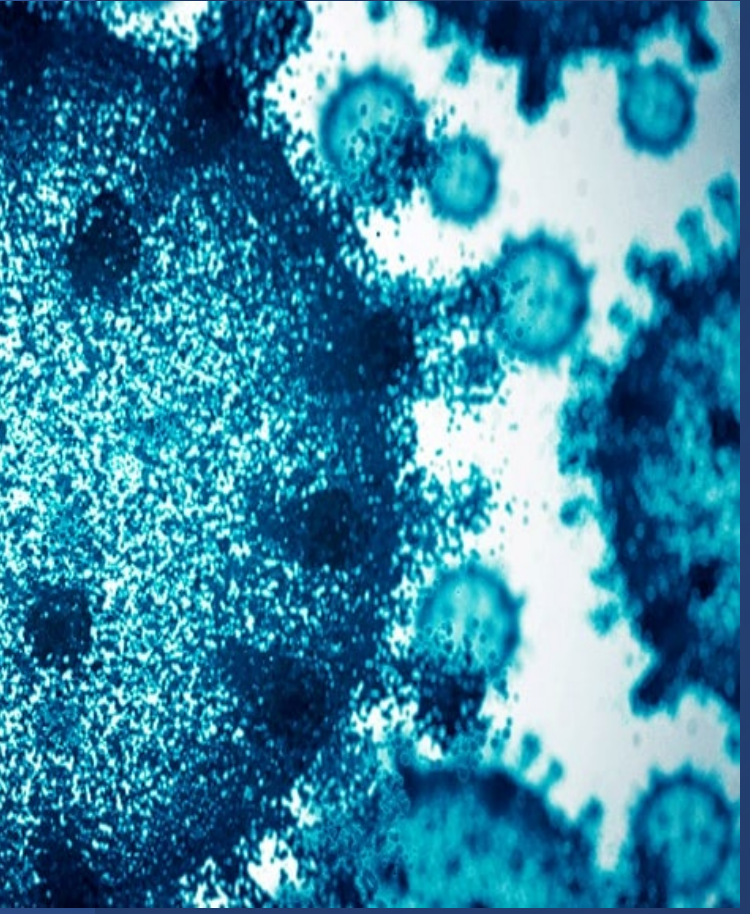


Post-COVID re-emerging pathogens: **Respiratory Pathogens**

Kimberly McCullor, PhD, MSc
Michigan Bureau of Laboratories

Amy Leber, PhD
Nationwide Children's Hospital

APHL ID Lab Con Pre-Conference Workshop

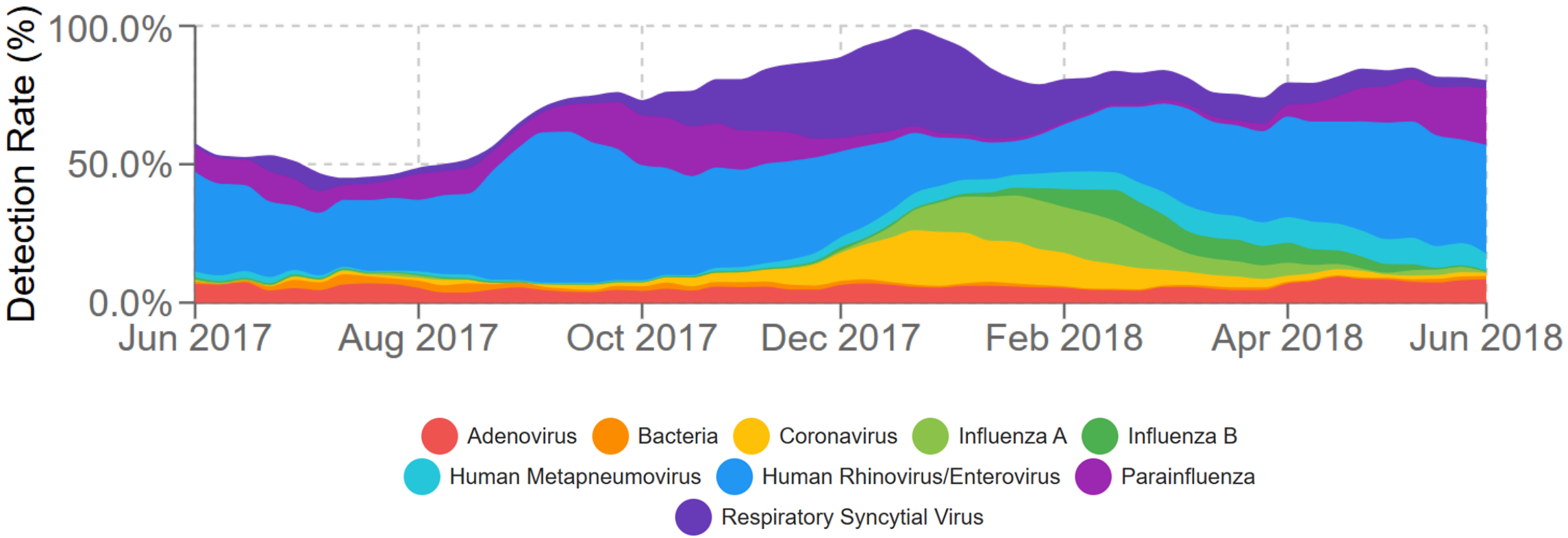
March 2025 Pasadena, CA

Outline:

- The scope of respiratory pathogen “reemergence”
- *Mycoplasma pneumoniae*
- *Bordetella pertussis*
- Influenza A H5

The Seasonality of Respiratory Infections

Nationwide Microbiology Lab



COVID Pandemic Timeline

Dec 8,2019
Cases with pneumonia like symptoms and with unknown etiology reported in Wuhan

Jan 7,2020
Chinese Center for Disease Control and Prevention identified novel coronavirus from admitted persons

Jan 9,2020
First death caused by SARS-CoV-2 reported in China

Jan 13,2020
First positive case reported outside China in Thailand

Jan 20,2020
282 laboratory-confirmed cases and 6 deaths reported by WHO in its SITUATION REPORT-1

Jan 30,2020
WHO declares SARS-CoV-2 outbreak as Public Health Emergency of International Concern (PHEIC)

Feb 2,2020
First death repo
outside China ir
Philippines

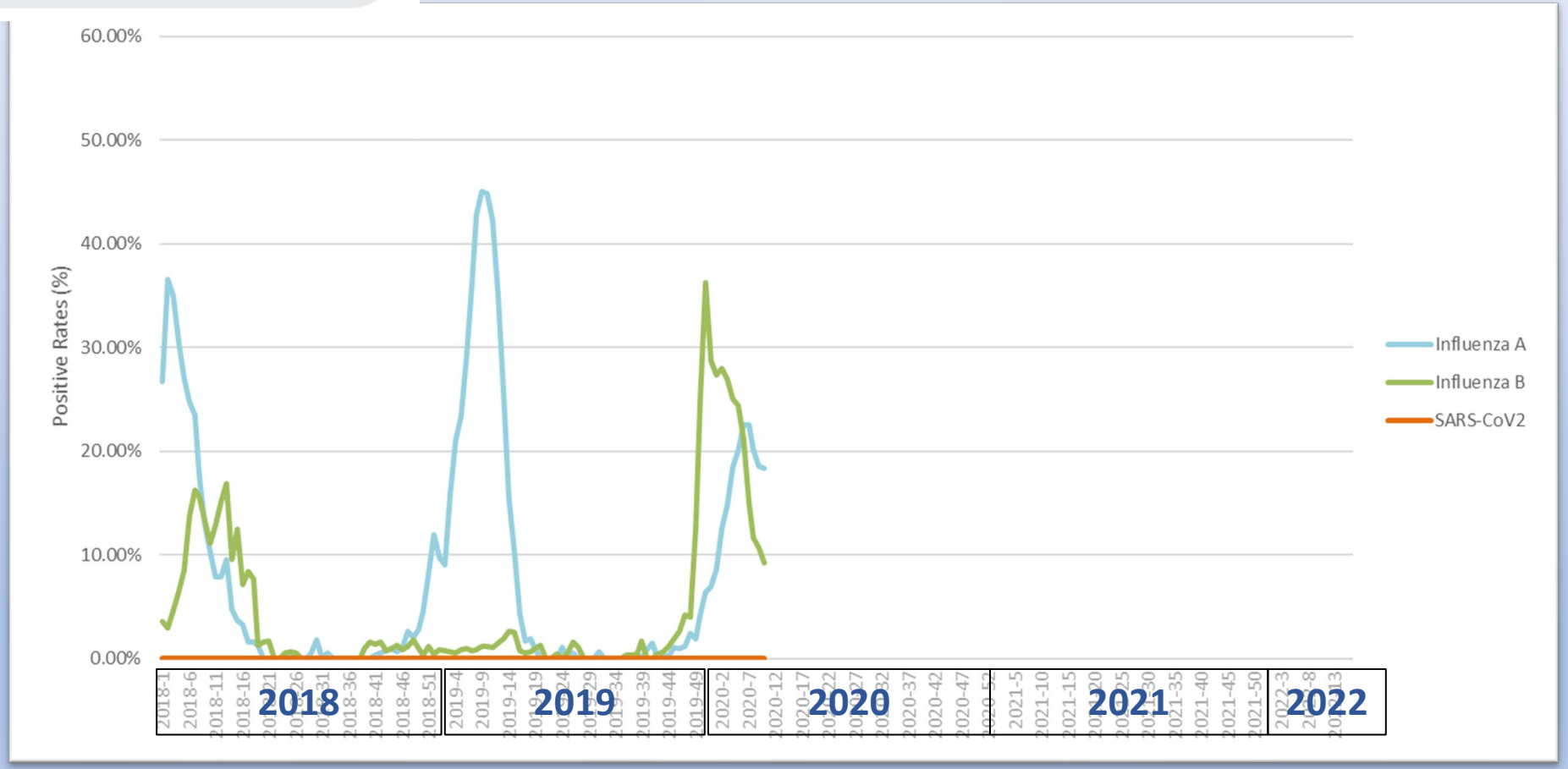
Feb 11,2020
Disease caused by SARS-CoV-2 is named as COVID-19

845
and 761,779 deaths were reported in WHO's SITUATION REPORT-209

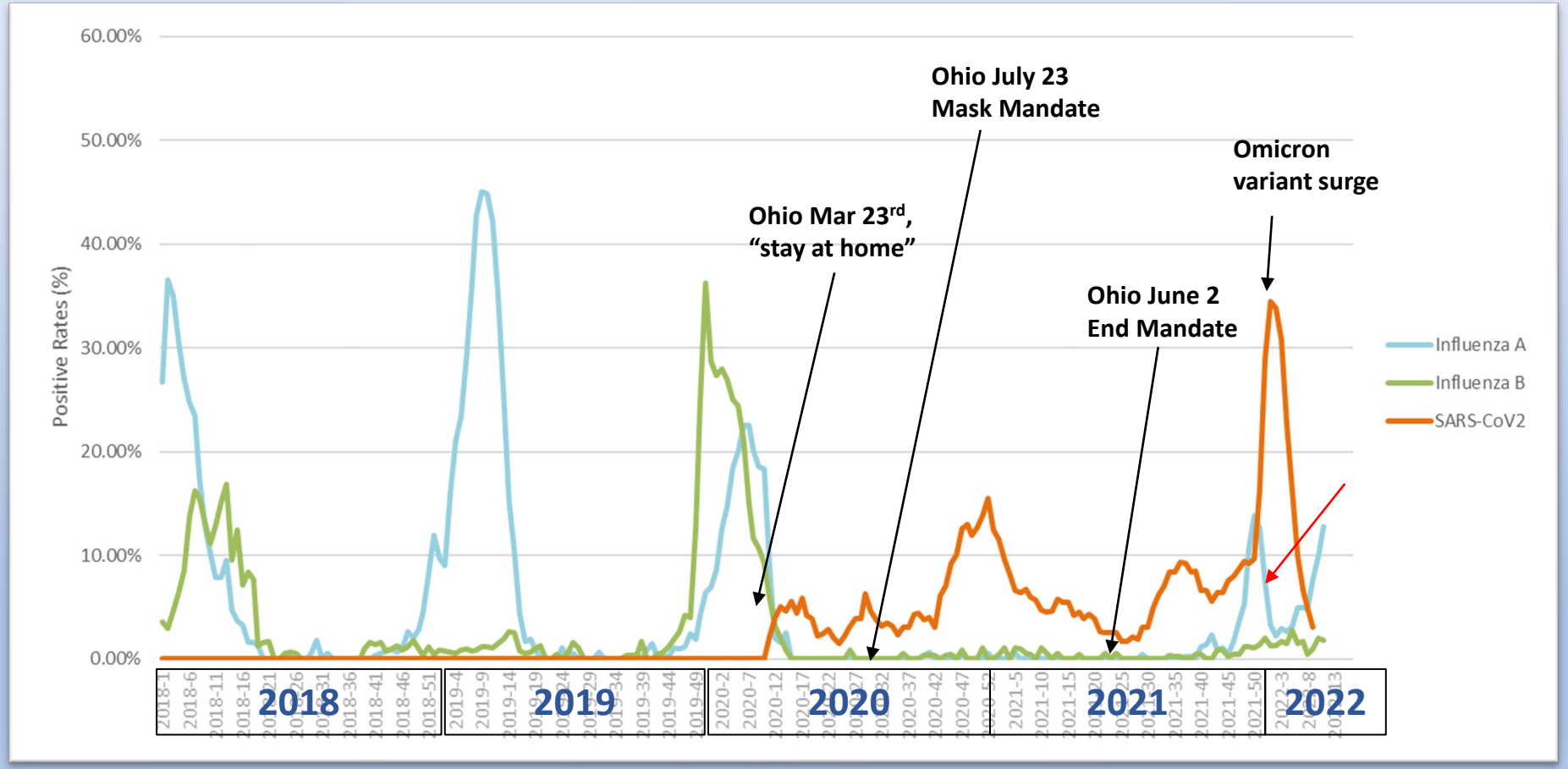
Mar 19, 2020
WHO released its SITUATI N REPORT-59 stating that the number of confirmed cases surpassed 200,000. It also mentioned that it took 3 months of time period to reach 100,000 and just 1 days to reach the next 100,000

