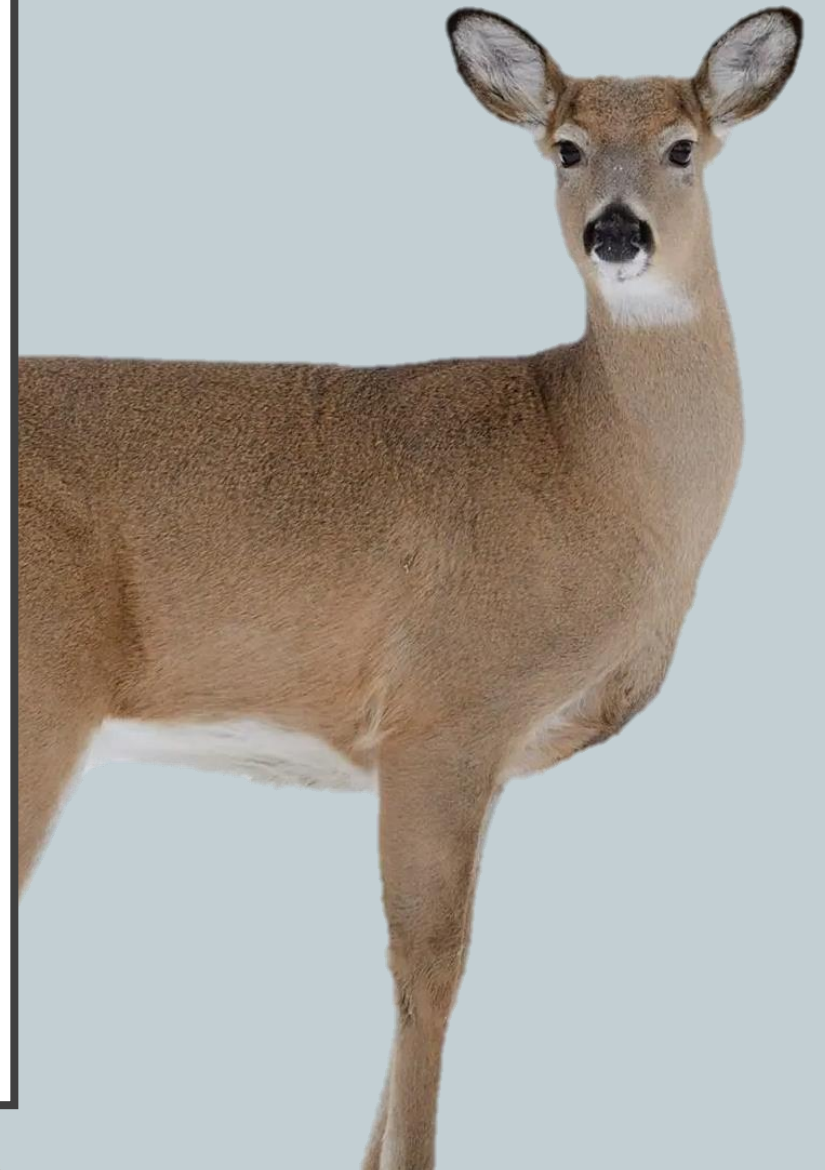


DIAGNOSTIC TIPS:

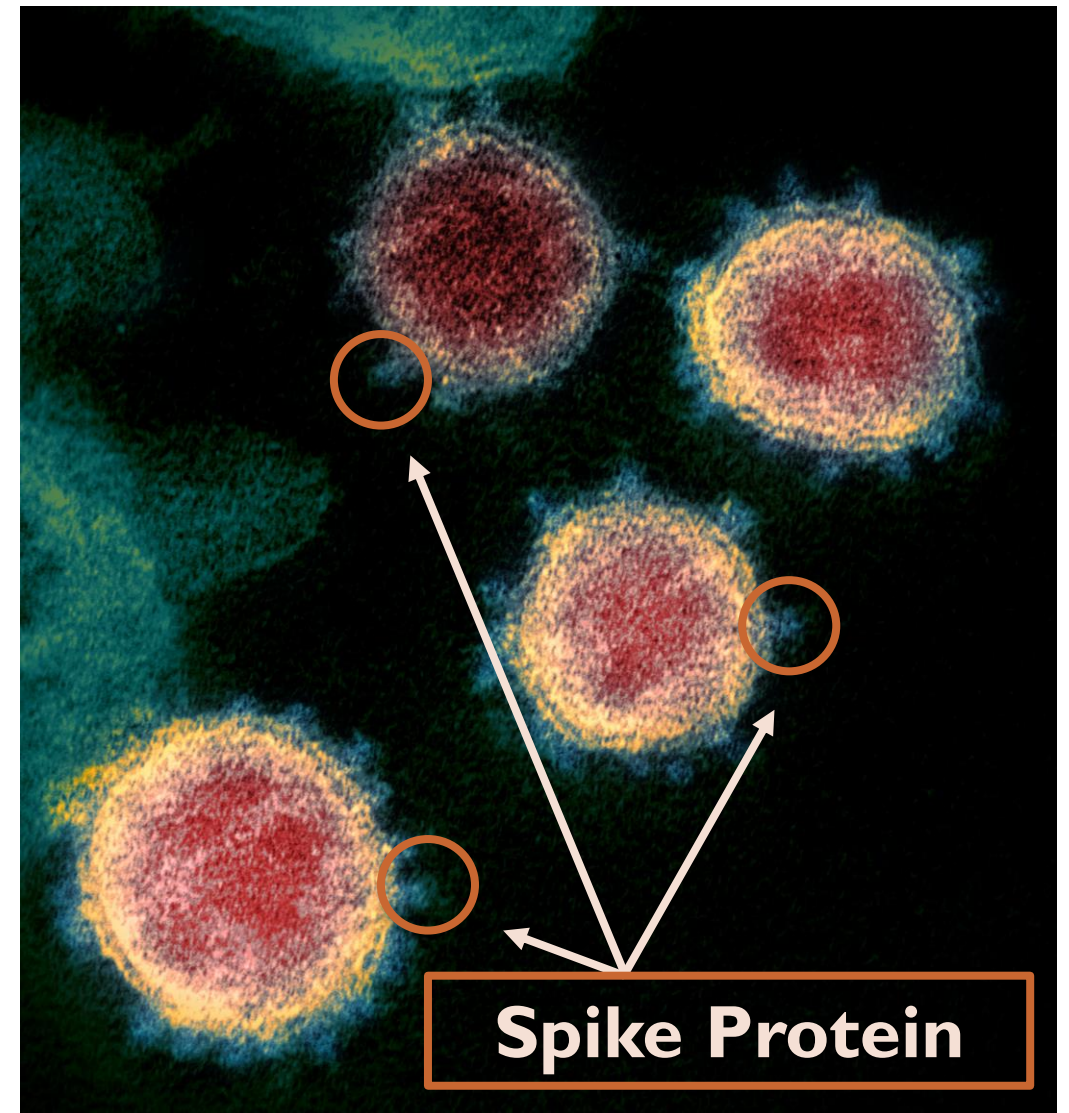
PERFORMANCE OF VARIOUS SARS-COV-2 DETECTION METHODS IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*)

RAQUEL FRANCISCO, SONIA M. HERNANDEZ,
ETHAN BARTON, MELANIE R. KUNKEL, KAYLA
ADCOCK, DANIEL G. MEAD, MICHAEL J. YABSLEY



CORONAVIRUSES

- Enveloped +ssRNA Virus
- Family: Coronaviridae
 - Subfamily: Orthocoronavirinae
 - Alpha, Beta, Gamma, Delta
- Spike Protein
 - Binding and Entry
 - Tissue Trophism



SARS-COV-2 IN WTD

- Highly susceptible
 - Become infected
 - Shed virus
 - ~3 to 5 days
- Develop neutralizing antibodies



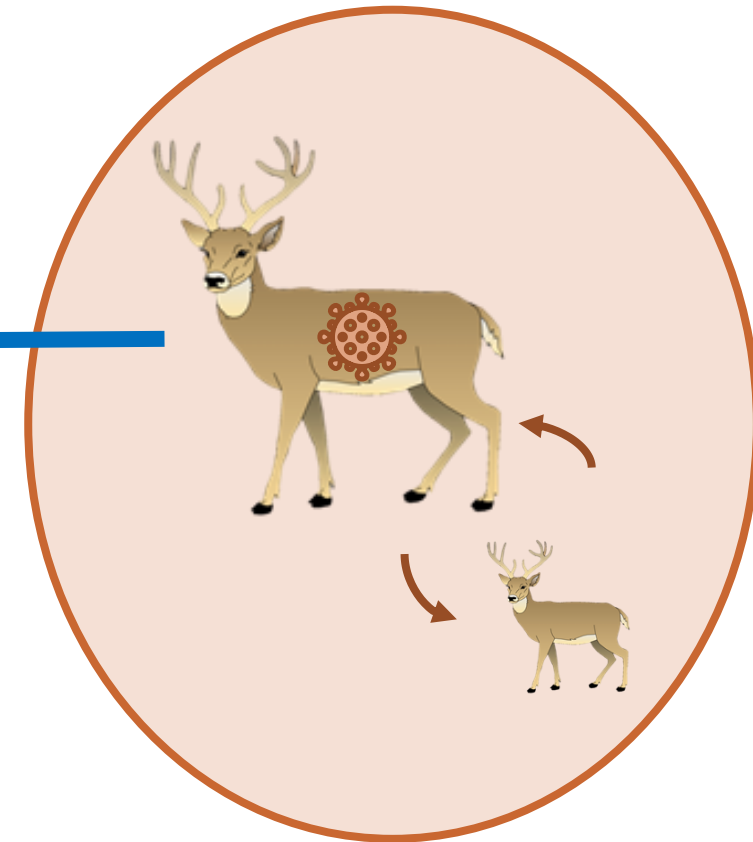
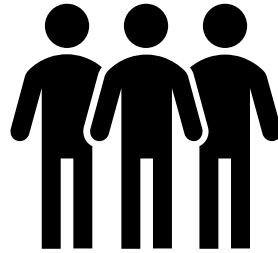
SARS-COV-2 IN WTD

