Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

COVID-19 Update

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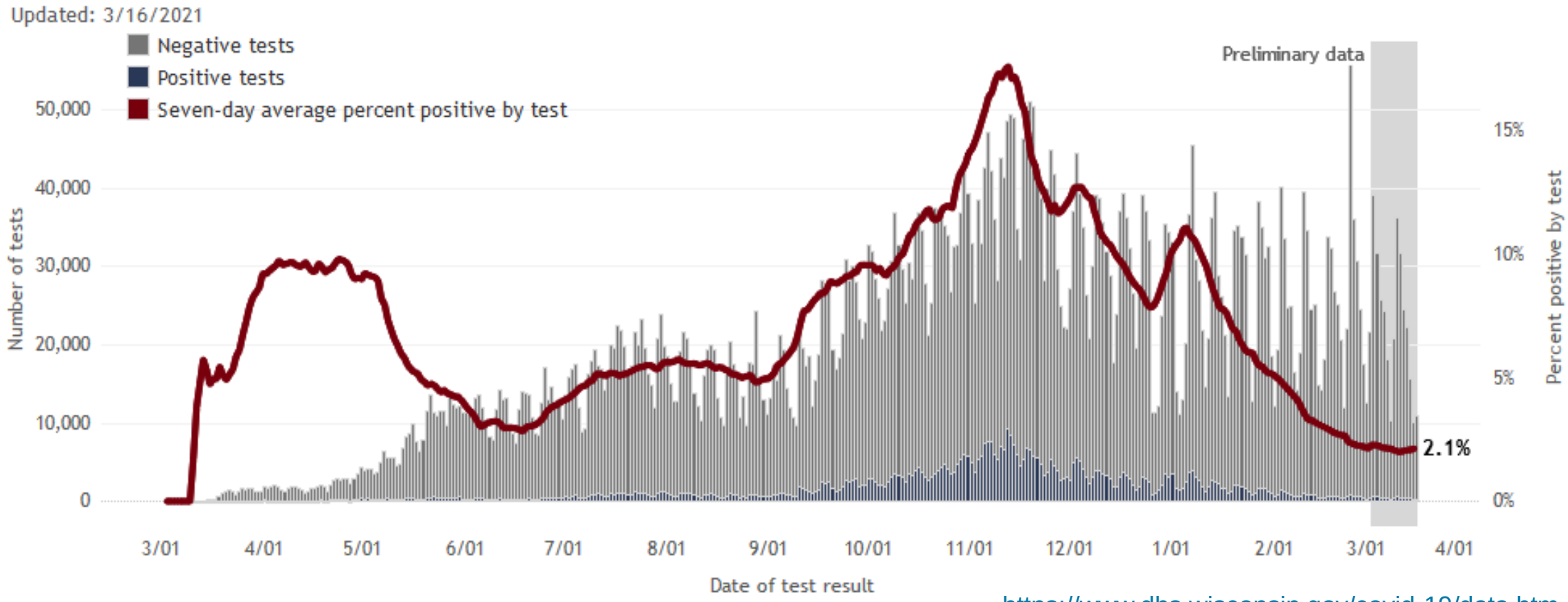
Contents

- How far we've come!
- Questionable results and disease prevalence
- Sequencing for variant surveillance


MARTY, WHATEVER HAPPENS
DONT EVER GO TO 2020!

The places we have been

7-day percent positive by test, total tests by day



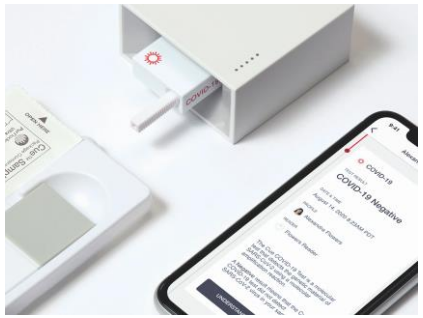
https://www.dhs.wisconsin.gov/covid-19/data.htm

COVID-19 At home tests

(S)=Symptomatic, (A)= Asymptomatic, (E)= Everyone

#	Test	Cost	Run time	Swab types	Device	Performance	Notes
1	Lucira Molecular (LAMP)	$50	30 min	Nasal	Single use device	Sens. 94.1%, Spec. 98% (N=101)	• By Prescription • 14 yrs and up at home, <14 by a clinician • No symptoms requirement • Physician based reporting
2	BinaxNOW Antigen	$25	15 min	Nasal	No device	Within 7 days of symptom onset Sens. 84.6%, Spec. 98.5% (N=460) Within 14 days of symptom onset Sens. 77.2%, Spec. 98% (N=167)	• By prescription • >15 yrs self collection, and 4-14 yrs by parental collection • Test within 7 days of symptom onset • Navica phone App, can take a picture and scan test barcode and report results
3	Ellume Antigen	$30	15 min	Mid-turbinate	Single use device	Sens. 96%, Spec. 100% (N=64) (S) Sens. 91%, Spec. 96% (N=134) (A) Sens. 95%, Spec. 97% (N=198) (E)	• No Prescription, over the counter • Self collected age 16+ or parent collect 2+. • No symptoms requirement • Phone app receives Bluetooth result from device
4	QuickVue Antigen	?	10 min	Nasal	No device	Sens. 84.8%, Spec. 99.1% (N=194)	• By prescription • Test within 6 days of symptom onset • Unobserved self-collection age 14+, adult collection age 8+.
5	Cue Molecular	?	20 min	Nasal	Re-usable device	Sens. 98.7%, Spec. 97.6%	• No Prescription, over the counter • People with or without symptoms as young as 2 years old • Results sent to phone app

Lucira


Cue

BinaxNOW

QuickVue

Ellume

FDA opens door to widespread at-home Covid-19 tests

“The Food and Drug Administration will allow some developers of Covid-19 tests to market their products for regular at-home use without first studying how well the tests perform in people without symptoms.”

“. . .the FDA's new policy takes into account that repeated testing over time, for screening purposes, can improve the overall accuracy of results.”

https://www.politico.com/news/2021/03/16/fda-at-home-covid-tests-476355

Ellume Health

***A lot remains unknown about test reporting or confirmation of results**

FDA Press announcement: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-takes-steps-streamline-path-covid-19-screening-tools-provides

Case

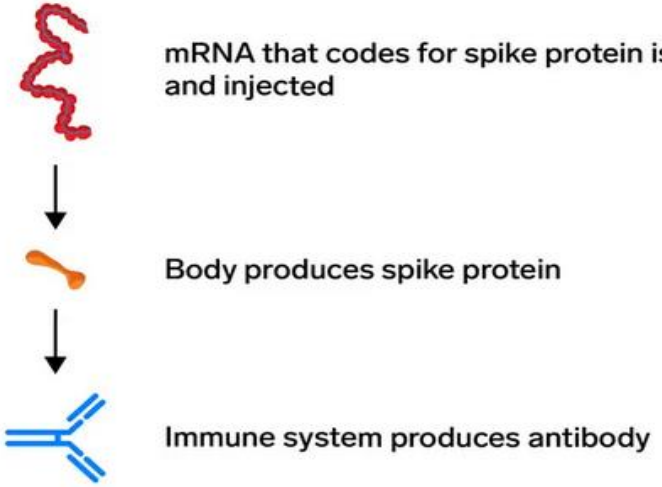
- Staff at a long term care facility are tested every week as part of a federally mandated plan
- Staff and resident vaccination rate is high (>85%)
- A nurse tests positive for SARS-CoV-2 by PCR
- They are vaccinated and have no known COVID-19 contacts or symptoms
- The facility hasn’t had a case in months

Possible reasons for a positive result

- The nurse is infected and a true positive
  - They could be Asymptomatic or Presymptomatic, even if vaccinated

Vaccination and Diagnostic Testing

- It is still possible, although rare, to get COVID-19 after being fully vaccinated
- Vaccines are effective at preventing severe disease and death
- Current vaccines will not cause positive molecular or antigen results



