonate
B	Elementary school-aged child	Rash, fatigue, headache, decreased appetite, fever	Pos NVO Ct: 35.82	Neg NVO Ct: >40**	NP	Tecovirimat
C	Infant	Diarrhea, lymphadenopathy, fever, rash	Pos NVO Ct: 34.67 OPVX Ct: 36.71	MPXV: Inconclusive¶ Neg OPXV Ct: >40 Neg NVO Ct >40	Neg	Tecovirimat

"If you obtain a high Ct value (generally ~34 or higher), CDC recommends to immediately re-extract and re-test to ensure there was no cross-contamination. CDC suggests this approach based on high Ct value alone, even in the absence of epidemiologic information."

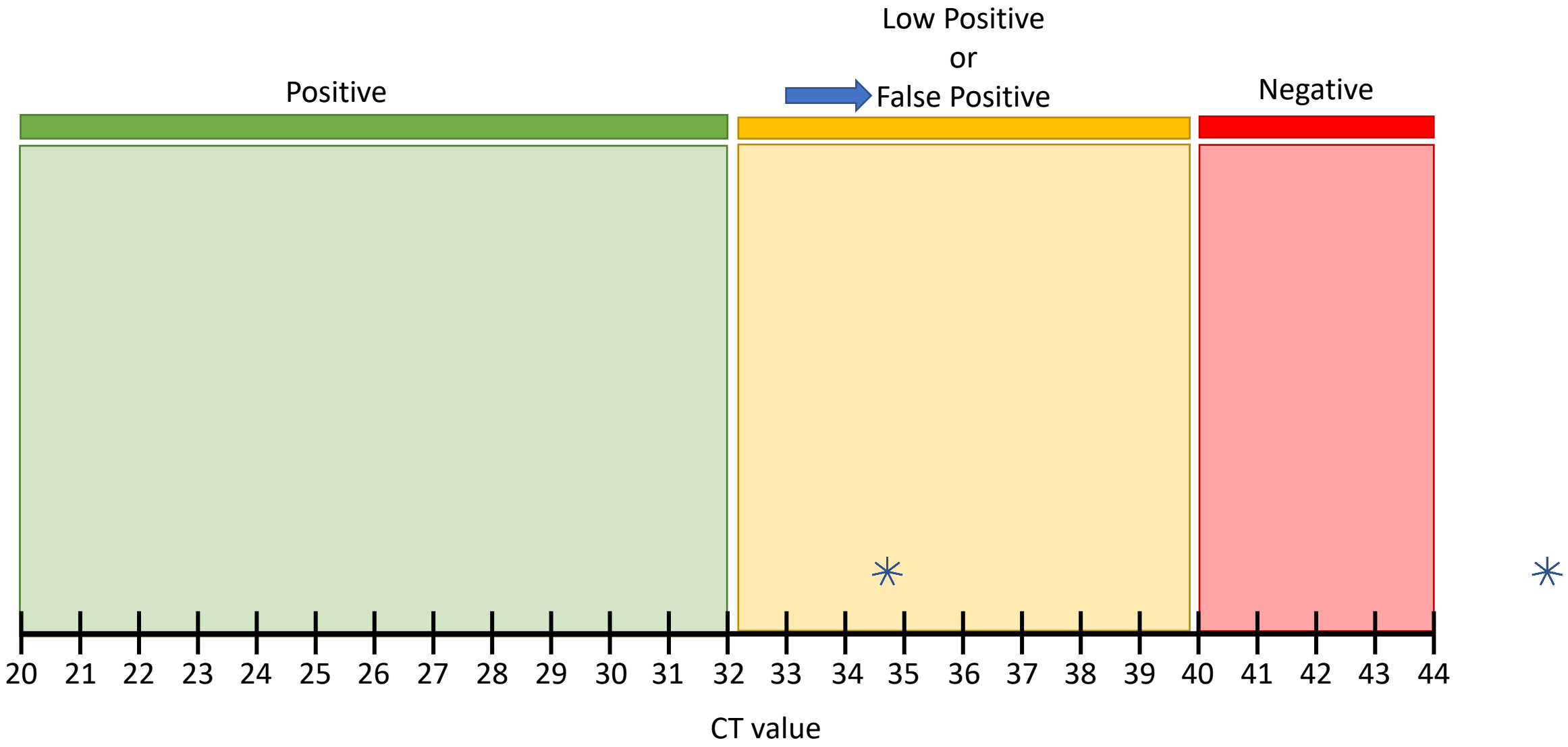
Theoretical MPXV PCR assay



Situation #1


Low Positive
or
False Positive
Positive
Negative
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value

