Emerging Viral Diseases: A 2025 Update for Laboratorians

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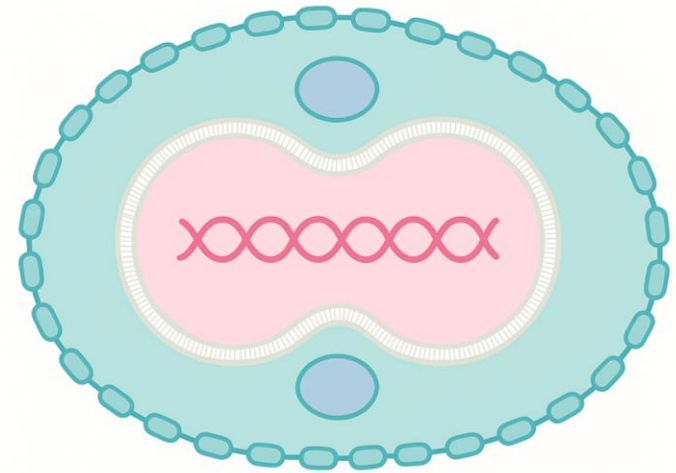
Learning objectives

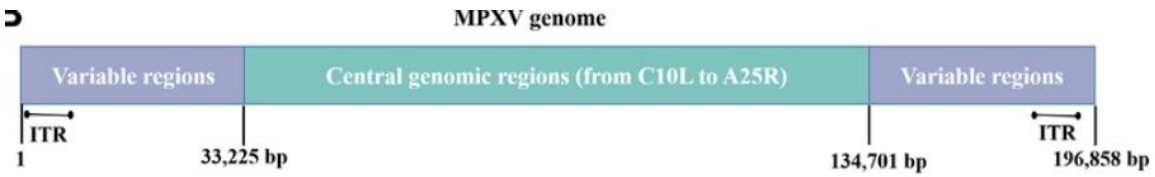
- List the environmental and societal causes that have led to the emergence and re-emergence of infectious agents
- Describe challenges encountered by laboratories who develop assays or perform testing for emerging and re-emerging pathogens
- Explore how clinical, commercial, and public health laboratories can work in unison to improve patient access to infectious disease testing

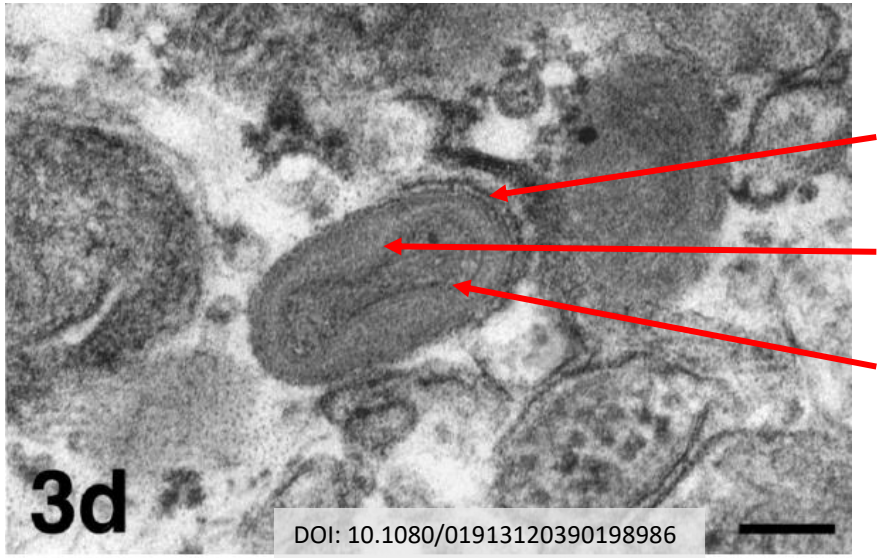
I. Mpox

Monkeypox virus (MPXV)

- Member of the Orthopoxvirus genus
  - Includes variola (smallpox), vaccinia, and cowpox viruses
  - 200kb linear ds DNA genome







Membrane

Lateral body

Core

Monkeypox virus (MPXV)

- Member of the Orthopoxvirus genus
- Historically divided into two clades

Clade I

- Formerly called “Congo Basin”
- Cases centralized in the DRC
- Higher mortality rate
- **Classified as a select agent**

Clade II

- Formerly called “West African”
- Cases centralized in Sierra Leone, Cote d'Ivoire, Nigeria
- Lower mortality rate
- Includes 2022 Clade IIb virus

Monkeypox virus (MPXV)

- Member of the Orthopoxvirus genus
- Historically divided into two clades
- Mpox vs Monkeypox
  - Disease name and virus name are overseen by two different groups

World Health Organization

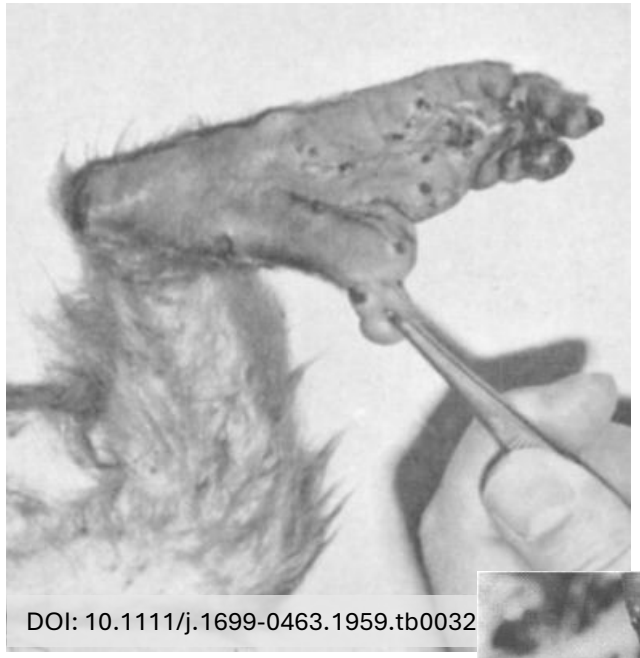
- Governs the **disease name**
- In 2022 monkeypox -> Mpox

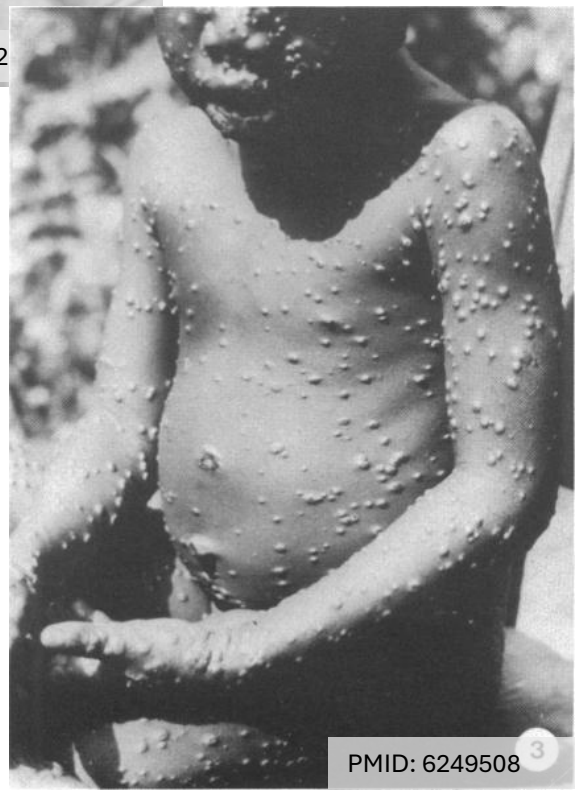
ICTV

- **Taxonomic name** should only be used when discussing the phylogenetic classification of viruses
  - In 2023 *Monkeypox virus* -> *Orthopoxvirus monkeypox*
- Whereas **virus name** is used to discuss clinical disease
  - Name for the virus remains monkeypox virus (MPXV)

Early Mpox outbreaks

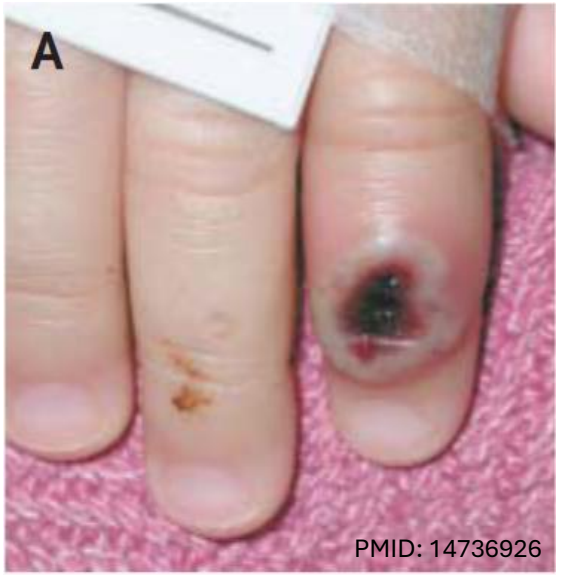
- 1950-2000 – Emergence
  - 1958: Mpox first described in imported primates with exposure to African rodents
  - 1970: First human case described
  - Found during smallpox surveillance efforts





Early Mpox outbreaks

- 1950-2000 – Emergence
  - 1958: Mpox first described in imported primates with exposure to African rodents
  - 1970: First human case described
  - Found during smallpox surveillance efforts
- 2003 – First U.S. cases of mpox
  - 43 cases from seven states linked to exposure to imported rodents
  - Virus spread between animals and from animals to humans
  - No human-to-human transmission was observed
- 2017 – Increasing cases and Kenyan outbreak

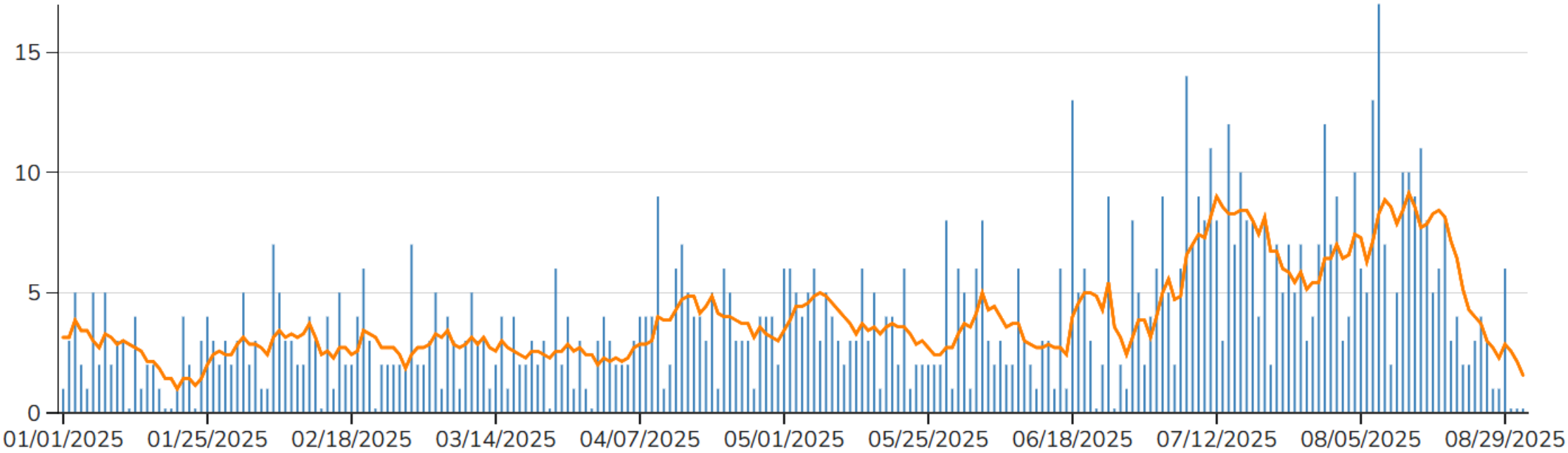


2022 Global Outbreak

- In May 2022, a cluster of mpox cases were identified in several European countries
- Genetic sequencing characterized the virus as clade IIb MPVX
- **These cases were very different from historical cases**
  - Cases were overwhelmingly observed in MSM populations
  - Spread was through close sexual contact
  - Mortality was exceedingly low
  - Genetic changes were ID'ed to suggested the virus had circulated in humans since 2016
- August 4th, 2022: U.S. declares a PHE. Over the course of the next year over 30,000 cases were identified in the US.

Clade IIb MPXV in the U.S. today

- Cases continue to be observed at low levels
  - 2024: 2,796 (7.6 cases/day)
  - 2025: 960 (4 cases/day)



Clade IIb MPXV in the U.S. today

- Testing infrastructure includes LDTs in clinical labs, CDC/PHL assays, and a commercial device
- MPXV should remain a part of the differential for genital ulcers (others include HSV, syphilis, VZV)
- In a survey of 47 samples with suspected mpox, there were 8 co-detections and 3 detections of other ulcer-causing viruses

Table 2. Description of Non-Mpox Pathogens Detected by the QIAstat-Dx Viral Vesicular Panel

Sample ID	Target Detected	Reference Method	Codetection With Mpox
8	HSV-2	Culture (–), PCR (+)	Yes
11	HSV-2	Culture (+), PCR (+)	Yes
17	HSV-2	Culture (+), PCR (+)	Yes[a]
18	VZV	PCR (+)	No
24	HSV-2	PCR (+)	Yes
29	HSV-2	PCR (+)	Yes
40[b]	Enterovirus	PCR (+)	Yes
41[b]	Enterovirus	PCR (+)	Yes
43	HSV-2	ND	No
44	HSV-2	ND	No
48	HSV-2	Culture (–)	Yes

PMC10051014

In nearly a quarter of samples, a pathogen other than mpox was detected

MPXV **Clade Ib** – a new global threat

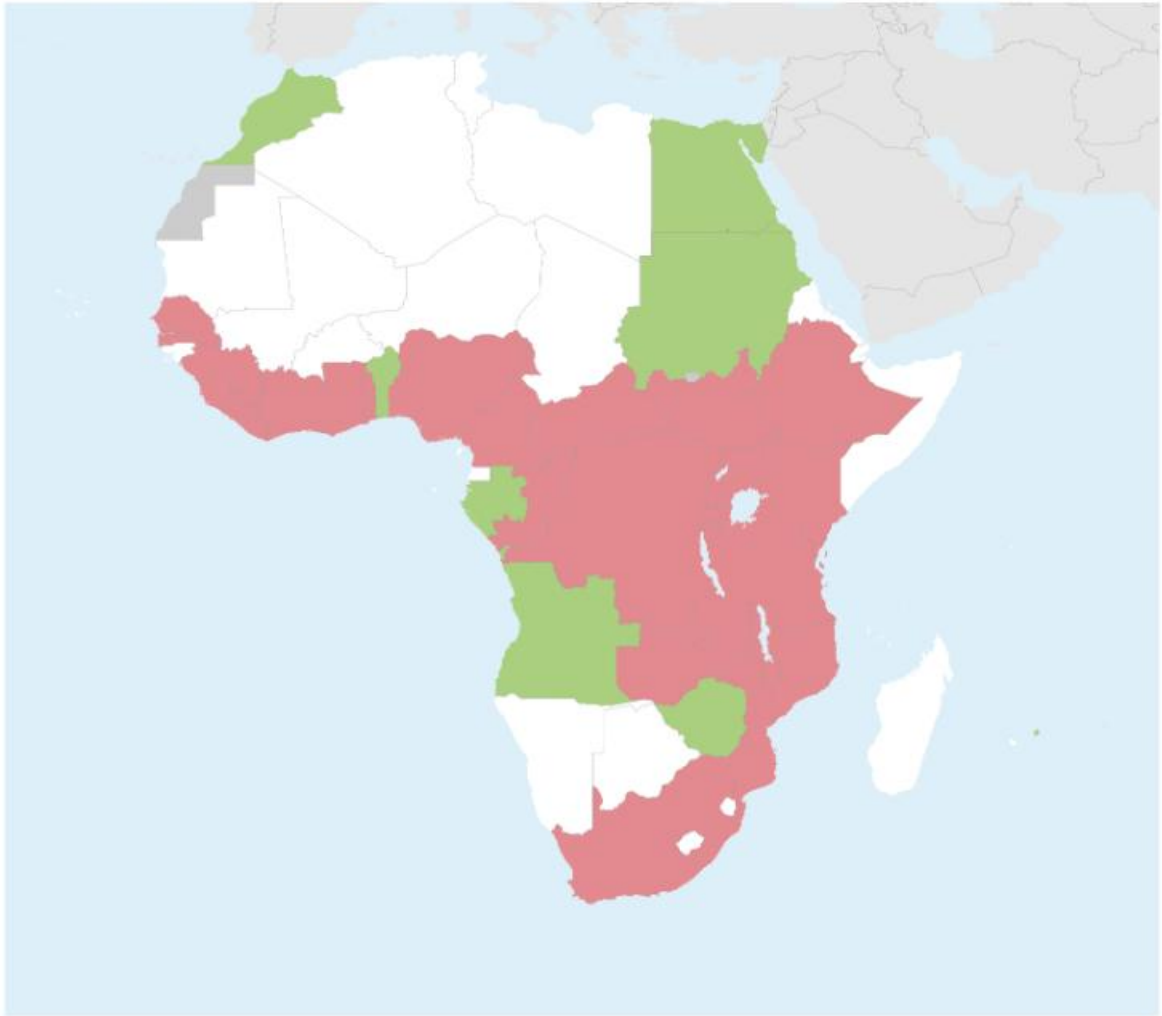
- First isolates ID'ed from September 2023 in the DRC
  - Rapid spread to neighboring countries then globally
  - Secondary transmission events in Germany, UK, Belgium
  - In the US, only 5 cases have been ID'ed all travel associated

- Genetic analysis classified the virus as Clade Ib MPXV
  - Mutations identified suggesting adaption to humans
  - Large deletion in portion of genome associated with virulence
  - Deletion also impacted ability to subtype clade I MPXV by the CDC