X Molecular test

X Antigen test

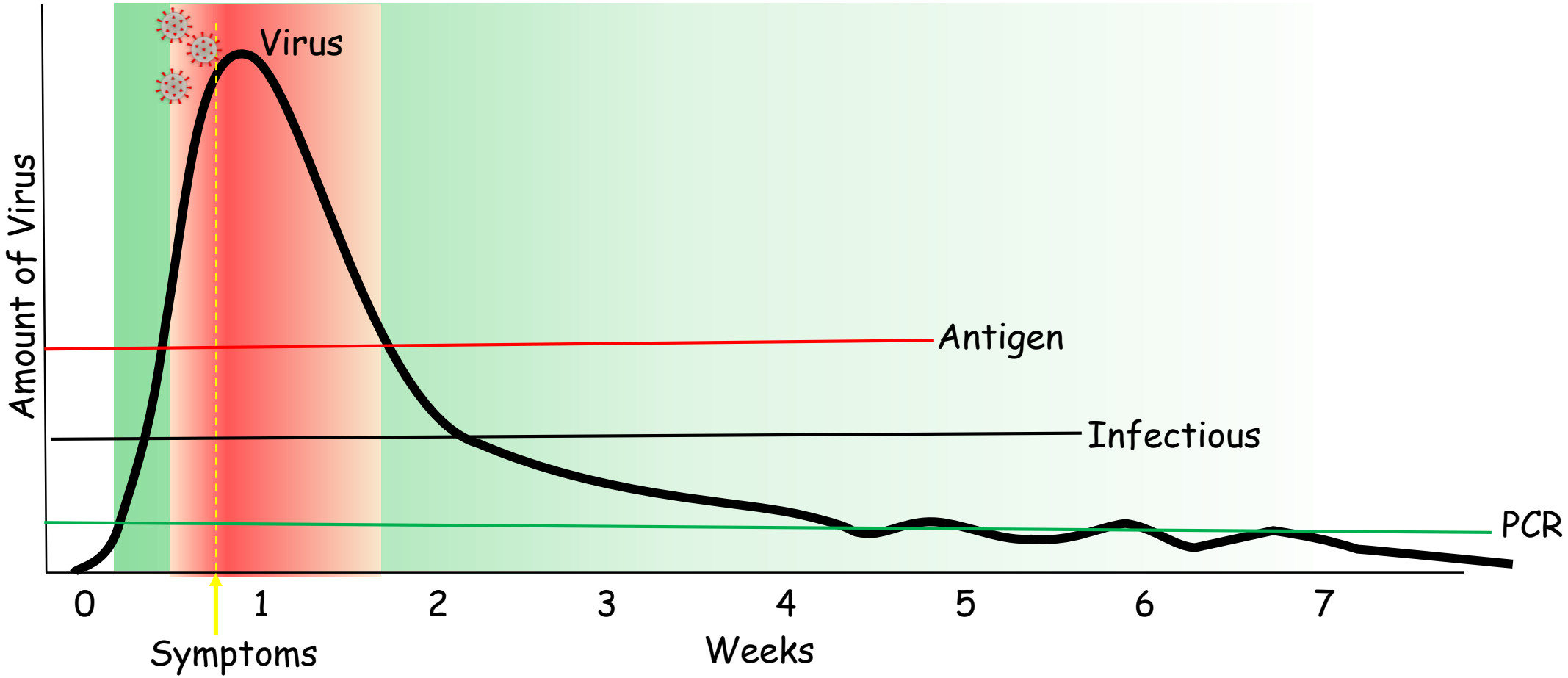
X/✓ Antibody test

https://www.cdc.gov/coronavirus/2019-ncov/vaccines/facts.html

Possible reasons for a positive result

- The nurse is infected and a true positive
  - They could be Asymptomatic or Presymptomatic
  - They could still shedding from a recent infection (long haulers)

Diagnostic Tests and Viral Load



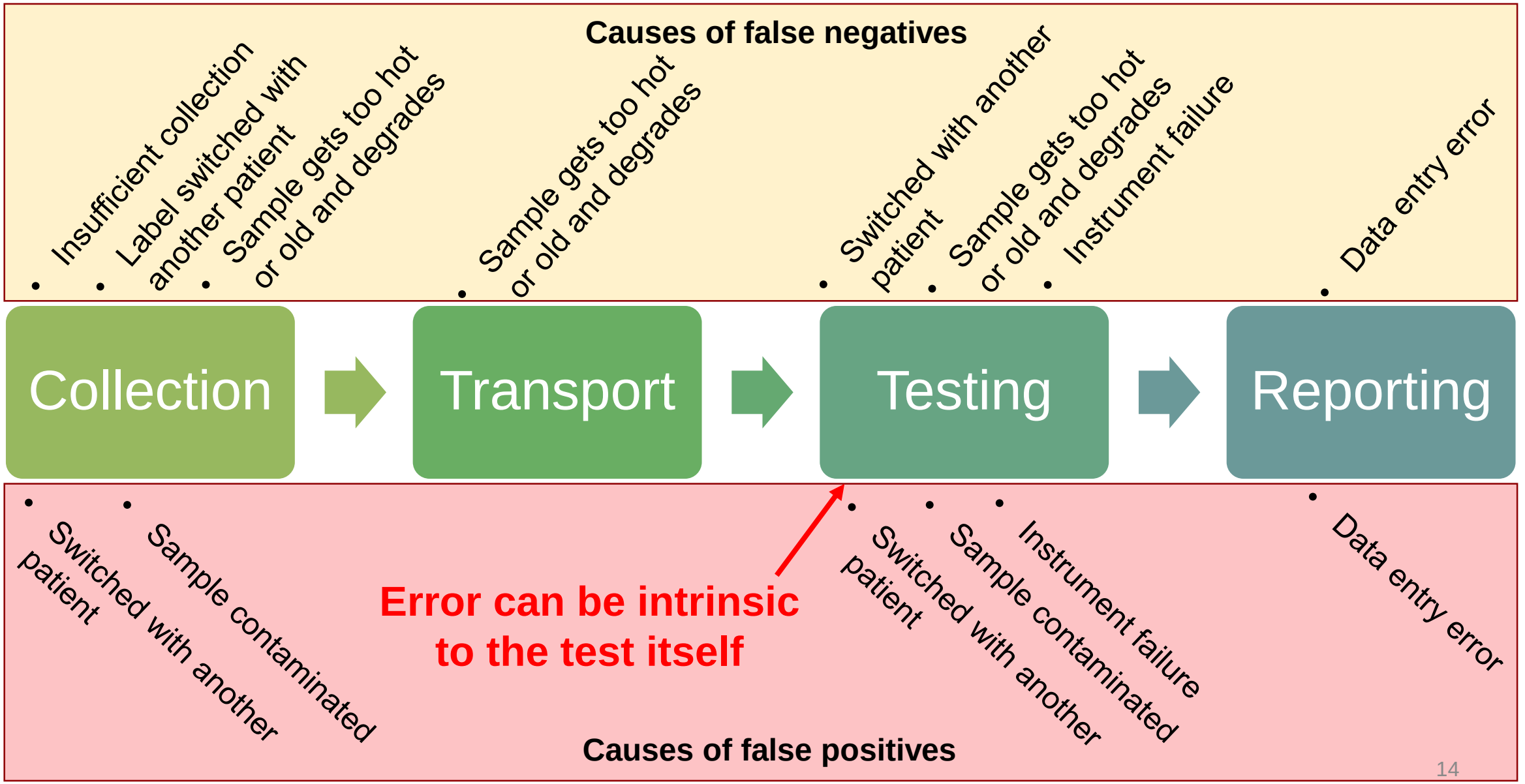
Possible reasons for a positive result

- The nurse is infected and a true positive
  - They could be Asymptomatic or Presymptomatic
  - They could still shedding from a recent infection (long haulers)
- The result was a false positive
  - Human error
  - Mechanical error
  - Random error

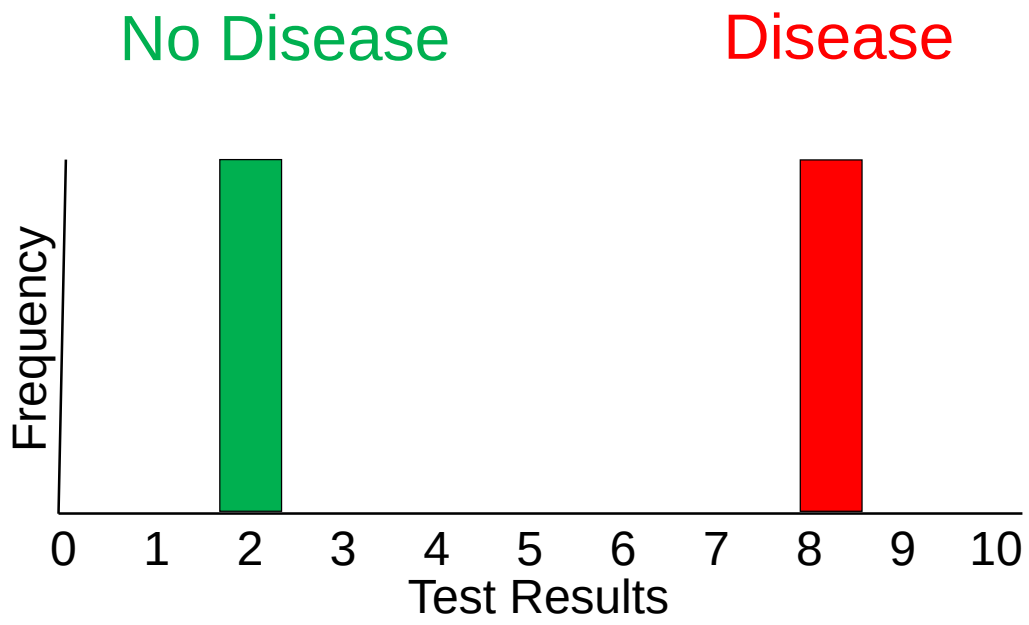
What should you do if you suspect a false positive?

- Do an internal investigation
  - Look for evidence of a specimen mix-up
  - Evaluate runs for mechanical failures
  - Check quality control metrics for signs of errors
  - Look within “runs” for signs of contamination
    - weak+ next to high+
    - Unusually high rate of positives in the run
  - Look for trends in reported false positives
  - Re-test the sample/run if you still have it
  - Do wipe-tests to look for contamination in the lab
  - If a problem is identified amend the report turning the result negative
- Recommend re-testing the patient
  - While additional molecular testing can support a positive diagnosis a negative result cannot erase the first positive
  - Two negative molecular tests, collected at least 24 hours apart, can release someone from isolation. But, they remain a recorded case.