- Surveillance in free-roaming populations
 - Reactive
 - Opportunistic
 - Focused around CWD protocols



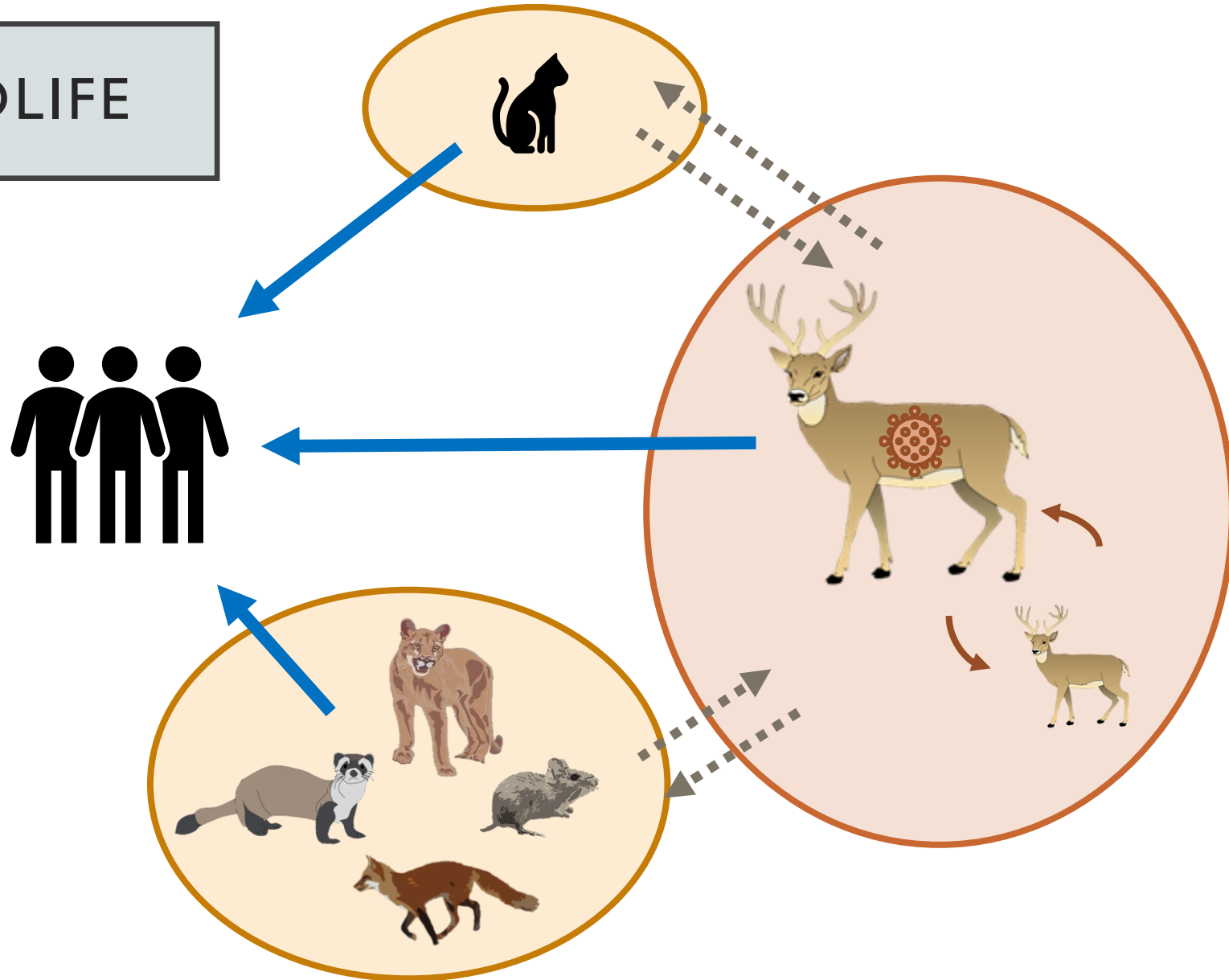
CONCERNS FOR WILDLIFE

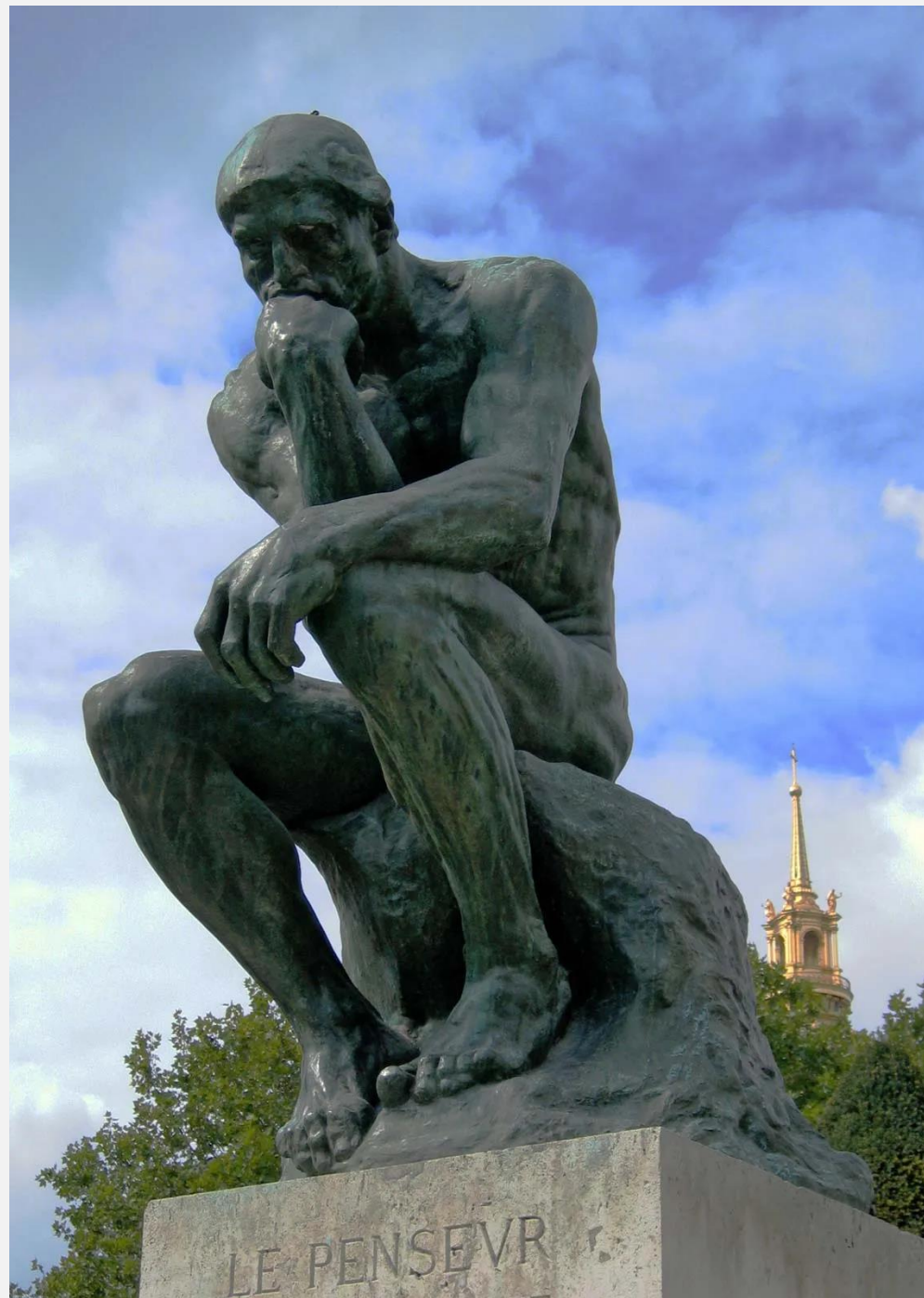
- Sylvatic Cycle
 - Reservoir
 - Zoonosis



