Wastewater Pathogen Surveillance



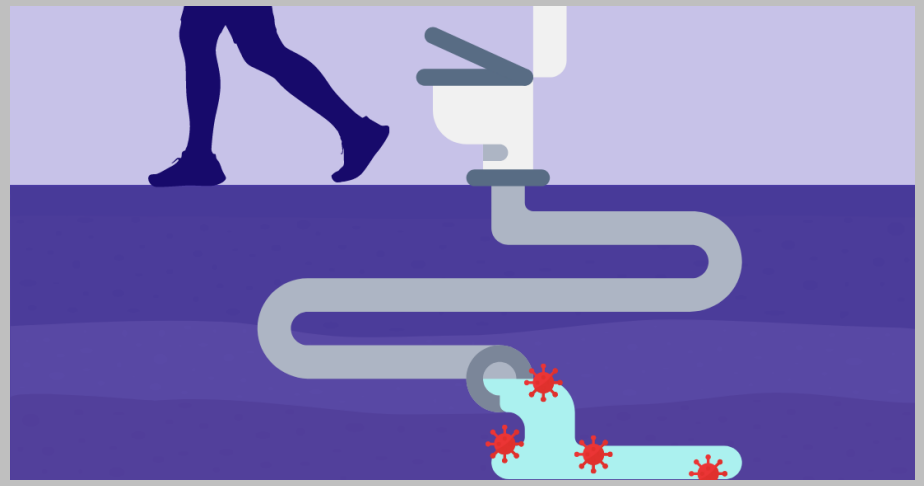
23 SEP 26

Mark J. Osborn, PhD

Associate Professor

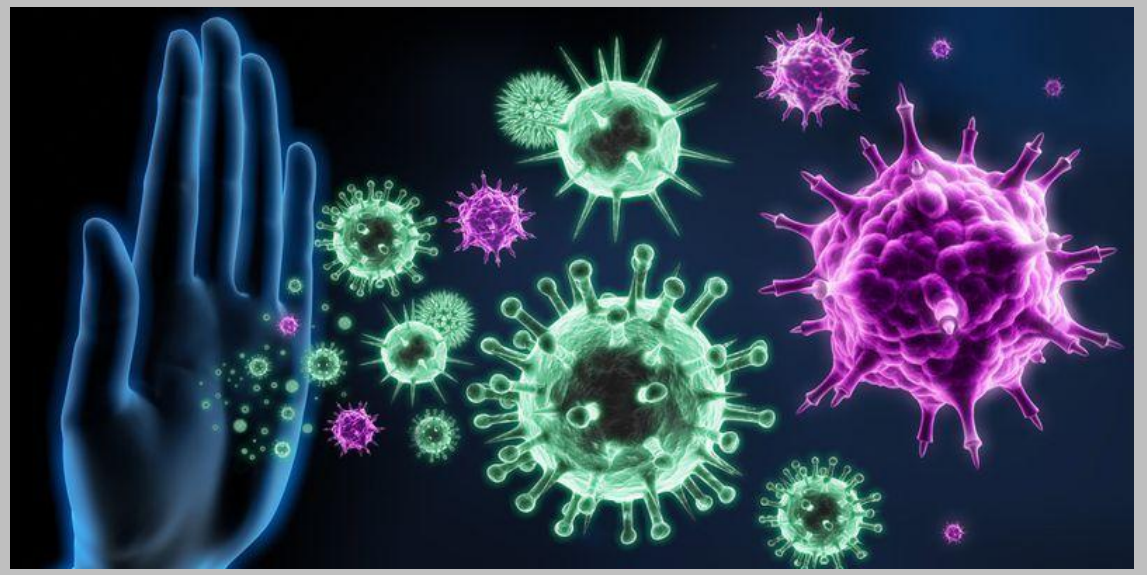