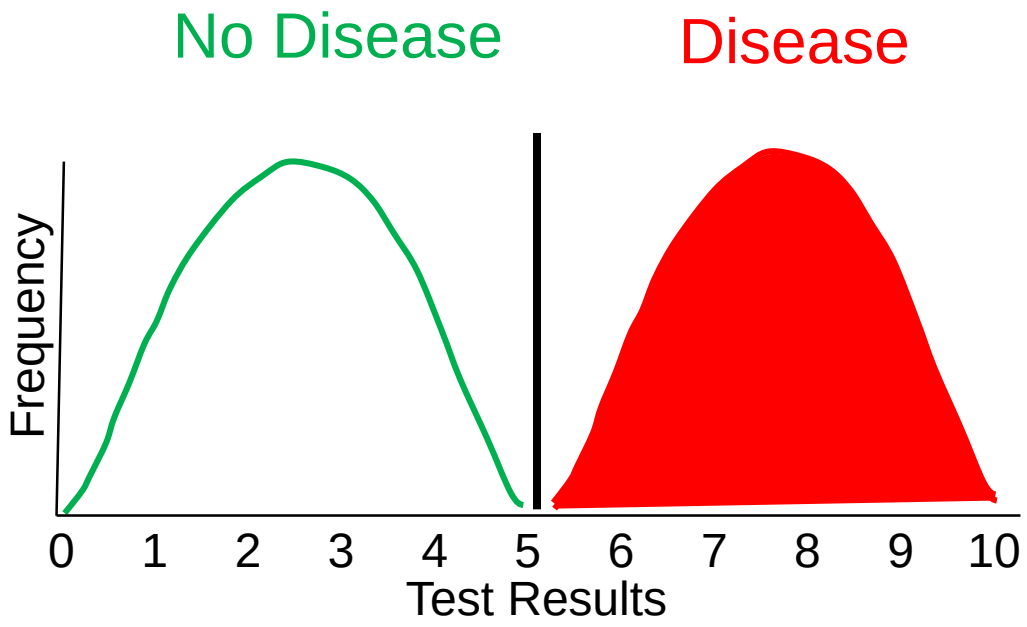
Causes of false results



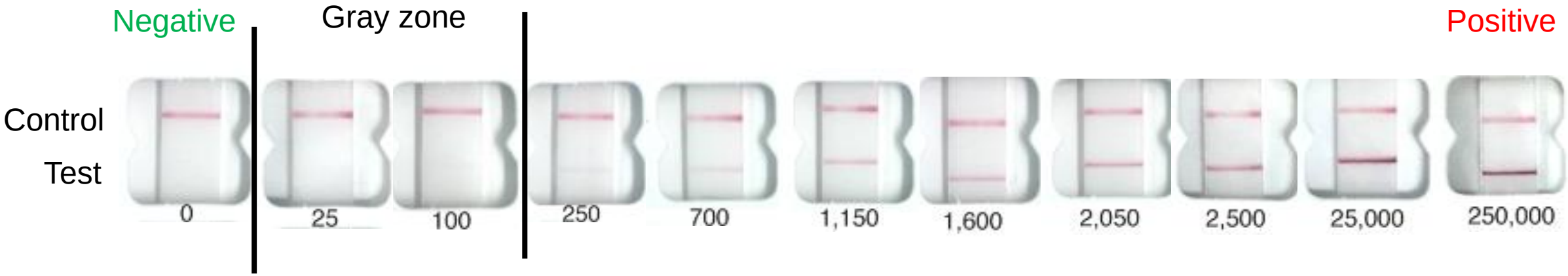
It’s it easy to tell them apart?

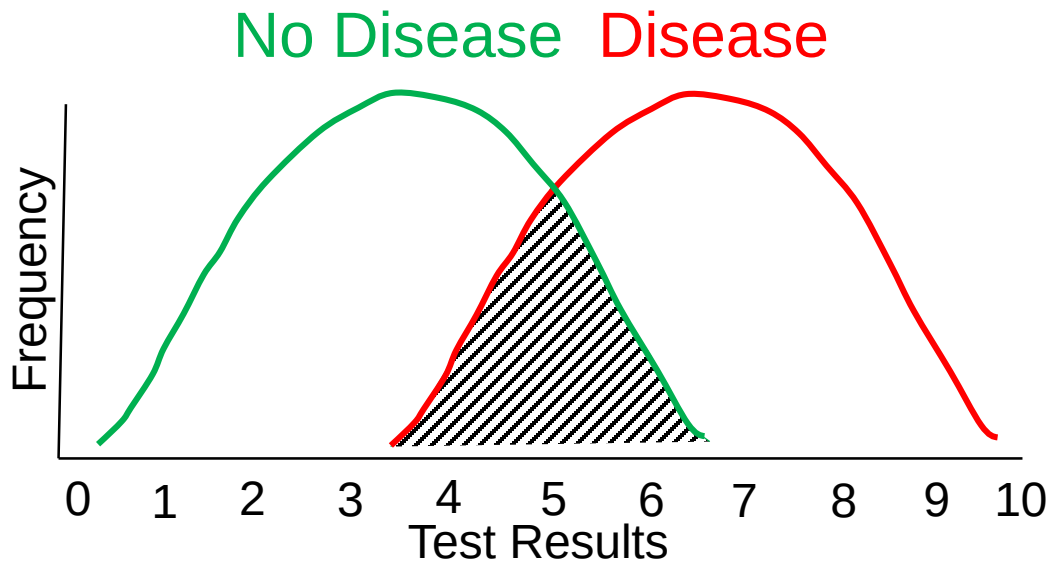


Biology Means Diversity

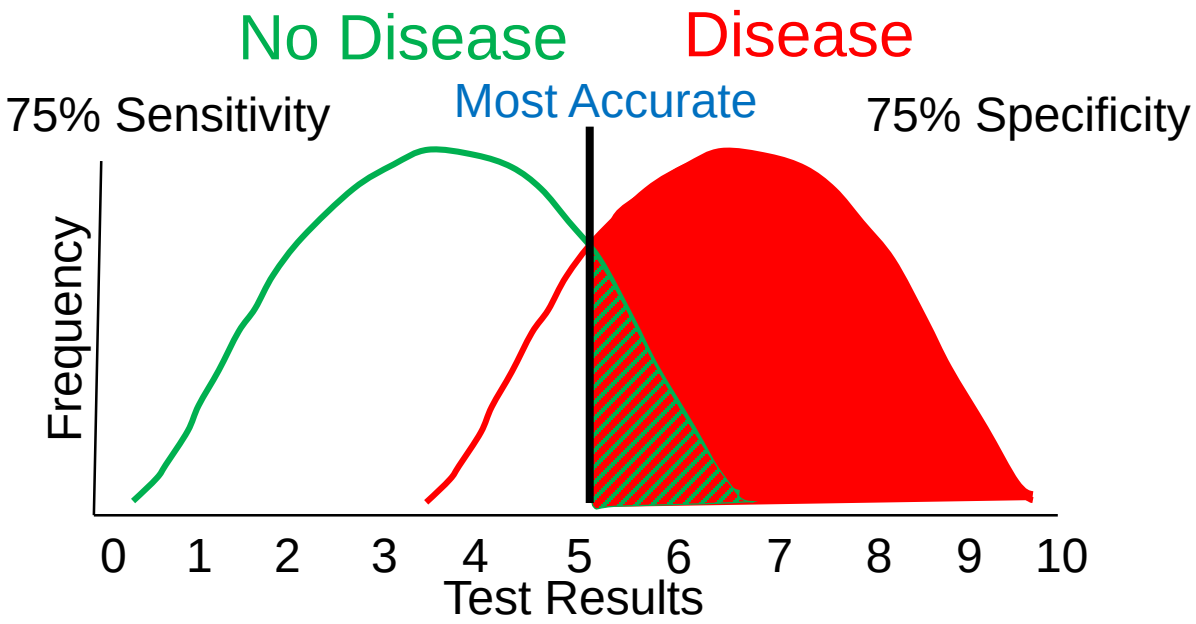


Finding the Gray Zone





Back to Basics



Sensitivity- How good is the test at detecting positives?
Specificity- How good is the tests at distinguishing true positives from false positives?
Accuracy- How good the test is overall at giving a correct diagnosis

Calculating Test Performance
Understanding the Chart

Truth

Test Results

	Patients **with** Disease	Patients **without** Disease	**Total test results**	
Positive Test	True positive	False positive	Total positive tests	PPV=% of positive results in people with disease
Negative Test	False negative	True negative	Total negative tests	NPV=% of negative results in people without disease
Total	Total people with disease	Total people without disease	Total People tested	

Sensitivity
(% of people with disease that have a positive test)

Specificity
(% of people without disease that have a negative test)

Prevalence= % of people tested that have disease

Calculating Test Performance

	Patients **with** Disease	Patients **without** Disease	**Total test results**
Positive Test	A % sensitivity x E	B (1-% specificity) x F	A+B
Negative Test	C (1-% sensitivity) x E	D % specificity x F	C+D
Total	E % prevalence x G	F (1-% prevalence) x G	G # of people tested

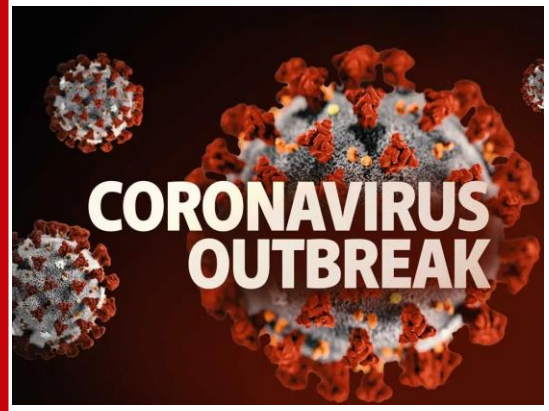
$$PPV = \frac{A}{(A+B)} \times 100$$

$$NPV = \frac{D}{(C+D)} \times 100$$

What you need to know

- Prevalence of the disease in the people you are testing
- The sensitivity and specificity of your test

Example 1: Testing a facility using a PCR test during an outbreak



	Patients **with** Disease	Patients **without** Disease	**All Patients**
Positive Test	147	4 False positive	151
Negative Test	3 False negative	846	849
Total	150	850	1000

PPV
97%

NPV
99%

98% Sensitivity

99.5% Specificity

15% Prevalence

Example 2: Routine testing by PCR of vaccinated employees who wear masks and distance

	Patients **with** Disease	Patients **without** Disease	**All Patients**
Positive Test	4.9	5 False positive	9.9
Negative Test	0.1 False negative	990	990.1
Total	5	995	1000

PPV
49%

NPV
99.9%

98% Sensitivity

99.5% Specificity

0.5% Prevalence

A test is only as good as the population tested

PPV/NPV Calculator

Prevalence	Sensitivity	Specificity
10%	**97%**	**85%**

Survey Question

Would you be interested in a tool/app that does the math for you?