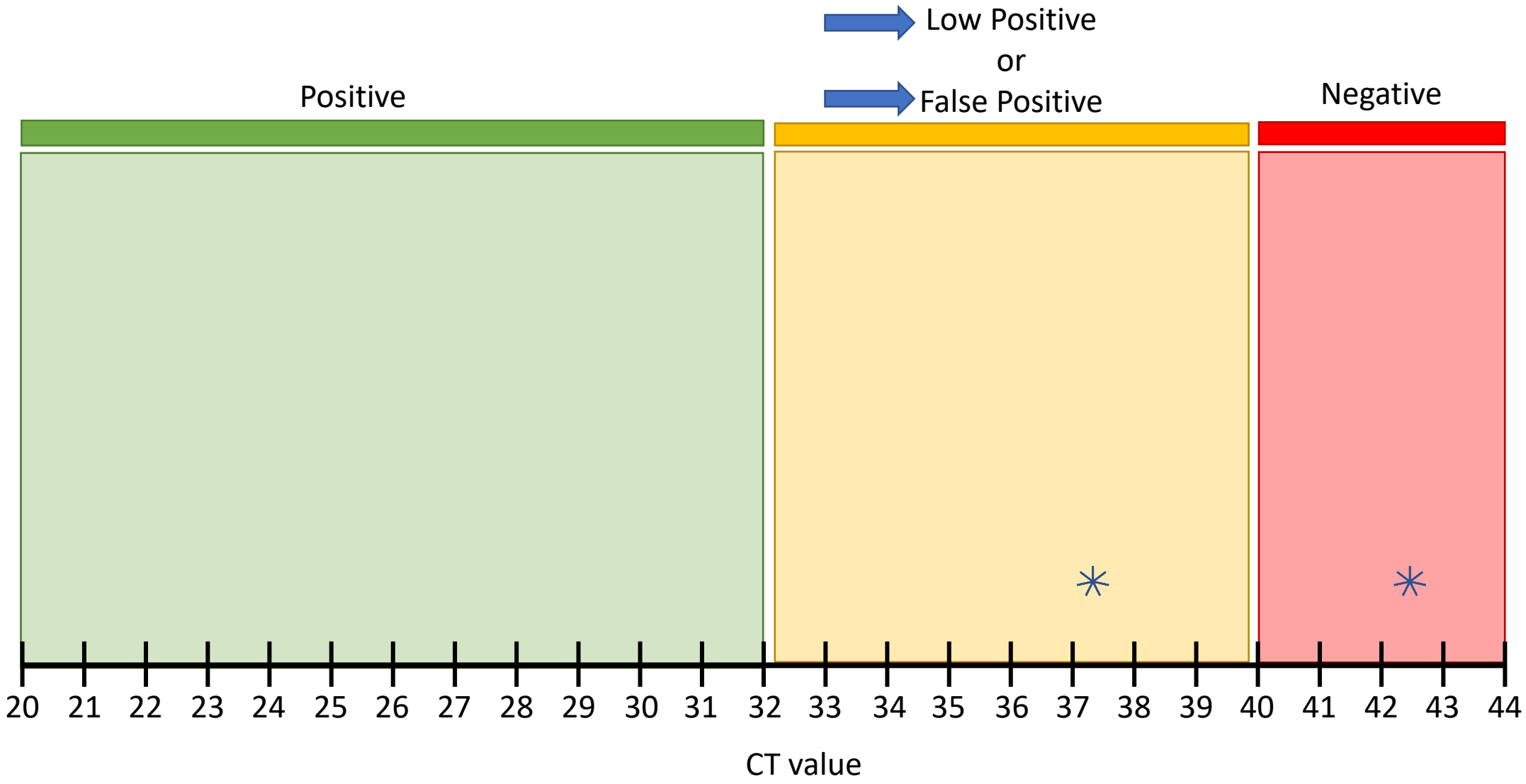
Situation #2

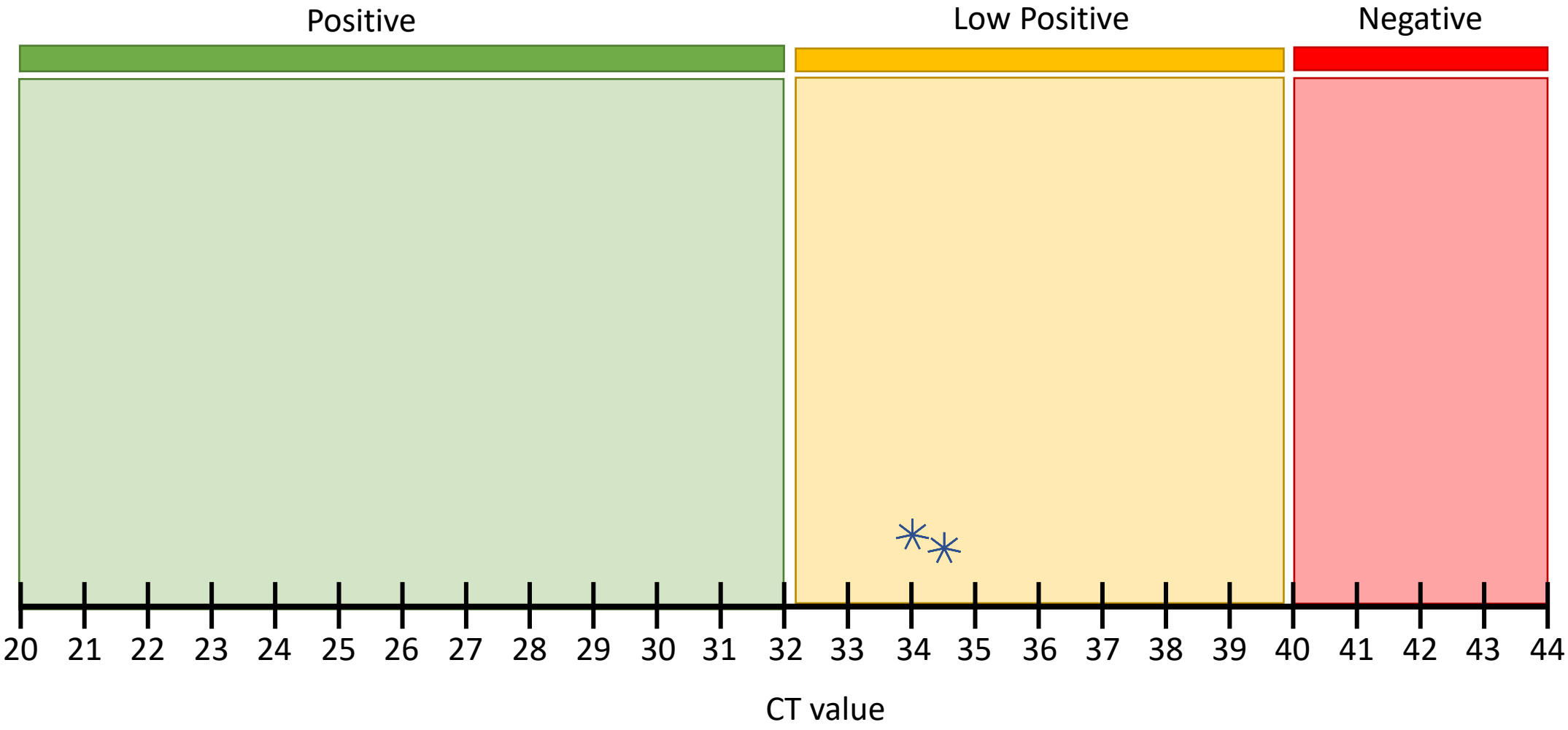

Low Positive
or
False Positive
Positive
Negative
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value