CONCERNS FOR WILDLIFE

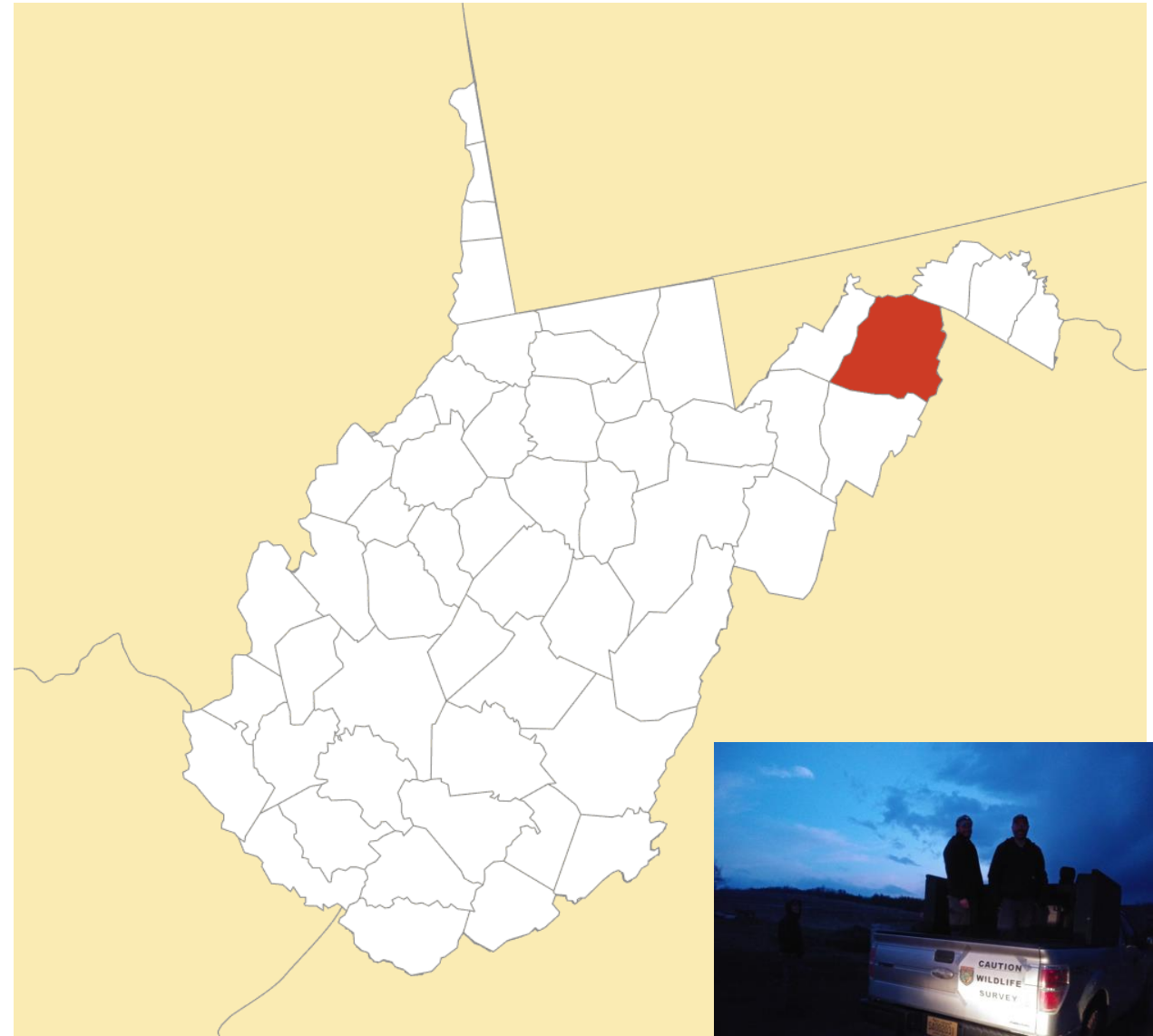
- Sylvatic Cycle
 - Reservoir
 - Zoonosis
 - Intermediate Hosts?





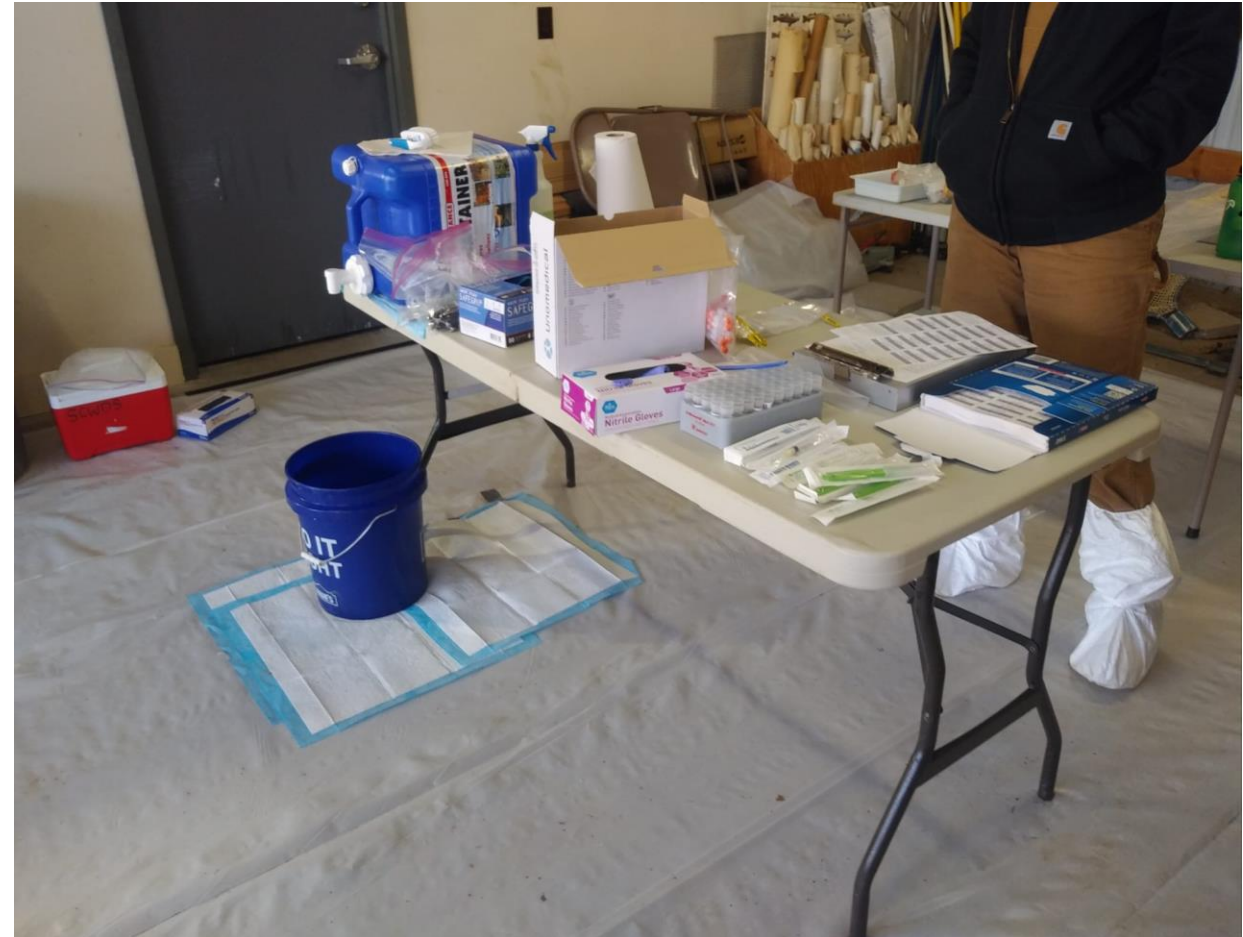
STUDY

- **Hampshire County, WV**
 - March 2022 to April 2022
- Targeted WTD cull
 - CWD endemic area
- Target of ~100 deer



STUDY

- 83 WTD Harvested
 - 6 samples per deer
 1. Retropharyngeal lymph nodes (RPLN)
 2. Tonsils
 3. Bilateral nasopharyngeal (NP) swab
 4. Peri-mortem serum
 5. Peri-mortem whole blood
 6. ~12hr Post-mortem thoracic cavity fluid (Juice)