A. No, thanks. I've got this!

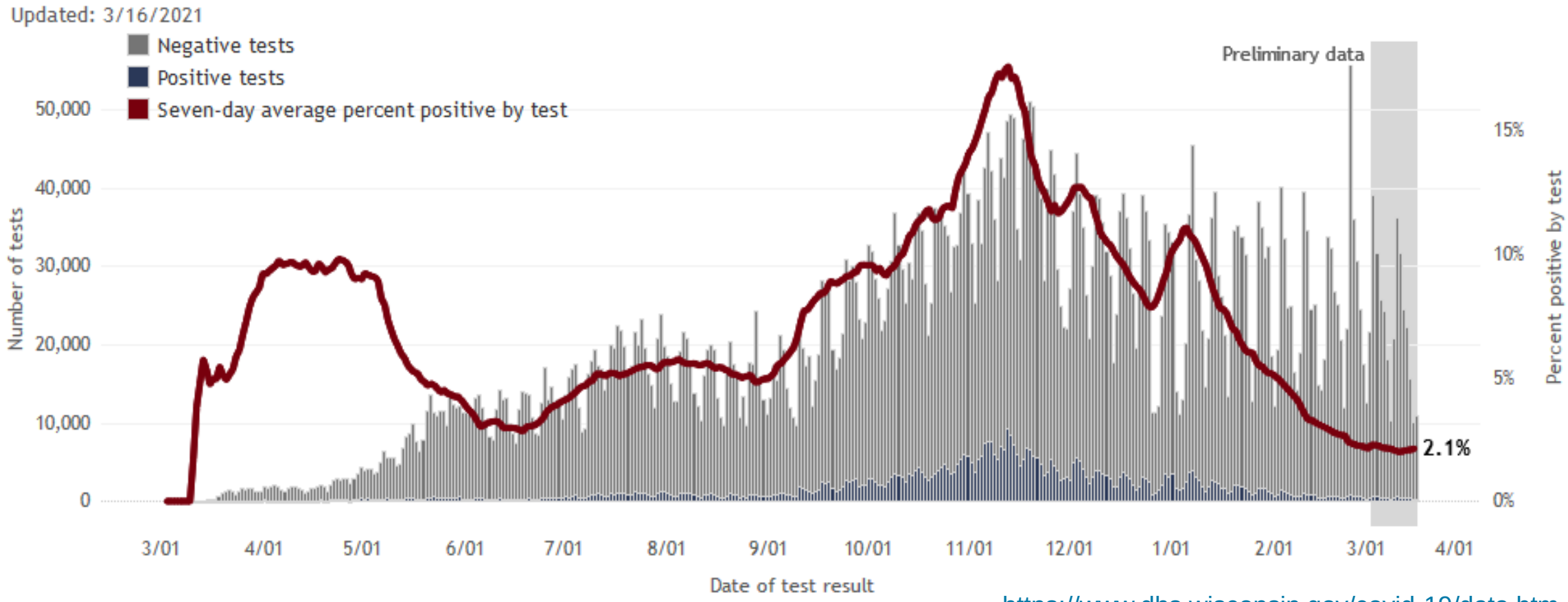
B. Eh, maybe?