osbor026@umn.edu

Wastewater Community Epidemiology and Pathogen Surveillance



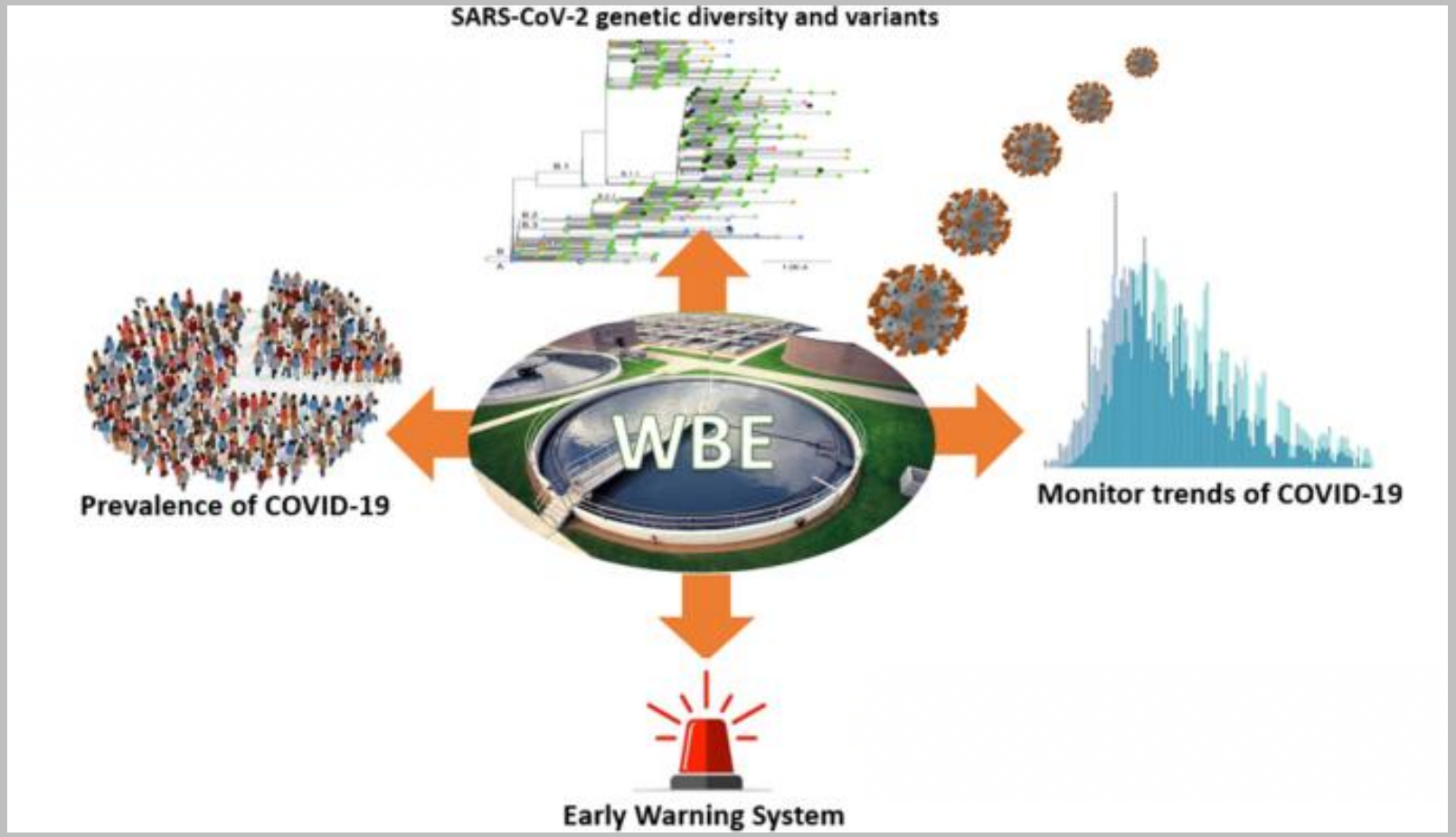






Overview/goals:

- **History**
- **Present**
- **Future**



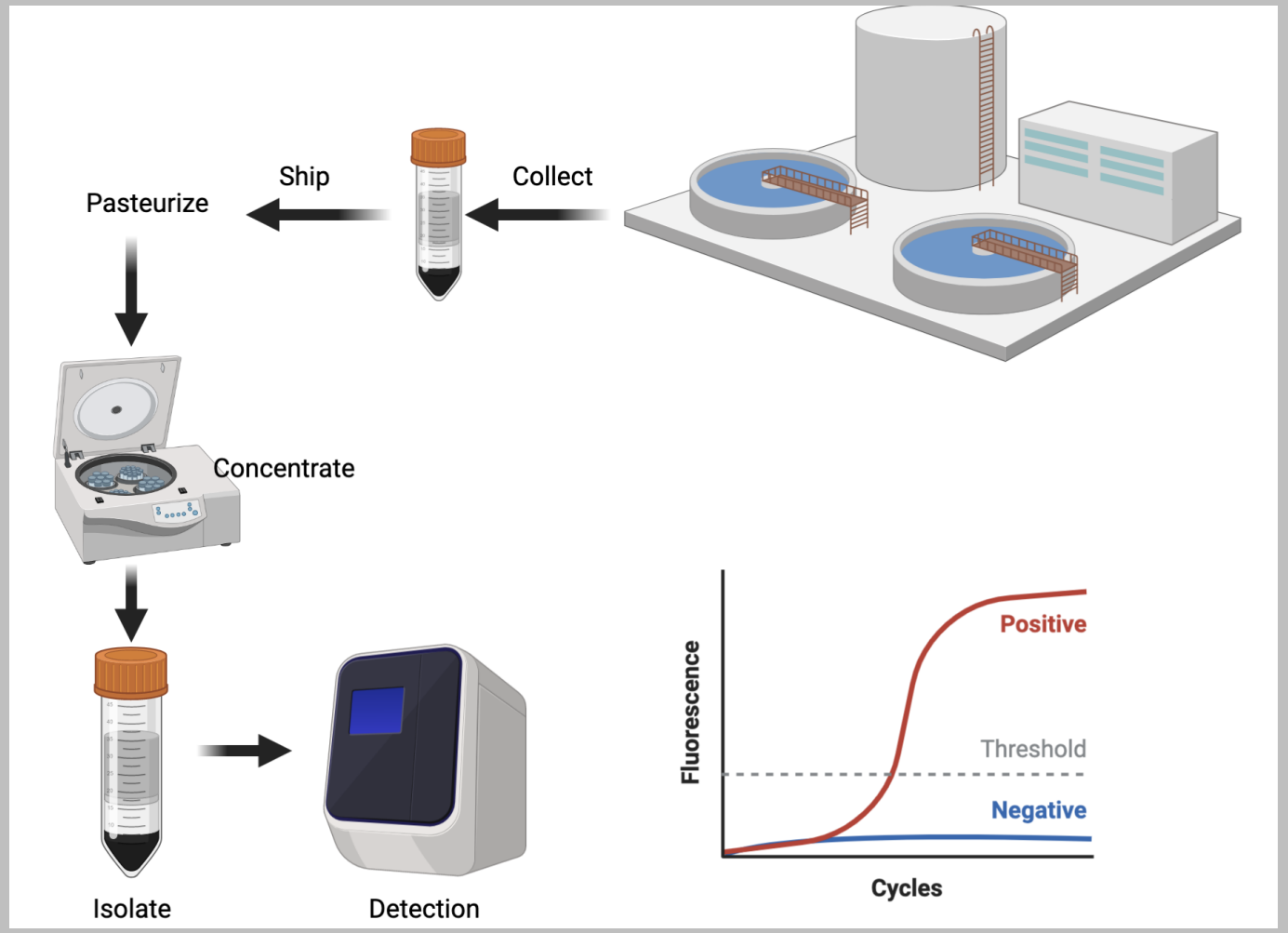
History