Situation #3

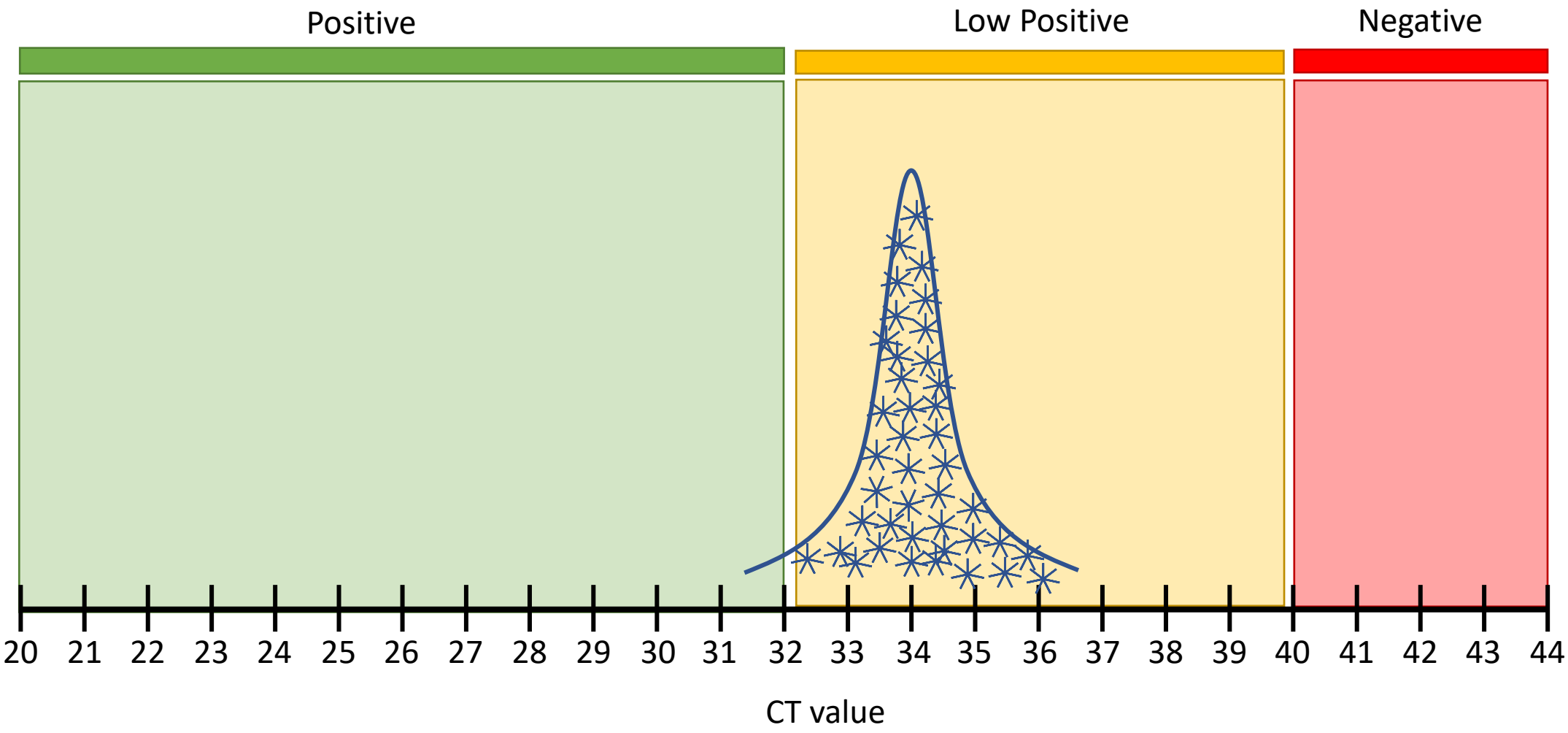
Post-analytic Question:

1. How to report samples near the LoD?

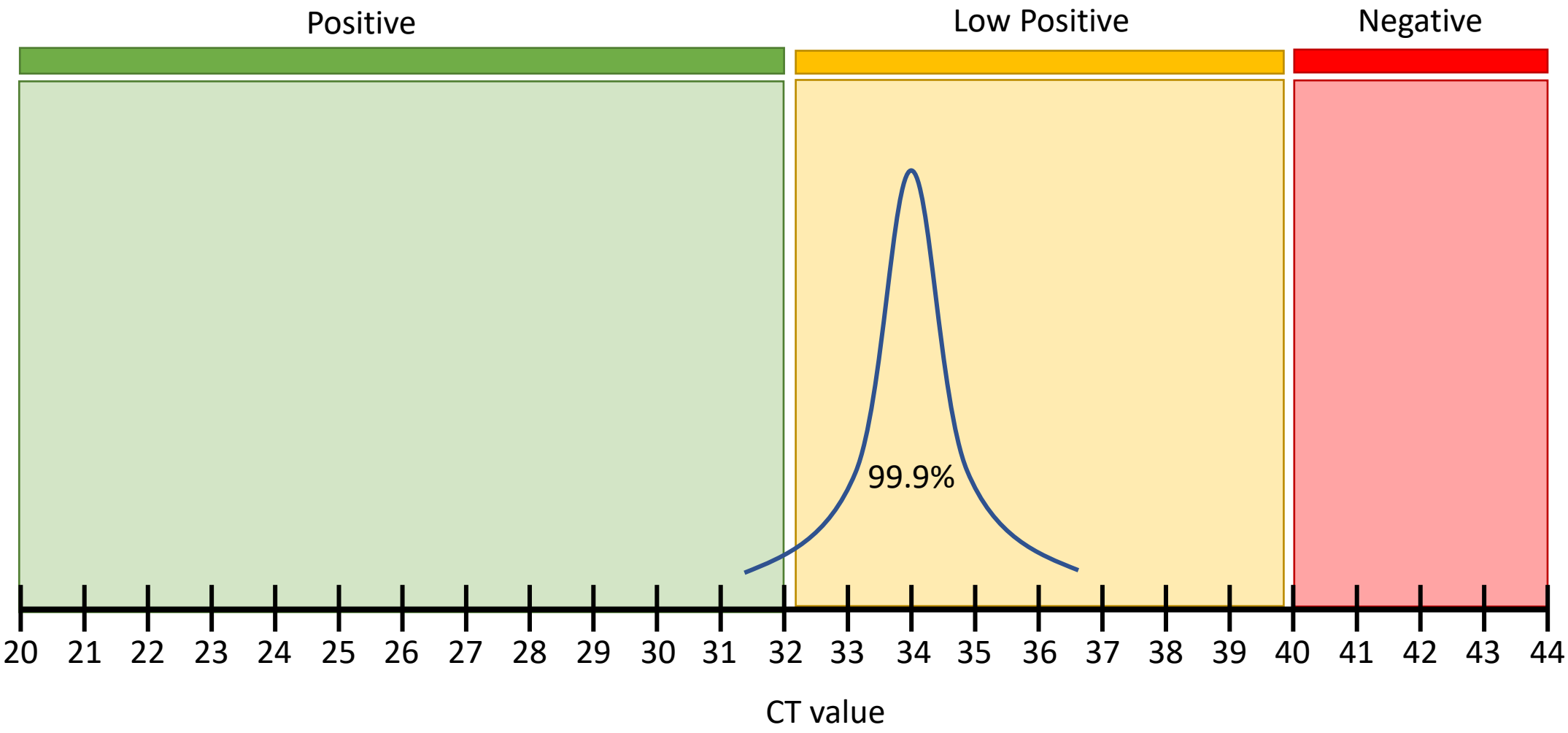


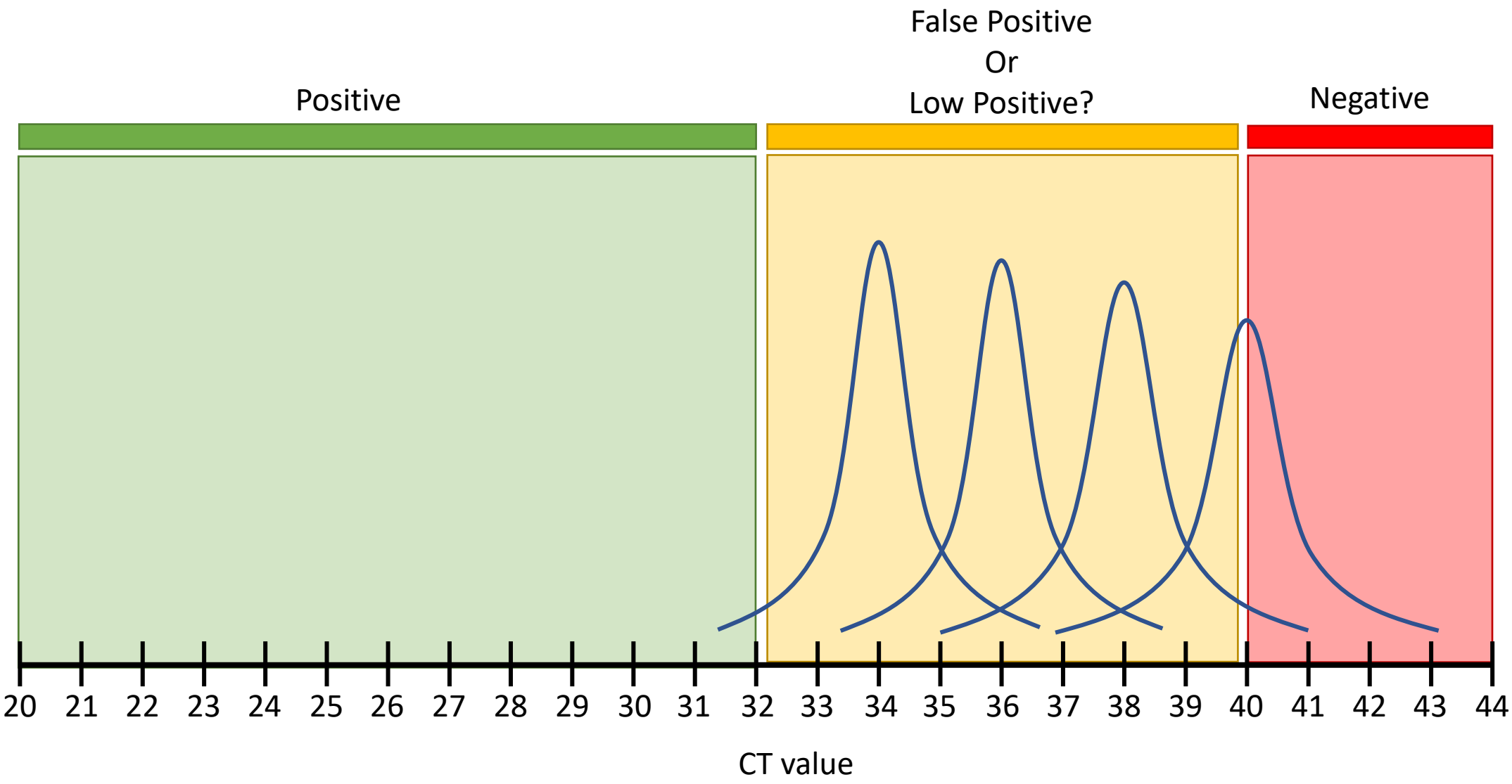

Positive
Low Positive
Negative
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value