Mpox: countries affected in Africa

from 1 Jan 2022, as of 24 Aug 2025

World Health Organization



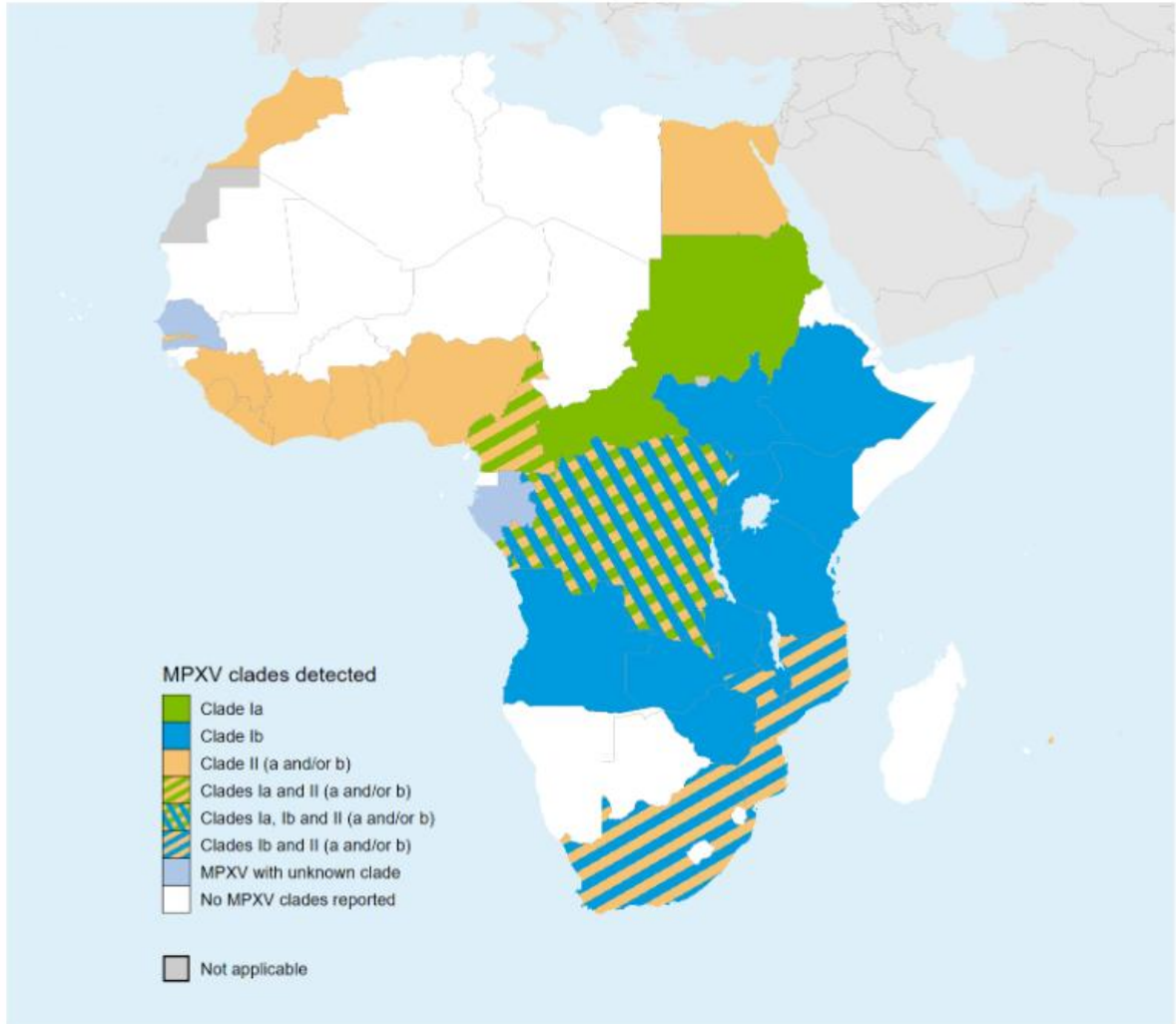
Outbreak status

- Active outbreak (cases reported in the last six weeks)
- Control phase (cases reported in the past 90 days)
- Cases reported since 2022, but not in last 90 days
- No mpox reported

MPXV clades detected globally

Includes imported cases; known distribution from 1 January 2022 to 24 Aug 2025

World Health Organization



Clade Ib vs Clade IIb – What's the difference?

Feature	**Clade Ib**	**Clade IIb**
Clinical Presentation	Rash, fever, lymphadenopathy; higher rates of complications (encephalitis, pneumonitis); more severe in children	Similar symptoms but generally milder; lower complication rates
Transmission	Human-to-human via sexual contact, household spread, and possibly environmental exposure; zoonotic spillover still occurs	Primarily sexual transmission among MSM networks; limited zoonotic involvement
Affected Populations	Broader demographic: men and women aged 20–30, children <15 years, sex workers, immunocompromised	Predominantly MSM aged 31–40; high HIV co-infection rates
Mortality (CFR)	~1.8% overall; higher in infants and young children (up to 6.3%)	~0.2% globally; higher in immunocompromised individuals

Overall, MPXV clade Ib causes more severe disease in a wider range of affected individuals

Targets for MPXV detection

Non-variola, orthopox

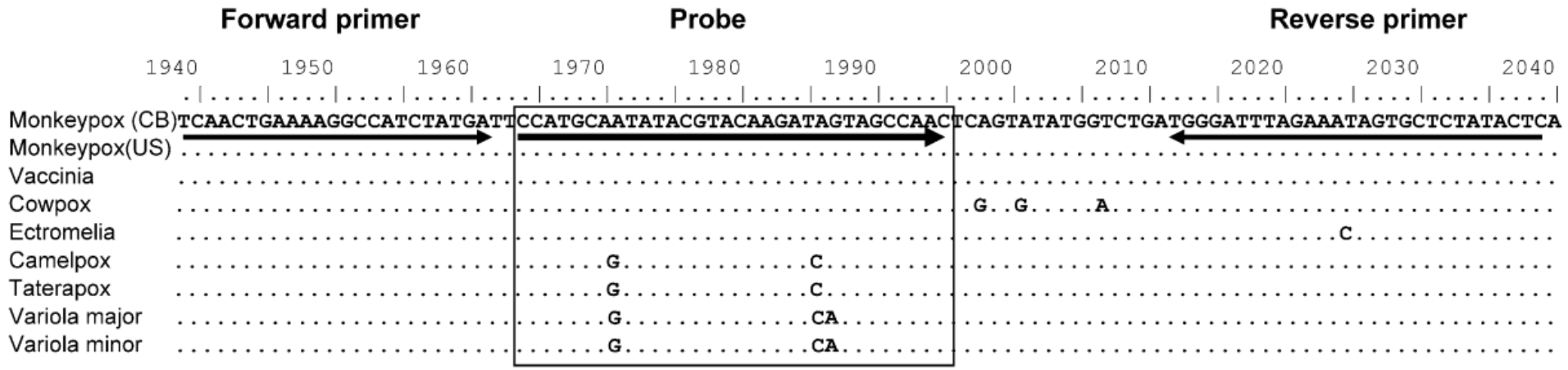
Advantages

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

Disadvantages

- Dx relies on epidemiological data

Monkeypox, Clade II specific



Targets for MPXV detection

Non-variola, orthopox

Advantages

- Can detect other orthopox infections (vaccinia, cowpox)
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Disadvantages

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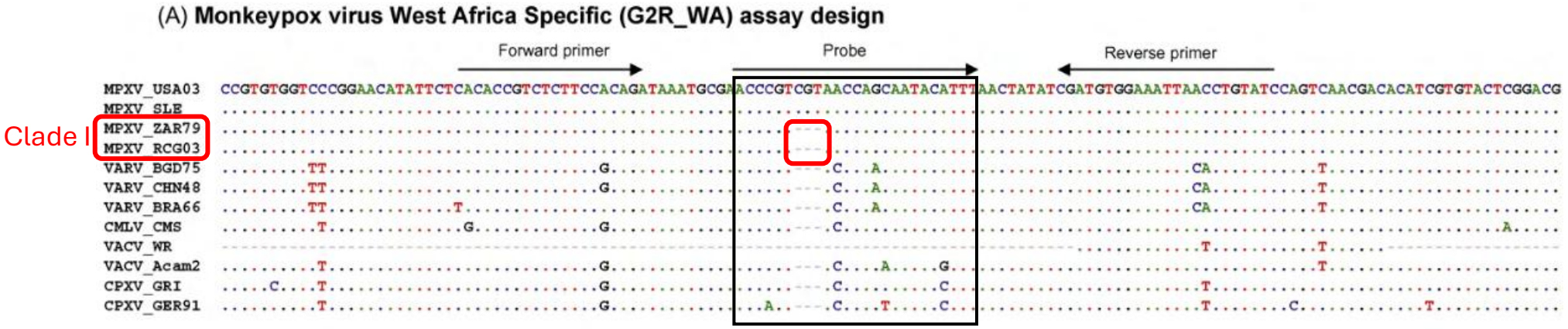
Monkeypox, Clade II specific

Advantages:

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

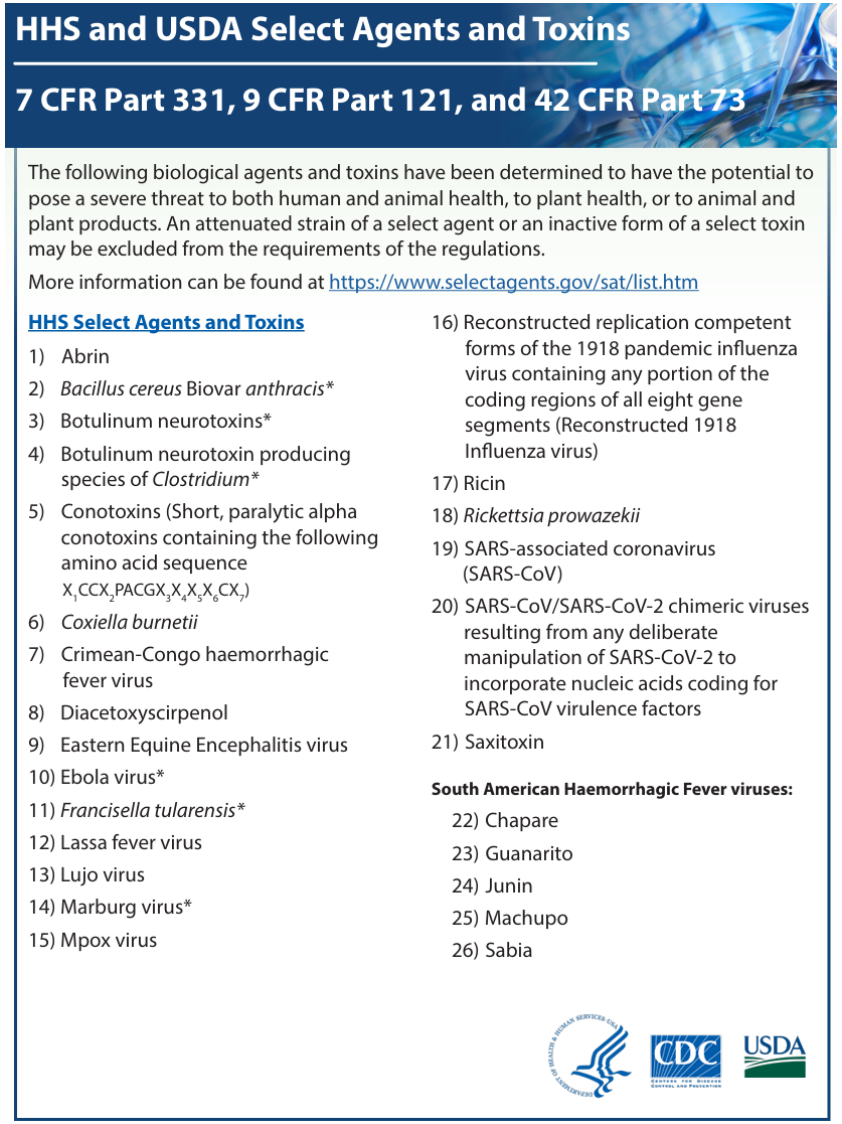
Disadvantages

- Challenging to find ideal target, Clade I and Clade II share >99% sequence identity
- 3 cases failed detection in CA



Why don’t we have assays for Clade 1a?

- **Clade I MPXV is considered a HHS Select Agent**
  - You need to have a special license and safety protocols
  - Needed to maintain positive specimens on site
  - Clinical labs must notify authorities and destroy specimen
- Clade II is not considered a select agent so it’s easier for clinical laboratories to develop assays



HHS and USDA Select Agents and Toxins

7 CFR Part 331, 9 CFR Part 121, and 42 CFR Part 73

The following biological agents and toxins have been determined to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products. An attenuated strain of a select agent or an inactive form of a select toxin may be excluded from the requirements of the regulations.

More information can be found at https://www.selectagents.gov/sat/list.htm

HHS Select Agents and Toxins

1) Abrin
2) *Bacillus cereus* Biovar *anthracis**
3) Botulinum neurotoxins*
4) Botulinum neurotoxin producing species of *Clostridium**
5) Conotoxins (Short, paralytic alpha conotoxins containing the following amino acid sequence $X_1CCX_2PACGX_3X_4X_5X_6CX_7$)
6) *Coxiella burnetii*
7) Crimean-Congo haemorrhagic fever virus
8) Diacetoxyscirpenol
9) Eastern Equine Encephalitis virus
10) Ebola virus*
11) *Francisella tularensis**
12) Lassa fever virus
13) Lujo virus
14) Marburg virus*
15) Mpox virus
16) Reconstructed replication competent forms of the 1918 pandemic influenza virus containing any portion of the coding regions of all eight gene segments (Reconstructed 1918 Influenza virus)
17) Ricin
18) *Rickettsia prowazekii*
19) SARS-associated coronavirus (SARS-CoV)
20) SARS-CoV/SARS-CoV-2 chimeric viruses resulting from any deliberate manipulation of SARS-CoV-2 to incorporate nucleic acids coding for SARS-CoV virulence factors
21) Saxitoxin

South American Haemorrhagic Fever viruses:

22) Chapare
23) Guanarito
24) Junin
25) Machupo
26) Sabia

CDC USDA

Mpox testing results and interpretation

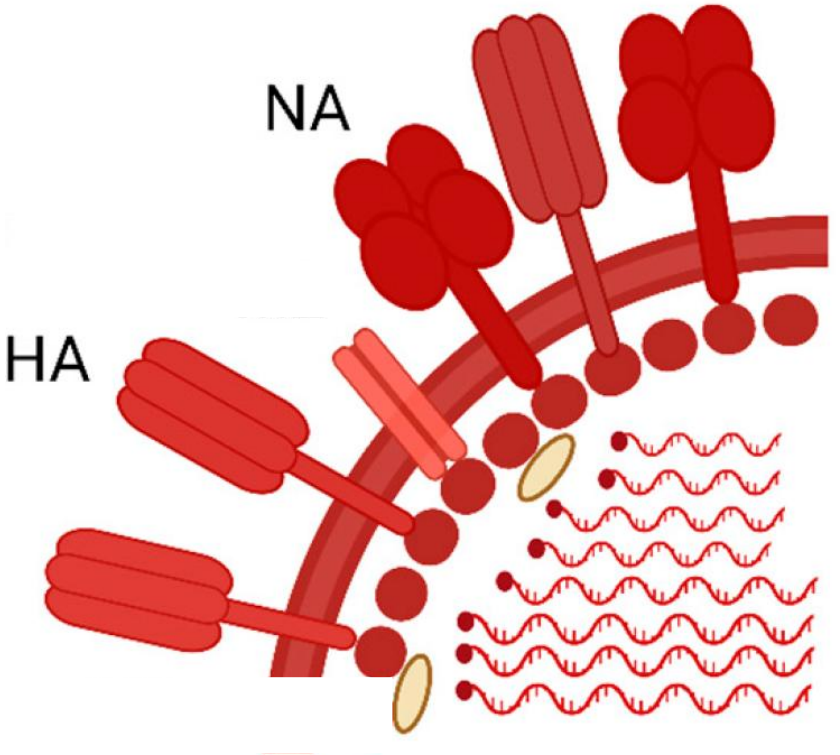
Assay target(s)	Clade IIb	Clade Ib	Comments
NVO			
Clade II			
NVO + Clade II			

Take home points

- Mpox is here to stay
- For genital ulcer disease testing: Consider mpox!
- While Clade Ib is rare in the U.S., results from mpox testing may give you a clue – **KNOW YOUR ASSAY!**

II. Avian Influenza A(H5)

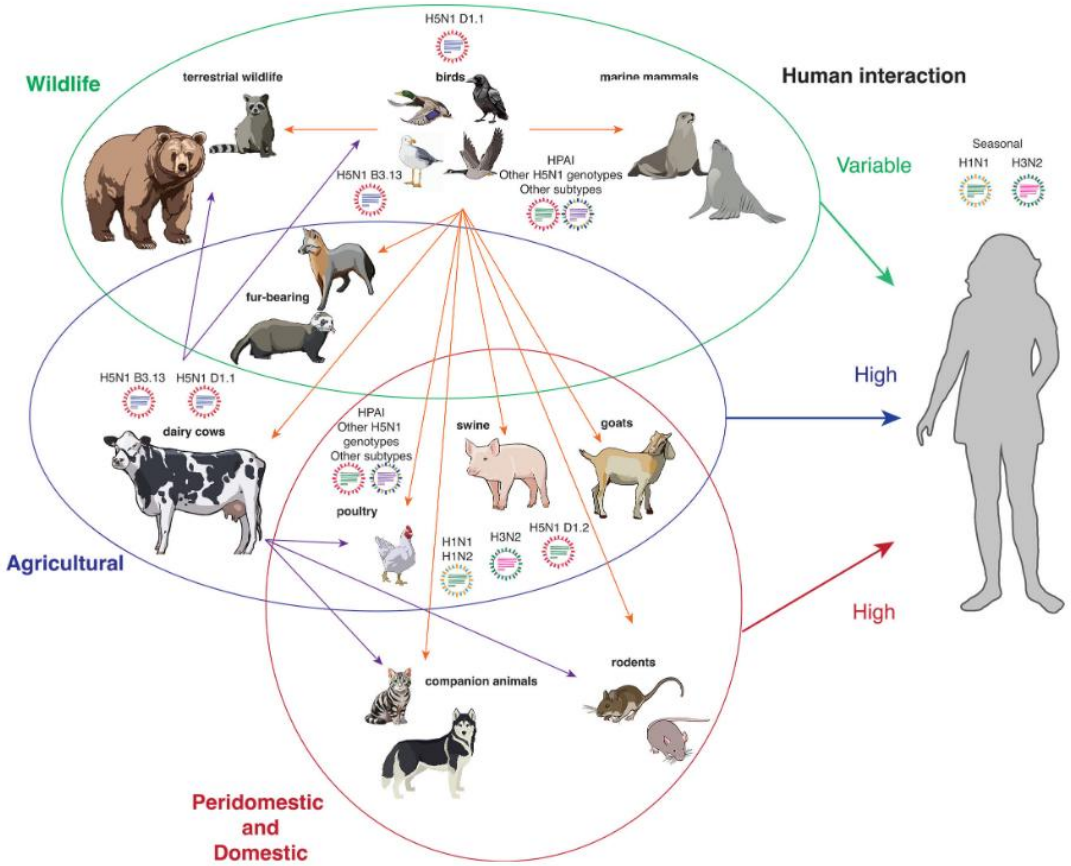
Influenza A virus



- Member of the Orthomyxoviridae family
- Genome is divided into eight segments
  - Key feature that affects pandemic potential
- HA and NA genes are most important
  - Used for naming virus (e.g. H5N1)
- Seasonal vs Novel
  - H1N1 and H3N2 are seasonal viruses
  - Novel viruses are non-seasonal strains that infect humans
    - Can have any of 18 HA subtypes
- **Highly pathogenic avian influenza**: any novel influenza A virus (H5, H7, etc.) that has high mortality in birds