Medical School
Duluth Campus

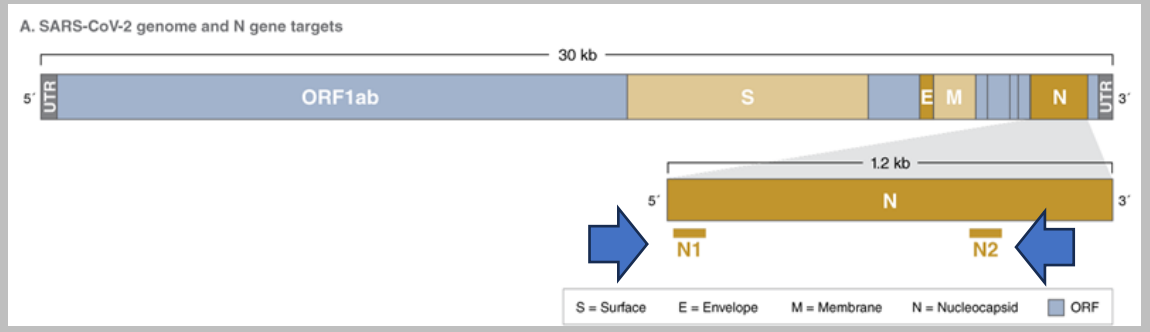