COVID19 pandemic changed Influenza activity

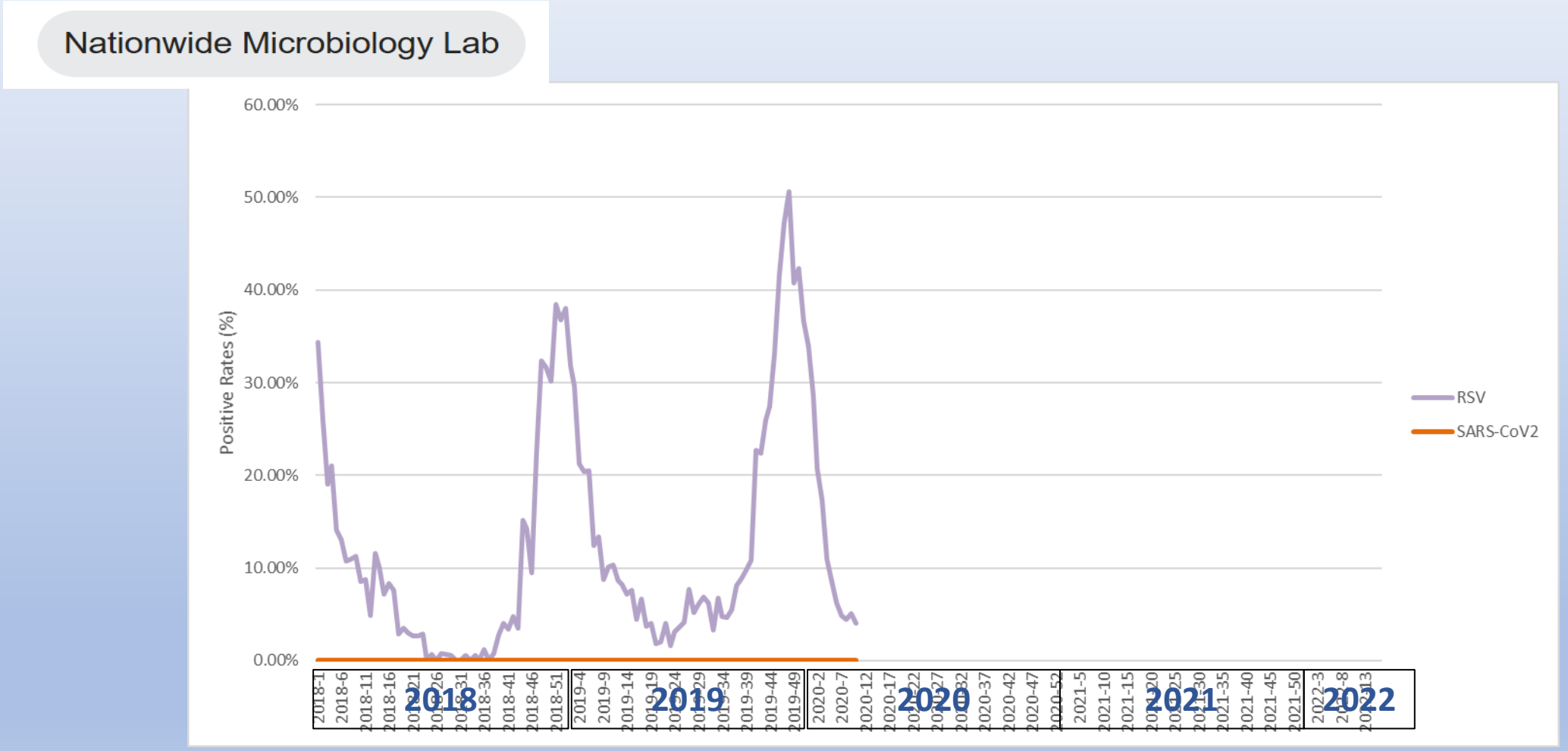
Nationwide Microbiology Lab



COVID19 pandemic changed Influenza activity

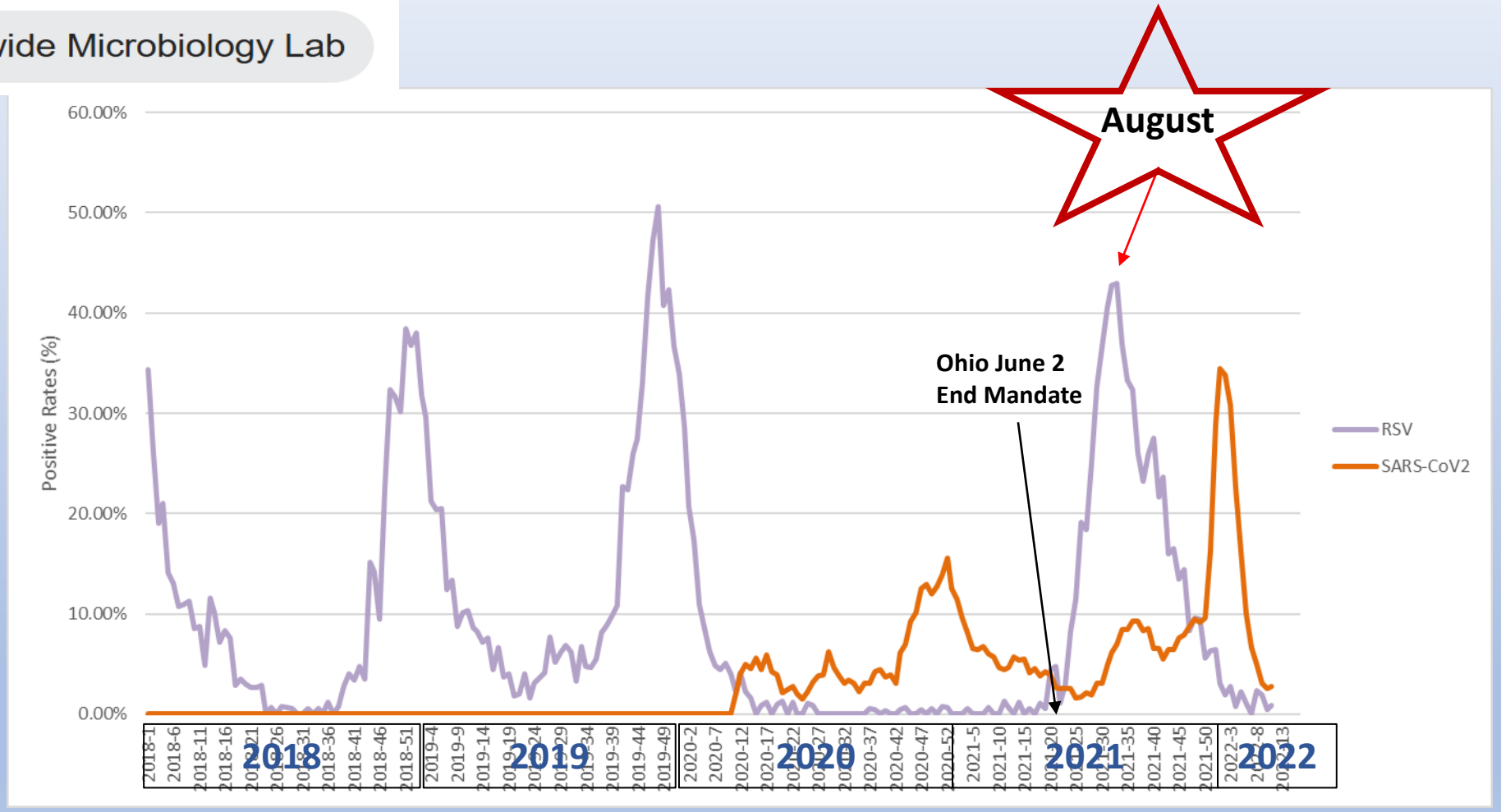


COVID19 pandemic changed RSV activity

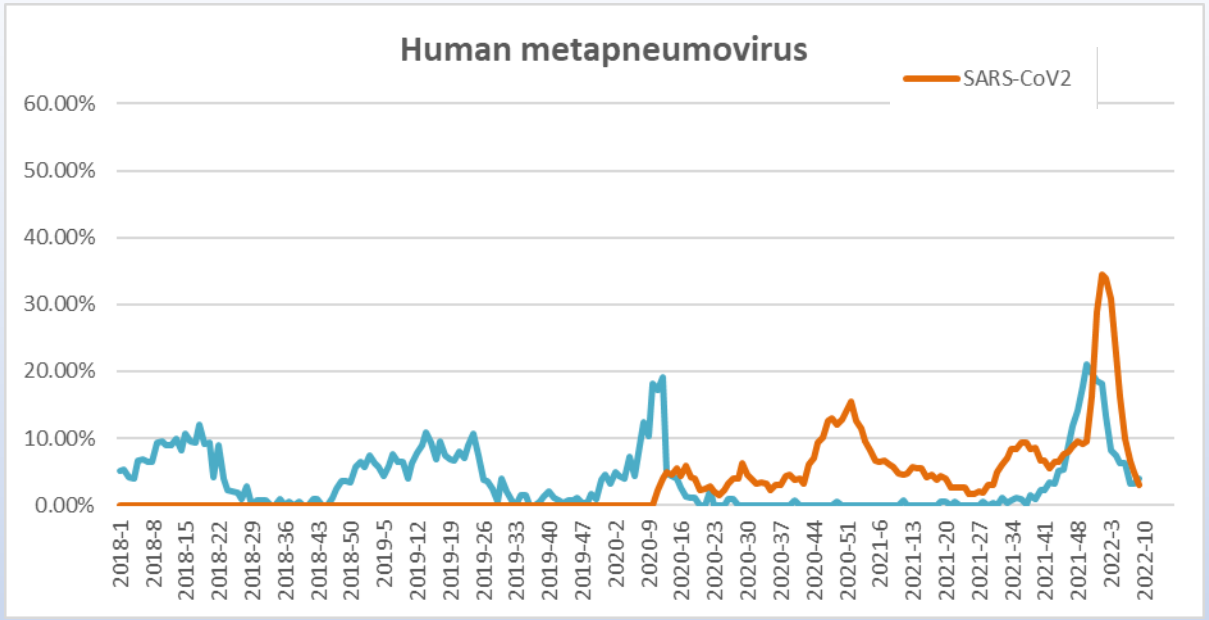


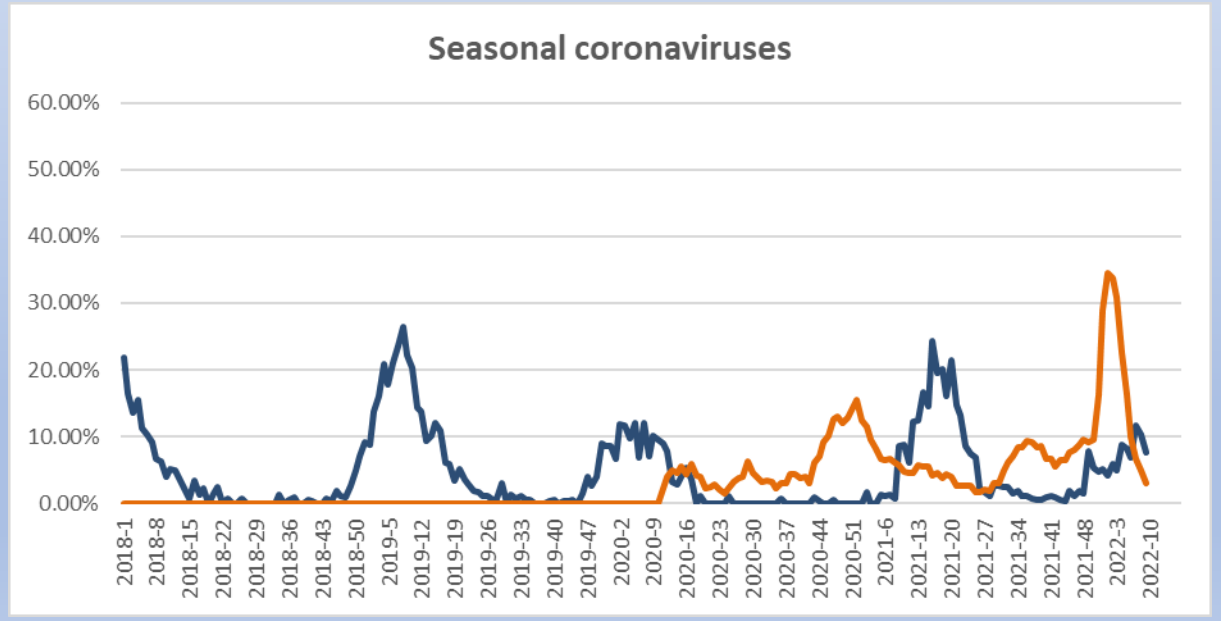
COVID19 pandemic changed RSV activity

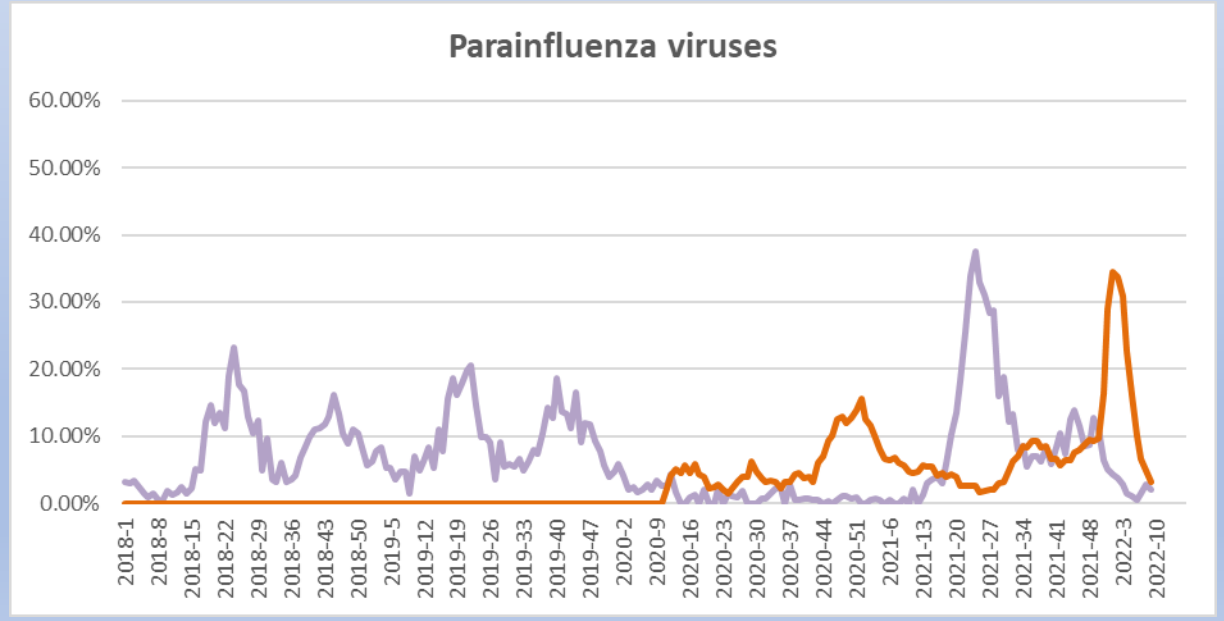
Nationwide Microbiology Lab



Other Viruses 2018-2022