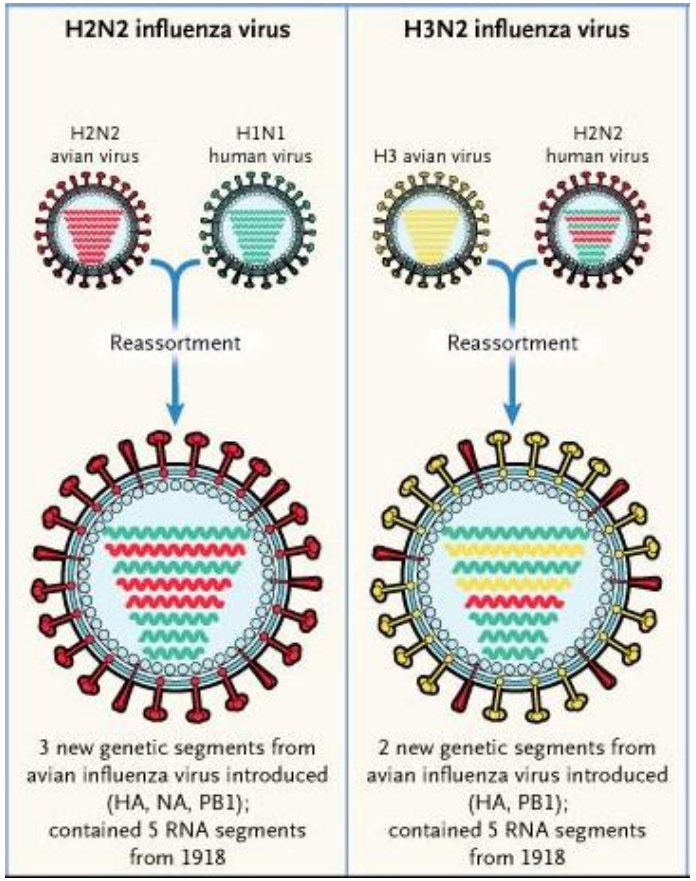
How do novel influenza outbreaks start

Zoonotic Transmission



- Close exposure to material with very high viral load
- Many undocumented events
- Dead-end infections lead to limited **epidemics**

Reassortment



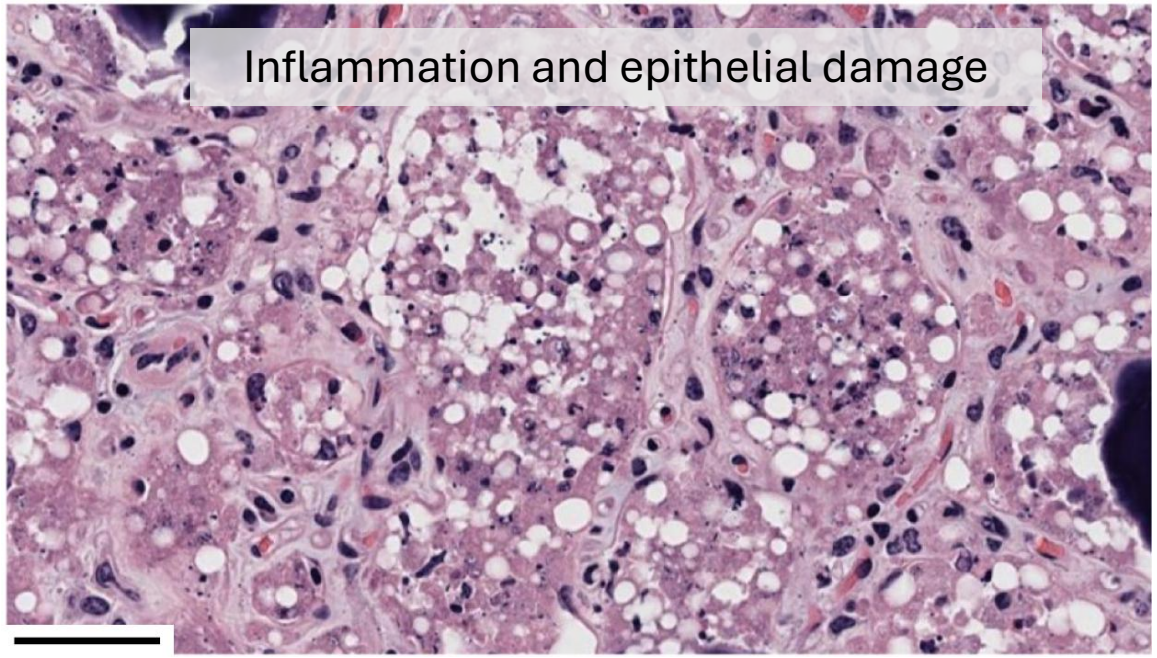
- Spreads between humans causing a **pandemic**
- High severity that decreases as the population gains immunity to the virus

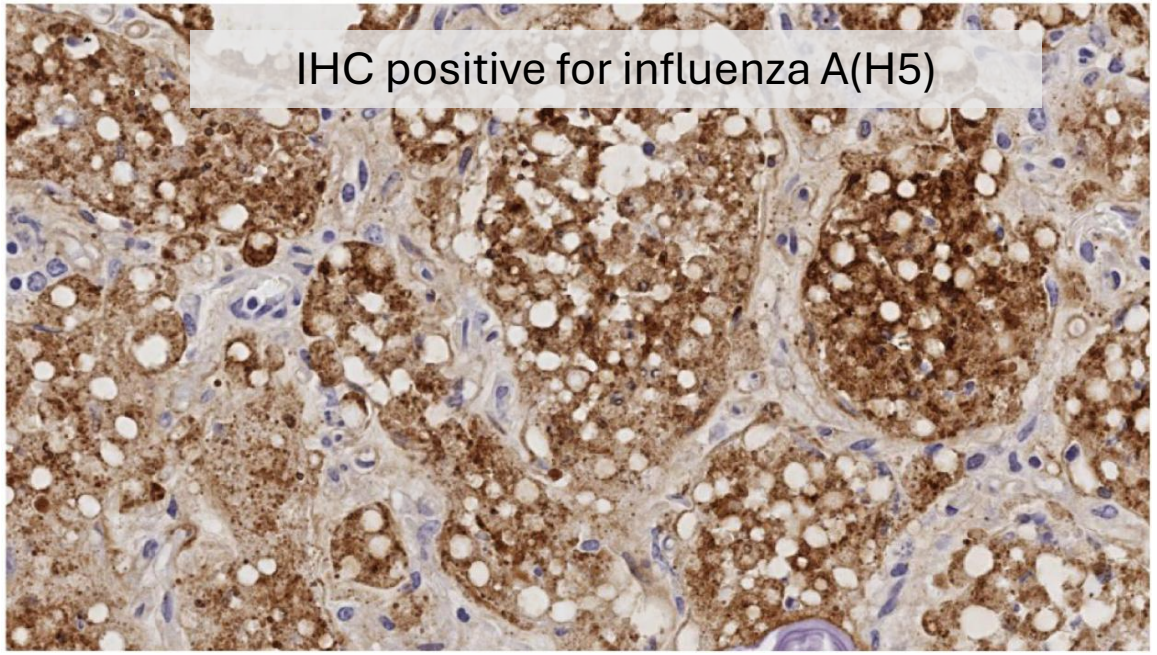
2024 H5N1 Outbreak - Origins

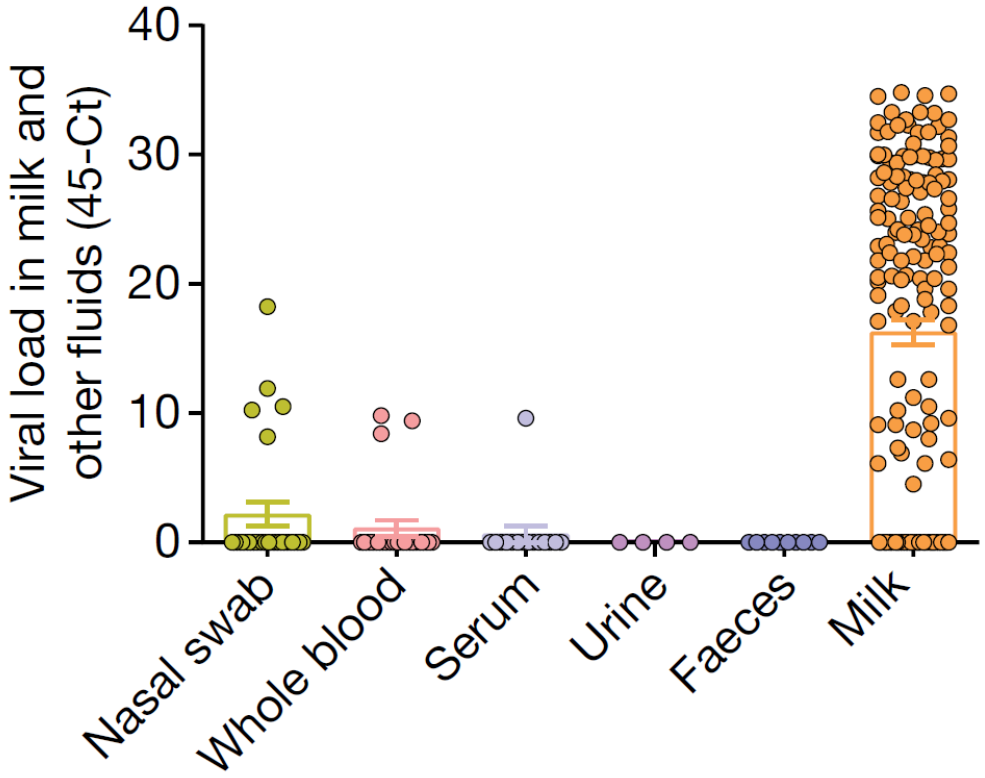
- In January, Texas farmers noticed an unknown illness spreading through their herds associated with poor milk production
- In March, the etiologic agent was identified as influenza A(H5)

<u>This was a very atypical outbreak of avian influenza:</u>

- Rare historical reports of influenza in cattle
- Documented cow-to-cow and cow-to-wildlife transmission events
- Animals had limited respiratory symptoms
- Primary route of spread appeared to be **via the mammary glands and milk**


Inflammation and epithelial damage


IHC positive for influenza A(H5)


Viral load in milk and other fluids (45-Ct)
40
30
20
10
0
Nasal swab
Whole blood
Serum
Urine
Faeces
Milk

Human cases of H5N1

- To date, 70 cases have been identified
  - Majority of cases linked to dairy farm
  - Other cases have been observed in association with poultry farms and backyard flocks
  - Clear exposure link in virtually all cases
- Conjunctivitis was major presenting symptom
  - Suggested likely infection route from splashes on handling infected milk
  - 93% of cases presented with conjunctivitis, fever was next most common
  - Respiratory symptoms present in a minority
- Serology studies suggest more infected
  - One small study found 14% (2/14) of individuals with mild symptoms positive for H5 antibodies
  - Challenging population for surveillance
- Low mortality rate vs historical outbreaks
  - 1 case fatality associated with co-morbidities

Variable	Overall (N=45)
Signs and symptoms	
Conjunctivitis — no. (%)	42 (93)
Measured fever or feeling feverish — no. (%)	22 (49)
Respiratory symptoms — no. (%)†	16 (36)
Cough	8 (18)
Sore throat	13 (29)
Shortness of breath	7 (16)
Myalgia — no. (%)	19 (42)
Headache — no. (%)	20 (44)
Fatigue — no. (%)	10 (22)
Nausea — no. (%)	6 (13)
Vomiting — no. (%)	2 (4)
Diarrhea — no. (%)	2 (4)

How concerned should we be?

- Sialic acid receptor preference
  - Alpha-2,3-linked in birds
  - Alpha-2,6-linked in humans
- Mammalian adaptation

Gene	Coding-region change	Functional type	Cattle with variant (no.)	Mean allele frequency	Consensus sequence	Low-frequency variants
HA	E91K	Mammal adapt.	1 (1)	0.05	0	1
HA	S137F	Mammal adapt.	1 (1)	0.376	0	1
HA	Q154R	Pathogenicity	5 (9)	0.013	0	6
HA	N209T	Mammal adapt.	1 (3)	0.012	0	1
HA	Q234K/R	Virulence	8 (32)	0.039	0	9
HA	G240R	Mammal adapt.	1 (1)	0.025	0	1
HA	S336N	Virulence	18 (245)	0.892	15	2
HA	P337L	Virulence	8 (21)	0.715	5	2
MP	R77K	Virulence	1 (7)	0.006	0	1
NA	T438A/I	Antiviral resist.	3 (9)	0.627	2	1
NA	R430K	Mammal adapt.	1 (82)	0.130	0	1
NS	D125N/G	Virulence	27 (20)	0.873	24	3
NS	E229K	Virulence	21 (85)	0.999	18	0
PB2	R389K/G	Mammal adapt.	2 (5)	0.012	0	2
PB2	E627K	Virulence/adapt.	1 (12)	0.329	0	1
PB2	D701N	Virulence	2 (3)	0.015	0	2

How concerned should we be?

- Vaccine exists
  - Some protection from seasonal N1 antibodies?
- NA inhibitors appear effective

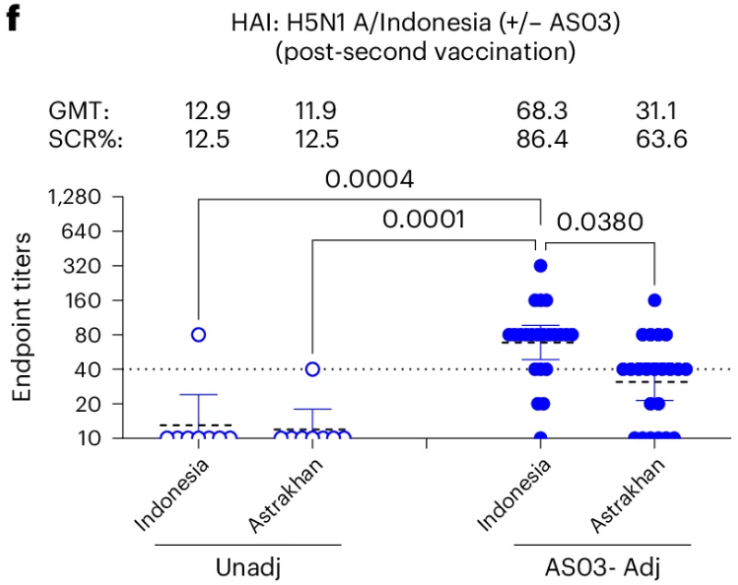


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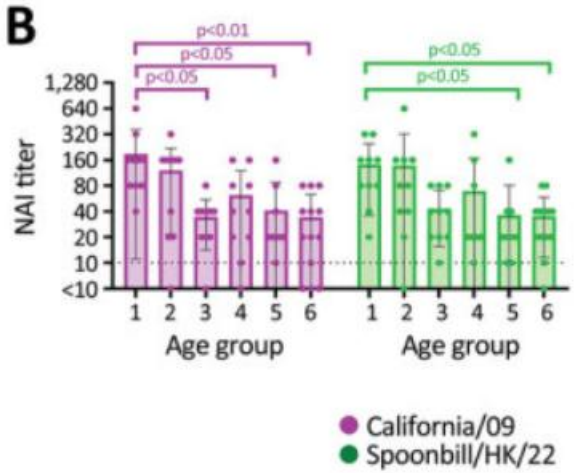
Table 3. NA inhibitor susceptibility of highly pathogenic avian influenza A(H5N1) viruses isolated from humans in fluorescent NA inhibition assay, 2023–2024 *

	Mean IC_{50}, nM (fold change)				
Influenza A(H5N1) virus	Oseltamivir	Zanamivir	Peramivir	Laninamivir	AV5080
Clade 2.3.4.4b, median IC_{50}, n = 7	3.61	0.20	0.08	0.16	0.04
A/Chile/25945/2023	2.98 ± 0.53	0.20 ± 0.02	0.09 ± 0.01	0.19 ± 0.03	0.04 ± 0.01
A/Texas/37/2024	3.16 ± 0.62	0.22 ± 0.03	0.10 ± 0.03	0.19 ± 0.03	0.04 ± 0.01
A/Michigan/90/2024	3.65 ± 0.71	0.19 ± 0.03	0.08 ± 0.02	0.16 ± 0.04	0.04 ± 0.01
A/Colorado/109/2024	3.99 ± 0.15	0.20 ± 0.02	0.08 ± 0.01	0.17 ± 0.03	0.03 ± 0.01
A/Colorado/137/2024	3.80 ± 0.15	0.19 ± 0.06	0.07 ± 0.00	0.16 ± 0.02	0.03 ± 0.00
A/Colorado/139/2024	3.51 ± 0.72	0.18 ± 0.03	0.07 ± 0.01	0.16 ± 0.03	0.03 ± 0.00
A/California/134/2024	3.61 ± 0.52	0.21 ± 0.02	0.07 ± 0.01	0.16 ± 0.02	0.04 ± 0.01
Control viruses†					
A/bald eagle/FL/2022 (H5N1)‡	3.07 ± 0.64	0.20 ± 0.04	0.09 ± 0.02	0.17 ± 0.03	0.04 ± 0.01
A/Illinois/45/2019 (H1N1)pdm09	0.34 ± 0.10	0.16 ± 0.04	0.05 ± 0.01	0.17 ± 0.01	0.07 ± 0.01
A/Alabama/03/2020 ((H1N1)pdm09, NA-H275Y	201.27 ± 44.79	0.25 ± 0.06	15.80 ± 2.74	0.38 ± 0.04	0.78 ± 0.14



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Who should be tested for H5N1?

2024–2025 Influenza Season: Surveillance For Novel Influenza A and Seasonal Influenza Viruses

Public Health
SEPTEMBER 20, 2024 • ESPAÑOL

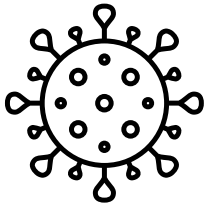
1 .Persons with known exposure to infected dairy cattle or poultry or exposed to potentially infectious animal products

2. Patients hospitalized with influenza when seasonal (H1/H3) fails to generate a result

- Minority of available assays will provide a seasonal H1 or H3 result
- Samples that fail seasonal subtyping may indicate a novel virus

What assays are available to identify H5?