SAMPLE COLLECTION

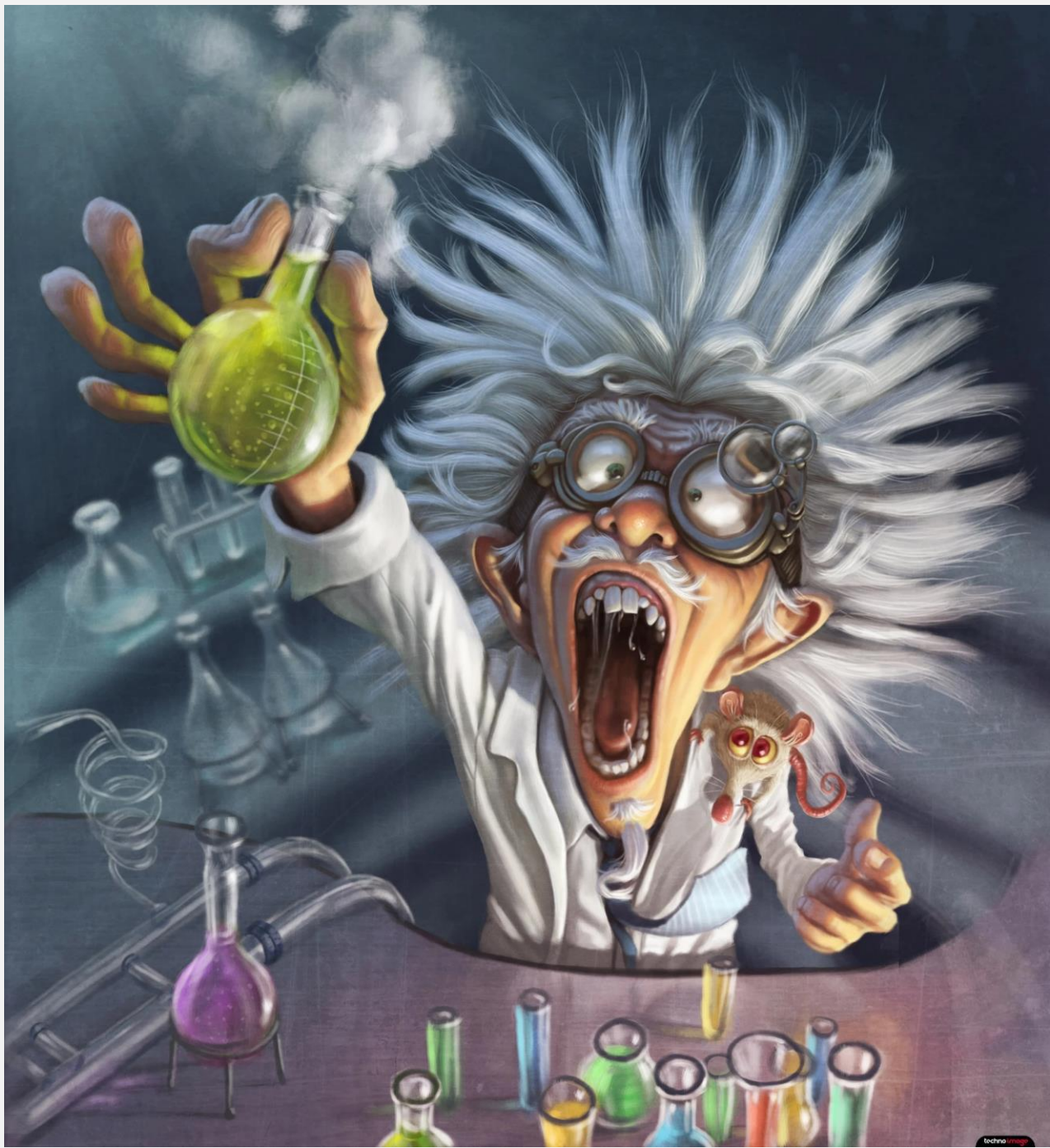


SAMPLE COLLECTION



SAMPLE COLLECTION

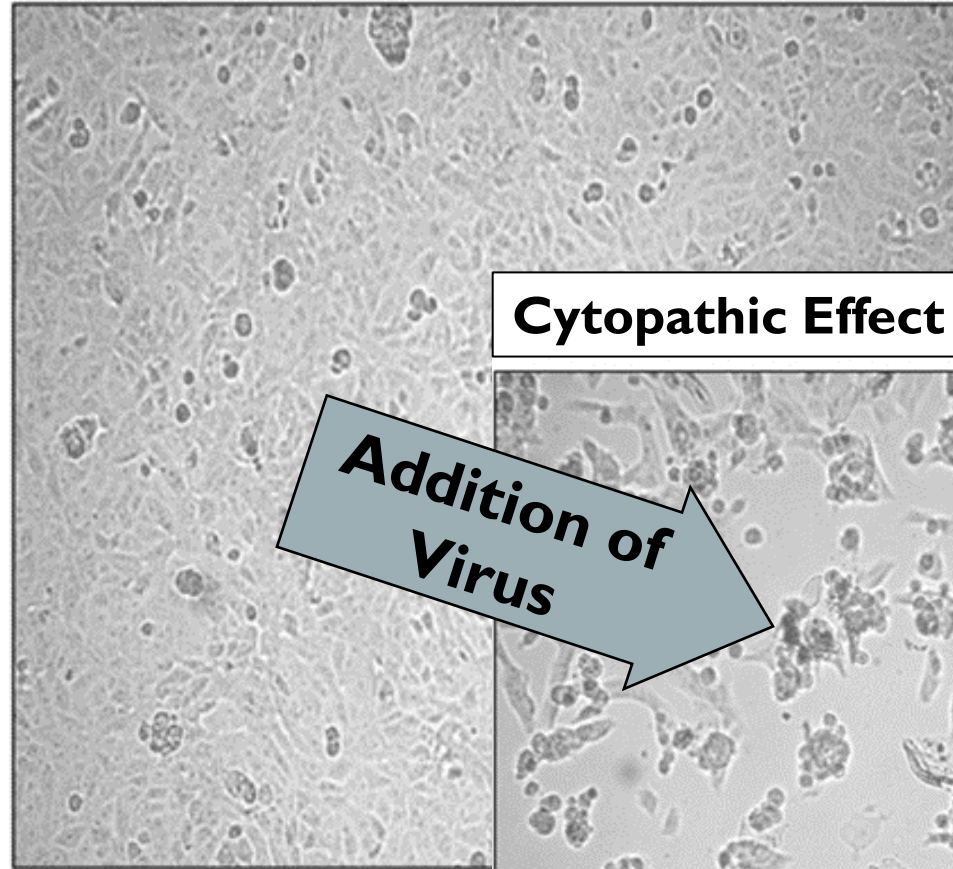




VIRUS ISOLATION

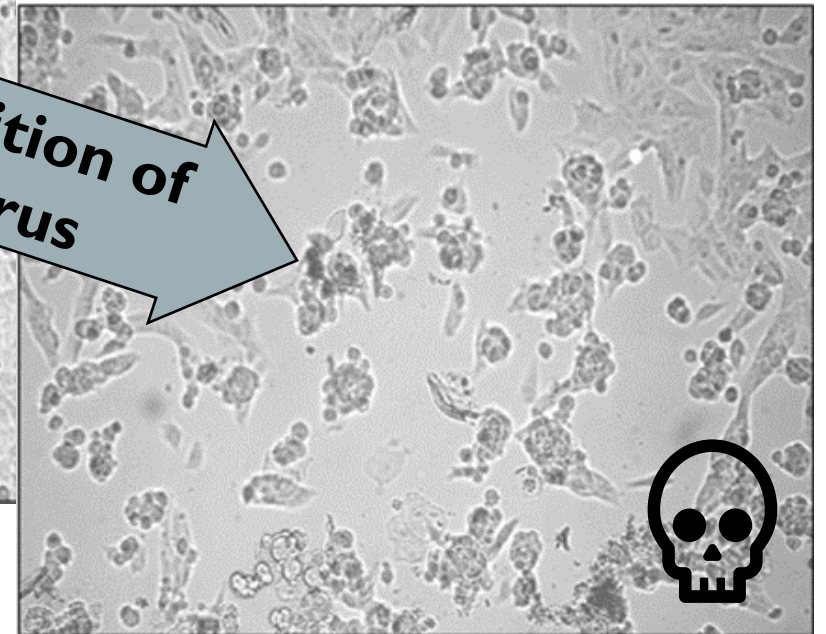
- Performed on
 - 82 Tonsils
 - 83 RPLNs
 - 83 NP Swabs
- Monitored for 14 days
 - 2 passages

Healthy Vero E6 Cell Culture



Cytopathic Effect (CPE)