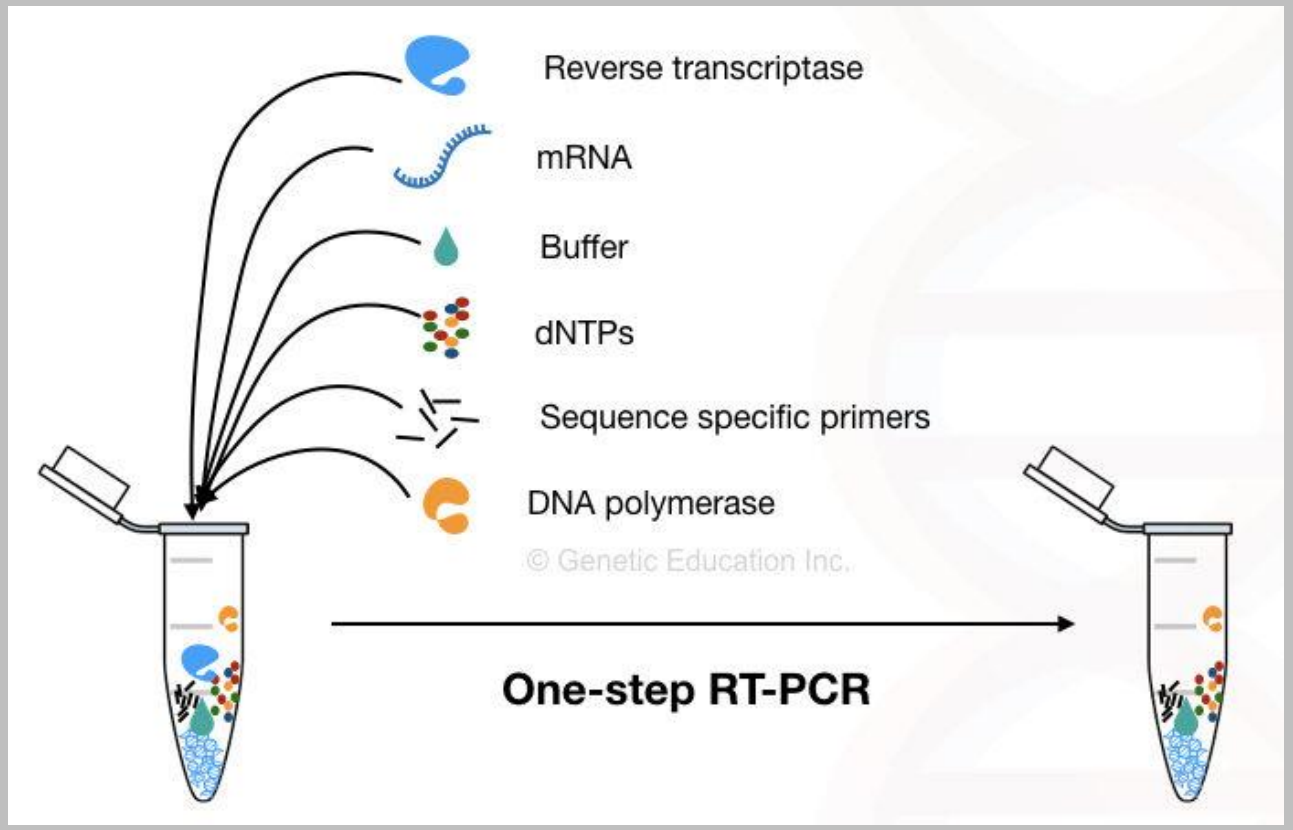


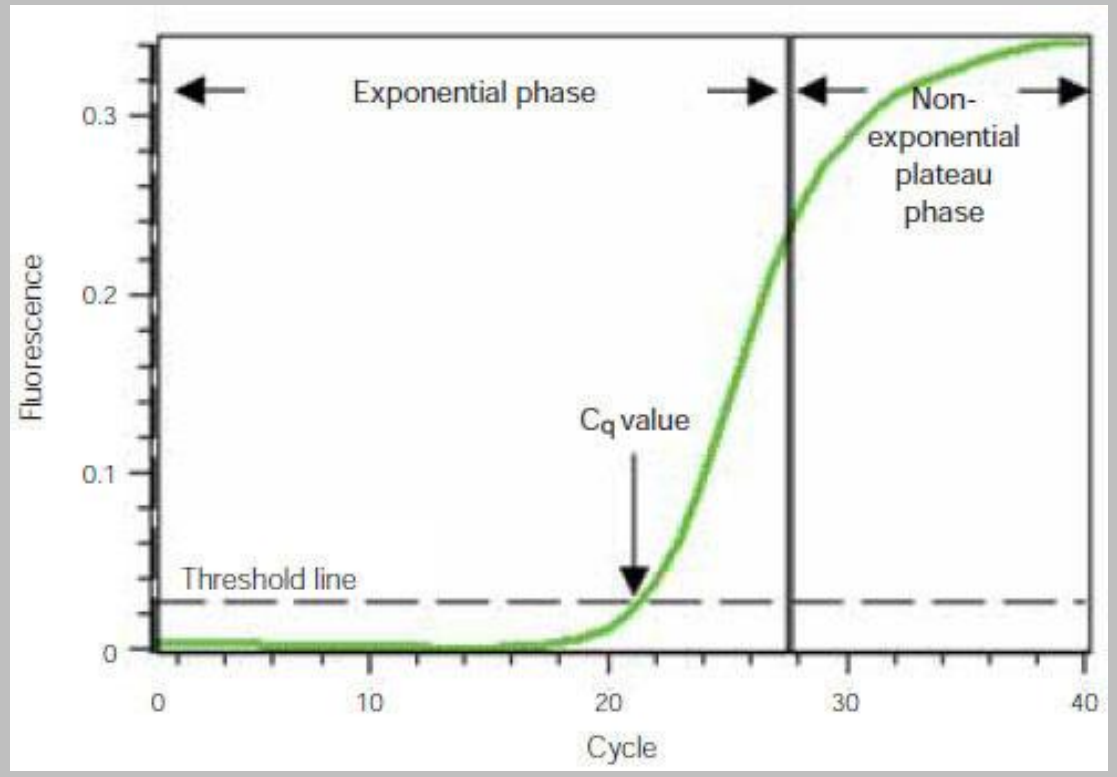




Methodology: qRT-PCR