1. Assays that detect H5

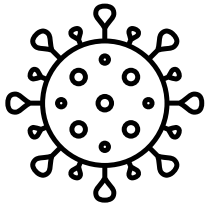


= Influenza A detected

H5N1

- Use conserved genes present in all HA subtypes
- Will not separate season from novel influenza viruses
- Many existing assays FDA-cleared or approved assays

2. Assays that subtype H5



= Influenza A(H5) detected

H5N1

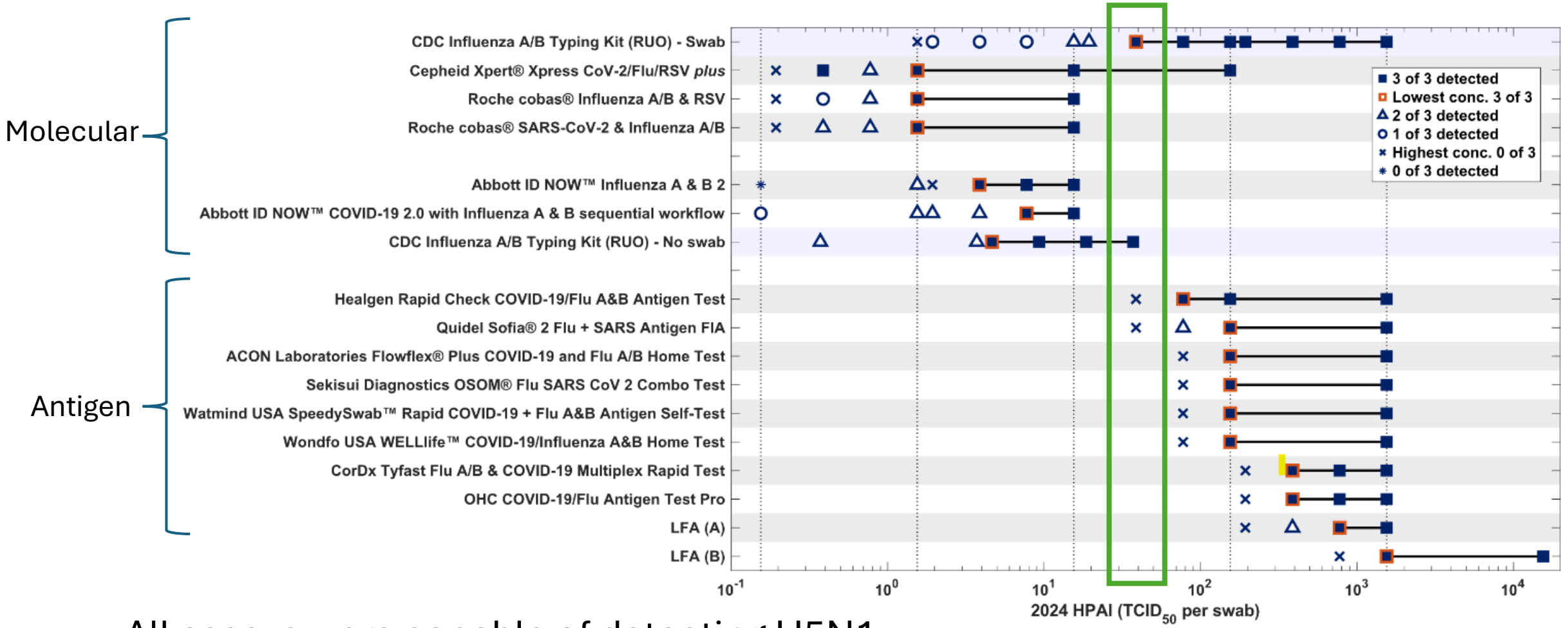
- Uses a region unique to H5
- Most useful as a reflex test following the identification of influenza A in a sample
- One FDA 510(k) cleared kit, the rest are LDTs

Commercial assays available for H5 detection?

Type of Test	Manufacturer	Test Name	Method	Seasonal influenza A Subtyping	H5 Detection	H5 Strains Evaluated According to Package Insert
NAAT (Syndromic Panel)	Biofire	Respiratory Panel 2.1	RT-PCR	Y	Y	**A/Chicken/Yunnan/1251/2003 (H5N1)** A/Northern pintail/Washington/40964/2014 (H5N2) A/Duck/Singapore/645/1997 (H5N3) A/Gyrfalcon/Washington/41088-6/2014 (H5N8)
	Biofire	Pneumonia panel	RT-PCR	N	Y	**A/Chicken/Yunnan/1251/2003 (H5N1)** A/Northern pintail/Washington/40964/2014 (H5N2) A/Duck/Singapore/645/1997 (H5N3) A/Gyrfalcon/Washington/41088-6/2014 (H5N8)
	Diasorin	VERIGENE Respiratory Pathogens Flex Test	RT-PCR	Y	Y	**A/Duck/Hunan/795/2002 (H5N1)** **A/Chicken/Korea/IS/2006 (H5N1)** **A/Scaly-breasted Munia/Hong Kong/2006 (H5N1)** A/Duck/Singapore/645/1997 (H5N3)
	Qiagen	QIAstat-Dx Respiratory SARS-CoV-2 Panel	RT-PCR	Y	Unknown	No Information Available
	Roche	cobas eplex Respiratory Pathogen Panel 2	RT-PCR	Y	Y	A/Duck/Singapore/645/1997 (H5N3)
NAAT (1-4 Targets)	Abbott	IDNow Influenza A&B 2	Isothermal	N	Y	A/Sichuan/26221/2014 (H5N6)
	Cepheid	Xpert Xpress CoV-2/Flu/RSV plus	RT-PCR	N	Y	**A/Duck/Hunan/795/2002 (H5N1)** **A/Vietnam/1194/2004 (H5N1)** **A/Anhui/01/2005 (H5N1)** **A/Japanese White Eye/Hong Kong/1038/2006** A/Mallard/WI/34/1975 (H5N2)
	Diasorin	Simplexa COVID-19 & Flu A/B Direct	RT-PCR	N	Y	**A/India/NIV/2006 (H5N1)-PR8-IBCDC-RG7** **A/Chicken/Vietnam/NCVD-016/2008 (H5N1)-PR8-** **A/Egypt/N03072/2010 (H5N1)-PR8-IDCDC-RG29** **A/Hubei/1/2010 (H5N1)-PR8-IDCDC-RG30** A/Pheasant/New Jersey/1355/1998 (H5N2)-PR8-
	Hologic	Panther Fusion FluA/B/RSV	RT-PCR	N	Y	**A/Hong Kong/486/1997 (H5N1)** **A/Vietnam/1203/2004 (H5N1)**
	Roche	cobas SARS-CoV-2 & Influenza A/B	RT-PCR	N	Y	**A/Cambodia/X0810301/2013 (H5N1)**
Antigen	Abbott	BinaxNOW Influenza A & B Card 2	Antigen	N	Unknown	No Information Available
	BD	BD Veritor Flu A+B assay	Antigen	N	Y	**A/Vietnam/1203/2004 (H5N1)** **A/Anhui/01/2005 (H5N1)** A/Pheasant/New Jersey/1355/1998 (H5N2) A/Northern Pintail/Washington/40964/2014 (H5N2) A/Gyrfalcon/Washington/41088-6/2014 (H5N8)
	Quidel	Sofia 2 Flu + SARS	Antigen	N	Y	Not Provided*

Toward diagnostic preparedness: detection of highly pathogenic avian influenza A(H5N1) in contrived nasal swab specimens using rapid antigen and point-of-care molecular tests

Leda Bassit,[1,2] Gregory L. Damhorst,[2,3] Heather B. Bowers,[1,2] Courtney Sabino,[1,2] Julie Sullivan,[2,4] Emily B. Kennedy,[5] Jacob Khouri,[6] Pamela Miller,[7] Eric Lai,[8] Raymond F. Schinazi,[1,4] Wilbur A. Lam,[2,4,9,10] Nira R. Pollock,[11] Anuradha Rao[2,4]



- All assays were capable of detecting H5N1
- Molecular assays had similar performance with antigen tests demonstrating lower sensitivity

What assays are available for H5 subtyping?

CDC and PHLs

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (CDC Flu rRT-PCR Dx Panel)

Influenza A/H5 Subtyping Kit (VER 4)

Instructions for Use Package Insert

	CDC Amplicon	Mismatches		
		Forward Primer(s)	Probe(s)	Reverse Primer(s)
A/Texas/37/2024	H5a	1	1	2
	H5b	0	1	0
2.3.4.4b strains^	H5a	0	1 (5.6%)	1 (40.3%)
	H5b	0	1 (5.8%)	0

^2.3.4.4b strains deposited in GISAID from 2022-2024. Only mismatches present at >5% prevalence

Figure 3. Influenza A/H5 Subtyping Kit: Example of Sample and Control Set-up

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	H5VC
B	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	H5VC
C	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	H5VC
D	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	H5VC
E	empty	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	empty
F	empty	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	empty
G	empty	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	empty
H	empty	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	empty

NOTE: HSC needs to be tested with each nucleic acid extraction run. If samples are from an extraction run already tested with HSC, repeat testing of HSC is not necessary.

Commercial

Avian Influenza Type A (H5) Primers and Probe Set

Targets Influenza A Clade 2.3.4.4b

World Health Organization

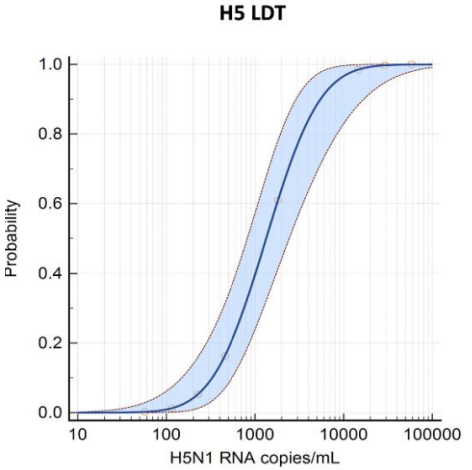
WHO information for the molecular detection of influenza viruses

Multiplex Dual-Target Reverse Transcription PCR for Subtyping Avian Influenza A(H5) Virus

Malaya K. Sahoo, Ingrid E.A. Morante, ChunHong Huang, Daniel Solis, Fumiko Yamamoto, Uzoamaka C. Ohiri, Daniel Romero, and Benjamin A. Pinsky

On This Page

Validation material



Automation

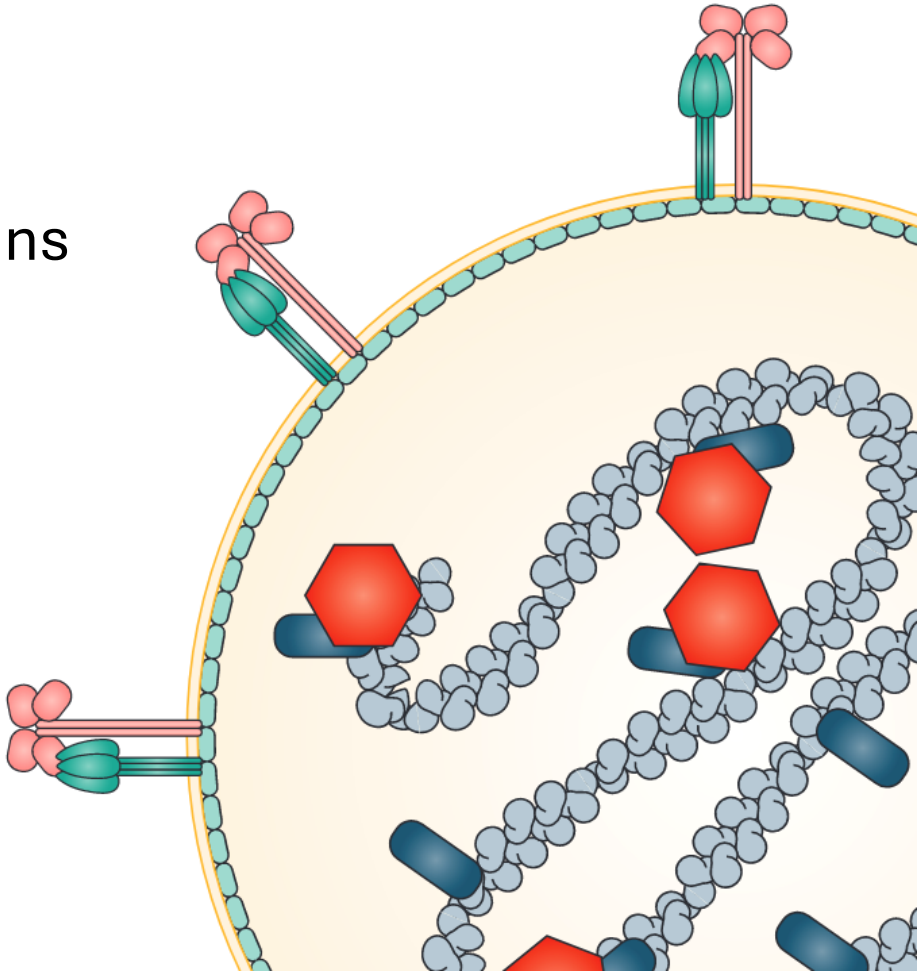
Take home points

- Influenza poses the greatest threat but we are also the best prepared to face it
- Many commercial assays have *in silico* or *in vitro* data which support the ability to detect (but not subtype) H5N1
- H5 subtyping is recommended only for patients with a known clinical exposure or if seasonal H1/H3 subtyping fails (CDC)

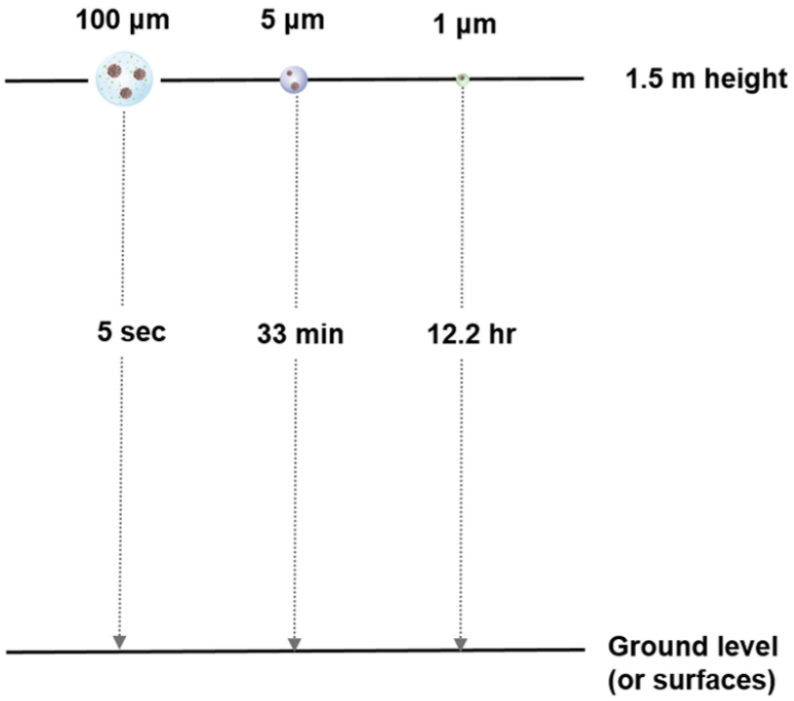
III. Measles

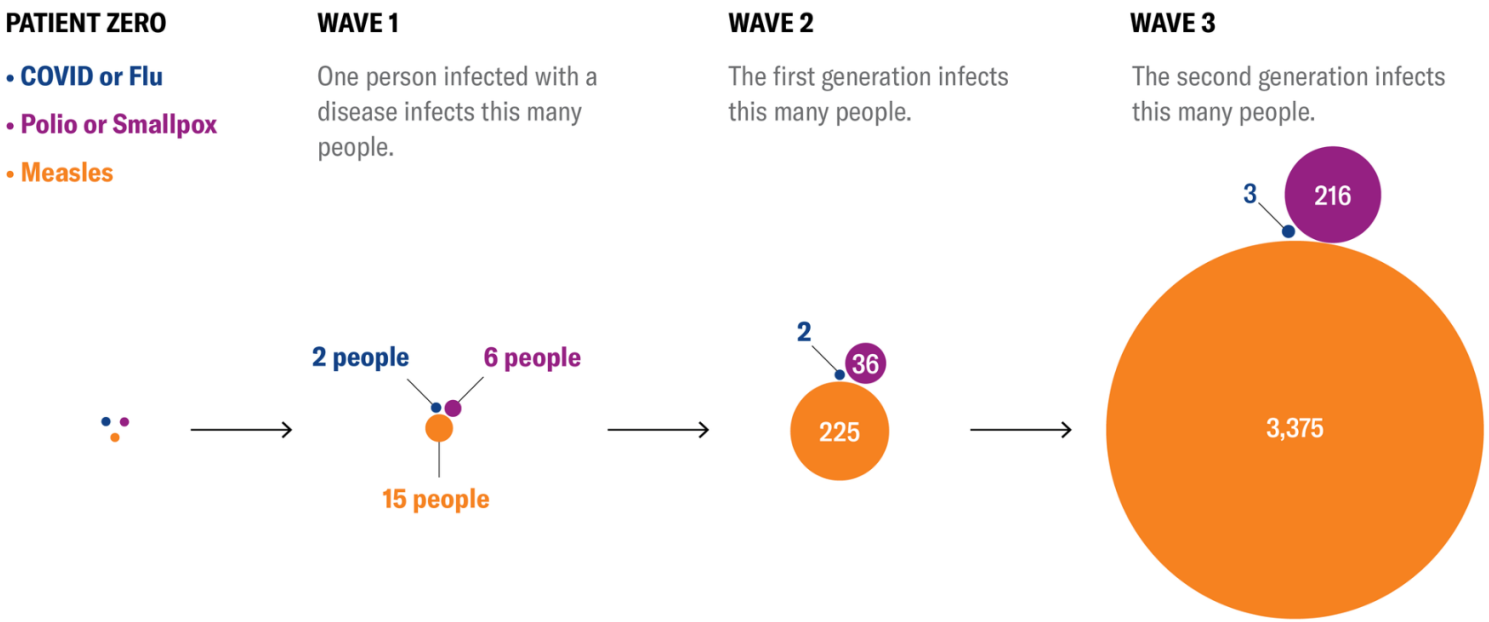
Measles virus

- Member of the paramyxoviridae family
  - Family includes mumps, RSV, parainfluenza
- 16kb single-stranded, (-) sense RNA virus
  - 6 structural proteins, and two non-coding proteins
  - Contains hemagglutinin (H) protein similar to influenza virus that binds host receptor
  - Fusion (F) protein allows viral entry
- One antigenic type
- Humans are the only reservoir for measles