**Addition of
Virus**



Vicenzi et al 2004

VIRUS ISOLATION RESULTS

- No significant CPE seen

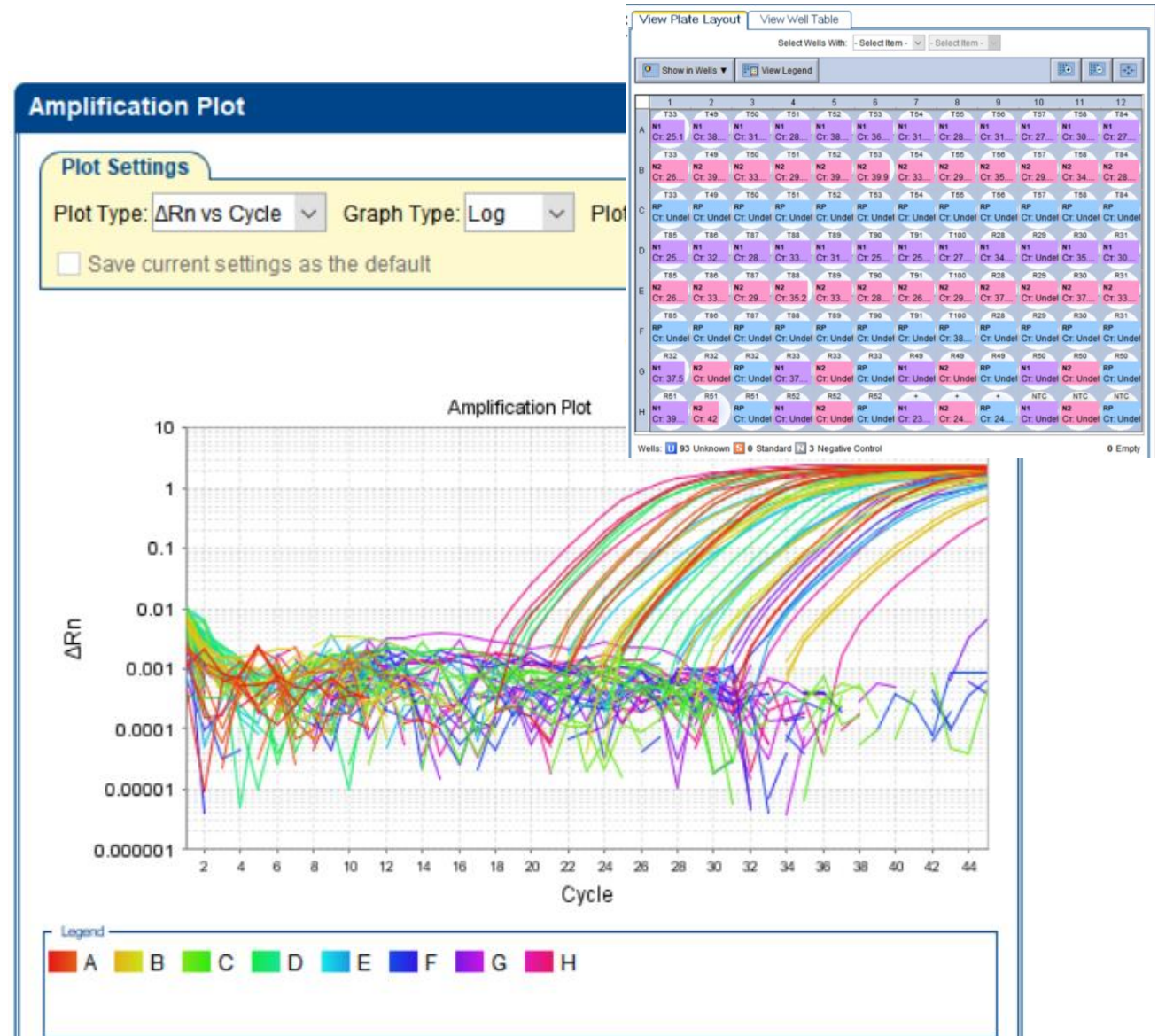


- No live virus detected



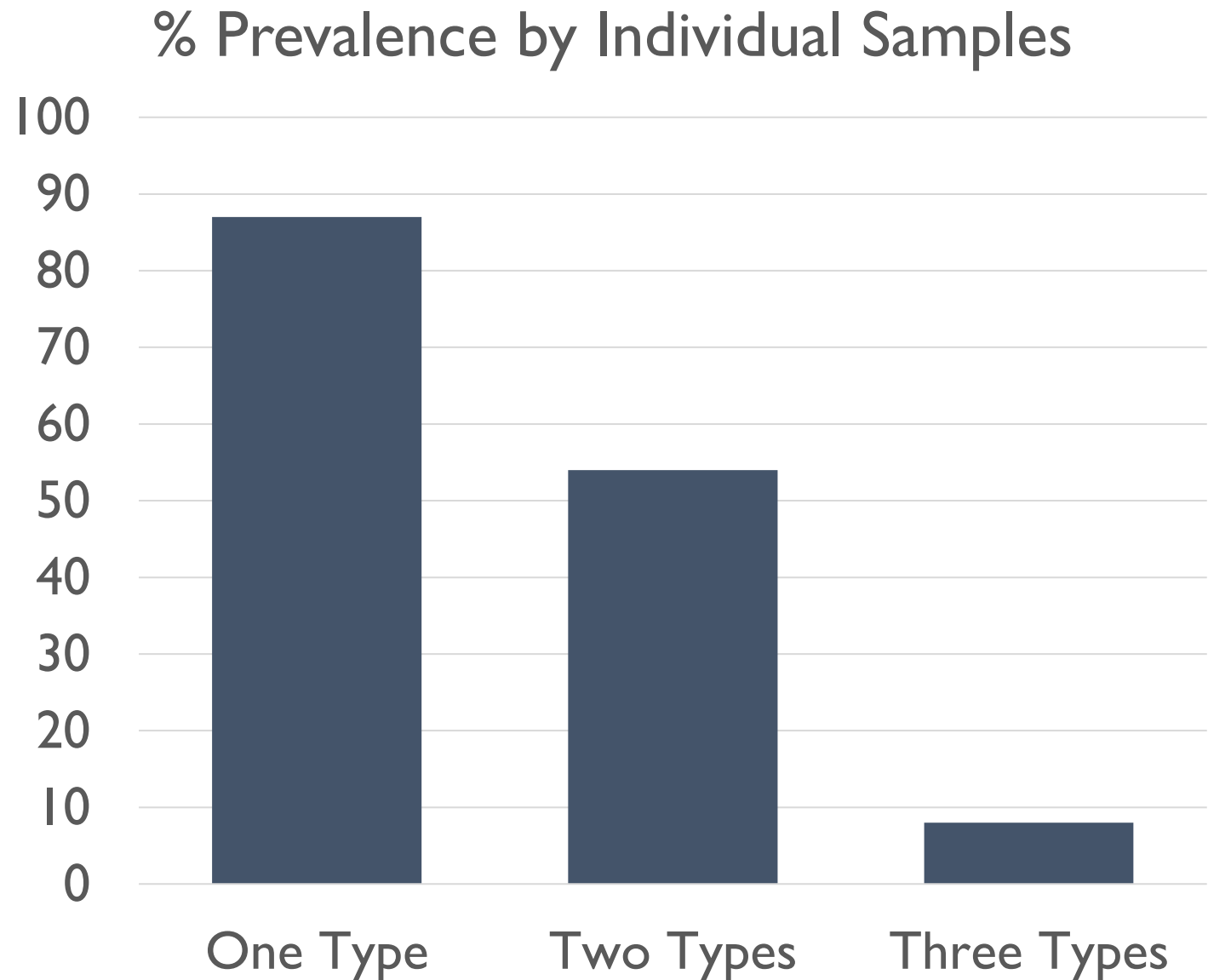
RT-PCR

- Performed on:
 - 82 Tonsils
 - 83 RPLNs
 - 83 NP Swabs
- Probe Targets
 - Nucleocapsid genes
 - N1 & N2
 - RP (Human RNase P gene)
- Positive = $C_t \leq 40$



RT-PCR RESULTS

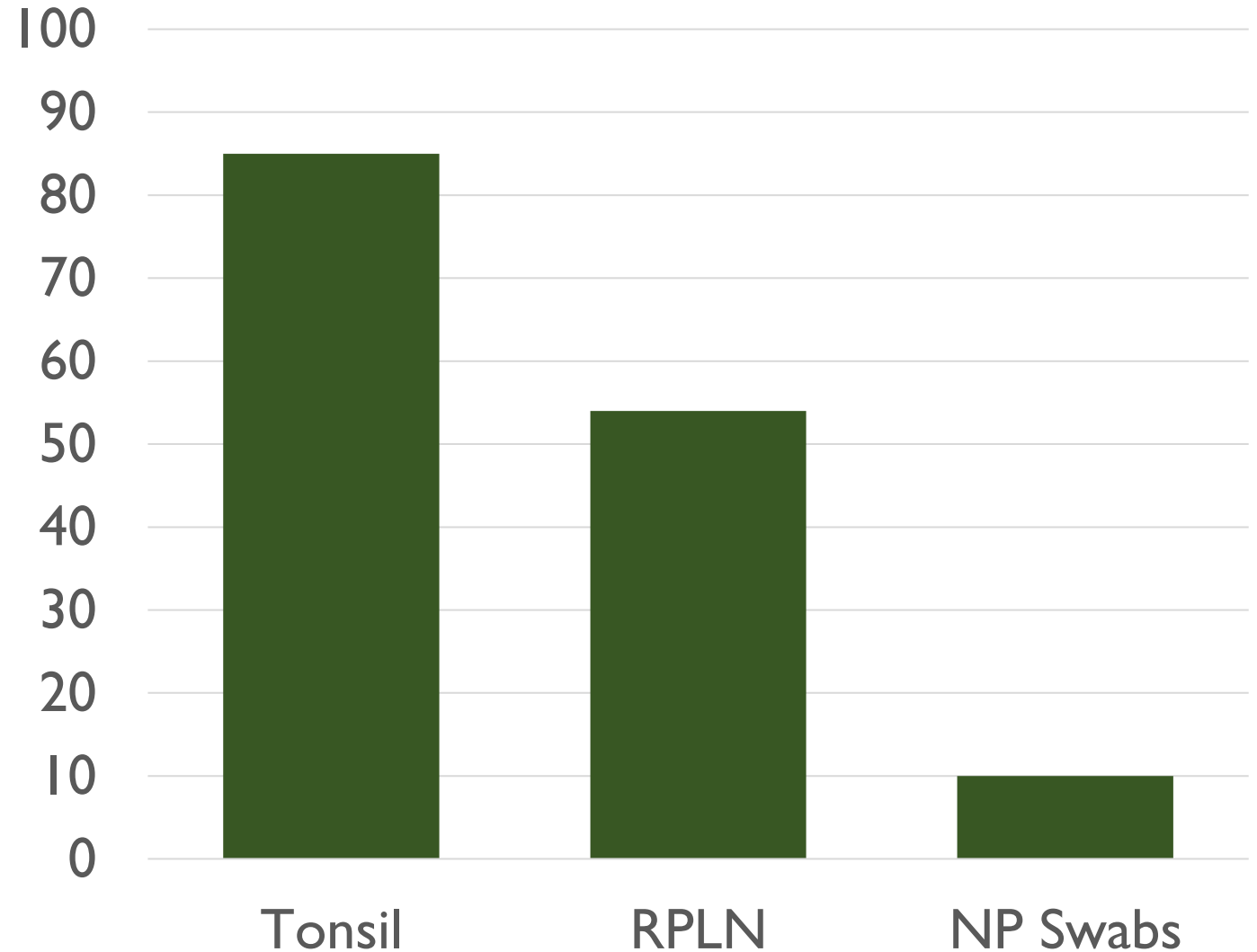
- 248 total samples
 - 72/83 positive in 1 type
 - 45/83 positive in 2 types
 - 7/83 positive in all 3 types