Situation #1

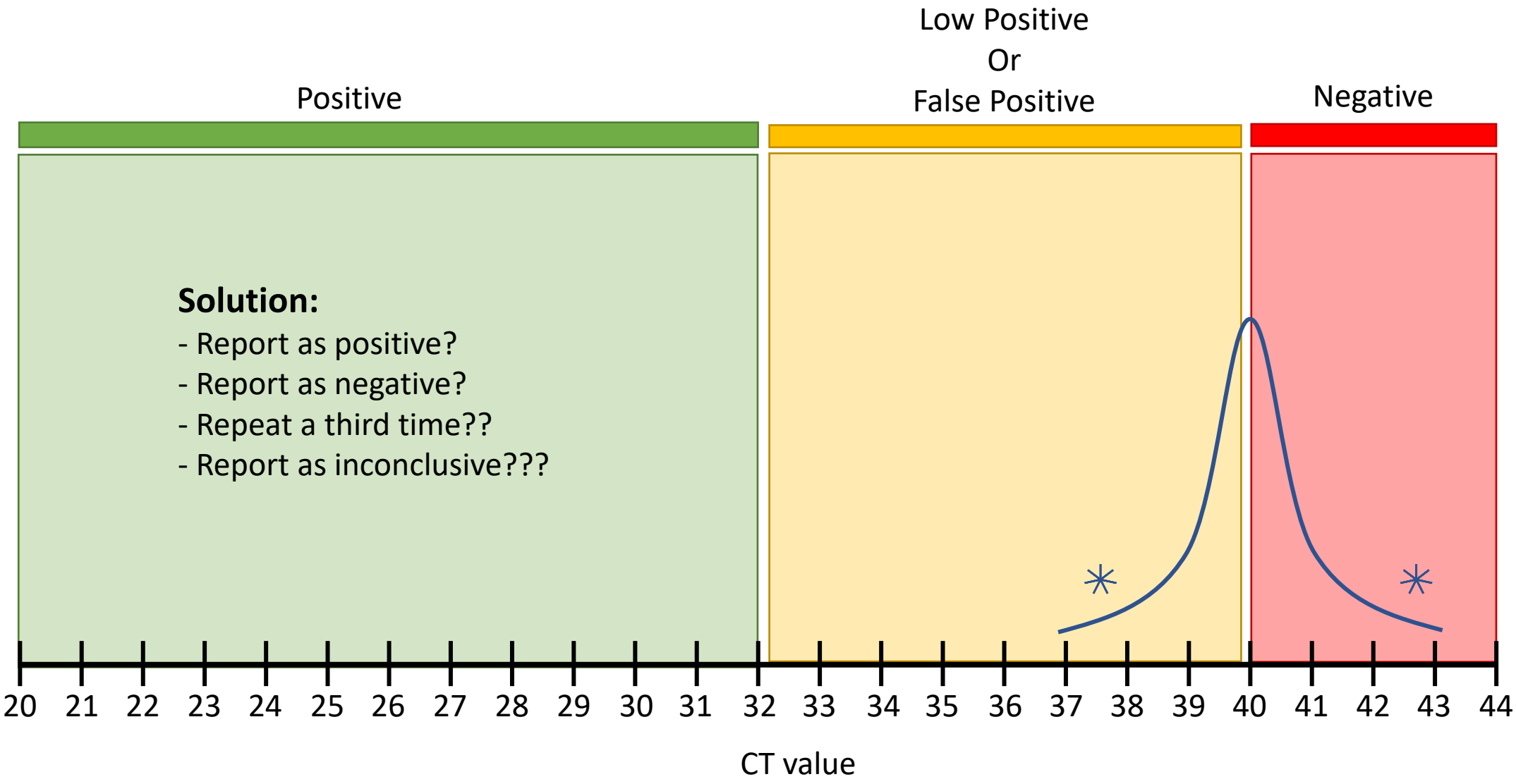

Positive
Low Positive
Negative
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value