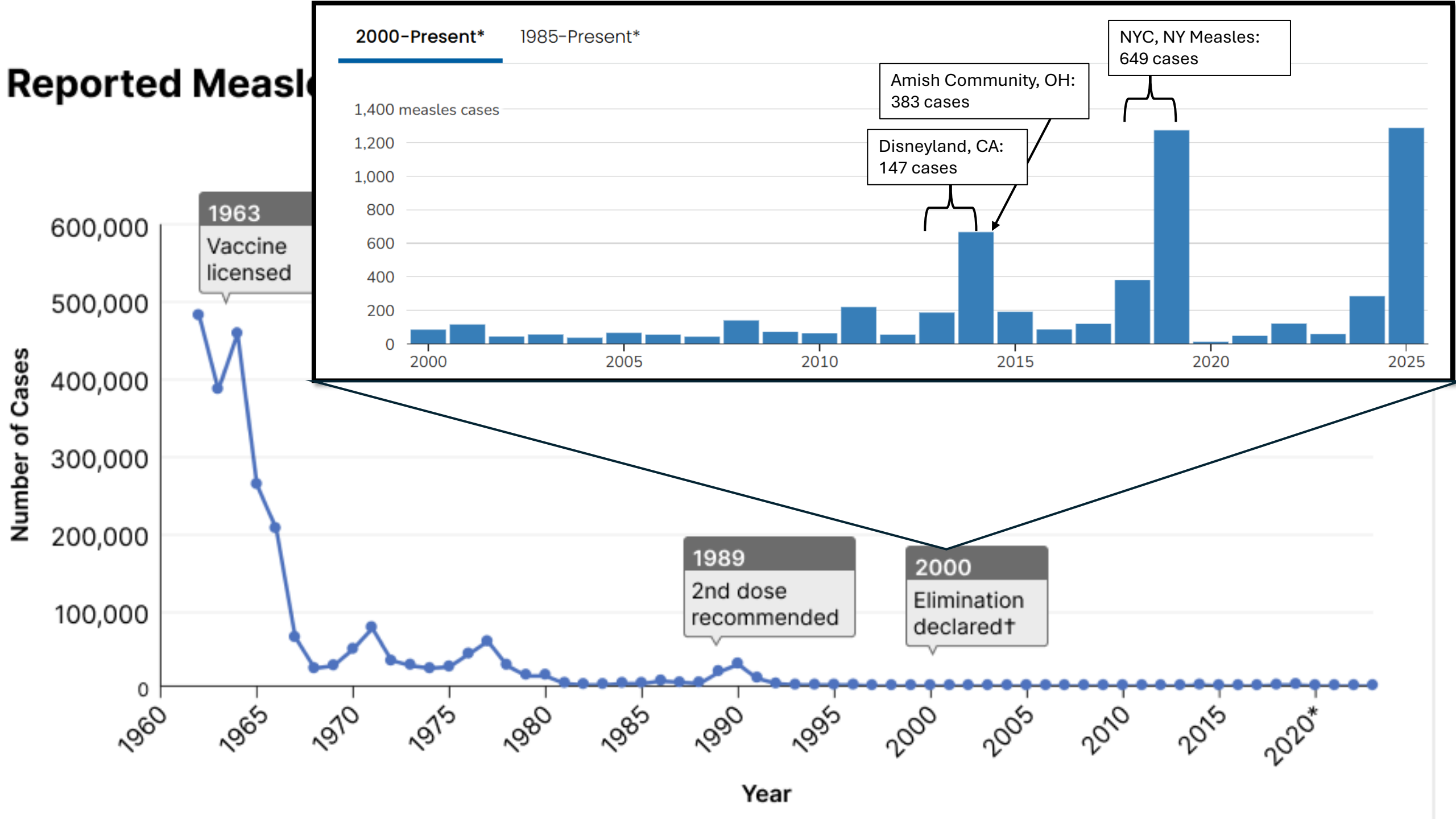


What makes measles so dangerous?





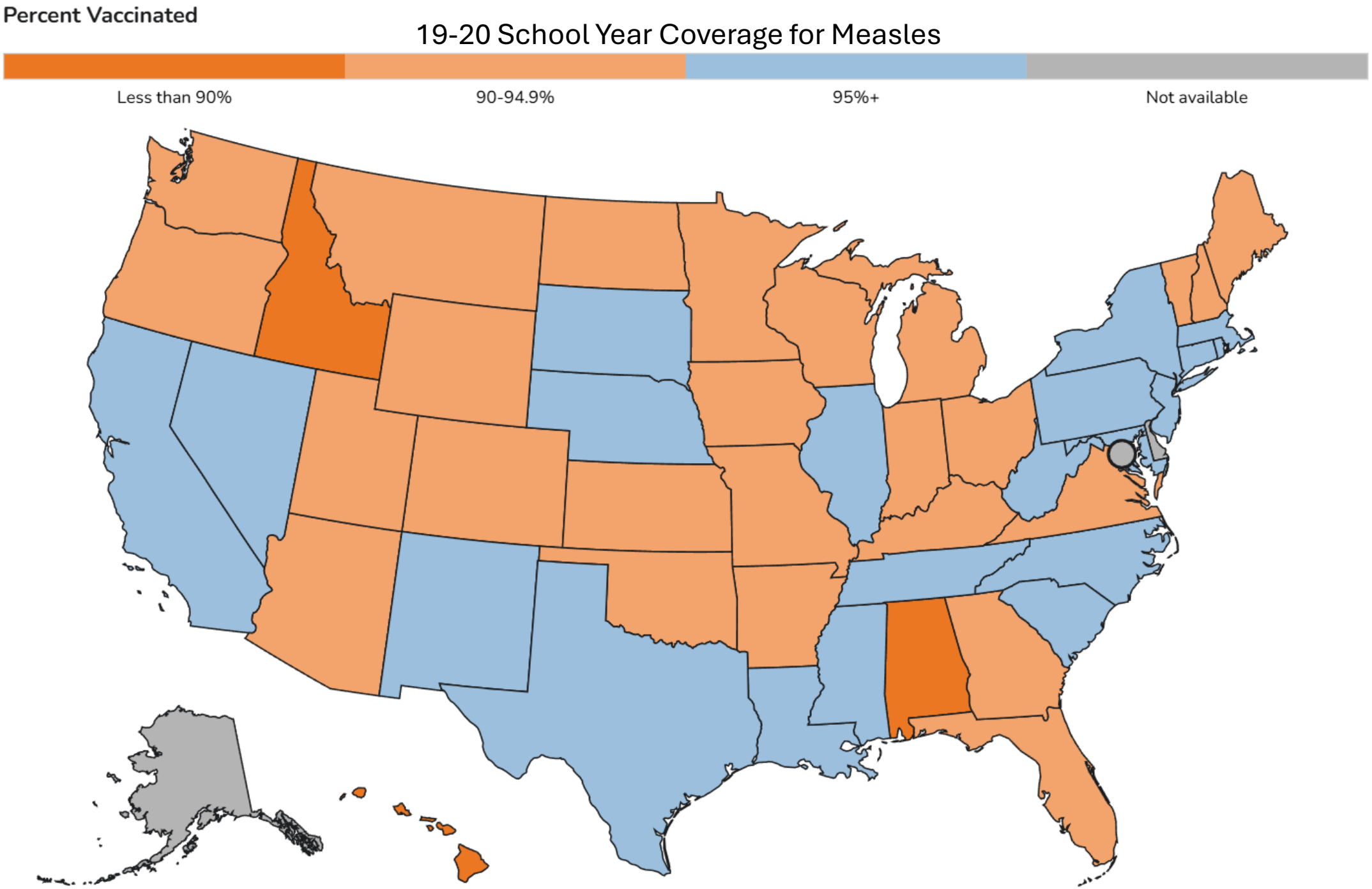
- Measles is transmitted an aerosol
  - Aerosolized particles can remain in the air for over 12 hours
- Don’t have to be in the room at the same time to become infected
- Highest Ro of any pathogen – one person can infect another 15!


Reported Measl
2000–Present*
1985–Present*
1,400 measles cases
1,200
1,000
800
600
400
200
0
2000
2005
2010
2015
2020
2025
Amish Community, OH:
383 cases
Disneyland, CA:
147 cases
NYC, NY Measles:
649 cases
1963
Vaccine licensed
1989
2nd dose recommended
2000
Elimination declared†
Number of Cases
600,000
500,000
400,000
300,000
200,000
100,000
0
1960
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
2020*
Year

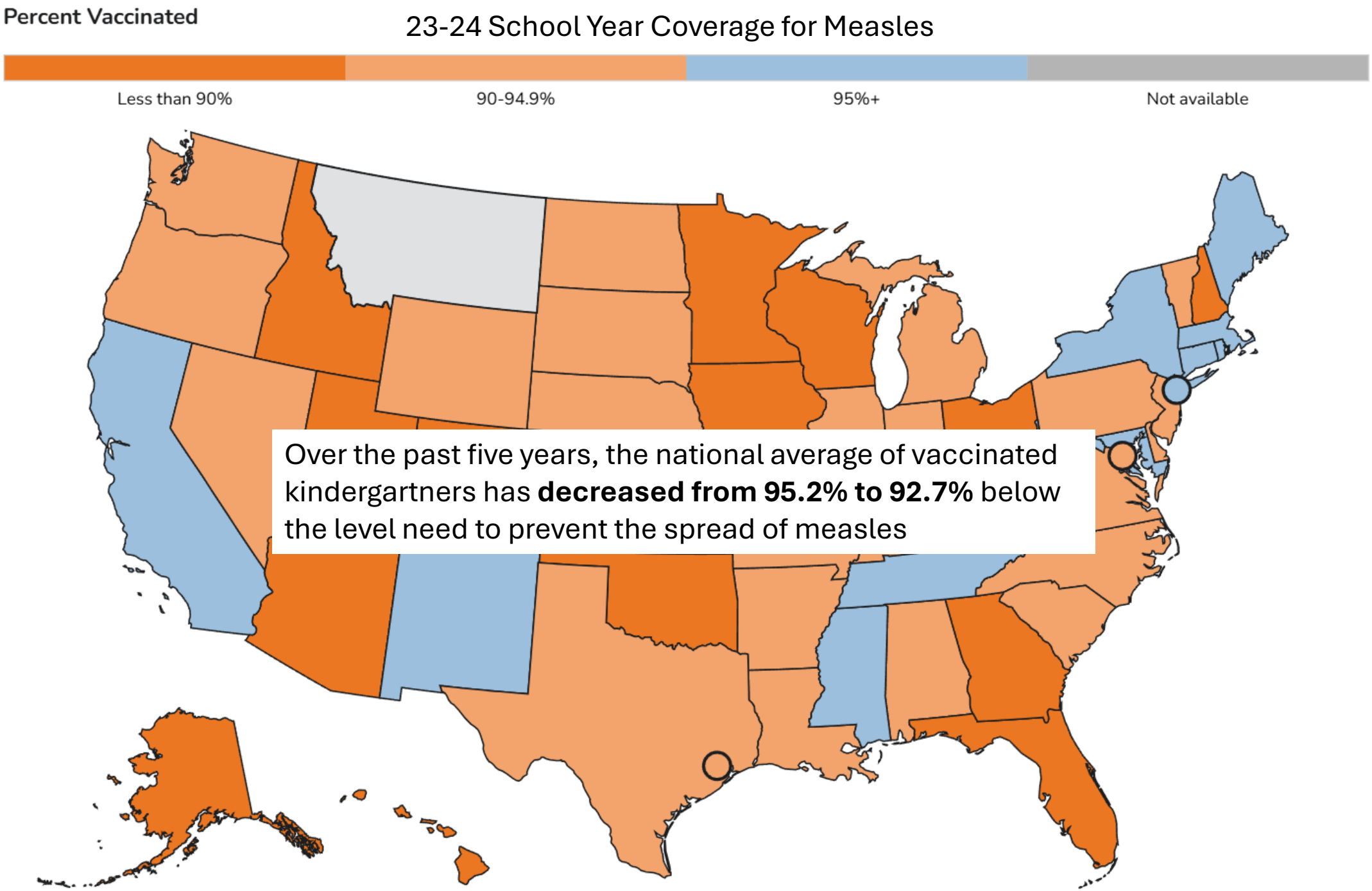
Why is this happening?

95%

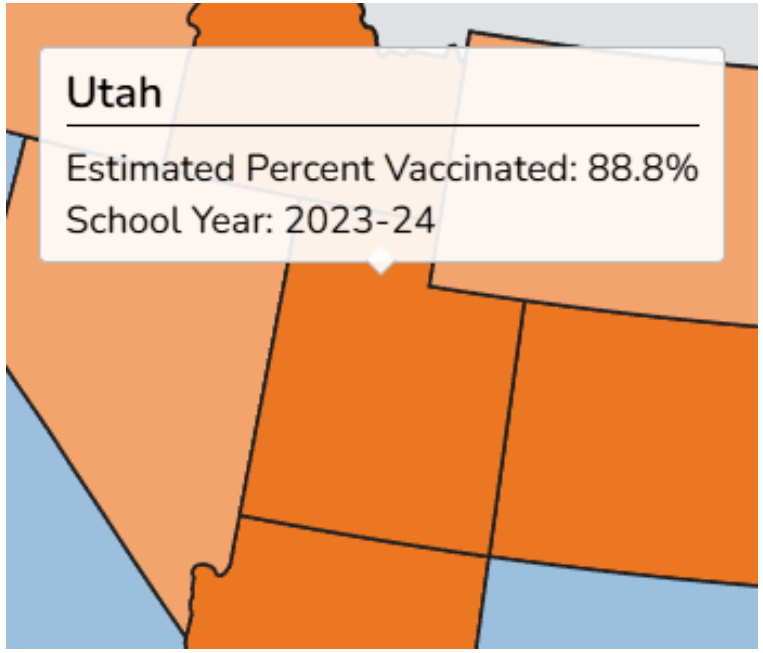
19-20 School Year Coverage for Measles



https://www.cdc.gov/measles/data-research/index.html


Percent Vaccinated
23-24 School Year Coverage for Measles
Less than 90%
90-94.9%
95%+
Not available
Over the past five years, the national average of vaccinated kindergartners has **decreased from 95.2% to 92.7%** below the level need to prevent the spread of measles

Pockets of under-vaccinated communities have driven the spread



Health District	Percent students missing DTaP	Percent students missing Polio	Percent students missing MMR
Southwest	19.5 %	19.3 %	19.3 %
Central	15.6 %	14.7 %	15.0 %
TriCounty	13.3 %	12.7 %	11.1 %
Southeast	11.8 %	12.0 %	12.0 %
Utah	12.1 %	11.9 %	11.8 %
San Juan	12.8 %	11.5 %	11.5 %
State of Utah, all students	11.7 %	11.5 %	11.2 %
Wasatch	11.7 %	11.4 %	11.2 %
Summit	10.7 %	10.7 %	10.7 %
State of Utah, in-person	10.5 %	10.2 %	9.9 %
Weber/Morgan	9.0 %	8.5 %	8.3 %
Davis	8.4 %	8.1 %	7.8 %
Salt Lake	8.1 %	8.1 %	7.4 %
Bear River	8.4 %	8.0 %	7.9 %
Tooele	7.8 %	7.2 %	6.7 %

Facility name	City	Adequately immunized %	Exempt %
All	All	All	All
Water Canyon School	Hildale	17.4	56.5
Canyon Grove Academy	Pleasant Grove	55.9	43.0
Mountain View Montessori	Washington	61.0	39.0
Valley Academy	Hurricane	57.3	34.7
Wasatch Waldorf Charter School	Holladay	67.6	31.1
Kanab School	Kanab	64.7	30.9
Bloomington School	St George	67.1	30.5
Timpanogos Academy	Lindon	69.9	30.1
John Hancock Charter School Pleasant Grove	Pleasant Grove	70.0	30.0
American Heritage School American Fork	American Fork	67.8	30.0