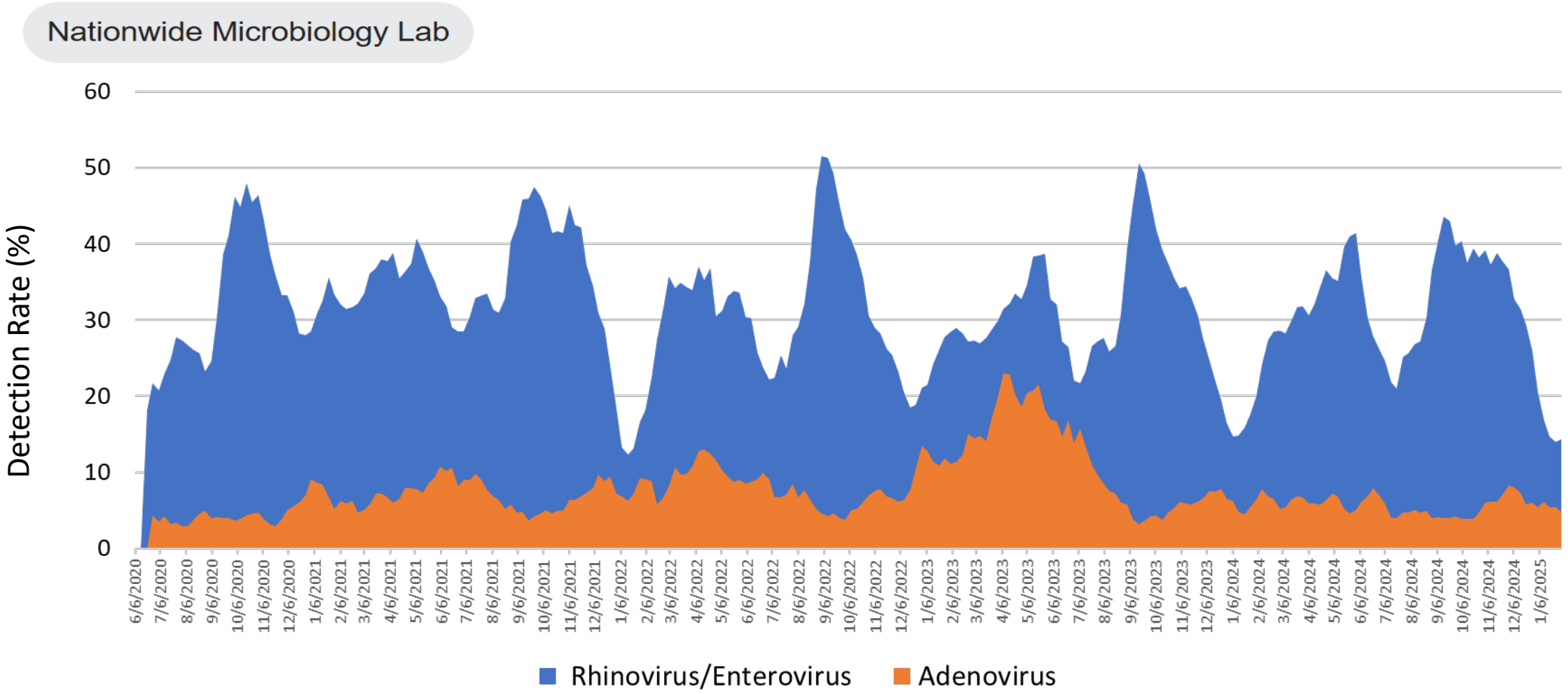




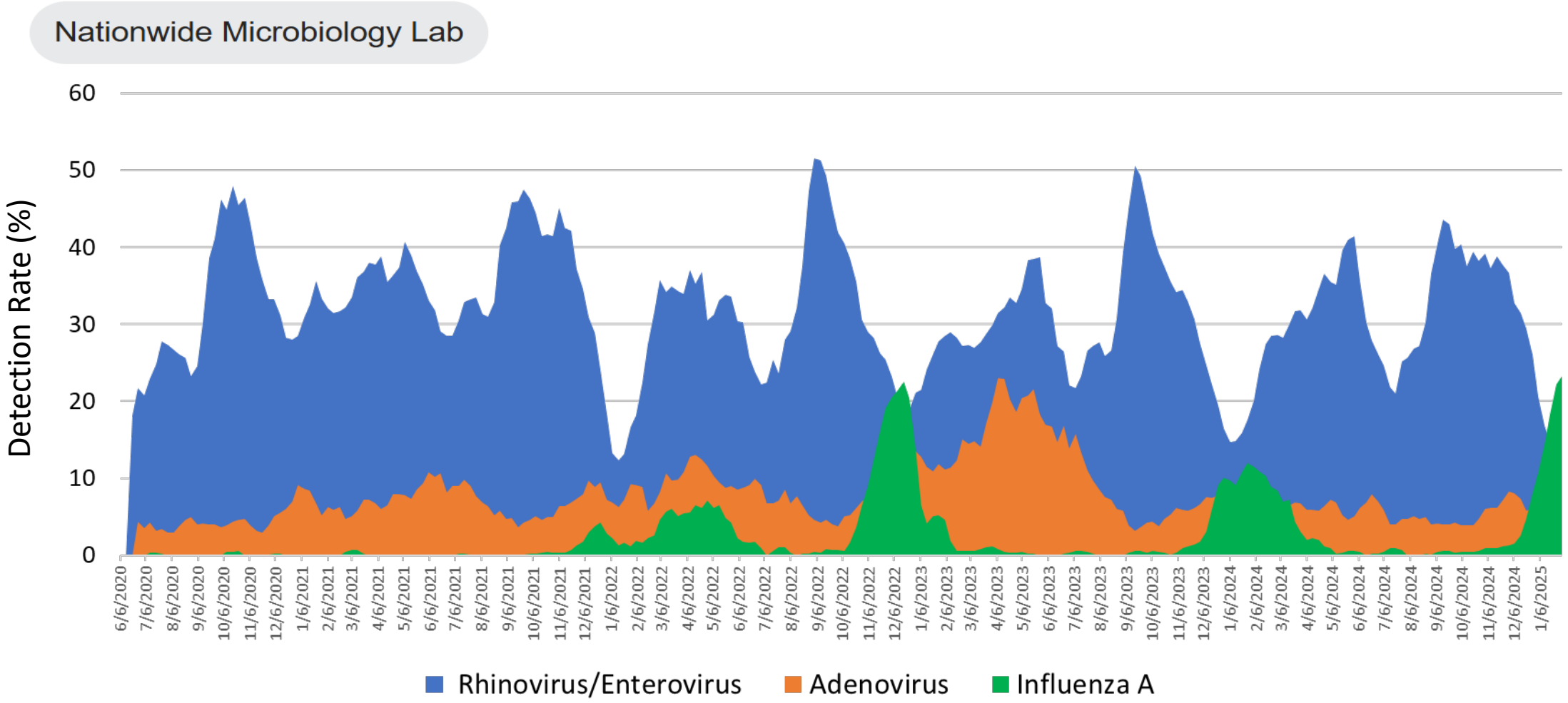
Why was Influenza & RSV activity impacted by the Pandemic?

- Public Health Measures (Social Distancing, Masking, and Hygiene)
- Changes in Travel Patterns
- Impact on Healthcare and Surveillance Systems
- Short & Long-Term Immunological Effects

Rhinovirus and Adenovirus 2020-2024



Rhinovirus and Adenovirus vs Flu A 2020-2024



Why did Rhinovirus & Adenovirus stay around?