RT-PCR RESULTS

- Tonsil tissue proved to be the most sensitive
 - Tonsils (70/82)
 - RPLN tissue (45/83)
 - NP swabs (8/83)

% Prevalence by Sample Type

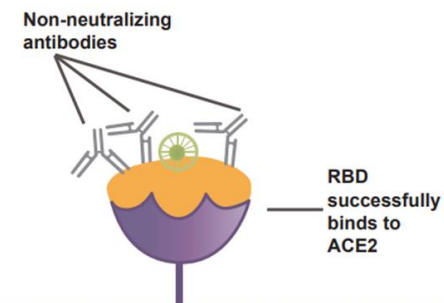


BLOCKING ELISA

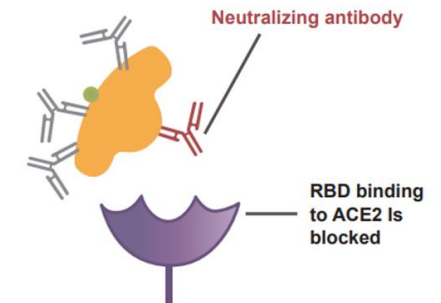
- Performed on:
 - 34x Serum
 - 34x Juice
 - 34x Eluted whole blood
- Validate additional WTD fluid types for antibody detection



svNT Negative Test



svNT Positive Test



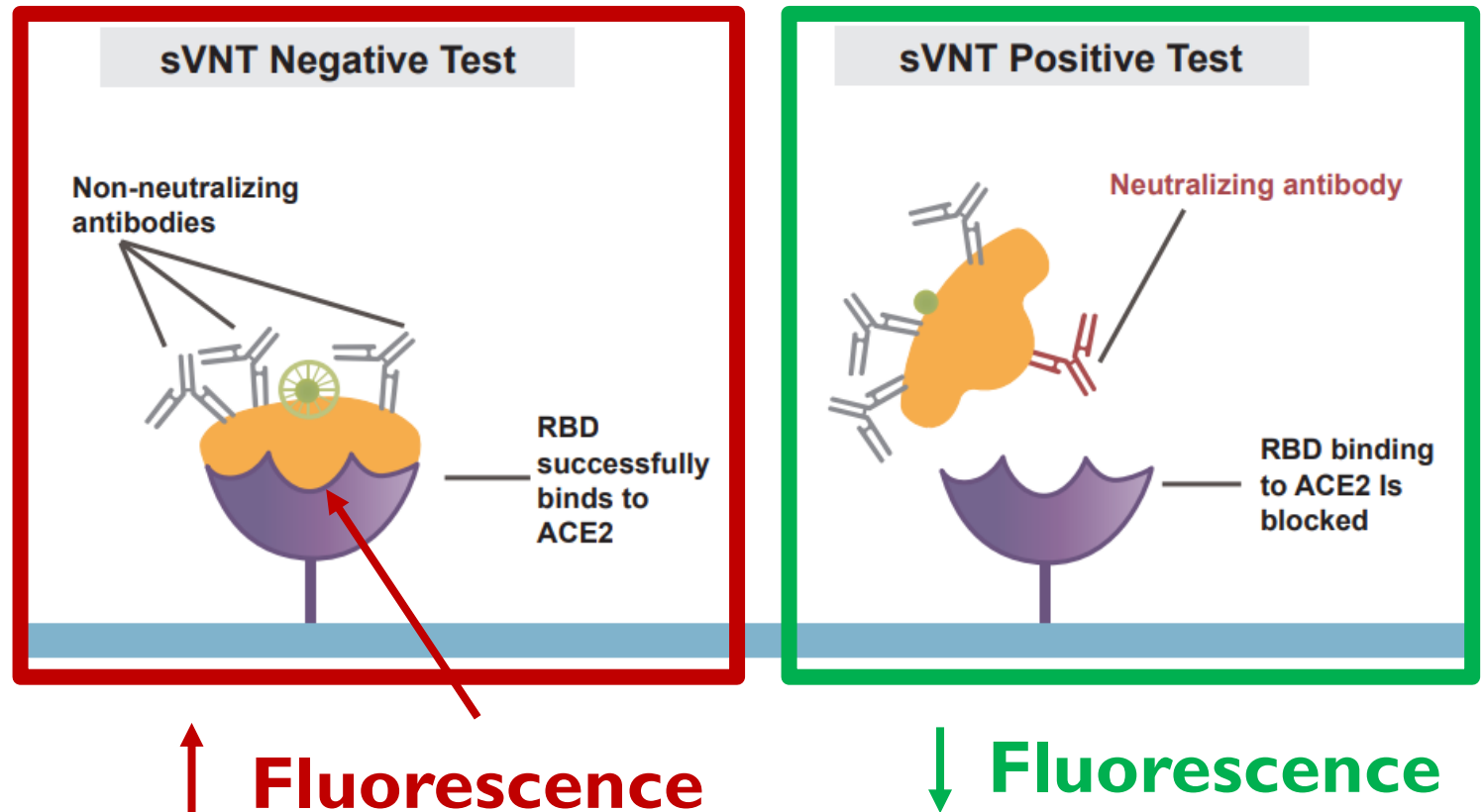
SARS CoV-2 Neutralization Antibody Detection Kit

Specifically detects neutralizing antibodies

BLOCKING ELISA

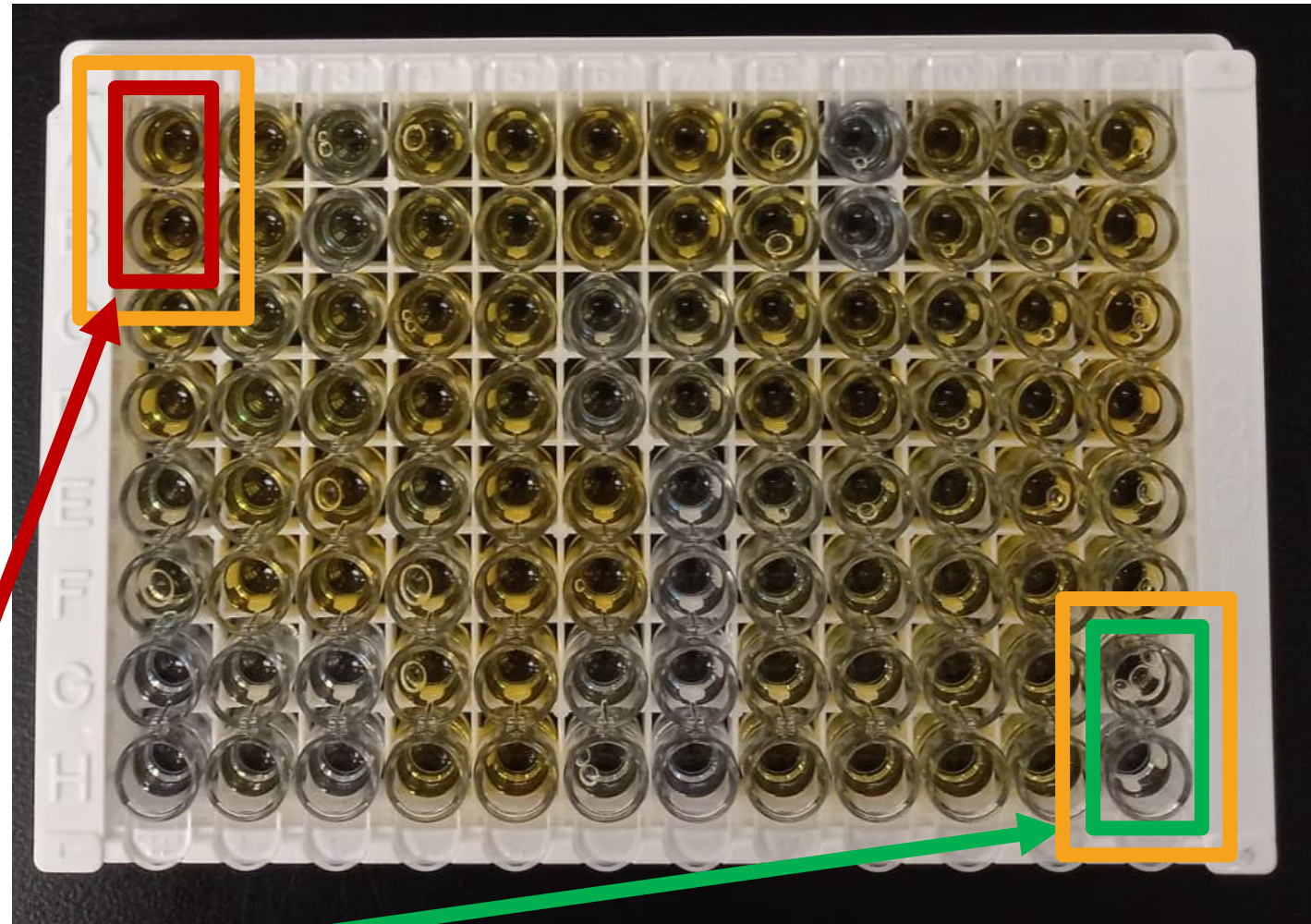
- SARS-CoV-2 Surrogate Virus Neutralization Test Kit
- cPass Genscript
- USDA Validated
- Chandler et al. 2021