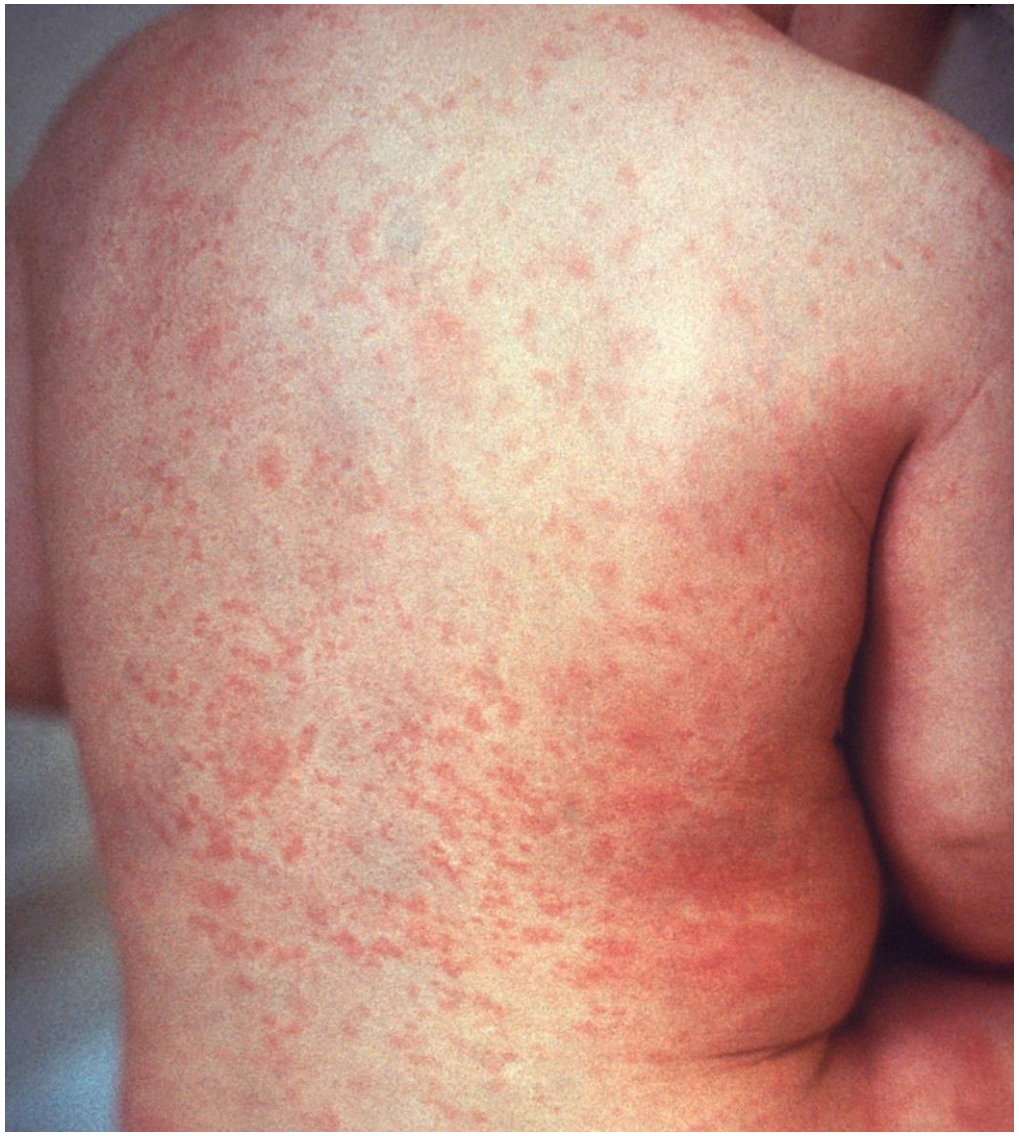
State level: ~11.2% unvaccinated

County level: ~19.3% unvaccinated

School level: >30% unvaccinated

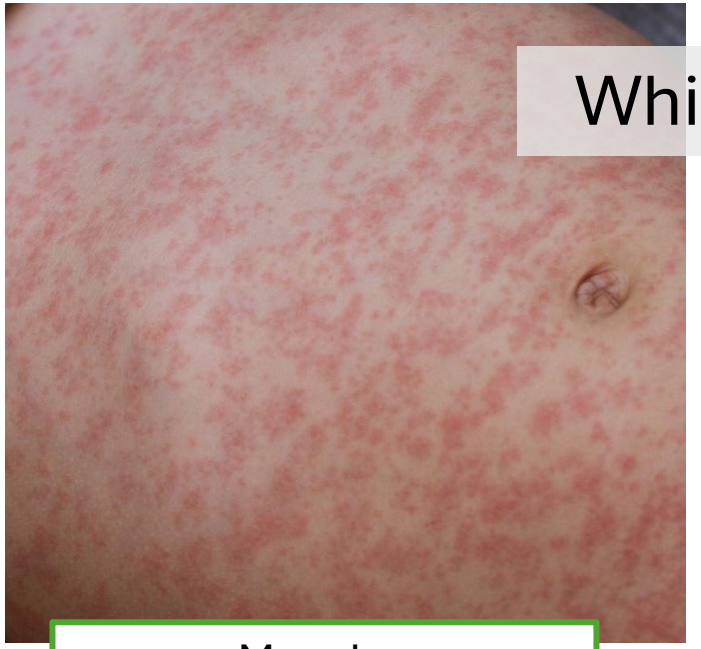
Clinical presentation of measles

- Incubation period
  - Symptoms begin 8-12 days after infection
- Prodrome begins with the 3 C’s
  - Cough, conjunctivitis, coryza
  - **Koplik spots** are pathognomonic
- Measles rash
  - Most diagnostically useful
  - Begins at the head and moved downward
  - **Patients are infectious 4 days before rash onset up to 4 days after**

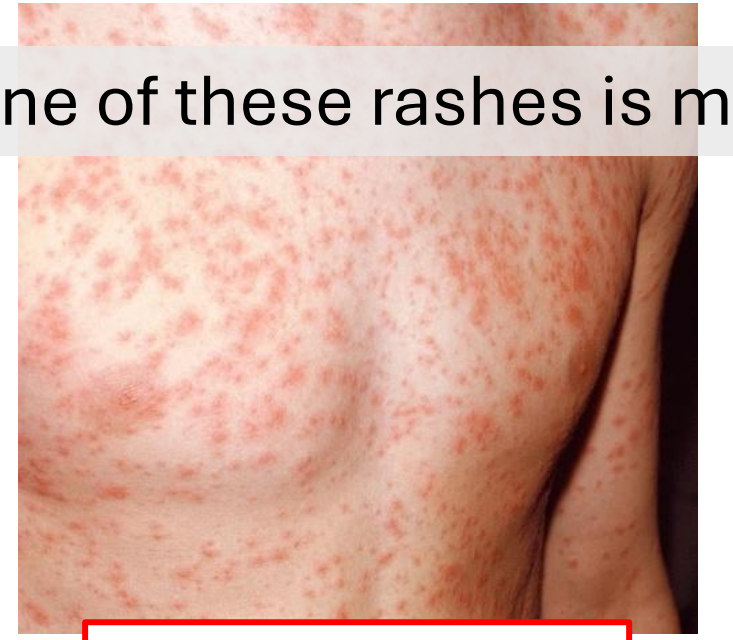


https://www.webmd.com/skin-problems-and-treatments/ss/skin-viral-rashes-guide

Which one of these rashes is measles?

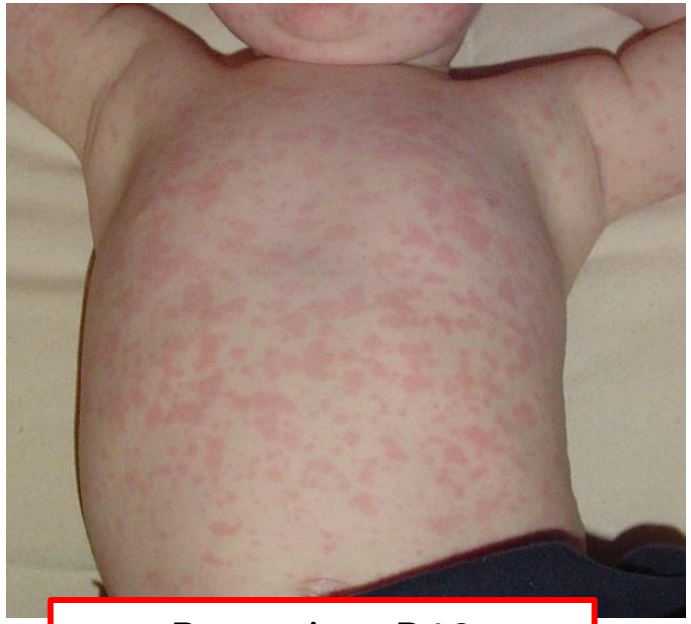


Measles

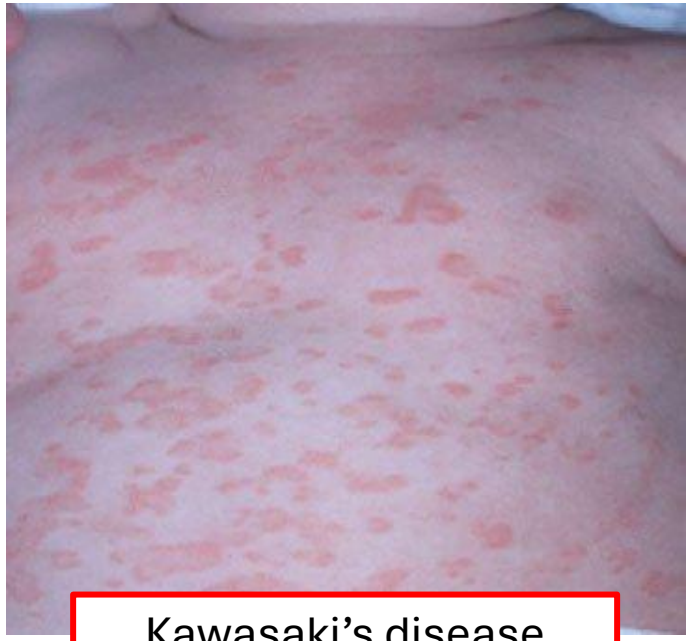


Rubella

HHV-6 (roseola)



Parvovirus B19



Kawasaki’s disease

Drug reaction

Measles is more than a mild rash

Severe complications

- Hospitalization (15-25%)
- Encephalitis: 1 per 1,000 cases (20% fatal)
- Death: 1–3 per 1,000 cases
- **Sub-acute sclerosing panencephalitis (SSPE)**
  - Progressively fatal disease that develops 5-10 years later

Community impact

- Pregnant women and children
  - 60% had adverse outcome
  - 10x higher maternal mortality
- Immunocompromised
  - Require high community coverage
- Immune amnesia
  - Increased susceptibility to other infectious agents

Current CDC testing recommendations

Measles – Recommendations for Testing for Clinicians

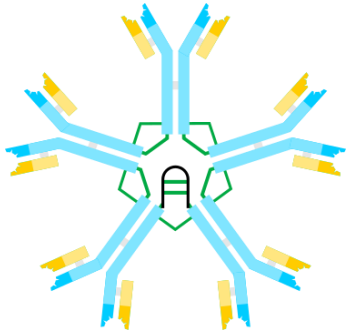
Measles is a mandatory, immediately notifiable disease. Please report confirmed and probable cases of measles to your local health department.

	Preference	Test	Specimen	Indication	Timing	Notes
ACUTE DISEASE	Preferred Test	RT-PCR	**Nasopharyngeal (NP) or throat (OP) swab** *(preferred)* Urine can be collected in addition to an NP/OP swab	Acute Disease	• A specimen for detection of virus should be collected as soon as possible upon suspicion of measles. • Specimen should be ideally collected within 3 days after rash onset but can be collected up to 10 days. • If >10 days since rash onset, PCR testing is generally not recommended.	• NP/OP swab collected <3 days after rash onset is the preferred specimen. Ideally, RT-PCR should be performed for all suspect measles cases identified within 10 days of rash onset. • Collecting a urine specimen along with an NP/OP swab may improve test sensitivity, especially if at the end of the RT-PCR detection window. • Contact your health department regarding where to send specimens for testing and genotyping, if appropriate.
	Preferred Test	IgM (with IgG)	Serum	Acute Disease	• Ideally, serology will be obtained for suspect measles cases, in addition to RT-PCR. • IgM is most sensitive 3+ days after rash onset and may be negative days 0–3 after rash onset. IgM can be detected for 6–8 weeks after acute measles.	• Detection of measles IgM can aid in the diagnosis of measles and can increase the detection window for acute cases. • Testing IgG for acute cases can provide evidence of pre-existing immunity, which can be helpful to differentiate rare instances of vaccine failure. • People with a history of measles vaccination may not have detectable IgM during an acute measles illness.
IMMUNITY	Only test for immunity	IgG only	Serum	Evidence of Immunity	• IgG can be detected approximately 2 weeks after measles vaccination.	• The presence of measles-specific IgG indicates a recent or prior exposure to measles virus or measles vaccine. • **IgM is <u>not</u> an appropriate test for immunity.**

* Viral culture is a valid way to confirm cases of acute measles disease; however, is not generally recommended as it takes longer to receive results than RT-PCR, which is widely available. Specimen collection and timing is similar to that for RT-PCR.
** Acute and convalescent phase serum specimen collection (separated by at least 2 weeks) to demonstrate a 4-fold increase in IgG titer can confirm measles infection but is generally not required to confirm measles infection.

Measles Serology Analytes

Immunoglobulin M (IgM)



- Pentameric antibody complex
- Produced by plasma cells early in infection

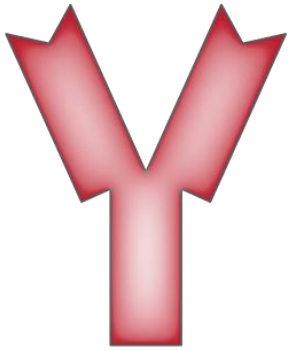
Diagnostic Criteria

Presence of IgM = acute infection

Limitations

- IgM may not be detected at rash onset
- IgM can persist for months
- False positives seen in EBV and Parvovirus B19

Immunoglobulin G (IgG)



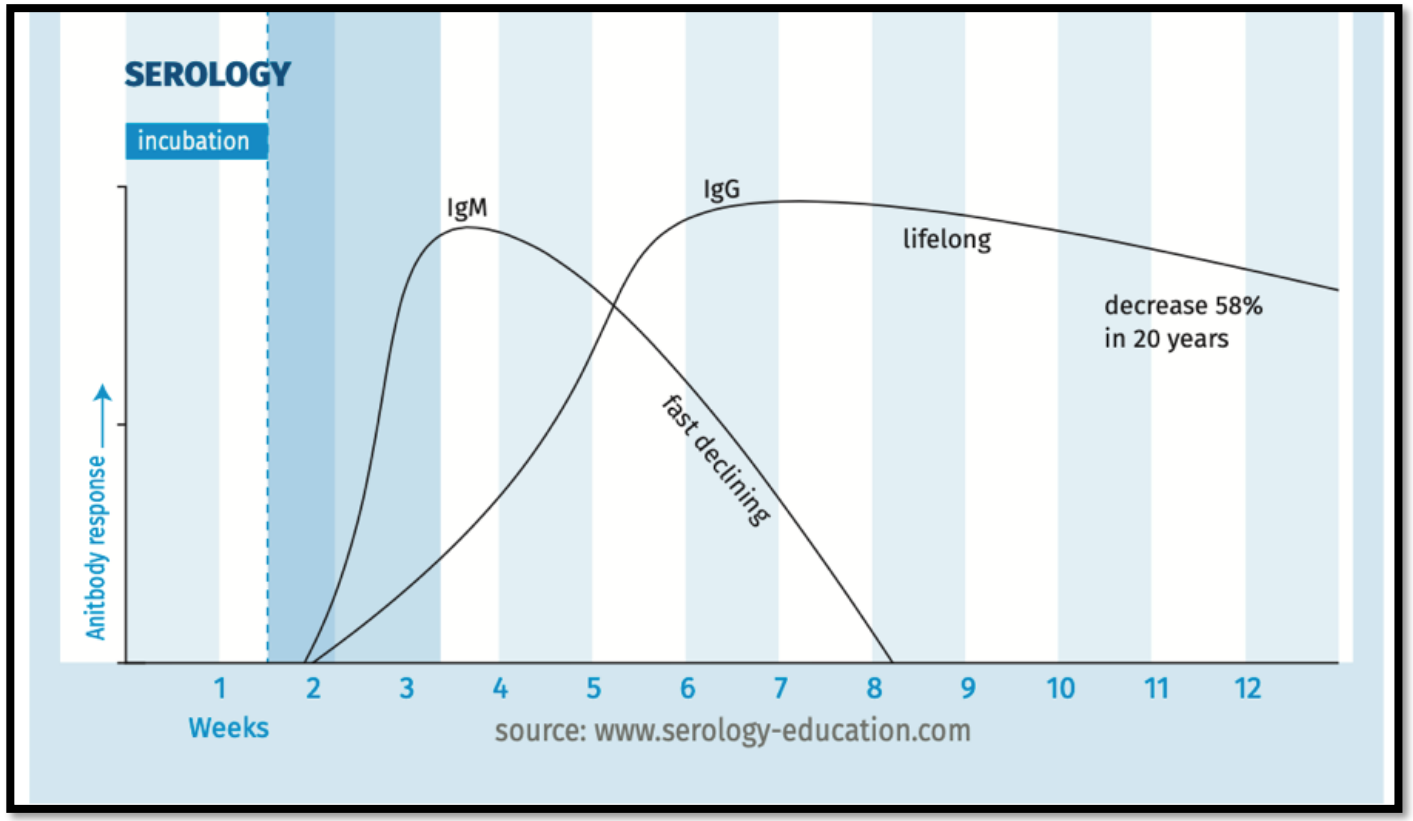
- Most common antibody found in circulation
- Single unit
- Part of long-term immunologic memory

4-fold increase in IgG titers
or
IgG seroconversion

IgG can also be used to test for prior infection/vaccination

- Requires two samples, collected weeks apart
- Not suitable for newborn dx
- Titers may not be available

Kinetics of measles virus serology



Immunoglobulin M:

- May be negative in the **first 3 days** of rash onset
- Highest sensitivity at **6 to 14 days** (94%-100%)
- Begin to **decline after 30 days** and are typically undetectable after 2-3 months

Immunoglobulin G:

- Delayed onset relative to IgM
- Sensitivity improved by **day 7-10** post rash
- Antibodies are typically considered to be **life-long**; however, <0.1% may serorevert per year
- Even if IgG antibodies aren't detectable by lab assays, individuals re-exposed to measles demonstrate a more rapid production of IgG

Molecular Testing

- Historically, measles PCR was available from public health labs and the CDC – however more labs are developing their own LDTs
- NP and OP swabs are typically recommended
  - Urine may improve detection, however it is dependent on the method
- Testing is most sensitive at the time the rash develops

TABLE 1. Analysis of measles virus RT-PCR positivity with clinical samples according to days postonset of rash (70 patients) or other clinical symptoms (4 patients)

Days post-onset of rash	Serum		PBLs		Urine		TS		All specimens	
	No. of samples[a]	No. (%; 95% CI) positive	No. of samples[a]	No. (%; 95% CI) positive	No. of samples[a]	No. (%; 95% CI) positive	No. of samples[a]	No. (%; 95% CI) positive	No. of samples	No. (%; 95% CI) positive
<0–3	64	22 (34; 23–47)	10	7 (70; 35–93)	15	10 (67; 38–88)	15	11 (73; 45–92)	104	50 (48; 38–58)
4–7	21	2 (10; 1–30)	12	5 (42; 15–72)	15	8 (53; 27–79)	15	10 (67; 38–88)	63	25 (40; 28–53)
8–13	7	0	5	3 (60; 15–95)	6	2 (33; 4–78)	6	4 (67; 22–96)	24	9 (38; 19–59)
14–20	4	0	6	2 (33; 4–78)	7	2 (29; 4–71)	5	1 (20; 0.5–72)	22	5 (23; 8–45)
>21	2	0	6	0	7	1 (14; 0.3–58)	7	0	22	1 (5; 0.1–23)
No. rash[b]	5	1 (20; 0.5–72)	3	1 (33; 0.8–91)	3	0	3	1 (33; 0.8–91)	14	3 (21; 5–51)
Total	103	25 (24; 16–34)	42	18 (43; 28–59)	53	23 (43; 30–58)	51	27 (53; 38–67)	249	93 (37; 31–44)

Molecular Testing

- Many different targets have been validated for PCR assays
  - N gene – Used by CDC and endorsed by WHO
  - Has excellent sensitivity and limit of detection
  - Detects both wild-type and vaccine strain (genotype A) measles
  - **When might you want to differentiate them?**

Table 2
Detection of Circulating MeV Isolates in Multiple Clinical Matrices.