No seasonality

Colonization/Prolonged Shedding

Route of transmission

SuperPowers

Non-enveloped

Multiple Serotypes

Other?

Effectiveness of Masking: Cornavirus, Influenza and Rhinovirus

- Coronavirus - Significant reduction in viral load in both droplets and aerosolized particles with a mask

- Influenza - Significant difference in the viral load for droplets but not aerosolized particles with mask

- Rhinovirus - No significant difference in the viral load in both droplets and aerosolized particles with or without a mask

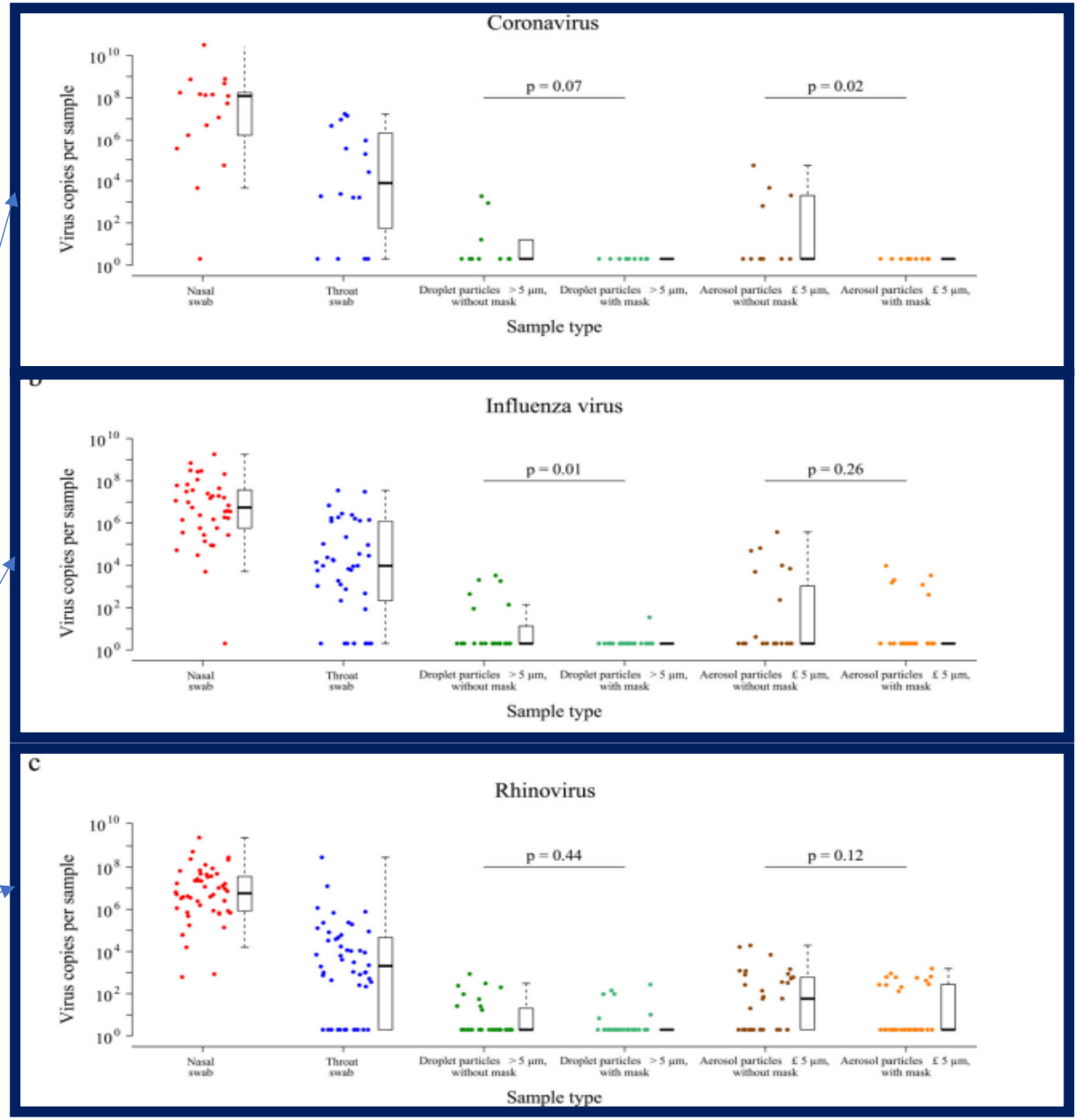
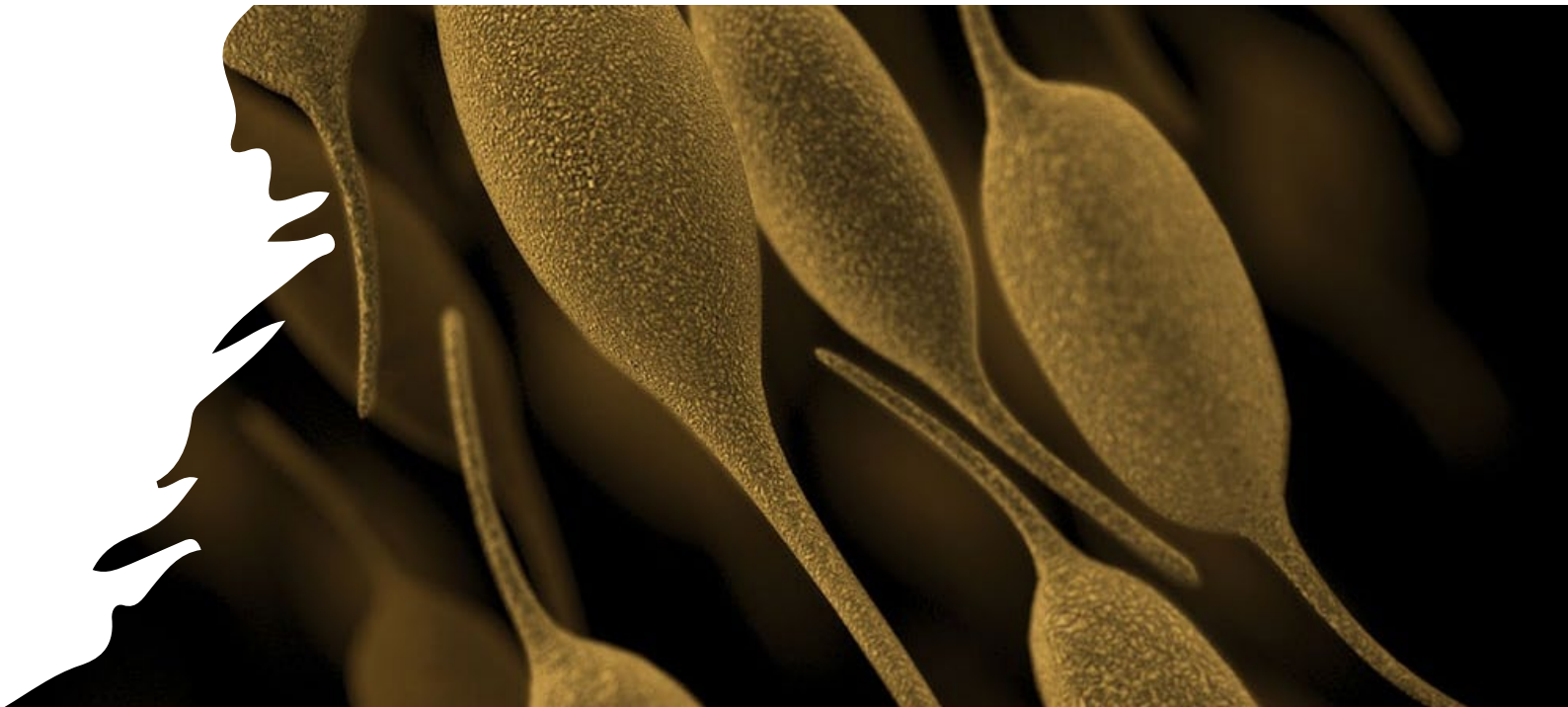


Figure 1. Efficacy of surgical face masks in reducing respiratory virus shedding in respiratory droplets and aerosols of symptomatic individuals with (a) coronavirus, (b) influenza virus or (c) rhinovirus infection.

Leung NHL, et al. Nat Med. 2020 May;26(5):676-680

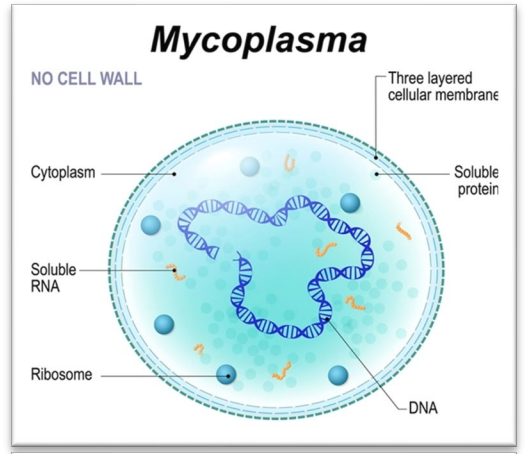
Mycoplasma pneumoniae

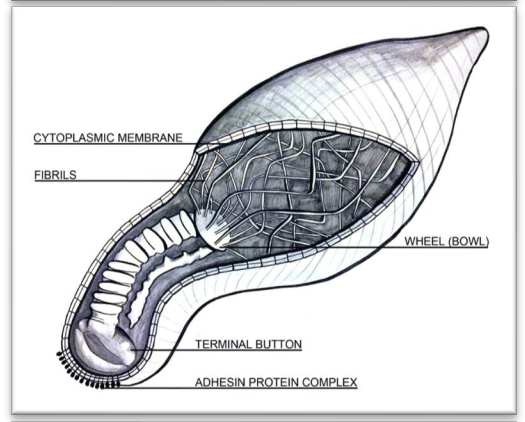
Reemergence post-Pandemic



Mycoplasma pneumoniae

- Bacteria lacking a cell wall, one of the smallest organisms capable of independent growth
- Leading cause of community-acquired pneumonia, especially in children and young adults
- Causes outbreaks of respiratory infections throughout much of the world *every 3-5 years*
- Macrolides are the first-line treatment





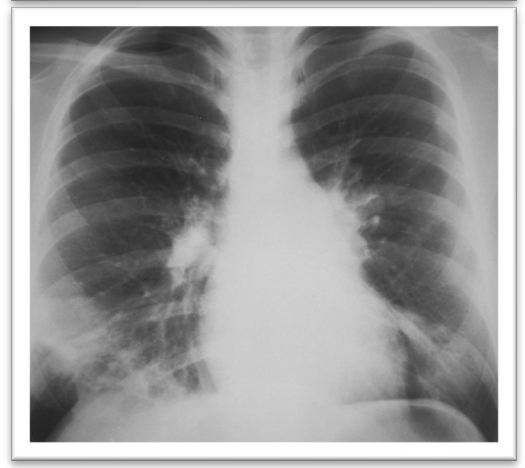
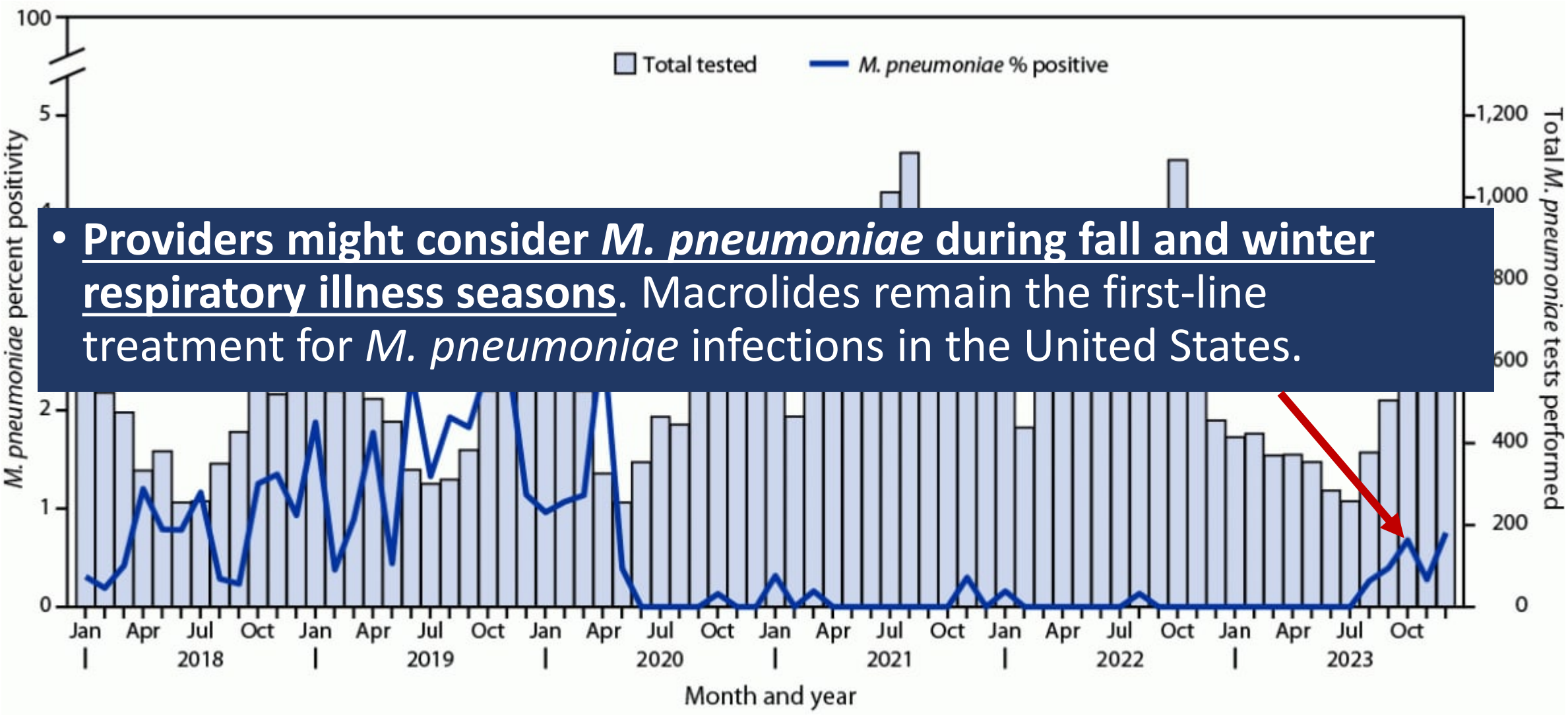