Assay Principle



DESIGN

- 102 Samples total
 - Ran in duplicate
- Positive = $\geq 30\%$ Inhibition

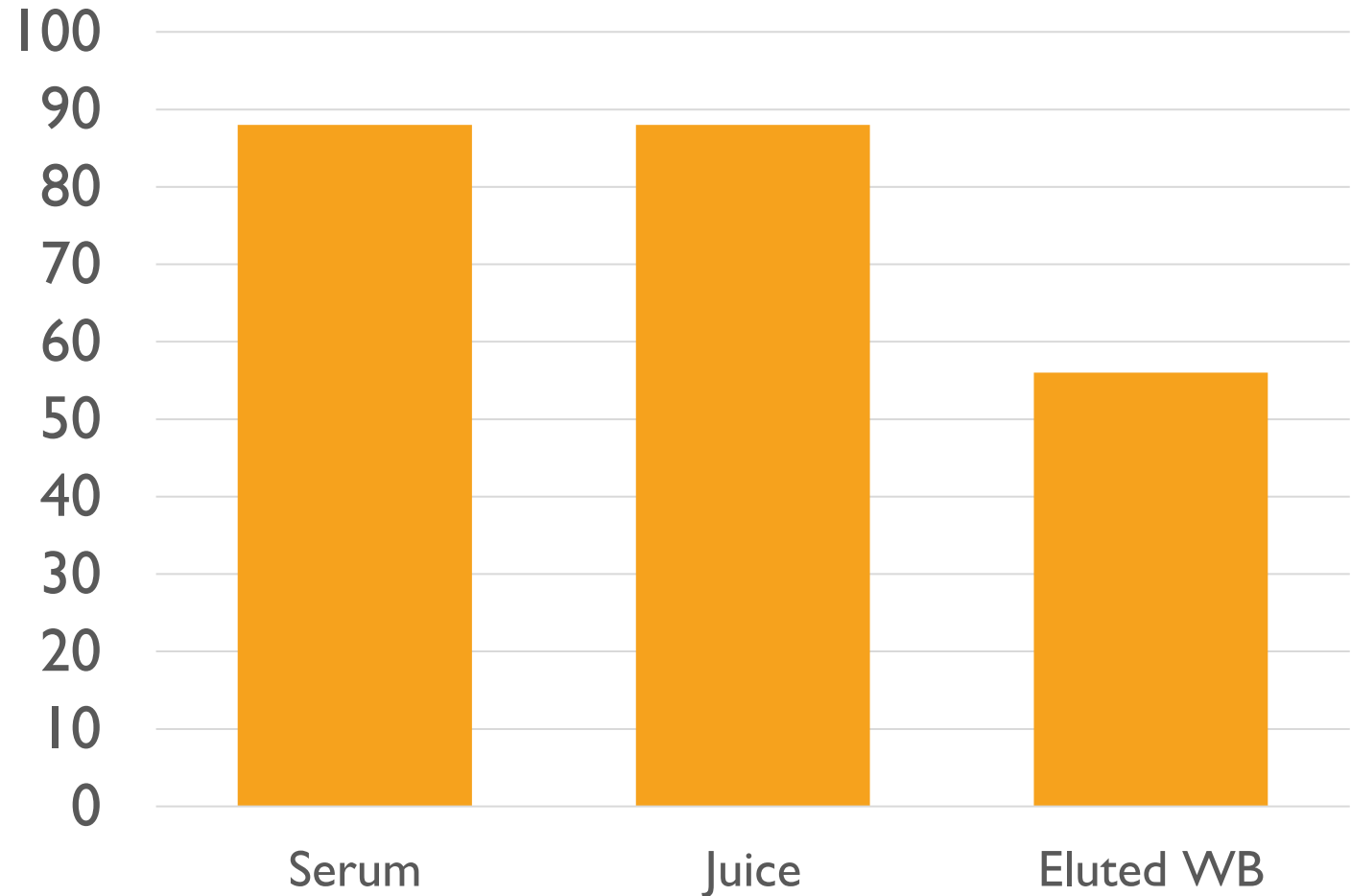
**Neg****Pos****Controls**

$$\text{Inhibition} = \left(1 - \frac{\text{OD value of Sample}}{\text{OD value of Negative Control}} \right) \times 100\%$$

RESULTS

- **Sample Performance**
 - Serum (30/34)
 - Juice (30/34)
 - Eluted whole blood (19/34)

% of WTD with Neutralizing Antibodies to SARS-CoV-2



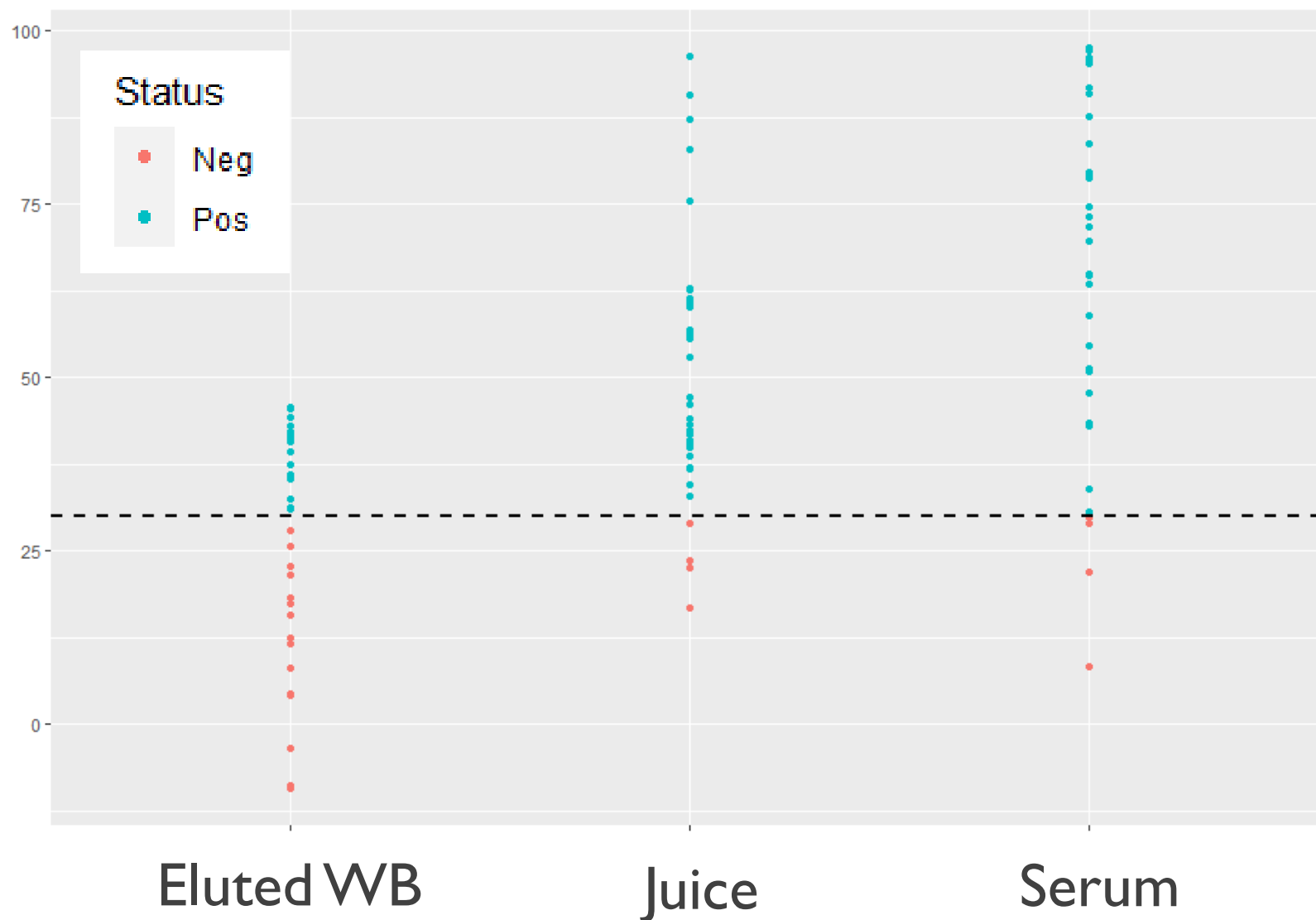
ELUTION VS SERUM

Apparent prevalence	0.56 (0.4, 0.7)
True prevalence	0.88 (0.7, 0.97)
Sensitivity	0.57 (0.4, 0.8)
Specificity	0.50 (0.1, 0.93)
PPV	0.89 (0.7, 0.99)
NPV	0.13 (0.02, 0.4)