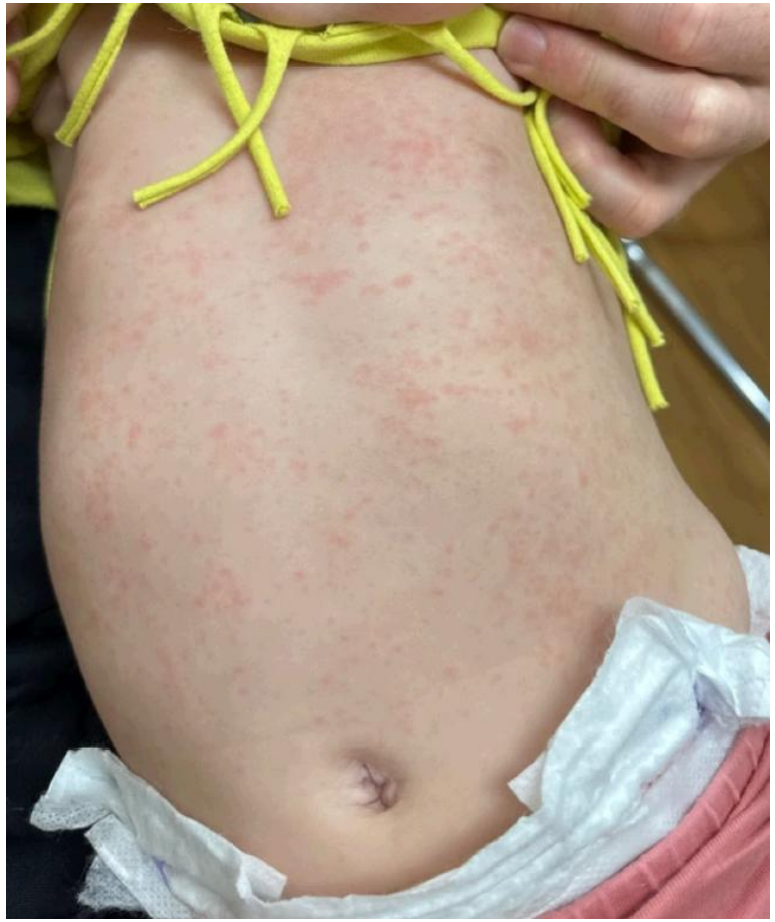
Virus	log_{10} copies/mL	NP			
		Two targets*	N	H	L
B3 Maryland USA 18.18	6.0–2.0	15/15	15/15	12/15	15/15
D8 Florida USA 35.18	7.0–2.0	18/18	18/18	18/18	18/18
D9 Ohio USA 17.14/3	5.0–2.0	11/12	12/12	11/12	11/12
H1 Utah USA 5.17	6.0–2.0	15/15	15/15	15/15	15/15
D4 New Mexico USA 6.11	6.0–2.0	15/15	15/15	7/15	15/15
	Total	74/75	75/75	63/75	74/75
	%	98.7	100.0	84.0	98.7
	95% **CI**	92.8–100	95.2–100	73.7–91.5	92.8–100

Table 4
MeV triple-target rRT-PCR 95% Lower Limit of Detection.

Virus	copies/mL	NP			
		Two Targets*	N	H	P
D8 Florida USA 35.18	1000	10/10	10/10	10/10	10/10
	500	10/10	10/10	9/10	10/10
	100	4/10	10/10	0/10	4/10
	95% LLOD	226	<100	520	226
	95% CI	101–352	UTC	UTC	101–352

Measles virus vaccine strain (MeVA)

- All measles vaccines are composed of genotype A (MeVA)
- Approximately 5% of individuals receiving the MMR vaccine will develop a post-vaccination rash that appears similar to measles
- Importantly, these individuals are not considered infectious and do not require infection control or isolation measures
- **Recently vaccinated patients will test positive for measles when using a pan-measles PCR assay**



Does this child have measles or a measles-vaccine associated rash?

Detection of vaccine strain measles virus

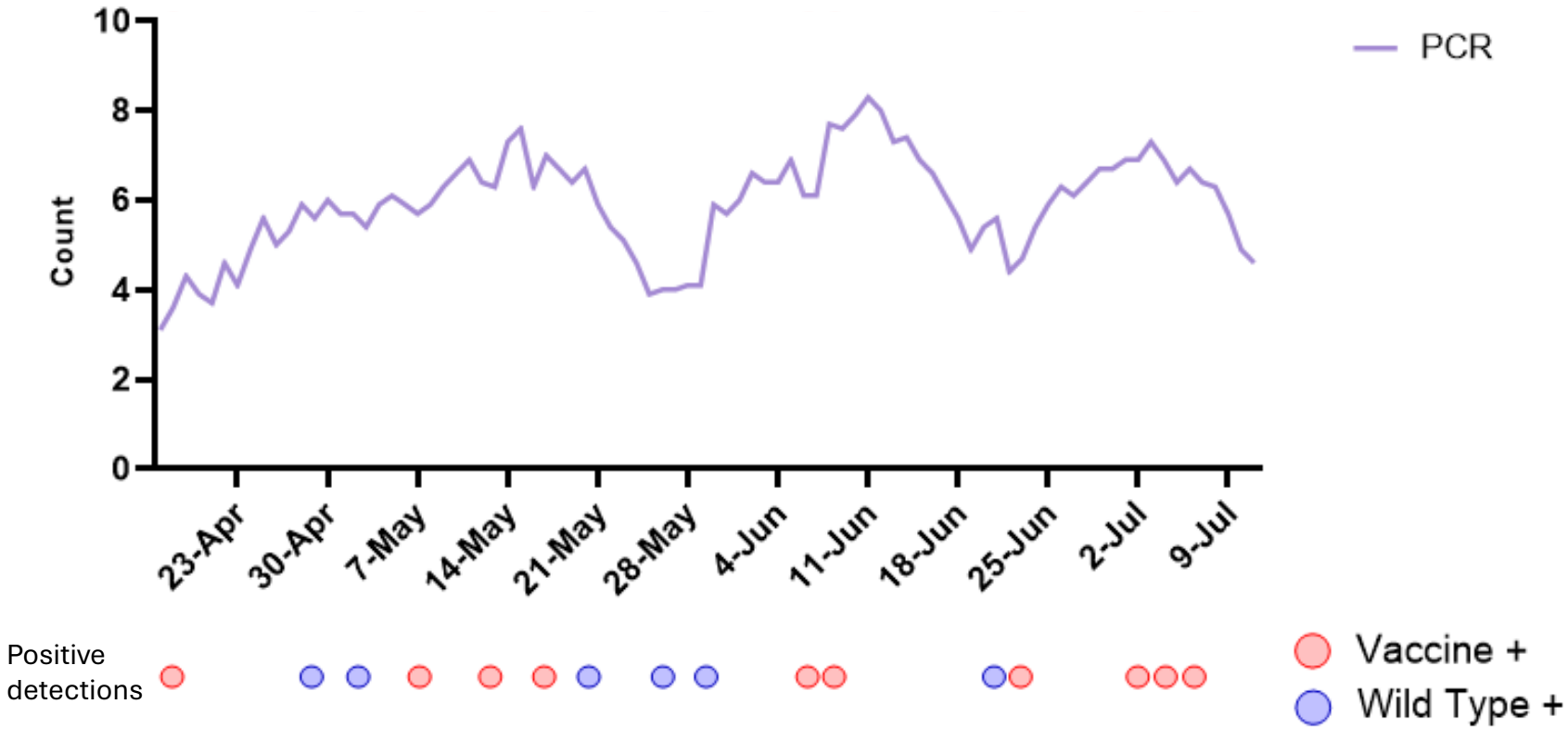
1. Sequencing
   - Performed via Sanger sequencing of a 450bp region
   - Pros: Allows for identification of exact strain (e.g. B3, D8)
     - Provides epidemiological clues about origin and spread of outbreak
   - Cons: Only available through PHLs, long turn-around time

2. Molecular testing
   - Real-time PCR test to detect SNPs specific to genotype A (MeVA)
   - Pros: Wider availability (reference labs, PHLs, CDC)
     - Can be run simultaneously with pan-measles virus PCR
   - Cons: Can not identify other genotypes, higher limit of detection than pan-measles virus target

Measles testing at ARUP

Volumes and Detection Rates

- Designed multiplex (MeV and MeVA) assay
- Runs on open channel of automated PCR instrument
- Launched test on April 17th, 2025



525 total tests (491 patients)

- Average 5.7/day
- Range: 1 – 14 tests
- Tests mostly collected M-F

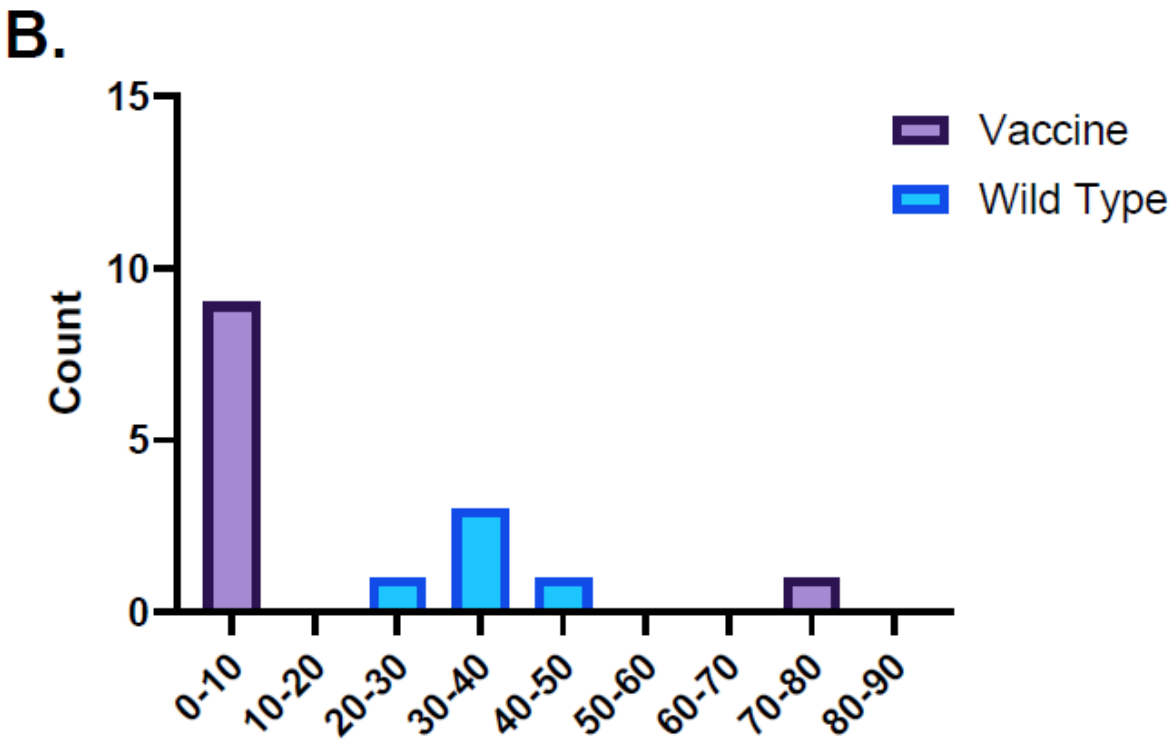
16 Positive PCR results

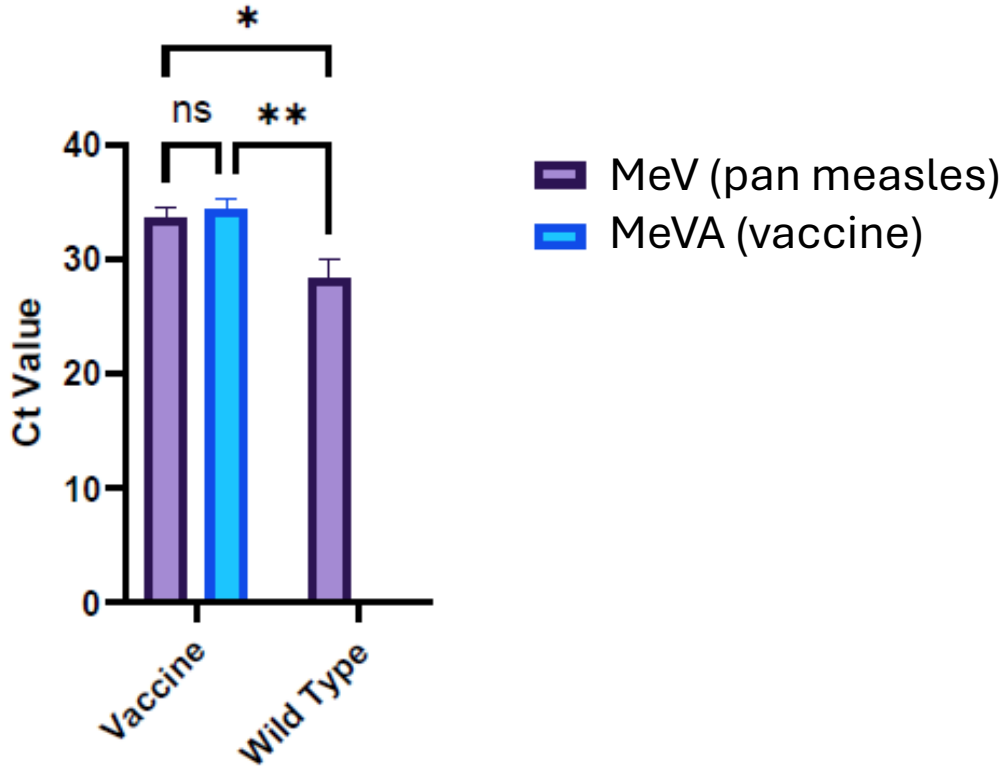
- 10 (62%) vaccine
- 6 (38%) wild type

Measles testing at ARUP

Age Ranges and Ct values

- Designed multiplex (MeV and MeVA) assay
- Runs on open channel of automated PCR instrument
- Launched test on April 17th, 2025





- 9/10 vaccine cases were identified in 0-10 year olds
- All wild-type cases were in patients 20-50 years-old
- One case of transplant patient who received vaccine

- MeV Ct values were later for vaccine cases versus wild-type cases (34.0 vs 28.7)
- For vaccine positive cases, the MeVA target (31.7) was slightly later than MeV target (31.0)

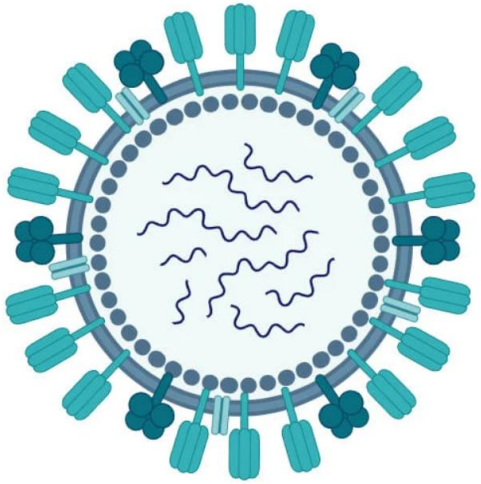
Take home points

- Despite being the most transmissible human virus, 95% vaccine coverage could eliminate measles
- Outbreaks are a local challenge with global impact
- Measles infections affect more than just the patient
- Laboratory testing relies on a combination of serological and molecular testing and may be essential to help separate vaccine-induced rashes from wild-type measles

IV. Pertussis

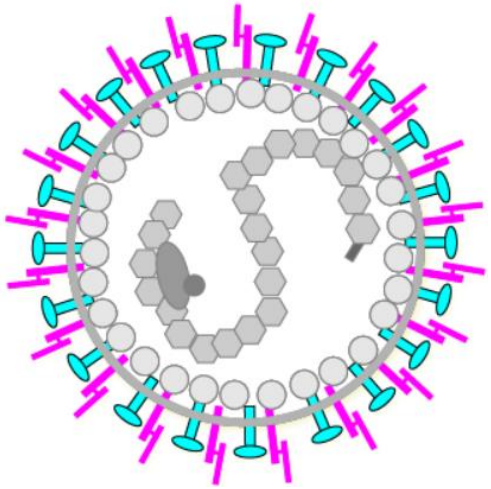
U.S. Case Counts

Avian Influenza H5N1
2024-now



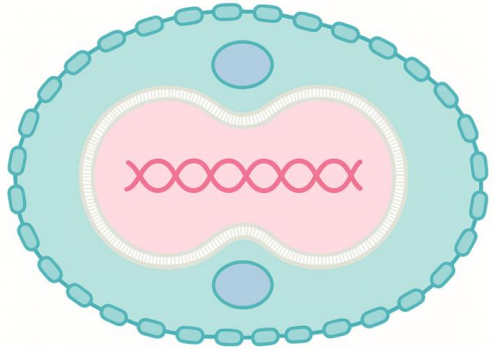
70 cases

Measles
2000-now



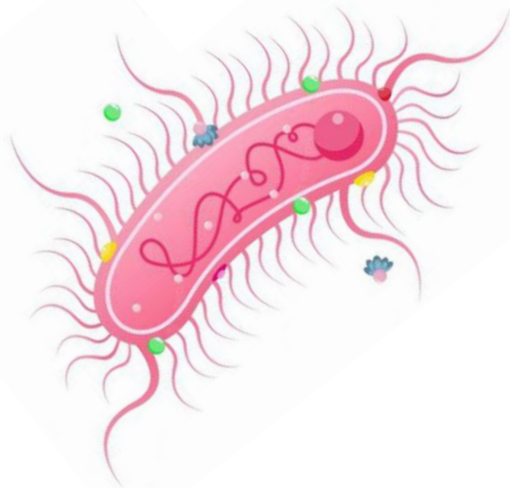
~6,000 cases

Mpox
2022-now



~35,000 cases

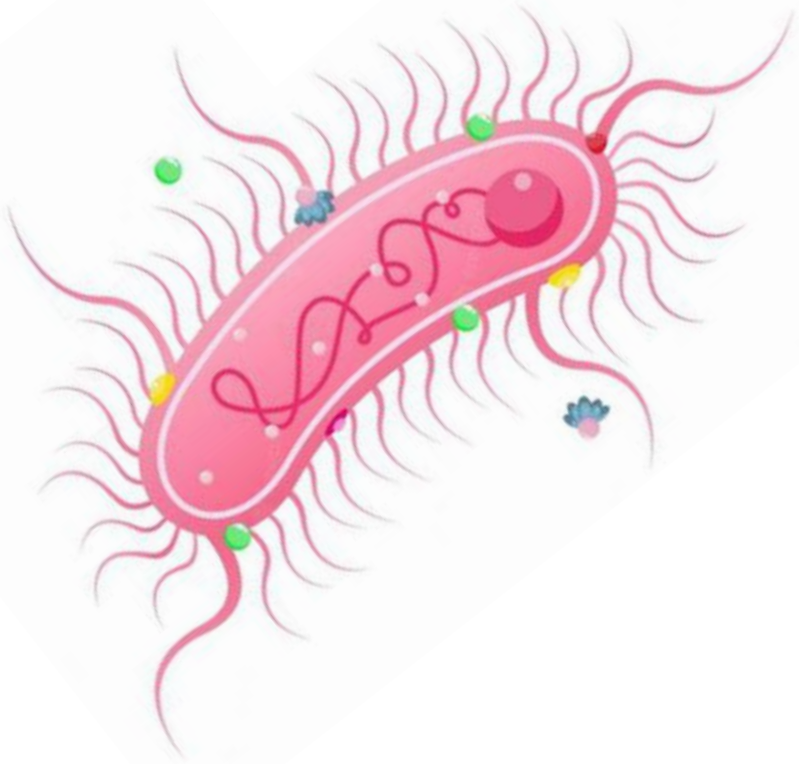
Pertussis
2024-now



~55,000 cases

- In 2024, 35,435 pertussis cases were reported to the CDC – a rate five times higher than the 7,063 cases reported in 2023
- 2025 is on track to have even more pertussis cases!