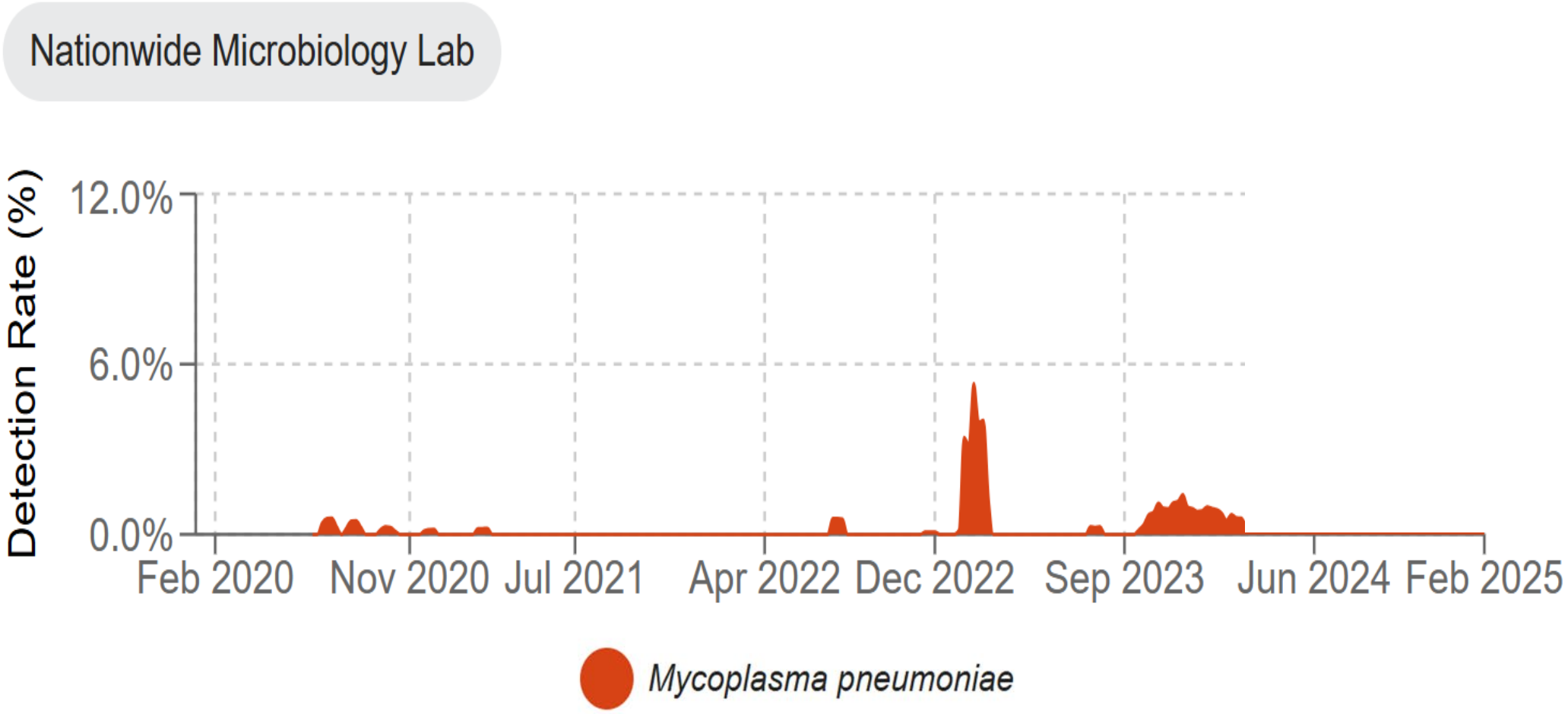
FIGURE. Monthly number of *Mycoplasma pneumoniae* tests performed and percentage of positive test results among children and adolescents with acute respiratory illness — four sites, New Vaccine Surveillance Network, 2018–2023



- **<u>Providers might consider *M. pneumoniae* during fall and winter respiratory illness seasons</u>**. Macrolides remain the first-line treatment for *M. pneumoniae* infections in the United States.

Notes from the Field: Reemergence of Mycoplasma pneumoniae Infections in Children and Adolescents After the COVID-19 Pandemic, United States, 2018–2024 | MMWR

Mycoplasma pneumoniae - June 2020 – Feb 2025
Multiplex Assay



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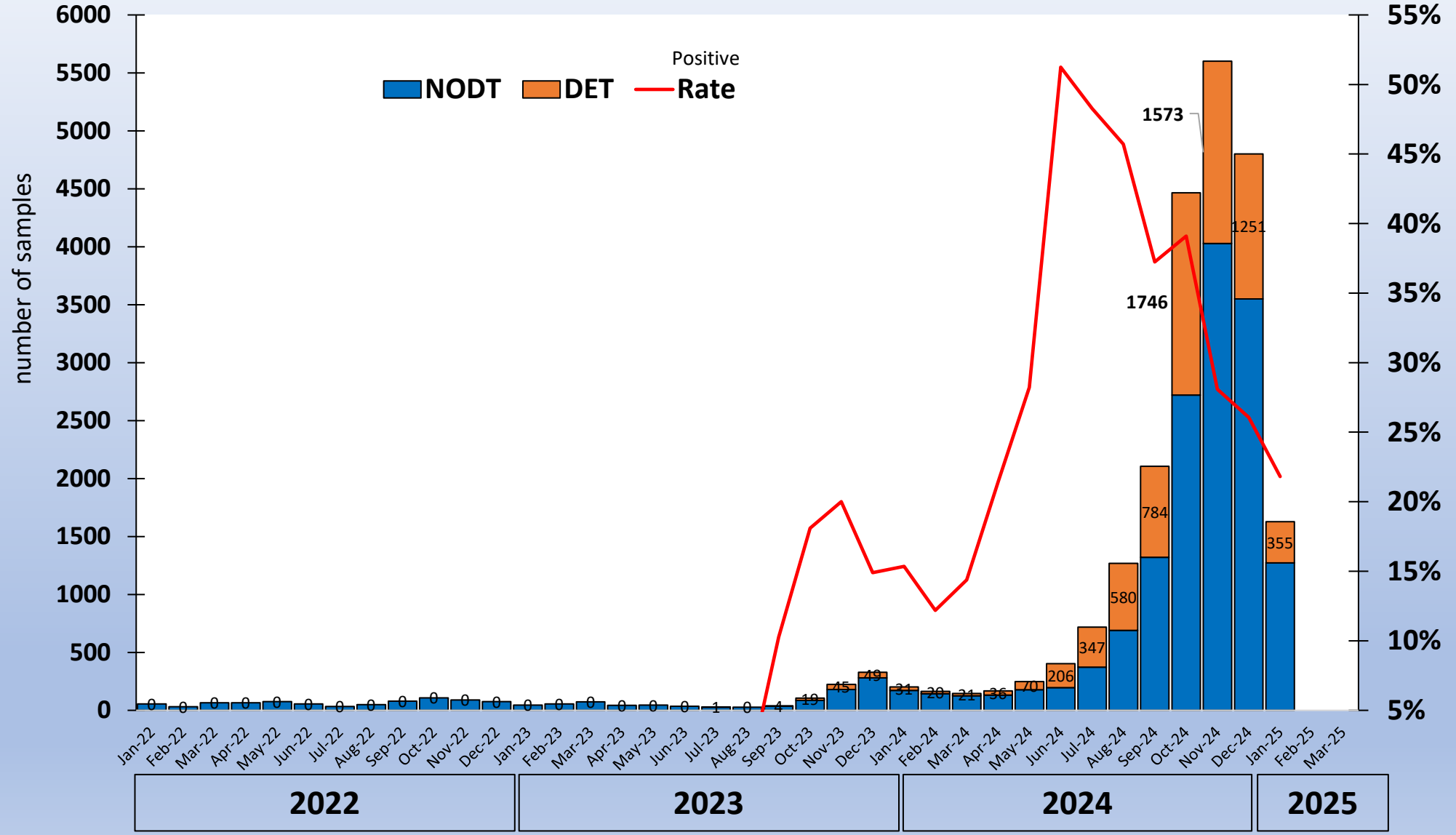
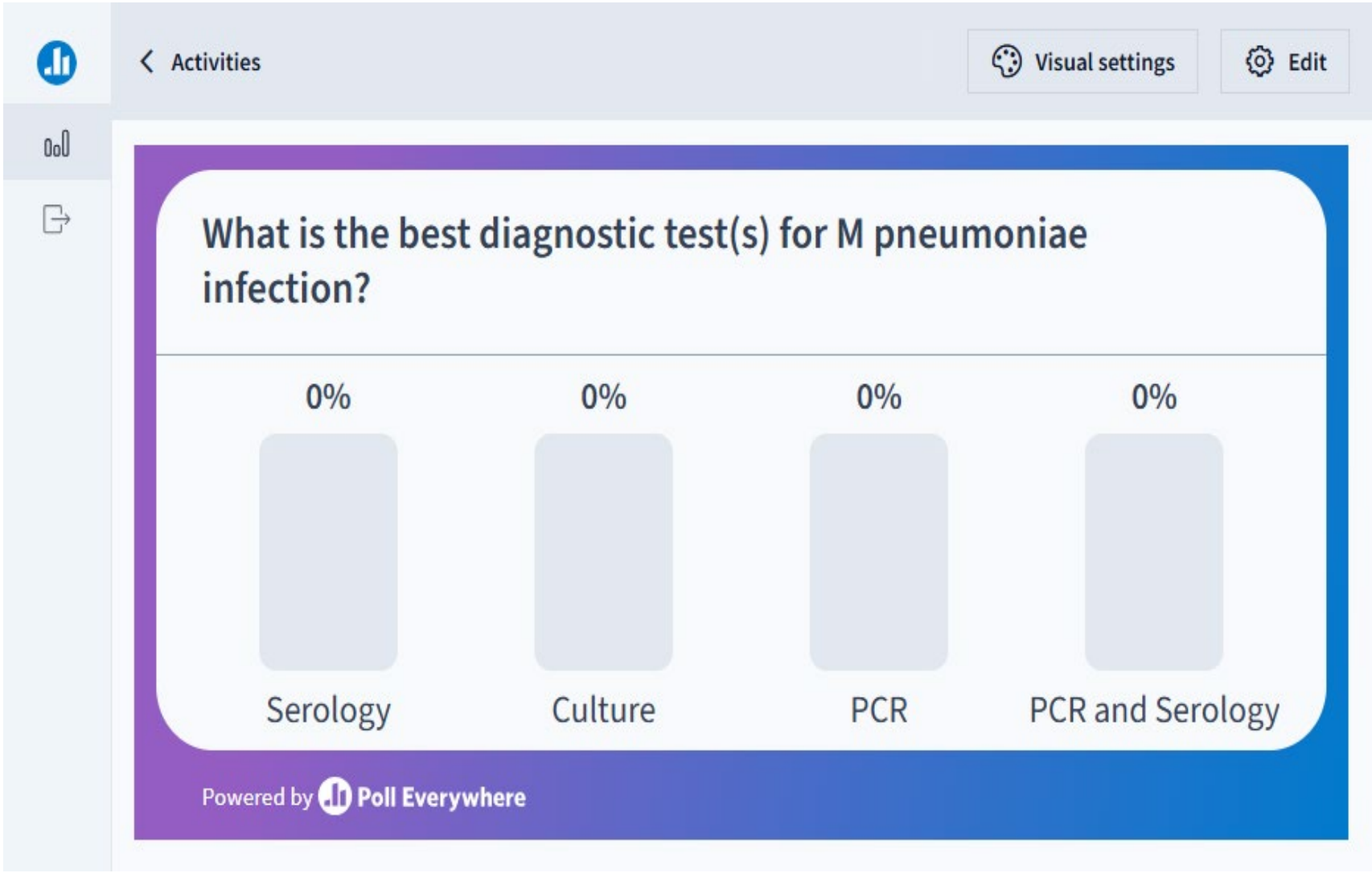
M. pneumoniae PCR Testing 2022 -2025