C. Yes, please!

D. OMG, YES! I will share it with everyone!!!

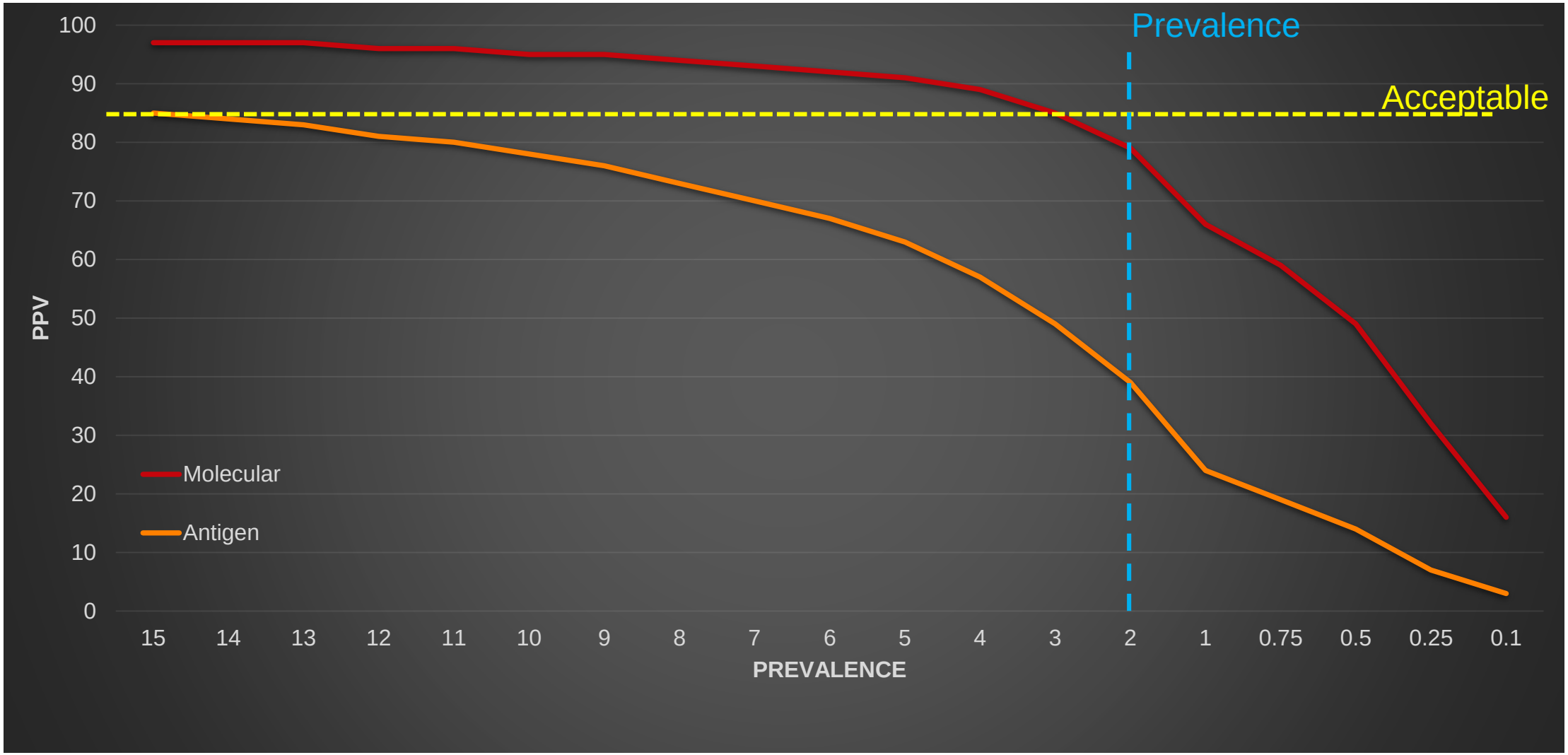
Prevalence has Dropped

7-day percent positive by test, total tests by day



https://www.dhs.wisconsin.gov/covid-19/data.htm

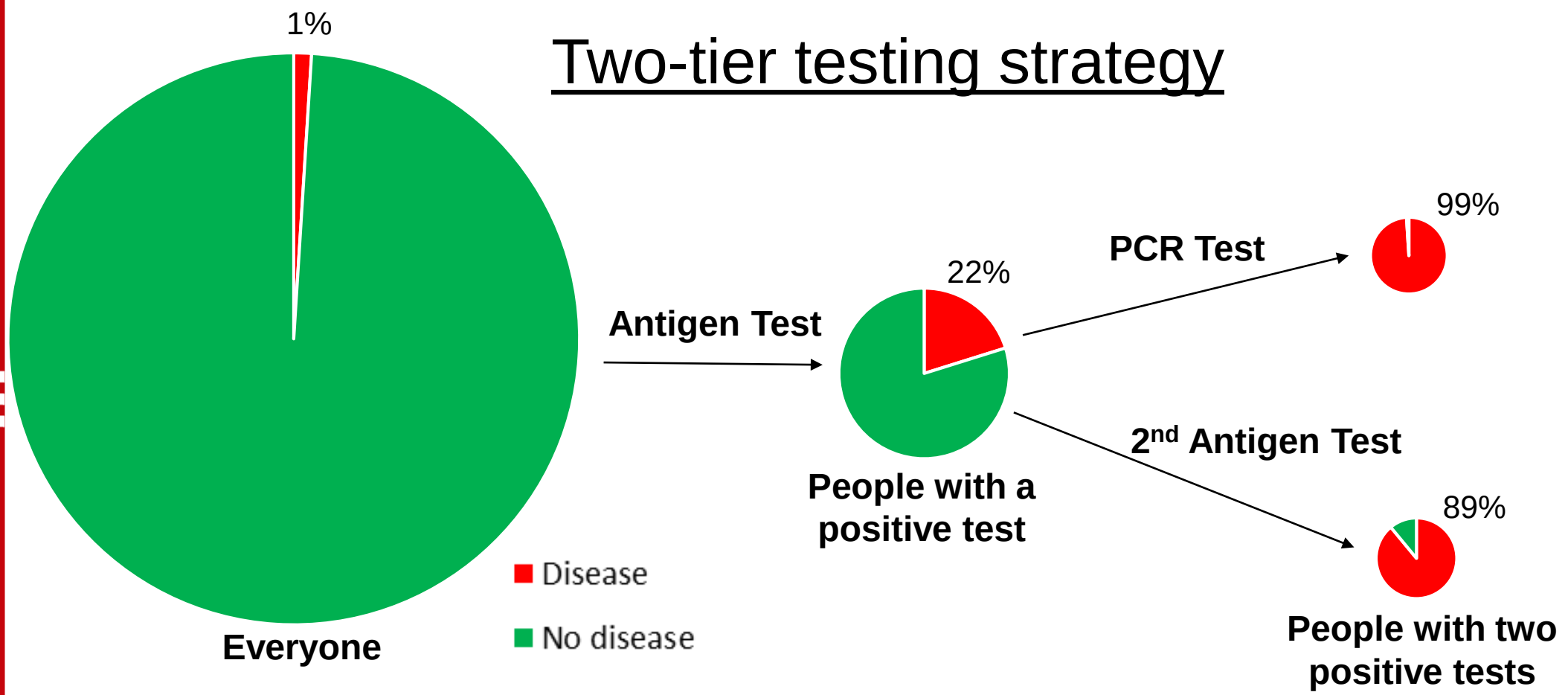
How Dropping Prevalence Impacts PPV



*Based on PCR with 99.5% specificity and antigen with 97% specificity.

How can we maintain confidence in testing?

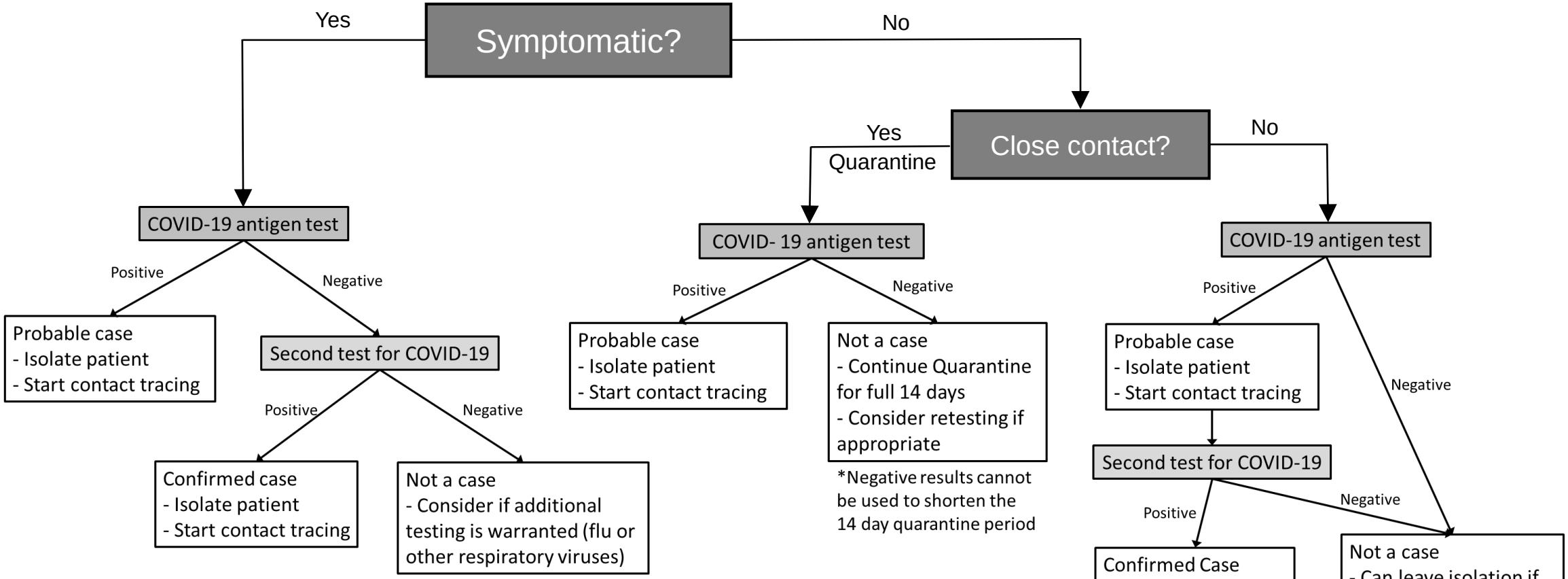
Two-tier testing strategy



Pro tip: If you use a 2 tiered testing system the first test should be the most sensitive, the second should be highly specific to produce the greatest accuracy.

Testing Strategy for Lower Accuracy Tests

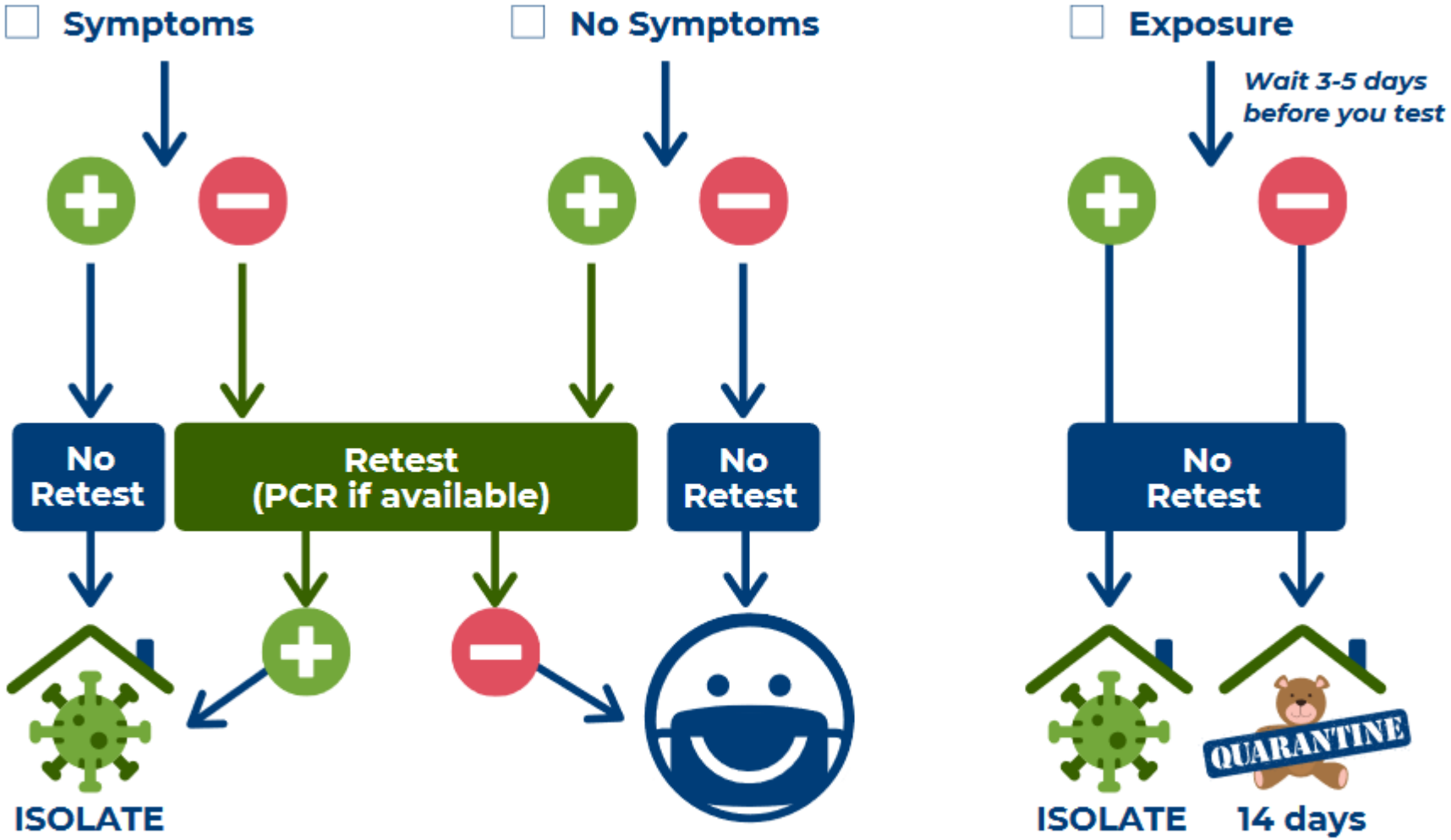
(most point of care tests)



Note: Confirmatory testing should be performed as soon as possible and within 48 hours. PCR as the second test is highly preferred for the greatest accuracy. Antigen tests can only result in probable cases, not confirmed cases.