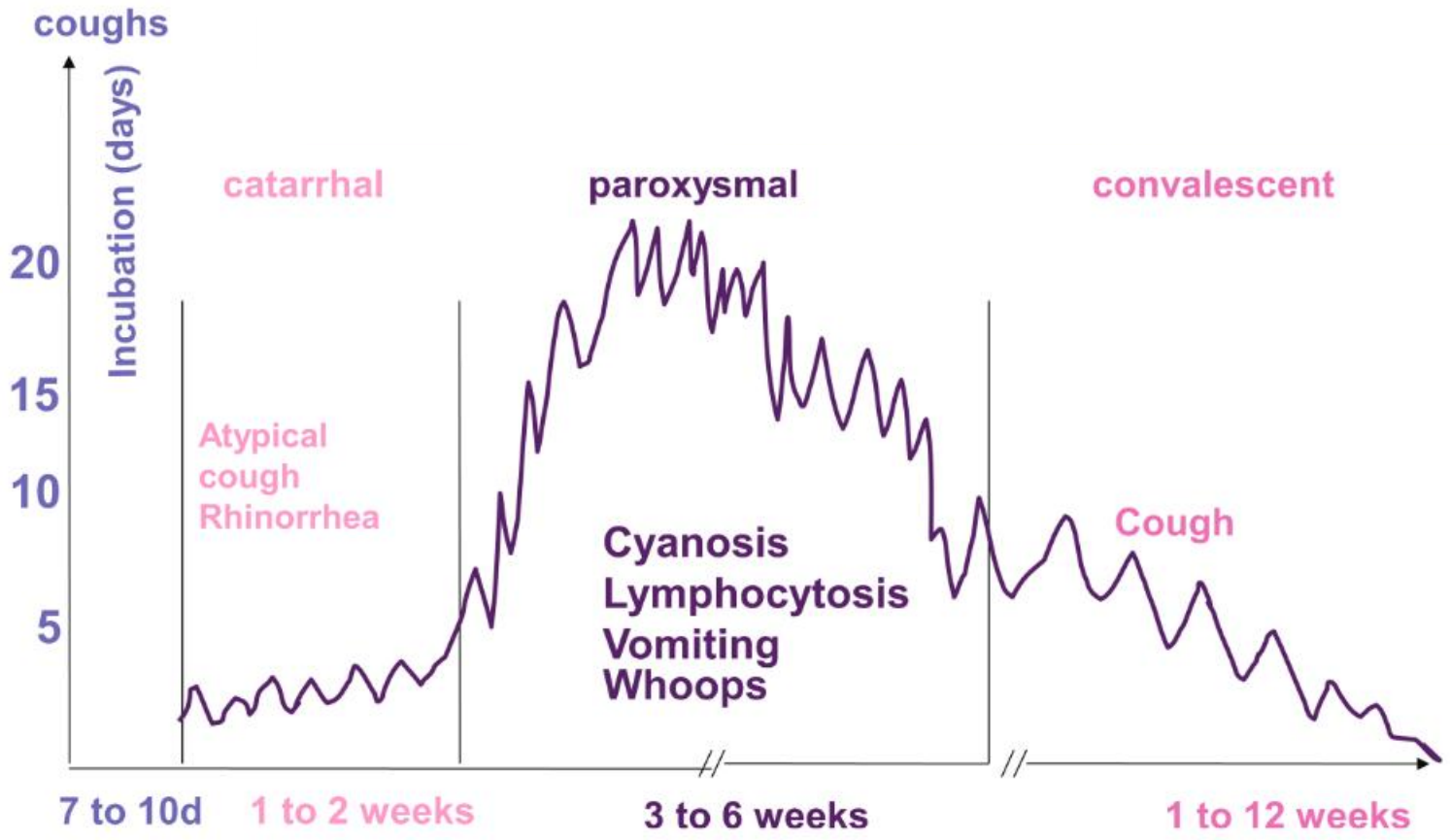
Pertussis



- Clinical disease caused by the organism *Bordetella pertussis*
- Most common of the *Bordetella* species to cause disease
  - *B. parapertussis*, *B. bronchiseptica*, *B. holmesii*
- **Very infectious**: 90% attack rate with infectious dose of 100 CFU
- Outbreaks observed during the summer and fall with a cyclical pattern of larger outbreaks every 2-5 years

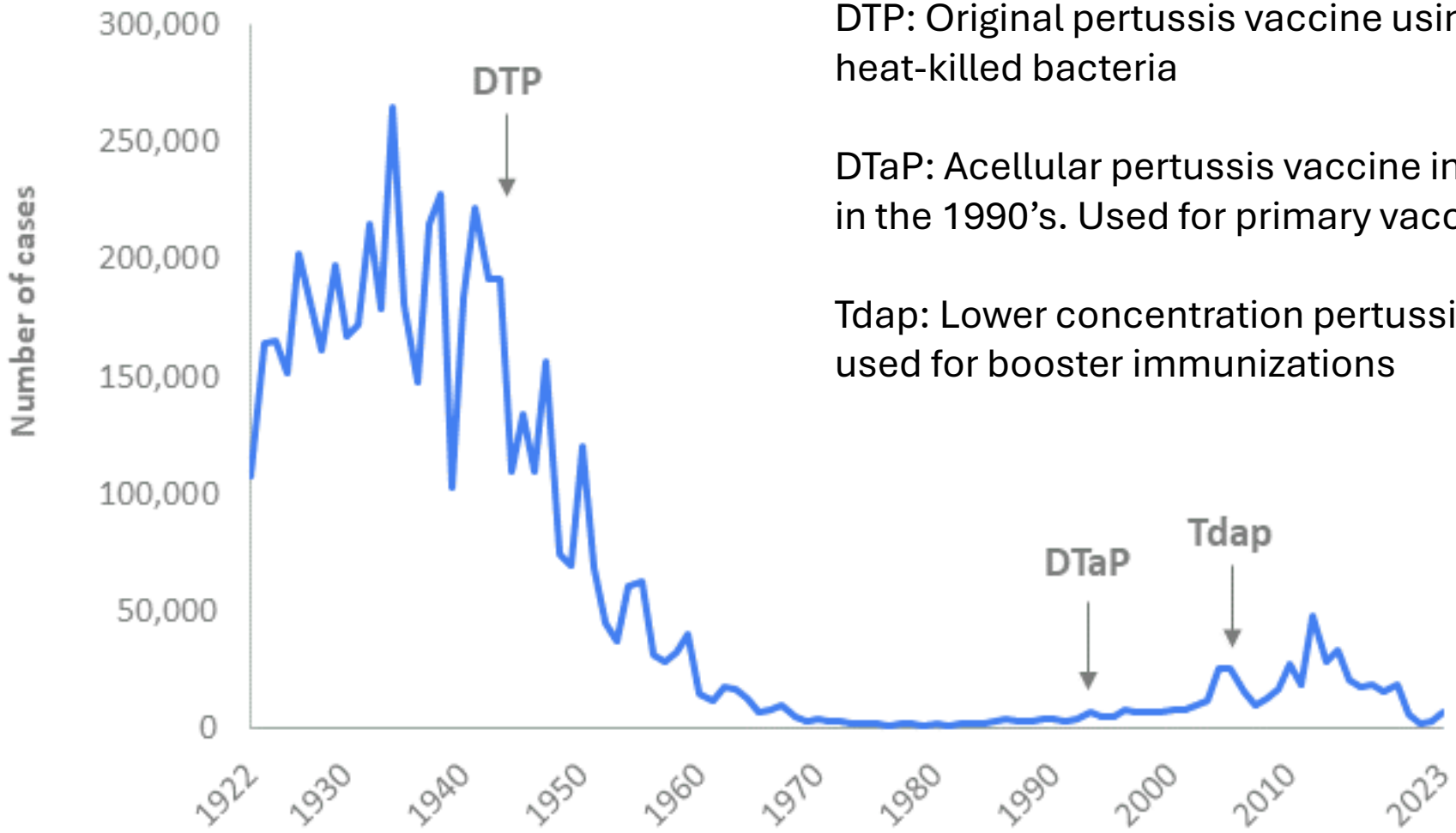
Clinical presentation

- Catarrhal
  - Non-specific, hard to dx pertussis on symptoms alone
  - Most infectious, and highest likelihood of testing positive
- Paroxysmal
  - Dangerous stage for young children and infants
  - Symptoms can persist for >8 weeks (100-day cough)
- Convalescent
  - No longer infectious



DOI: 10.1002/9781683670438.mcm0048

Reported NNDSS pertussis cases: 1922-2023



DTP: Original pertussis vaccine using whole, heat-killed bacteria

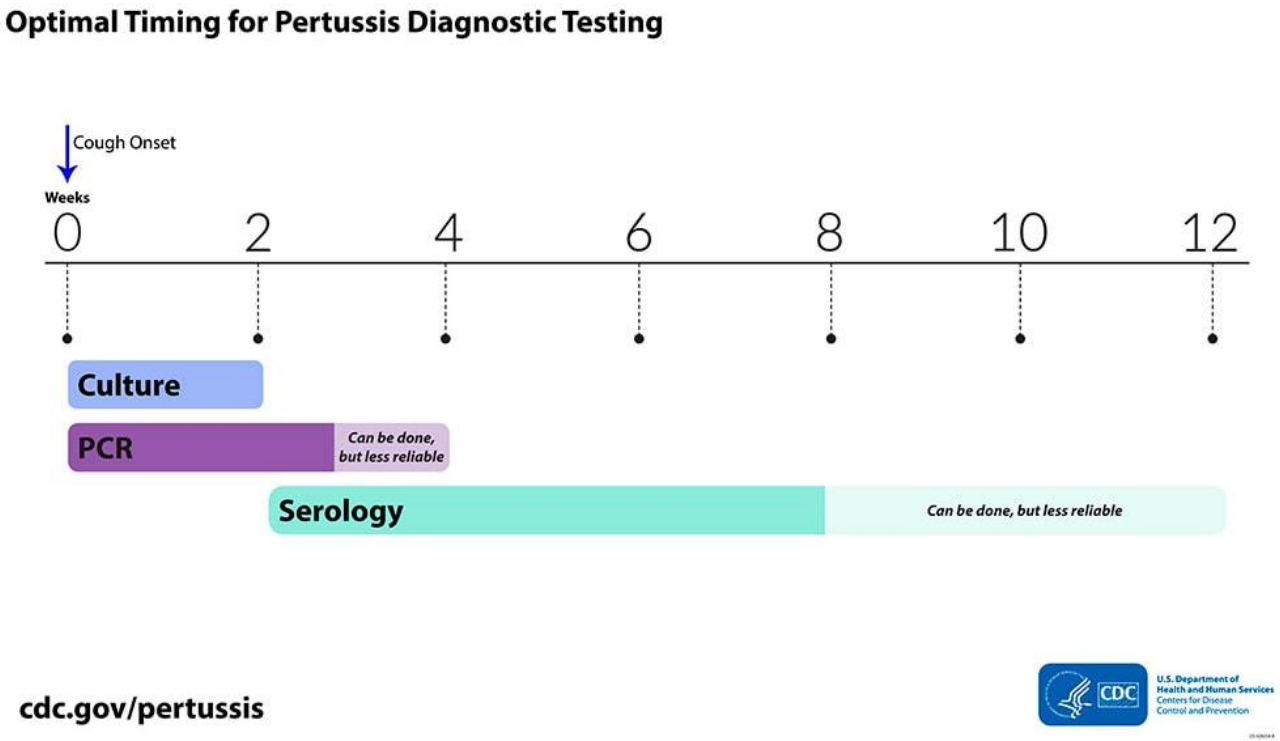
DTaP: Acellular pertussis vaccine introduced in the 1990's. Used for primary vaccination

Tdap: Lower concentration pertussis vaccine used for booster immunizations

SOURCE: CDC, National Notifiable Diseases Surveillance System

Pertussis testing

- Culture
  - Pros: Gold standard, highest specificity
  - Cons: Lower sensitivity, media requirements
- Molecular
  - Pros: Most sensitive technique
  - Cons: Limited availability, specificity questions
- Serology
  - Pros: Useful for epi tracing
  - Cons: Poorly standardized, not recommended for diagnosis



Molecular testing

- Sensitivity ranges from 70-99%
  - Twice as sensitive as culture when used in the same patients
- Available as stand-alone LDTs or part of syndromic panels
- Two most common B. pertussis targets
  - *IS481*: Most sensitive, but present in other species
  - *ptxP*: Only found in B. pertussis, less sensitive

TABLE 2 Possible targets for detection of *Bordetella* DNA by real-time PCR

Target	Organism in which target is present	~Copy no./genome (reference)	Comments
IS*481*	*B. pertussis*	~200	
	B. holmesii	~10	
	B. bronchiseptica	<5	<1% of isolates
IS*1001*	*B. parapertussis*	~20	
	B. bronchiseptica	<5	~50% of isolates
IS*1002*	*B. pertussis*	~10	
	B. parapertussis	<5	
hIS*1001*	*B. holmesii*	5–20 (155)	
ptxP	*B. pertussis*	1	
recA	*B. holmesii*	1	

Table 2. Analytical sensitivity in LOD_{95}.

Strain	Target	LOD_{95} (CFU/ml)
***B. pertussis* BAA-589**	IS*481*	6.01
	ptxA-Pr	147.55
***B. pertussis* BORD1705**	IS*481*	13.21
	ptxA-Pr	181.62

PMID: 38759432

Is specificity a major problem?

TABLE 3 Comparison of PCR results from 3,984 clinical specimens at two U.S. commercial laboratories and at CDC laboratory

Species interpretation at commercial laboratories	Species interpretation at CDC laboratory	No. of specimens (% of lab) with the indicated PCR results		
		Lab A (n = 2904)	Lab B (n = 1080)	Both labs (n = 3984)
IS481 positive (B. pertussis)	*B. pertussis*	2,180 (81.6%)	944 (95.2%)	3,124 (85.3%)
	B. holmesii	37 (1.4%)	14 (1.4%)	51 (1.4%)
	Potential *B. bronchiseptica*	0 (0%)	1 (0.1%)	1 (0.1%)
	B. pertussis and *B. holmesii*	3 (0.1%)	1 (0.1%)	4 (0.1%)
	B. pertussis and *B. parapertussis*	2 (0.1%)	1 (0.1%)	3 (0.1%)
	Indeterminate *B. pertussis*	380 (14.2%)	21 (2.1%)	401 (10.9%)
	Negative for all targets	69 (2.6%)	10 (1.0%)	79 (2.2%)
	Total Tested	2,671	992	3,663
	Any *B. holmesii* detected[a]	40 (1.5%)	15 (1.5%)	55 (1.5%)
	Total Matching[b]	2,222 (83.2%)	959 (96.7%)	3,181 (86.8%)

2024 Study comparing reference lab results to CDC for B. pertussis

- Over 85% of results agreed between the two assays
- Majority of discordant results were due to indeterminate or negative CDC results
- *B. holmesii* or *B. bronchiseptica* accounted for 1.5% of total detections

Challenge case

- In December, a vaccinated 12-year-old presents to the pediatrician
- Four days ago, the child developed fever and a cough
- There have been several children at the school sick with influenza
- A syndromic PCR panel is done and the results are:
  - Influenza A: **Detected**
  - All other targets are negative
  - B. pertussis: **Detected**

- **What are some features that make you suspicious of a false positive result?**
- **What is a potential explanation?**

Aerosolized Vaccine as an Unexpected Source of False-Positive *Bordetella pertussis* PCR Results

- Investigation began following repeated late positive results from a single clinic
- Wipe testing was performed at the clinic and samples of the vaccine were tested
- Contamination was found in the environment and on the providers' hands
- CDC recommends using separate areas and frequent changes of gloves

TABLE 1 *Bordetella pertussis* PCR results of original patient specimens and other samples

Source and specimen	CT ± SD[a]	Interpretation
Original pediatrician's office		
Original patients (n = 13) (mean CT)	39.33 ± 0.98	Positive[b]
Nasal wash solution	—[c]	Negative
Bulb	—	Negative
Pentacel DTap-IPV washing[d]	29.13	Positive
Unopened vaccine bottles[d]		
Adacel (1:100)	17.7 ± 0.2	Positive
Pentacel DTap-IPV (1:100)	15.1 ± 0.13	Positive
Infanrix (undiluted)	—	Negative
Pediatric vaccine clinic[e]		
HCP A (nares; before work)	—	Negative
HCP A (nares; after work)[f,g]	40.3	Positive
HCP A (hand; before work)	—	Negative
HCP A (hand; after work)[f,g]	36.1	Positive
HCP B (nares; before work)	—	Negative
HCP B (nares; after work)[f,g]	39.8	Positive
HCP B (hand; before work)	—	Negative
HCP B (hand; after work)[f,g]	37.0	Positive
Computer keyboard (wipe)[f,g,h]	36.8	Positive
Preparation table (wipe)[f,g,h]	32.5	Positive
Examination table (wipe)[f,g,h]	34.0	Positive
Fridge handle (wipe)[f,g,h]	34.6	Positive
Wall above prepn table (wipe)[f,g,h]	32.0	Positive

Take-home points

- Even ‘routine’ vaccine preventable diseases are seeing a surge in activity that require enhanced laboratory testing
- PCR offers superior detection of *B. pertussis* relative to culture
- **Know your assay’s target**
- Any suspected false positive warrants investigation


Thank you!
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