Situation #1

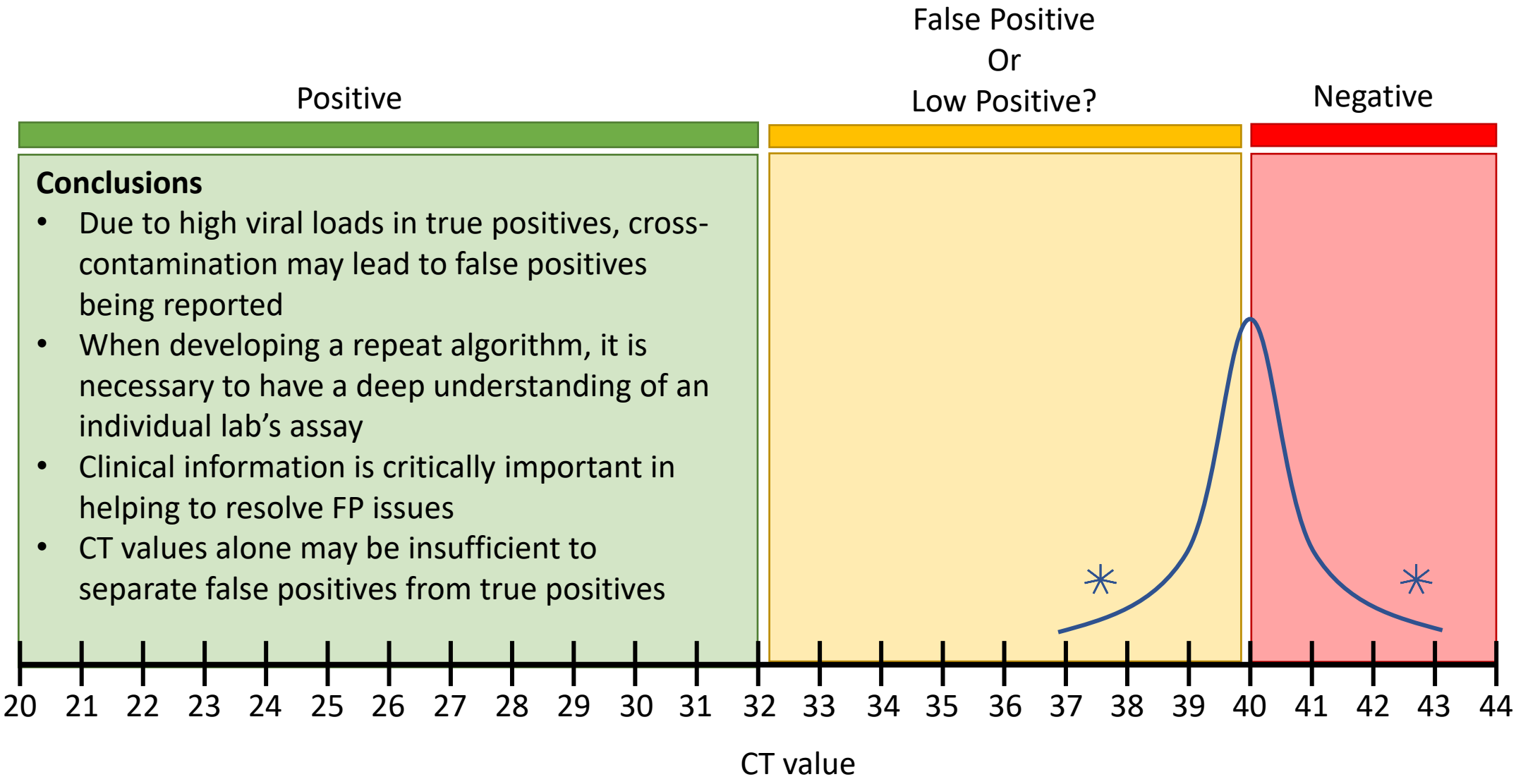

Positive
Low Positive
Negative
99.9%
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value


Positive
False Positive
Or
Low Positive?
Negative
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value

Situation #3

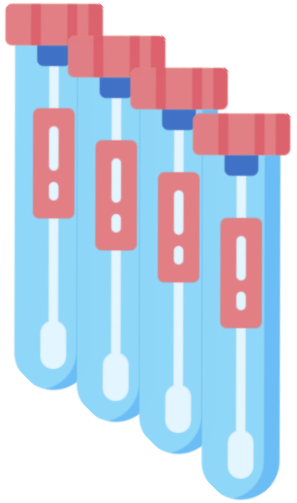

Positive
Low Positive
Or
False Positive
Negative
Solution:
- Report as positive?
- Report as negative?
- Repeat a third time??
- Report as inconclusive???
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value

Situation #3


Positive
False Positive
Or
Low Positive?
Negative
Conclusions
• Due to high viral loads in true positives, cross-contamination may lead to false positives being reported
• When developing a repeat algorithm, it is necessary to have a deep understanding of an individual lab's assay
• Clinical information is critically important in helping to resolve FP issues
• CT values alone may be insufficient to separate false positives from true positives
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44
CT value