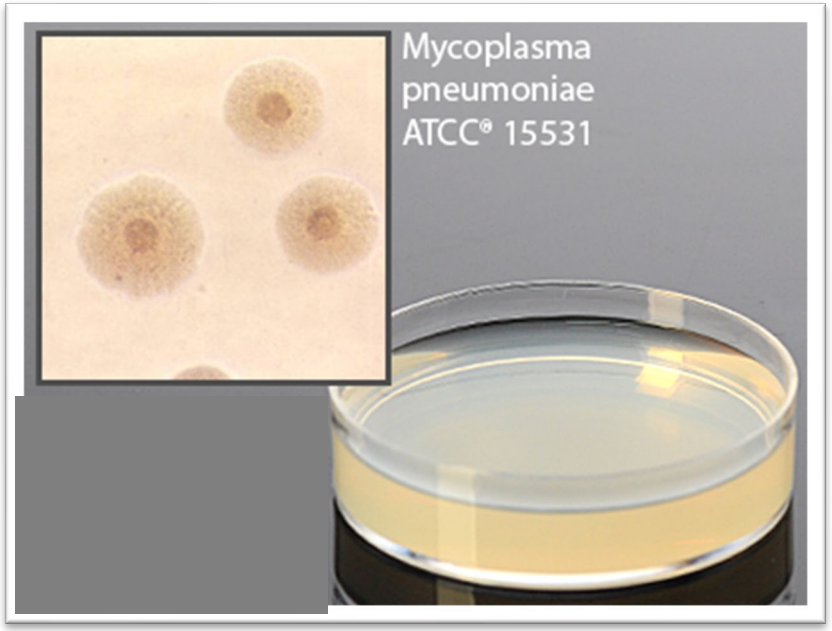
What is the best diagnostic test(s) for *M pneumoniae* infection?

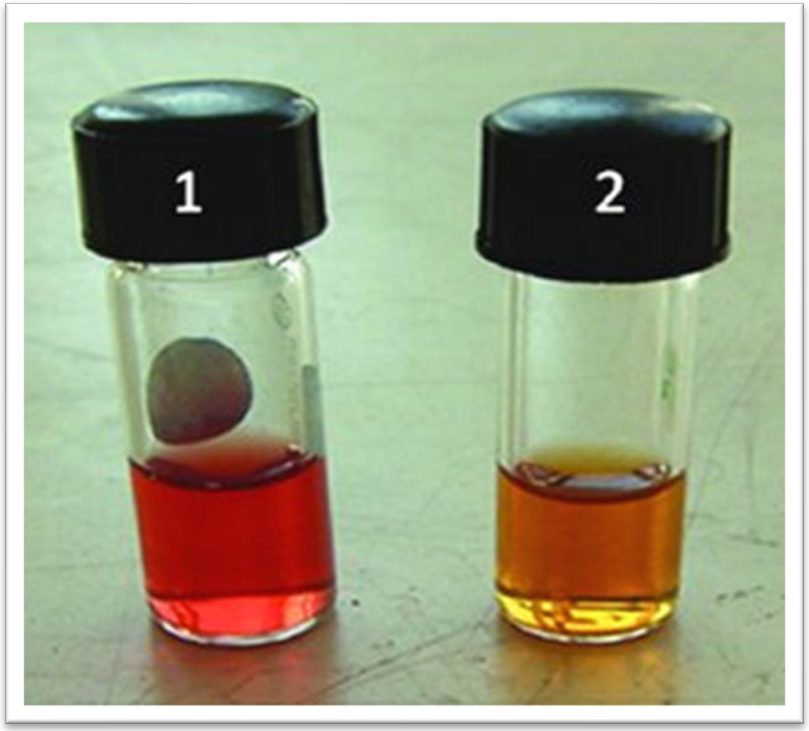


Mycoplasma Culture

- Fastidious and grows slowly in culture media
- Strictly aerobe
- Optimum growth at 35-37°C
- Grow on enrichment media with 20% human or horse serum
- Requires cholesterol or sterol and nucleic acid precursors for growth
- Sensitivity/Specificity ~ 40-80%, 95%

Not considered a primary diagnostic tool

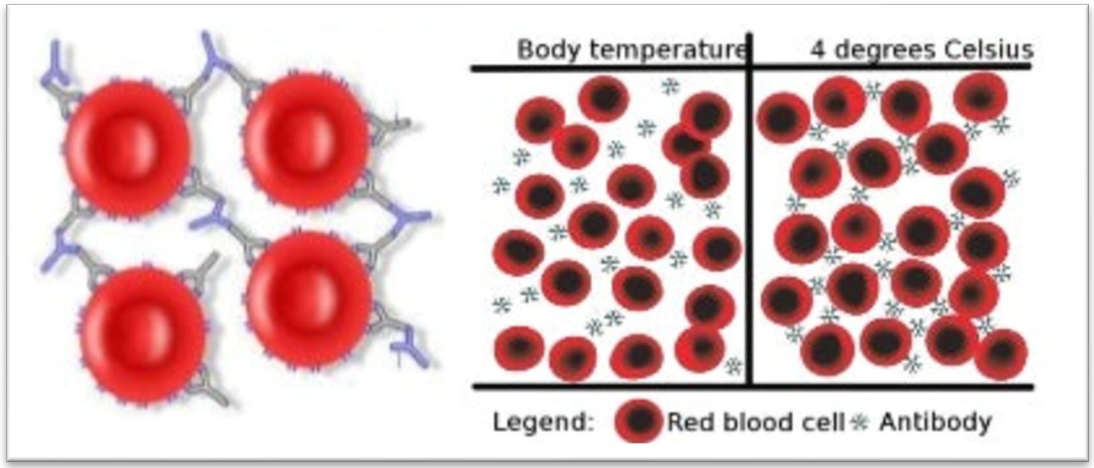




Serologic Diagnosis of *M pneumoniae*

Cold agglutinins

- Seen in patents infected with M pneumoniae
- Patient's sera agglutinates human O group erythrocytes at 4°C, the agglutination is reversible at 37°C



Low sensitivity: 20-50%

Moderate specificity: 80%

Not considered a primary diagnostic tool

Serologic Diagnosis of M pneumoniae

Table 1. Results of PCR Technique and Paired Serology for *Mycoplasma Pneumoniae* in Children With CAP

	Paired serology (IgM or IgG)		
Test	Positive*	Negative	Total
Positive PCR	14	40	54
Negative PCR	3	419	422

*Quadruplication of titers (IgM or IgG).

PCR showed 91% concordance with paired serology

Copete, A. et al. The Pediatric Infectious Disease Journal 39(7):p e104-e108, July 2020.

Serology vs PCR for M pneumoniae

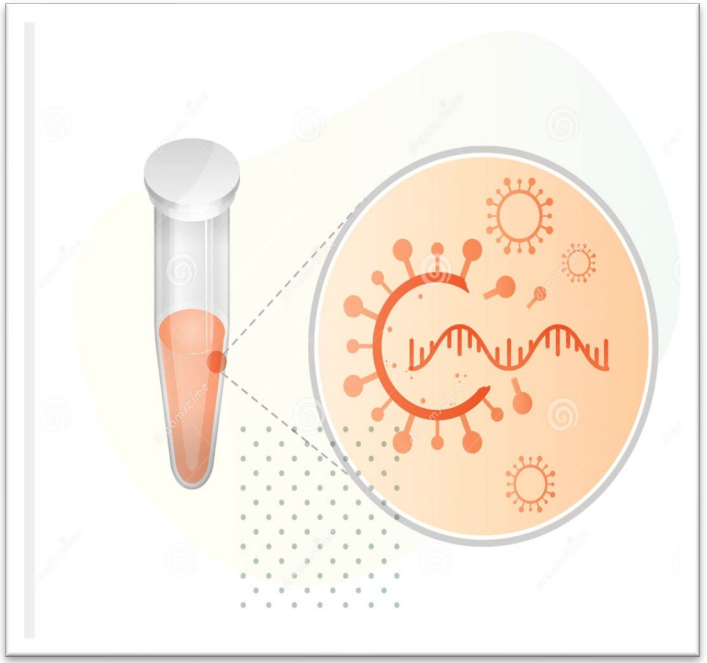
Test Type	Sensitivity	Specificity	Advantages	Disadvantages
PCR	85-95%	95-98%	• Early detection, • High sens/spec	• Limited availability • Increased cost
Serology (IgM/IgG)	50-80% (IgM)	80-95%	• Easy to perform • Lower Cost	• Lower sensitivity • Cross-reactivity • Delayed results

Should serology be discontinued as a primary diagnostic for *M. pneumoniae*?

- Yes
- No
- Maybe

Nucleic Acid Amplification Tests

- FDA-Cleared Assays
  - Alethia Mycoplasma Direct (LAMP) – Throat swab
  - FilmArray Respiratory Panel – NP swab
  - QIAstat-Dx Respiratory Panel – NP swab
  - ePlex Respiratory Panel – NP swab
- Lab-developed PCR
  - NCH – targets P1 adhesin molecule gene
    - Throat, NP, lower resp, CSF