DHS Guidance- HAN #17 https://content.govdelivery.com/accounts/WIDHS/bulletins/2a24fd9

Testing strategy for lower accuracy tests



For more information on COVID-19 testing in Wisconsin, visit:
www.dhs.wisconsin.gov/testing

What the right amount of testing?

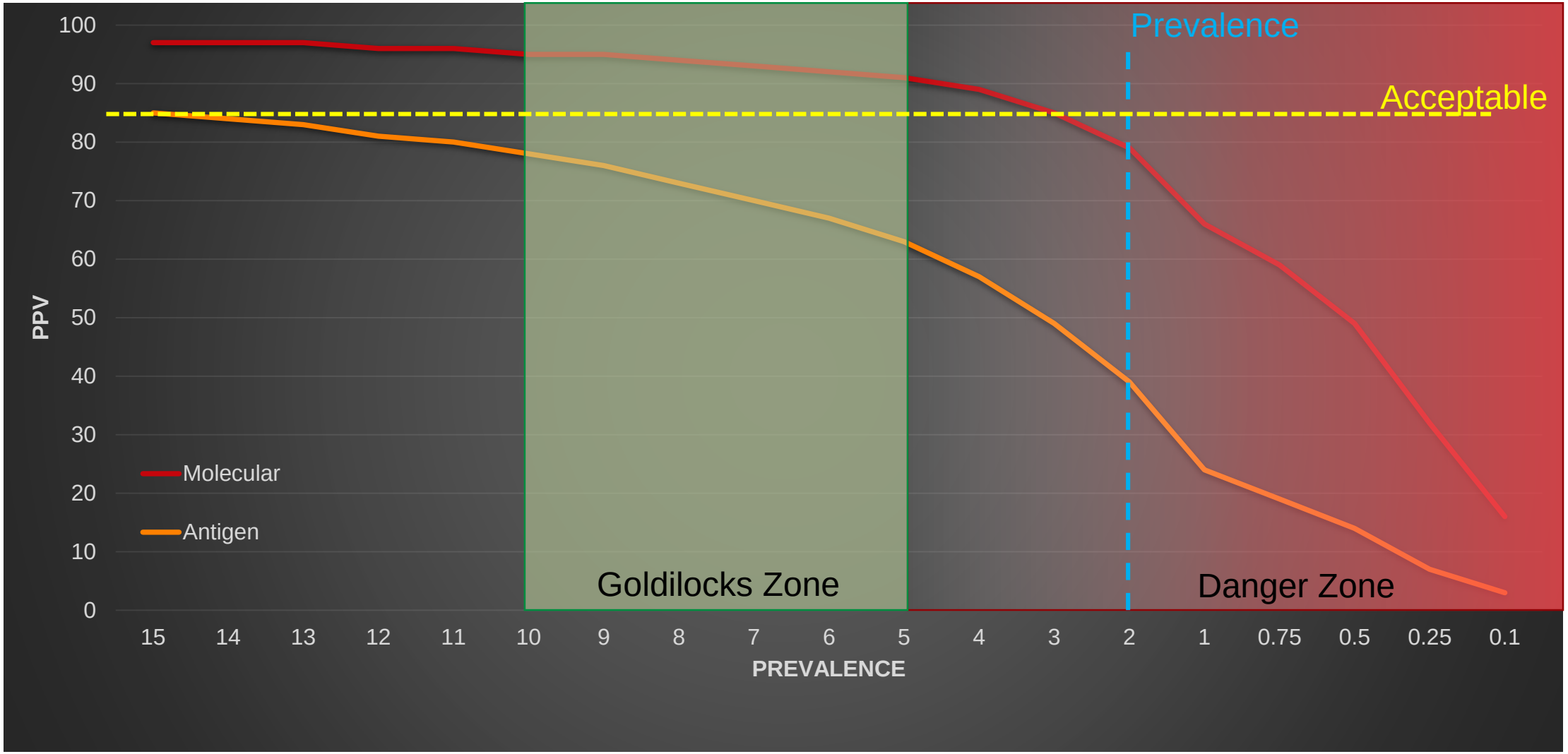
- Depends on the goal
  - Identify all cases
  - Monitor for new variants
  - Understand disease prevalence

- Aim to have 5-10% of tests be positive
  - Too high and you might be missing positives
  - Too low and positives will be more likely to be false

Adjusting Prevalence

- Prevalence is about who you are testing which can be changed by targeting different patient populations
- Ways to increase prevalence (too many false positives)
  - Only test people at higher risk of disease
    - Congregate settings
    - Outbreak investigations
    - Close contacts
    - People with symptoms of COVID-19
- Ways to decrease prevalence (missing too many cases)
  - Test more people
  - Test the same people more often

How Dropping Prevalence Impacts PPV



*Based on PCR with 99.5% specificity and antigen with 97% specificity.

Symptomatic

Example 2: ~~Routine~~ testing by PCR of vaccinated employees who wear masks and distance

	Patients **with** Disease	Patients **without** Disease	**All Patients**
Positive Test	147	4 False positive	151
Negative Test	3 False negative	846	849
Total	150	850	1000

PPV
97%

NPV
99%

98%
Sensitivity

99.5%
Specificity

15% Prevalence

Example 3: ~~Routine~~ antigen testing of unvaccinated school kids who wear masks

Symptomatic

	Patients **with** Disease	Patients **without** Disease	**All Patients**
Positive Test	148	20 False positive	168
Negative Test	2 False negative	830	832
Total	150	850	1000

PPV 88%

NPV 99.7%

98.7% Sensitivity

97.6% Specificity

15% Prevalence

Example 3: Routine testing by antigen test of unvaccinated school kids who wear masks

with two-tier testing

Test #1 (antigen)

	Patients **with** Disease	Patients **without** Disease	**All Patients**	
Positive Test	19.7	23.5 False positive	43.2	PPV 45.6%
Negative Test	0.3 False negative	956.5	956.8	NPV 99.9%
Total	20	980	1000	

98.7% Sensitivity

97.6% Specificity

2% Prevalence

Test #2 (PCR)

	Patients **with** Disease	Patients **without** Disease	**All Patients**	
Positive Test	19.3	0.1 False positive	19.4	PPV 99.5%
Negative Test	0.4 False negative	23.4	23.8	NPV 98.3%
Total	19.7	23.5	43.2	