JUICE VS SERUM

Apparent prevalence	0.88 (0.7, 0.97)
Sensitivity	0.87 (0.7, 0.96)
Specificity	0.00 (0.0, 0.6)
PPV	0.87 (0.7, 0.96)
NPV	0.00 (0.0, 0.6)

Average Inhibition of Different WTD Samples



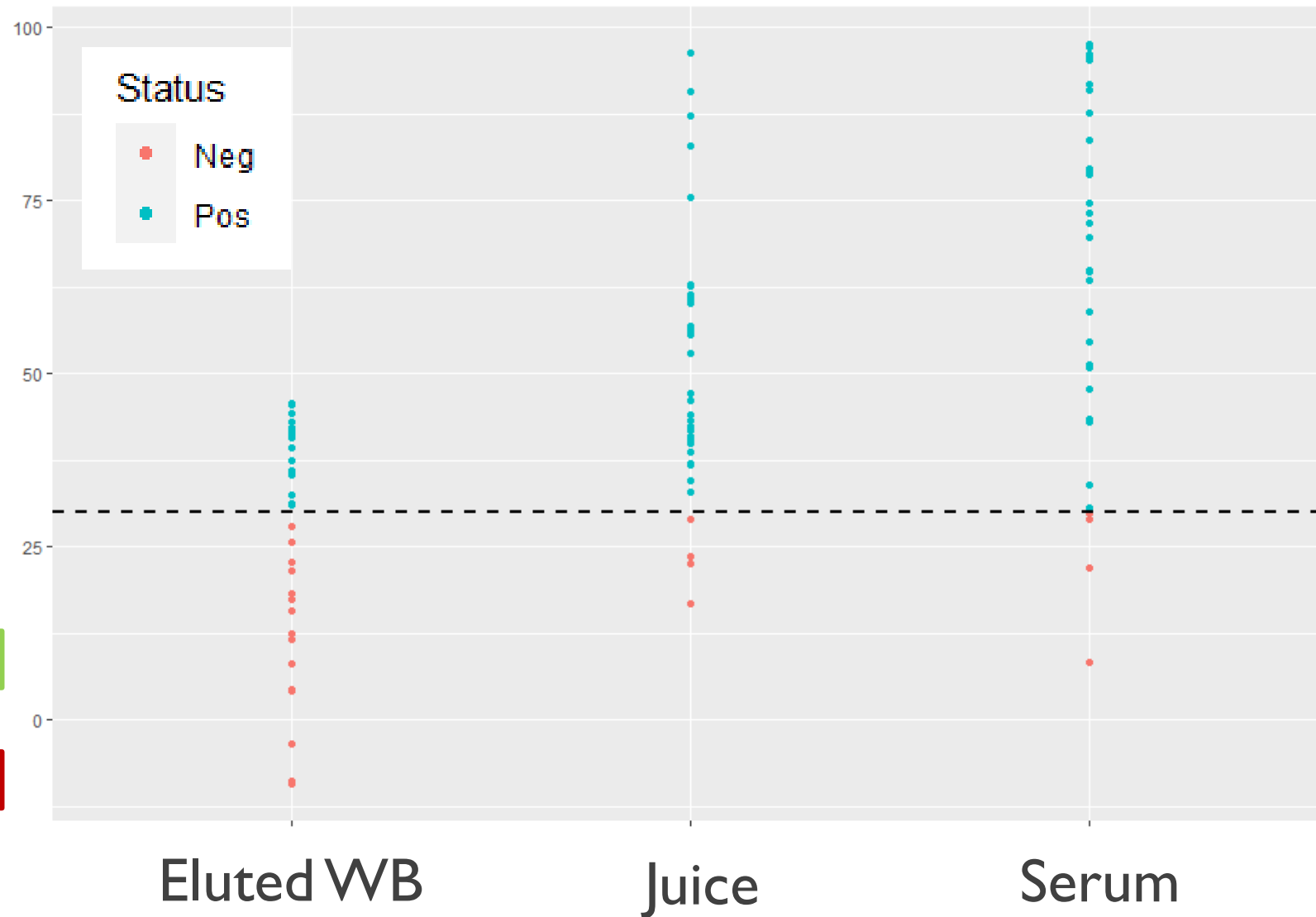
ELUTION VS SERUM

Apparent prevalence	0.56 (0.4, 0.7)
True prevalence	0.88 (0.7, 0.97)
Sensitivity	0.57 (0.4, 0.8)
Specificity	0.50 (0.1, 0.93)
PPV	0.89 (0.7, 0.99)
NPV	0.13 (0.02, 0.4)

JUICE VS SERUM

Apparent prevalence	0.88 (0.7, 0.97)
Sensitivity	0.87 (0.7, 0.96)
Specificity	0.00 (0.0, 0.6)
PPV	0.87 (0.7, 0.96)
NPV	0.00 (0.0, 0.6)

Average Inhibition of Different WTD Samples



CONCLUSION

Juice and eluted blood are hot garbage...



CONCLUSION

- Be careful with sample selection
- Be conservative about interpretation
- Serum still appears to be the most accurate for seroprevalence



ACKNOWLEDGMENTS

WV DNR BIOLOGISTS

SONIA M. HERNANDEZ

DANIEL G. MEAD

NICOLE M. NEMETH

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KAYLA G. ADCOCK



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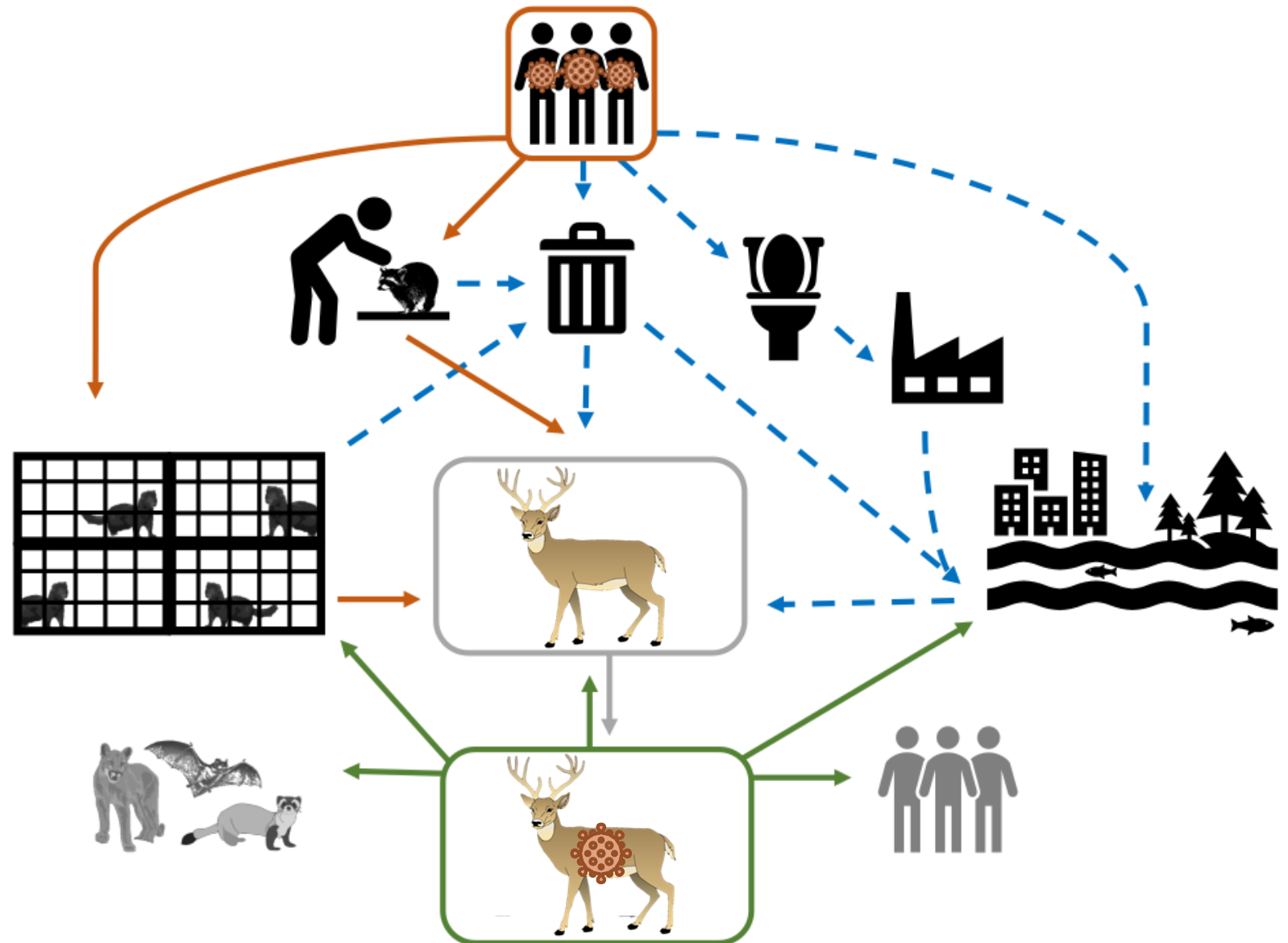
HUMAN URBAN WILDLIFE INTERFACE

Figure Key

Direct Contact Transmission
(Human to Animal)

Indirect Contact Transmission
(Human to Animal)

Direct Contact Transmission
(Animal to Animal/Human)



SAMPLE PREP