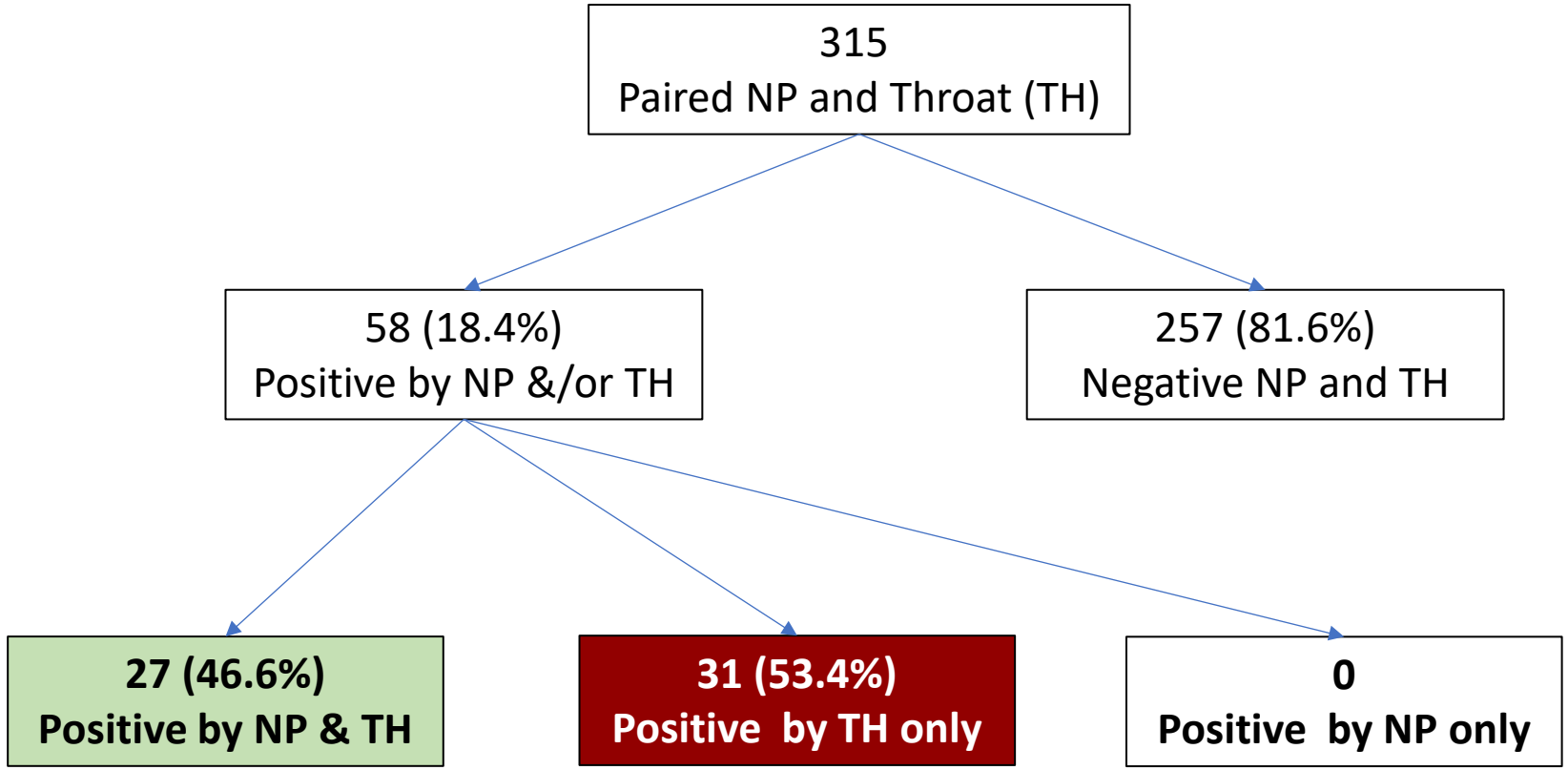


Is a multiplex molecular panel appropriate for diagnosis of M pneumoniae?

- Yes
- No
- Sometimes

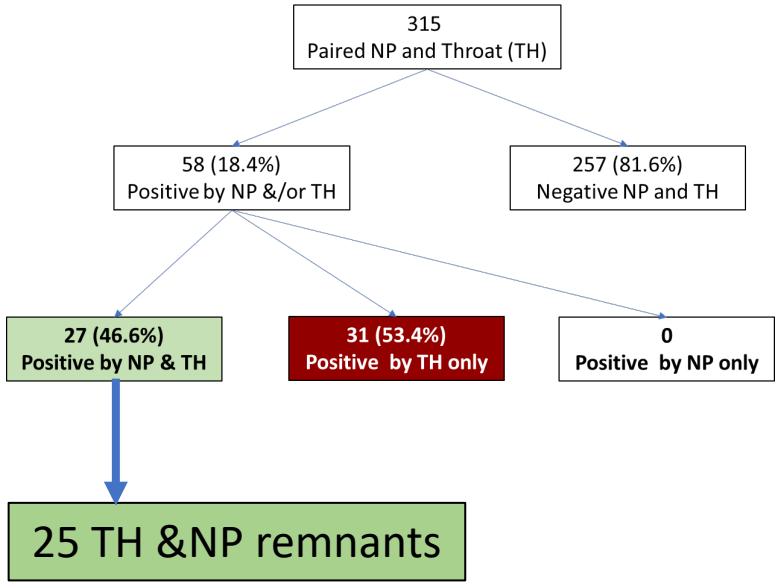
Detection of *M pneumoniae* in Paired Samples

- 315 patients with both a NP and Throat sample collected within 48 hrs
- **NP** tested on a **multiplex panel**
- **Throat** tested on LDT **PCR** for M. pneumoniae



Testing NP with Multiplex could miss >50% of Positives

Detection of *M pneumoniae* in Paired Samples



LDT MPN PCR

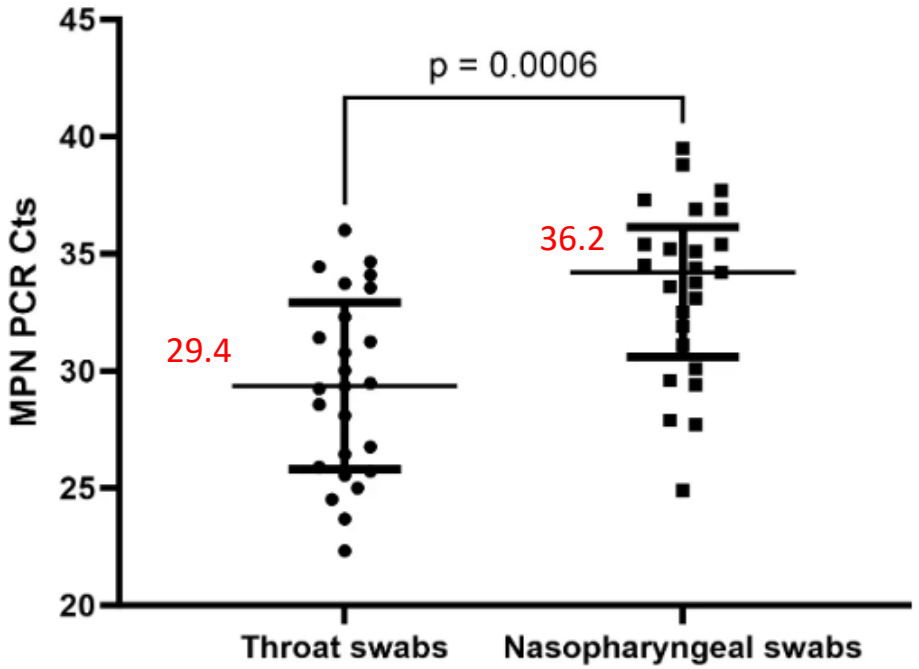
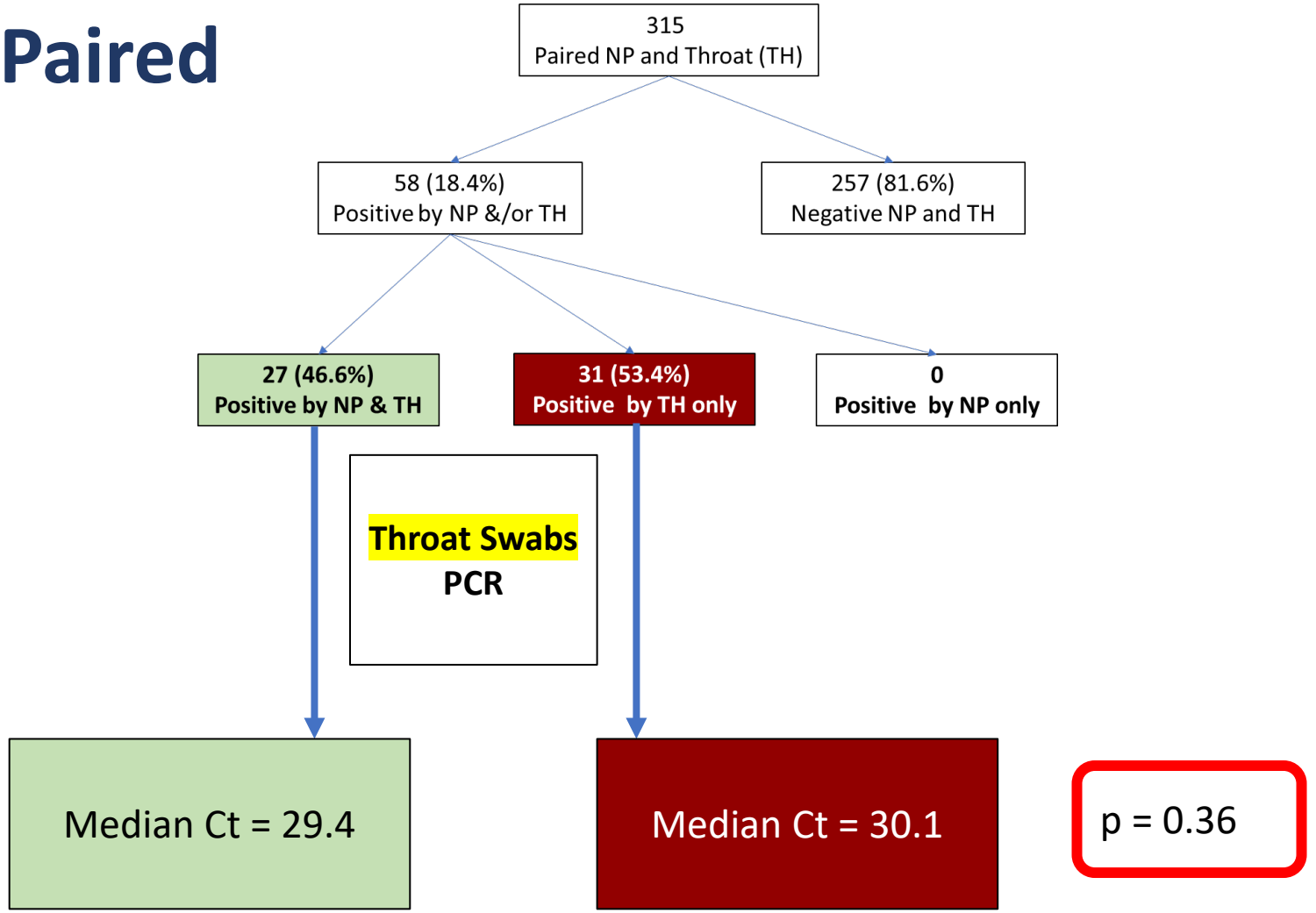


FIG 1 Comparison of MPN-PCR Ct values between throat swabs and nasopharyngeal swabs from the same patient. The bars represent the median with interquartile ranges.

The bacterial load is higher in the throat

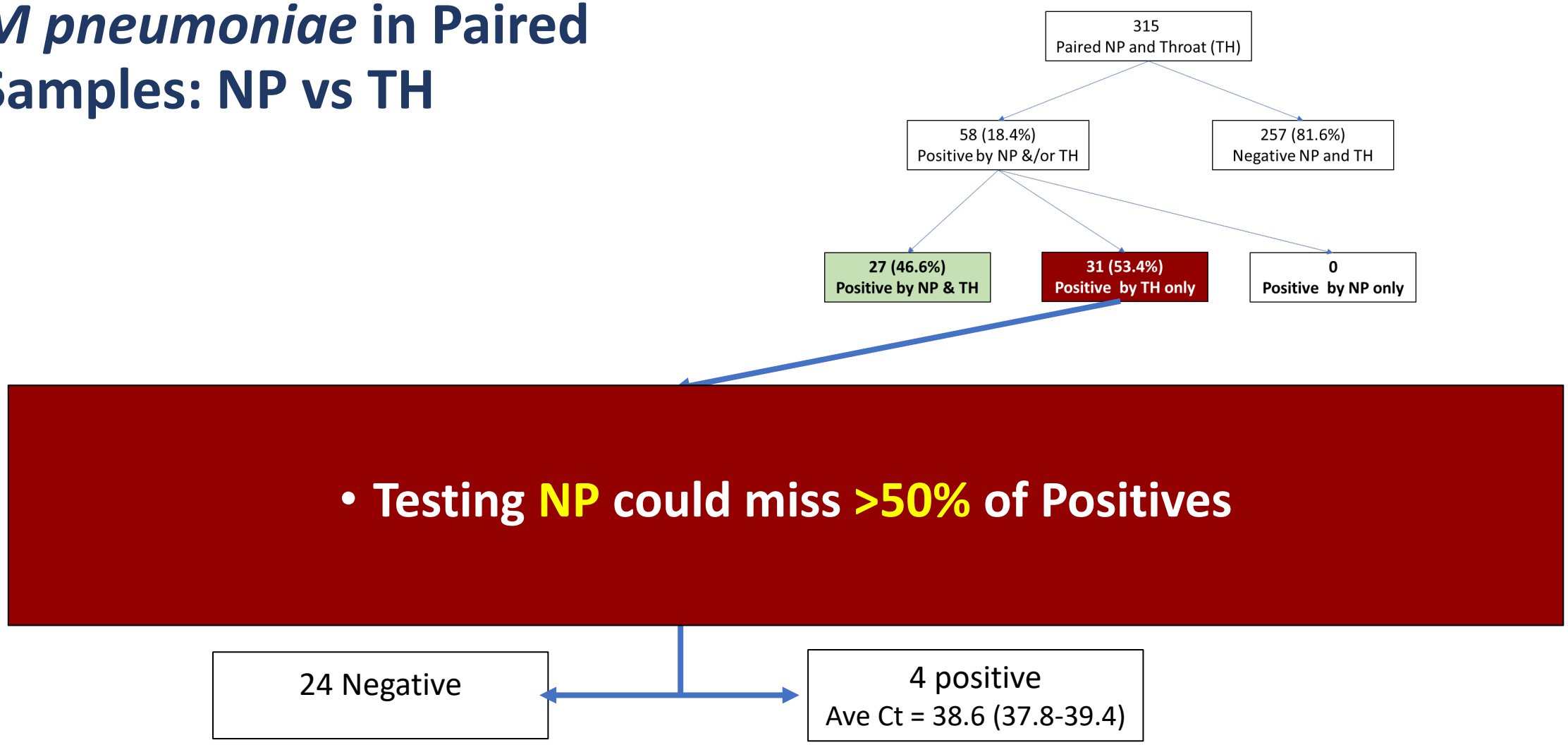
M pneumoniae in Paired Samples: NP vs TH