What the Lab Needs to Know about MPXV Major Conclusions

Pre-analytical



- Skin lesions are most sensitive
- Collect early, but virus may be recovered from healing skin
- 2 swabs are virtually as sensitive as 3 or more

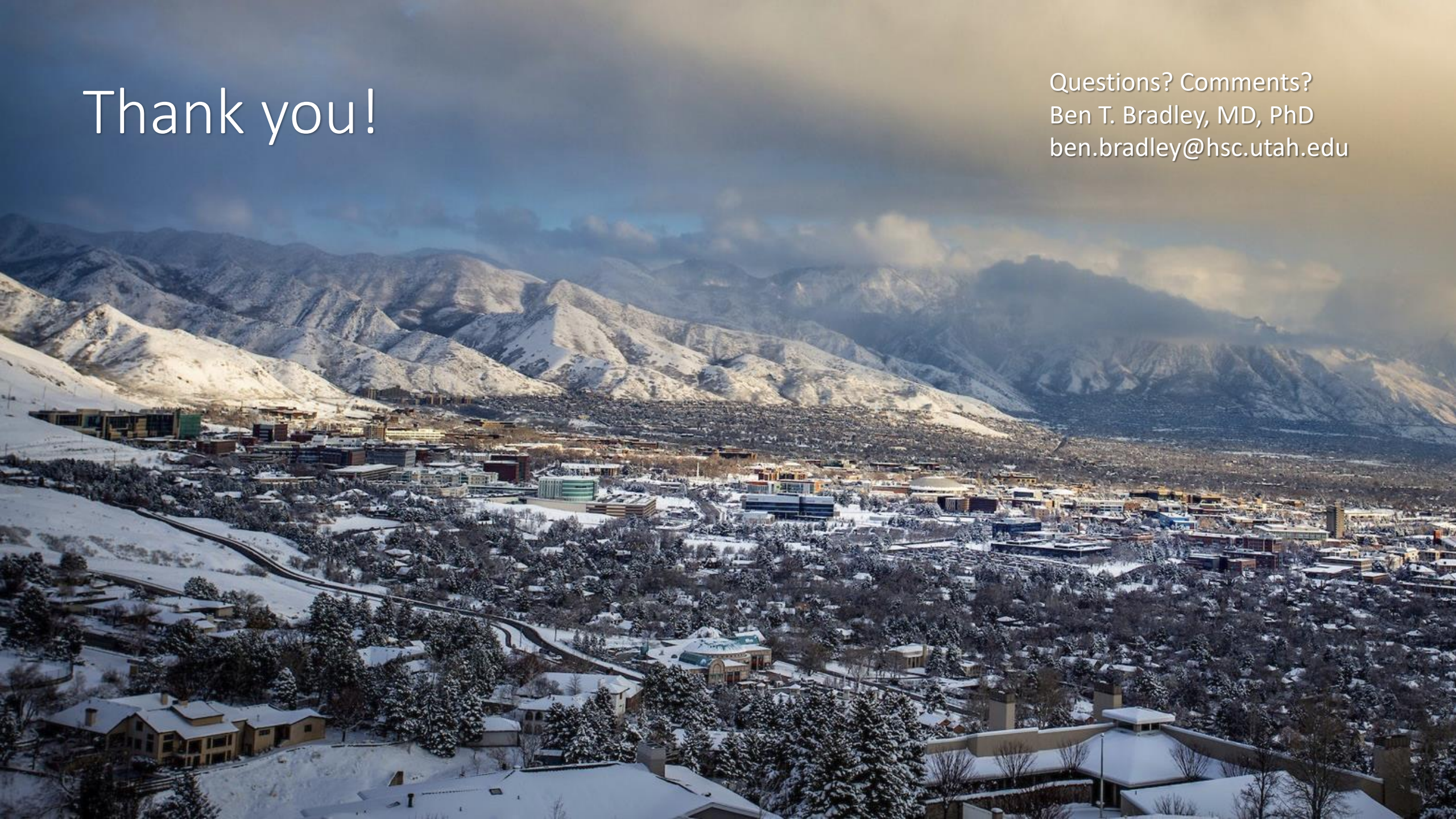
Analytical

- OPXV and MPXV targets have similar analytic performance
- Infectious virus is present even in specimens with late Ct values
- Viral culture assays may incidentally recover MPXV

Post-analytical

- Samples with late CTs may raise concern for FP results
- Careful consideration must go into creating repeat algorithms

Thank you!



Questions? Comments?
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ben.bradley@hsc.utah.edu