98% Sensitivity

99.5% Specificity

45.6% Prevalence

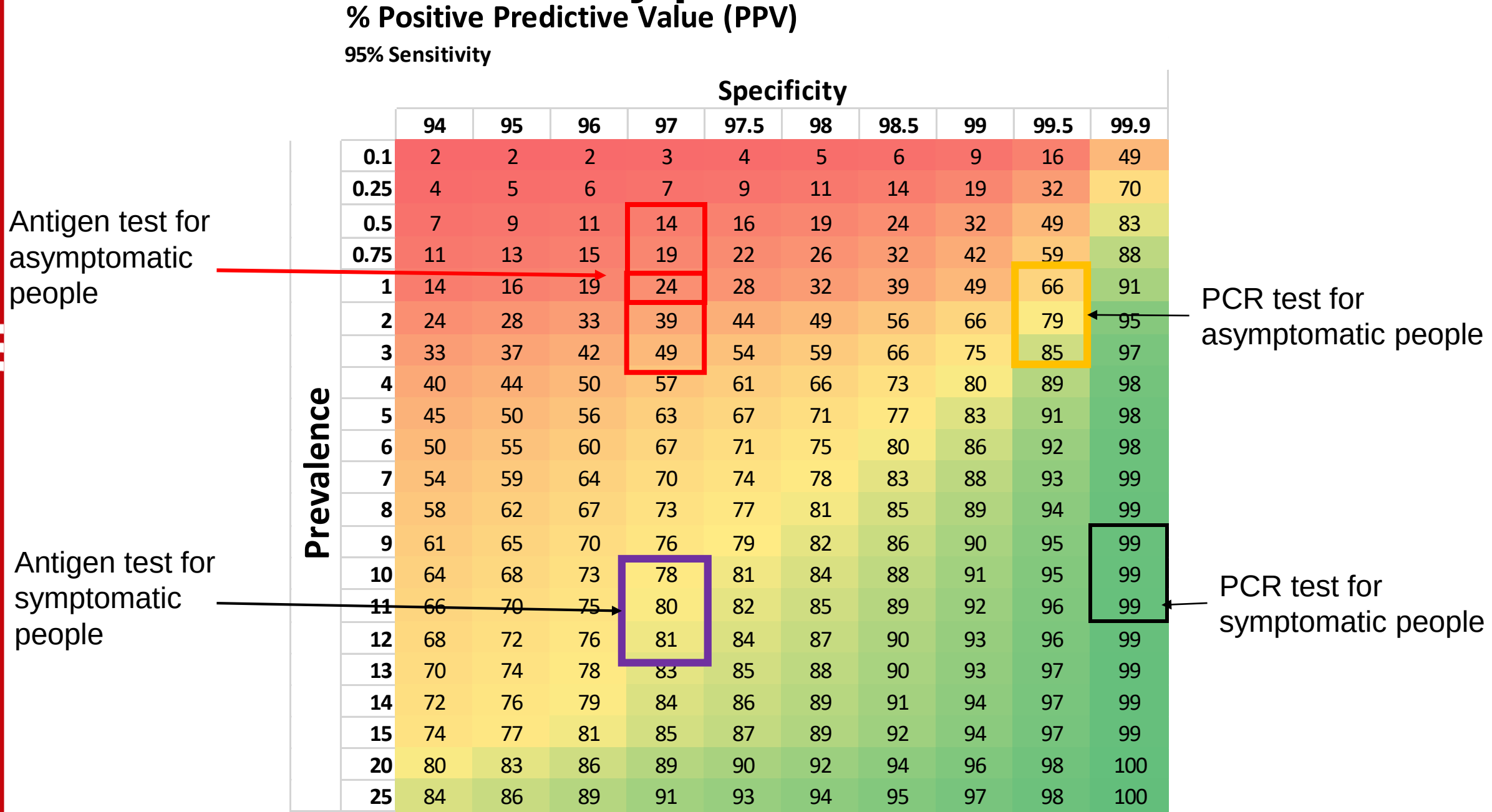
Summary

- No test is perfect, errors will happen
- The error rate goes up as prevalence goes down
- Prevalence in WI has dropped a lot
- Strategically altering the prevalence in the people you test allows for greater accuracy

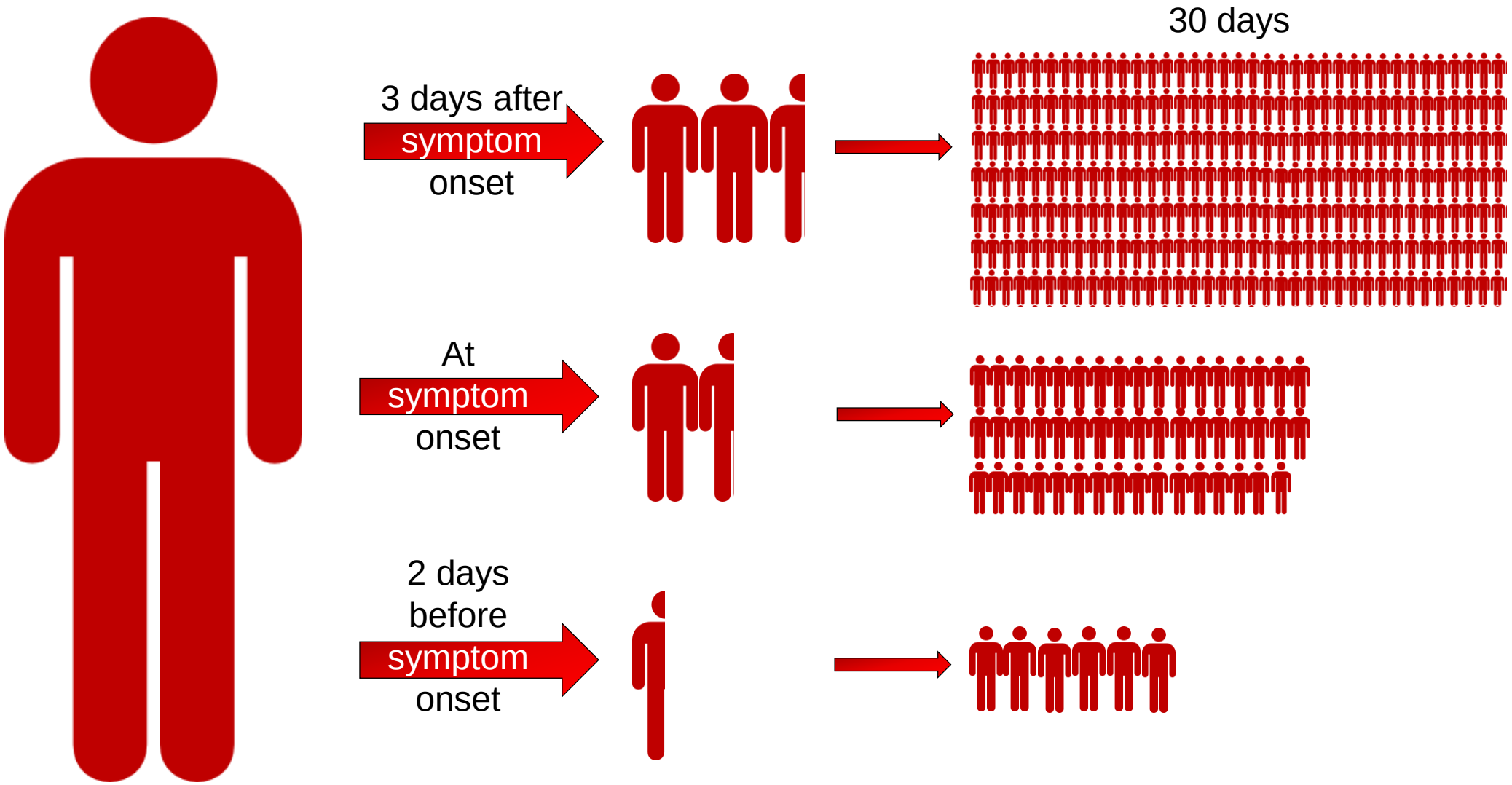
What the right amount of testing?

- Right size testing developed for influenza epidemic
  - Provides a number of tests per capita for adequate surveillance
  - Considers
    - Population size
    - Transmissibility of the virus
    - Current prevalence based on illness reports in clinics
  - 3000 symptomatic people/day to detect novel strains with 95% confidence
    - Currently testing ~30,000 people/day

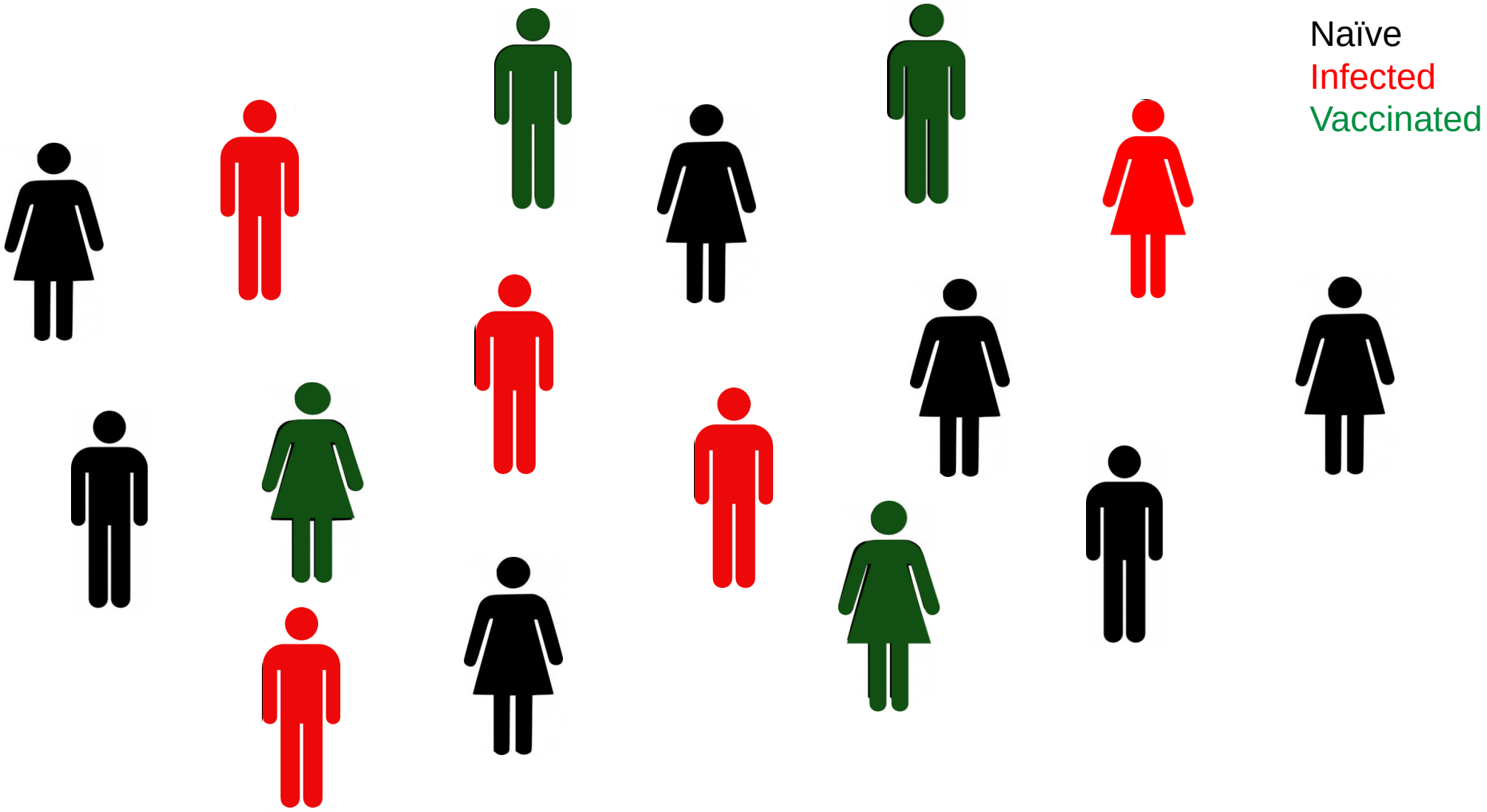
What are the chances my positive test result is correct?