- TH samples from both groups
- **Tested on the LDT MPN PCR**



The bacterial load in the throat is similar in both groups

M pneumoniae in Paired Samples: NP vs TH



Testing Cost and Reimbursement

- Proprietary Laboratory Analyses (PLA) Codes
- CPT codes for specific tests created by the AMA
- 0202U INFCT DS BCT/VIR Respir DNA/RNA 22 TRGT

Certain codes are prohibited to be billed on the same date of service:

- >35 Mutually exclusive procedures
  - Testing for specific pathogens on the panel
  - General codes for procedures
    - *87798 DETECT AGENT NOS DNA AMPLIFIED*

Not reimbursed for additional Mycoplasma PCR testing or other PCR!

There is More to Test Choice than Performance!?

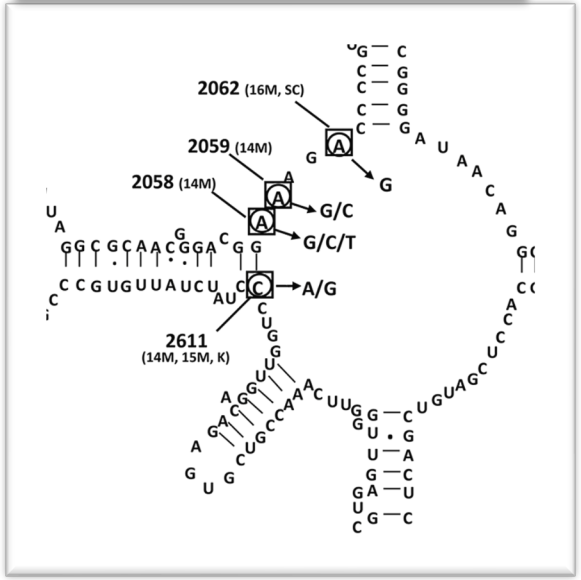
Is a multiplex molecular test, using NP source, sufficient for diagnosis of *M pneumoniae*?

- Yes
- No
- Sometimes

Macrolide Resistance in *M pneumoniae* (MRMp)

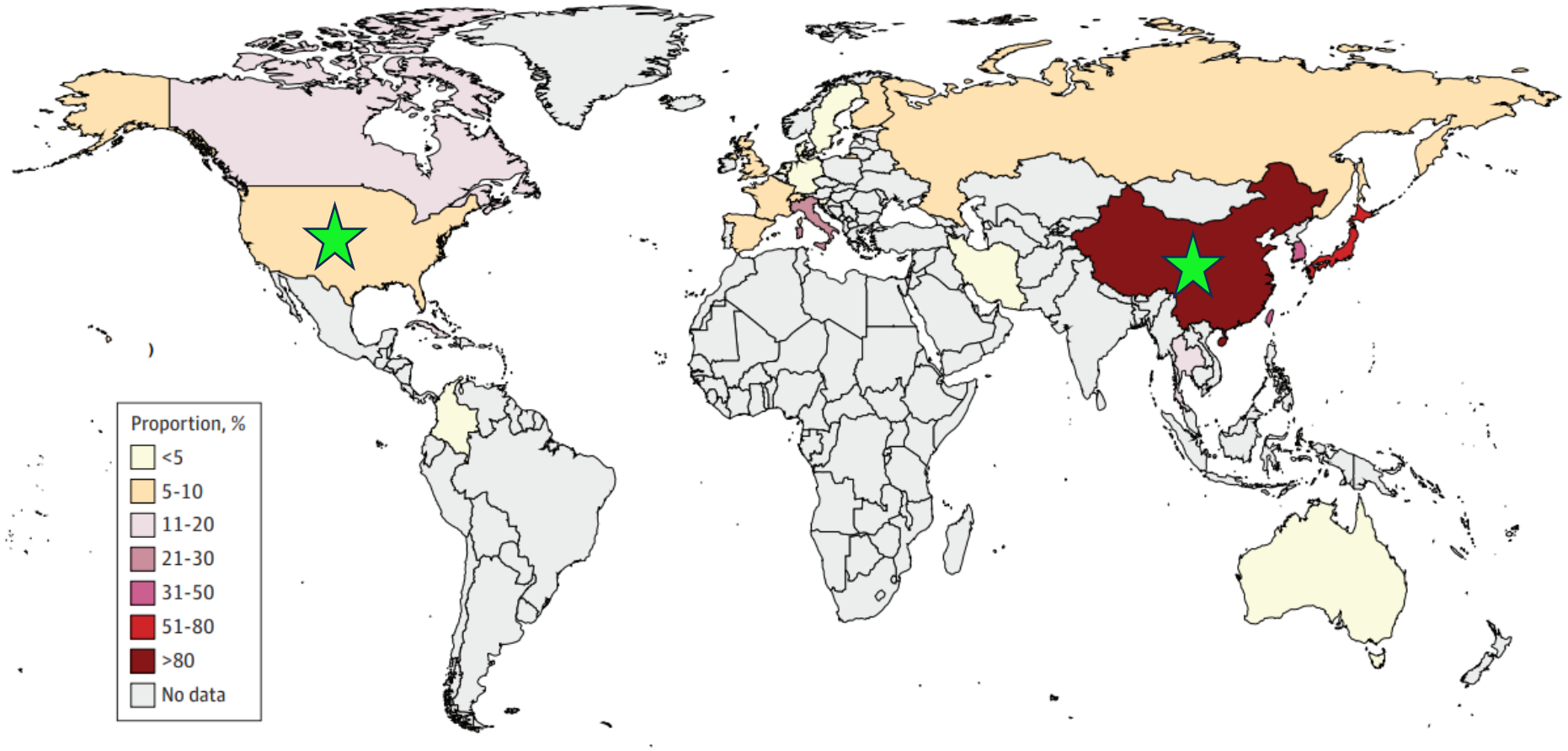
- Resistance Mechanisms
  - Primarily due to mutations in the **23S rRNA gene**, which interferes with the drug's ability to bind and inhibit protein synthesis
  - **Point mutations** in this gene, especially at positions 2063 and 2064, are responsible for most cases of resistance

- Signs of Treatment Failure
  - **Persistent or worsening symptoms** despite the initial macrolide treatment.
  - **Fever** that does not resolve after the expected time
  - **Radiological progression** (worsening pneumonia on imaging)
  - Possible **delayed or incomplete recovery** due to ineffective antibiotic action



Resistance to Macrolides in MPN

Figure 2. Map of the Proportion of Macrolide-Resistant *Mycoplasma pneumoniae* Infections by Country



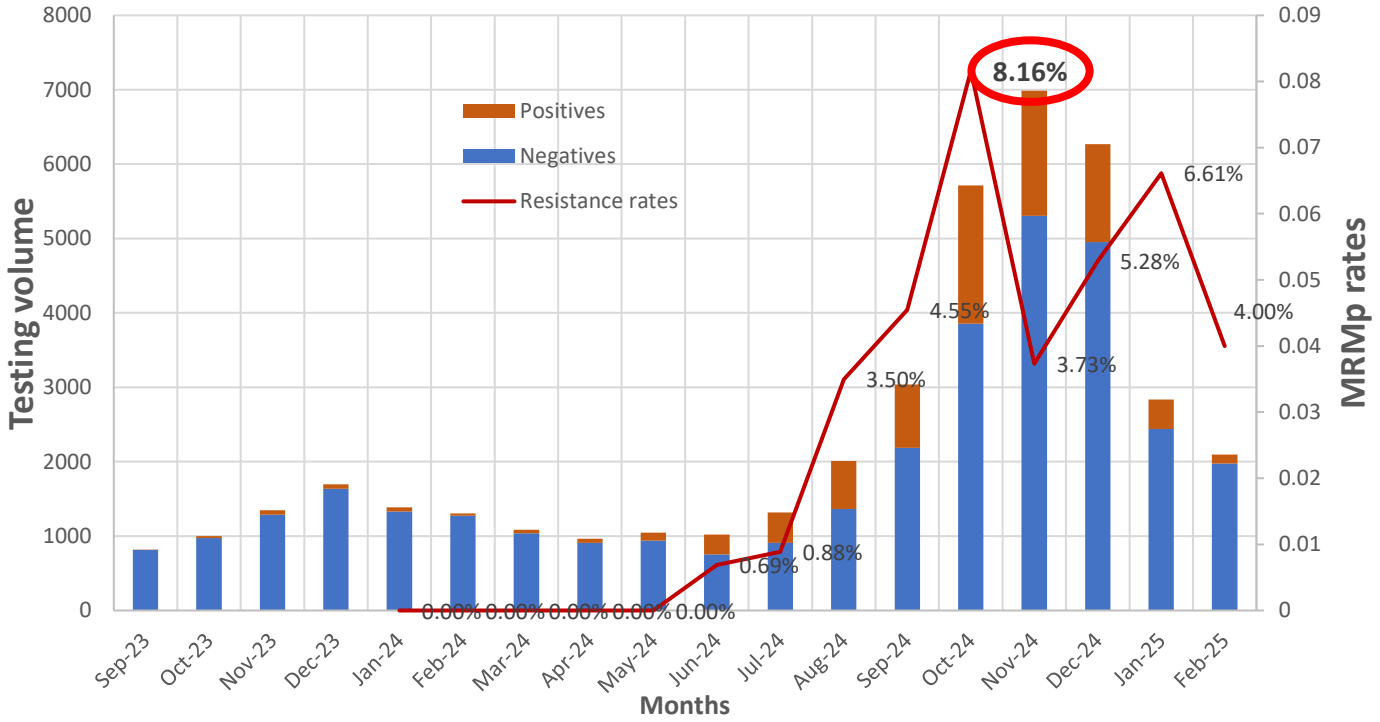
Local Resistance to Macrolides: Central Ohio

Pre-COVID[1]

- **2.8%** resistance
- More likely exposure to Macrolides
- 5-fold greater odds of PICU admission

Post-COVID outbreak[2]

- Increasing resistance, up to **8.1%**
- Declined as the outbreak subsided
- Clinical findings under study



[1] Emerg Infect Dis. 2021 Jun;27(6):1588-1597.

[2] Emerg Infect Dis. In Press

Should Macrolide Resistance testing be standard?:

- Yes
- No
- Maybe

Summary:

- Post-COVID, respiratory pathogens have reemerged, some causing “outbreaks”
- *M. pneumoniae* reemerged and led to outbreak in 2023-24
  - Molecular detection is the best diagnostic
  - Detection is dependent on sample type with throat being most sensitive
  - Multiplex testing using NP swabs can miss a significant number of M pneumoniae infections
  - Resistance to Macrolides is still relatively low

Acknowledgments:

Dr Huanyu Wang

Dr Sophonie Oyeniran

Thank you!