The Power of Early Detection



Natural Infection and Vaccination provide protection



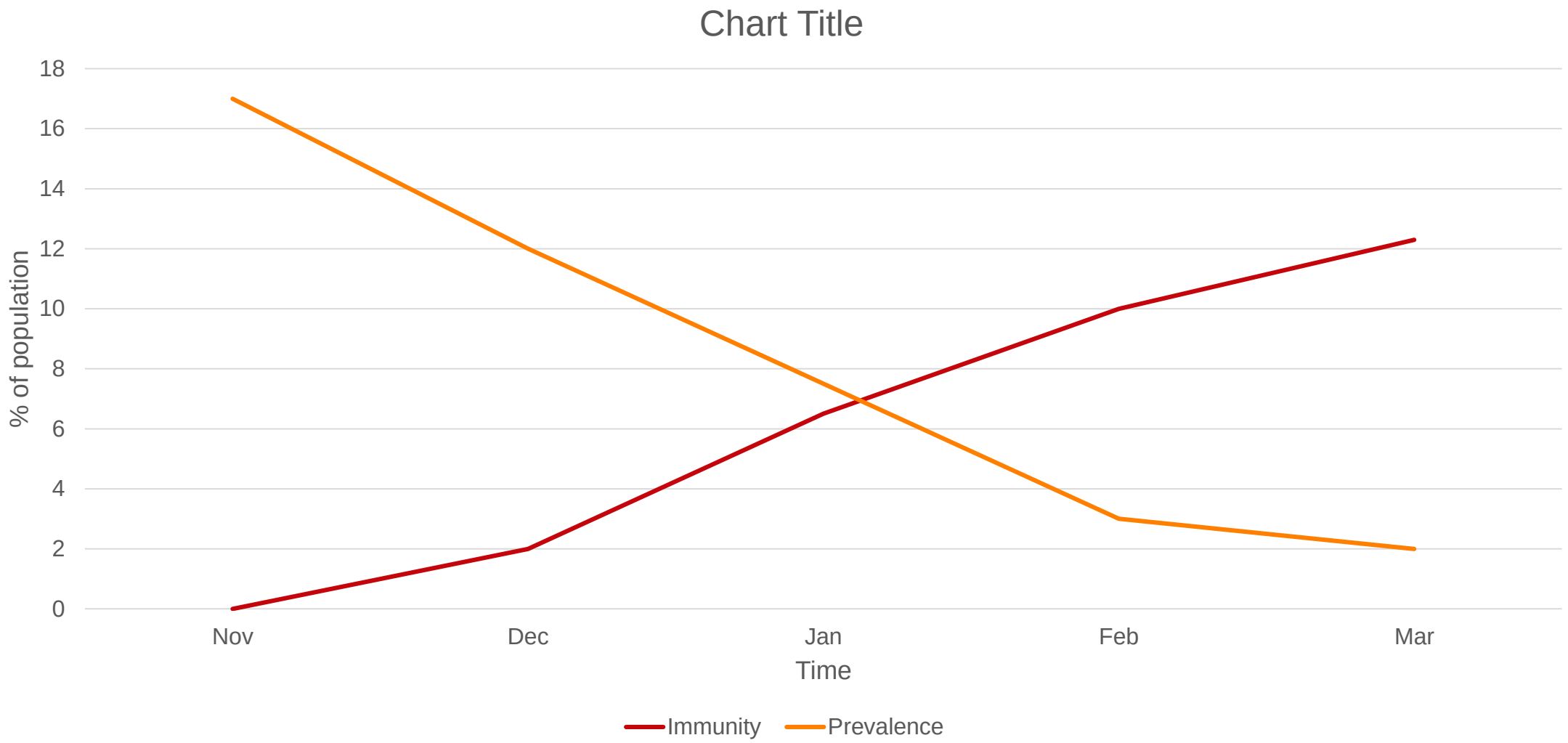
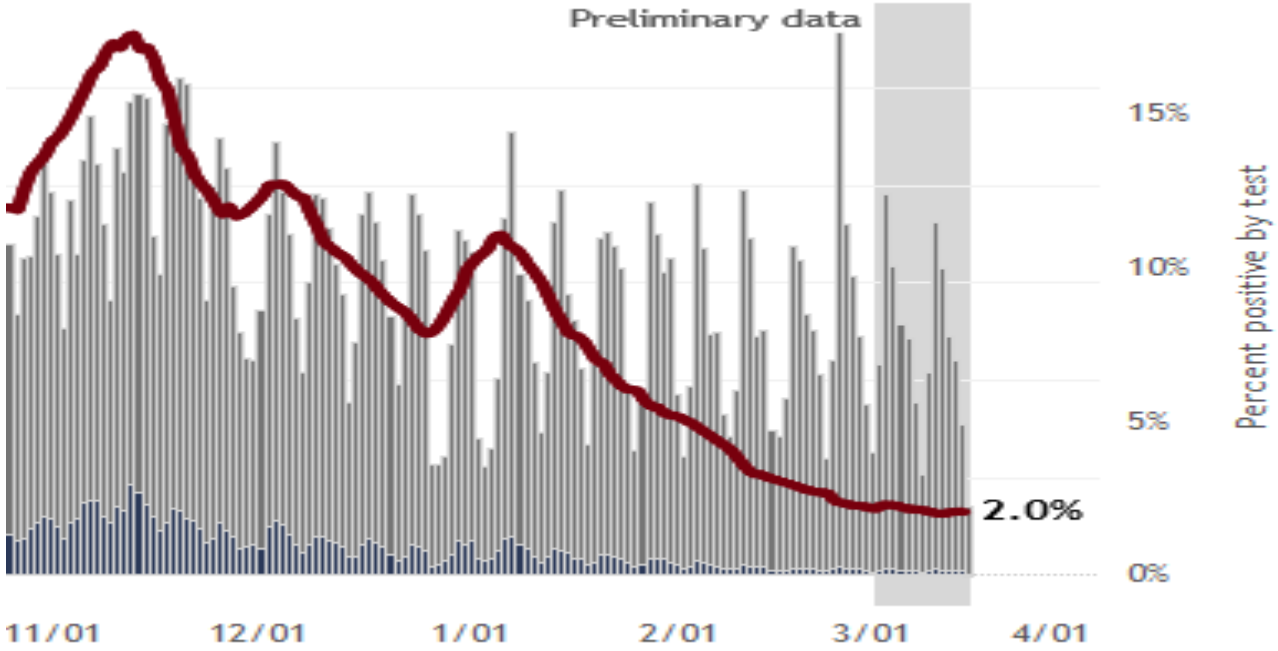
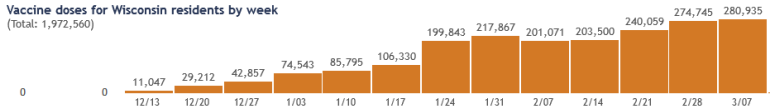


Chart Title
18
16
14
12
10
8
6
4
2
0
% of population
Nov
Dec
Jan
Feb
Mar
Time
Immunity
Prevalence


Preliminary data
15%
10%
5%
0%
Percent positive by test
2.0%
11/01
12/01
1/01
2/01
3/01
4/01


Vaccine doses for Wisconsin residents by week
(Total: 1,972,560)
0
0
11,047
29,212
42,857
74,543
85,795
106,330
199,843
217,867
201,071
203,500
240,059
274,745
280,935
12/13
12/20
12/27
1/03
1/10
1/17
1/24
1/31
2/07
2/14
2/21
2/28
3/07

What should you do if you suspect a false positive?

- Notify the lab
  - They can do an internal evaluation for a problem
    - Look for trends in reported false positives
    - Look within “runs” for signs of contamination
    - Do wipe-test to look for contamination in the lab
    - Look for evidence of a specimen mix-up
    - Evaluate runs for mechanical failures
    - Check quality control metrics for signs of errors
    - Re-test the sample if they still have it (1-7 days)
  - If a problem is identified they can amend the report turning the result negative
- Re-test the patient
  - While additional molecular testing can support a positive diagnosis a negative result cannot erase the first positive
  - Two negative molecular tests, collected at least 24 hours apart, can release someone from isolation. But, they remain a recorded case.

Sequencing can not be used for patient management

CLIA SARS-CoV-2 Variant Testing Frequently Asked Question
Date: 3/10/2021

Does a facility that performs surveillance testing to identify SARS- CoV-2 genetic variants need a CLIA certificate?

CMS is temporarily exercising enforcement discretion under CLIA for SARS-CoV-2 genetic variant testing on identified specimens in which patient-specific results are reported to State or local Public Health Departments. As defined by Centers for Disease Control and Prevention (CDC), public health surveillance testing for SARS-CoV-2 is intended to monitor community- or population-level outbreaks of disease, or to characterize the incidence and prevalence of disease. Public health surveillance testing is performed on de-identified specimens, and thus results are not linked to individuals. Public health surveillance testing cannot be used for individual decision-making. See CDC's Testing Strategies for SARS-CoV-2 (Frequently Asked Questions about Coronavirus (COVID-19) for Laboratories).

https://www.cms.gov/files/document/clia-sars-cov-2-variant.pdf