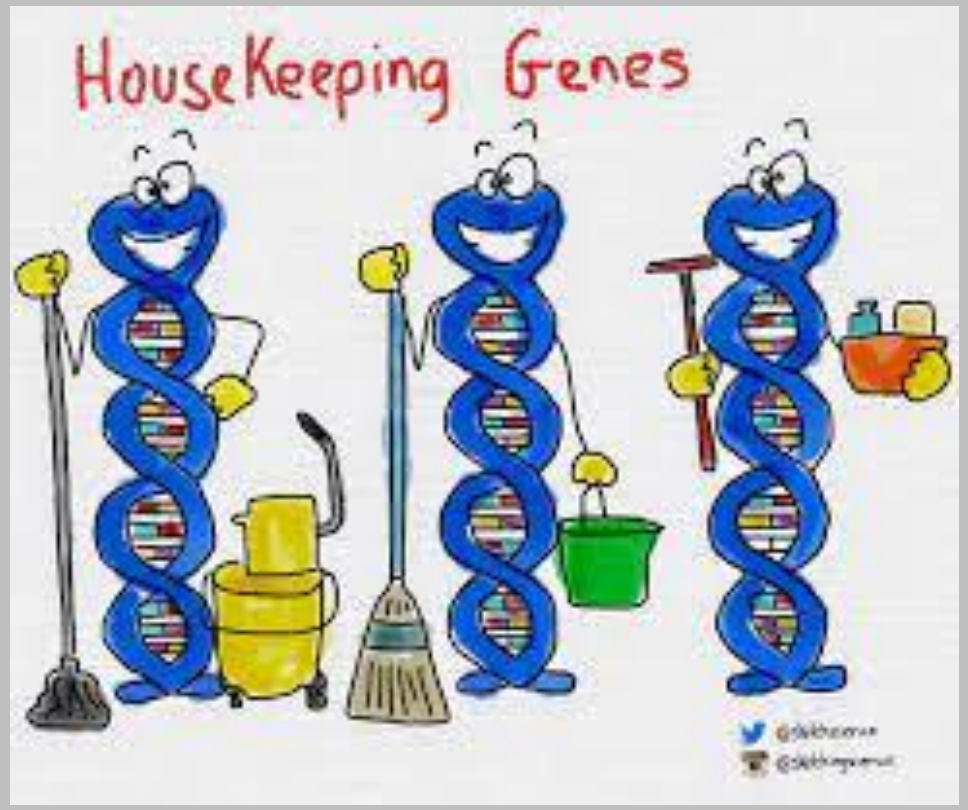


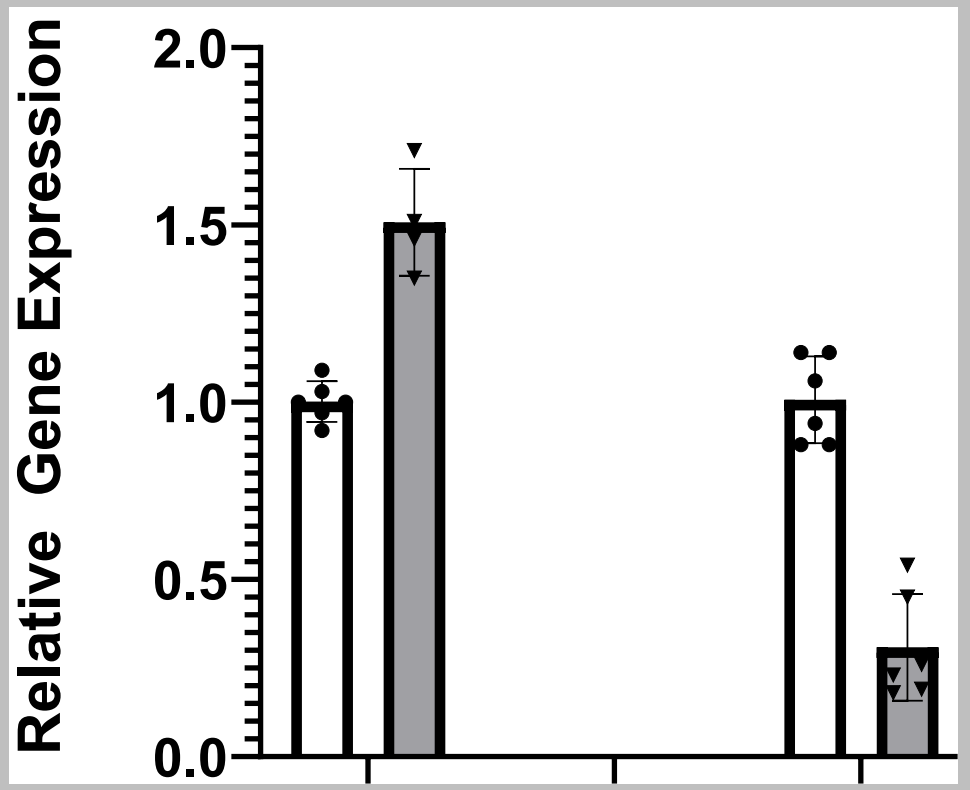




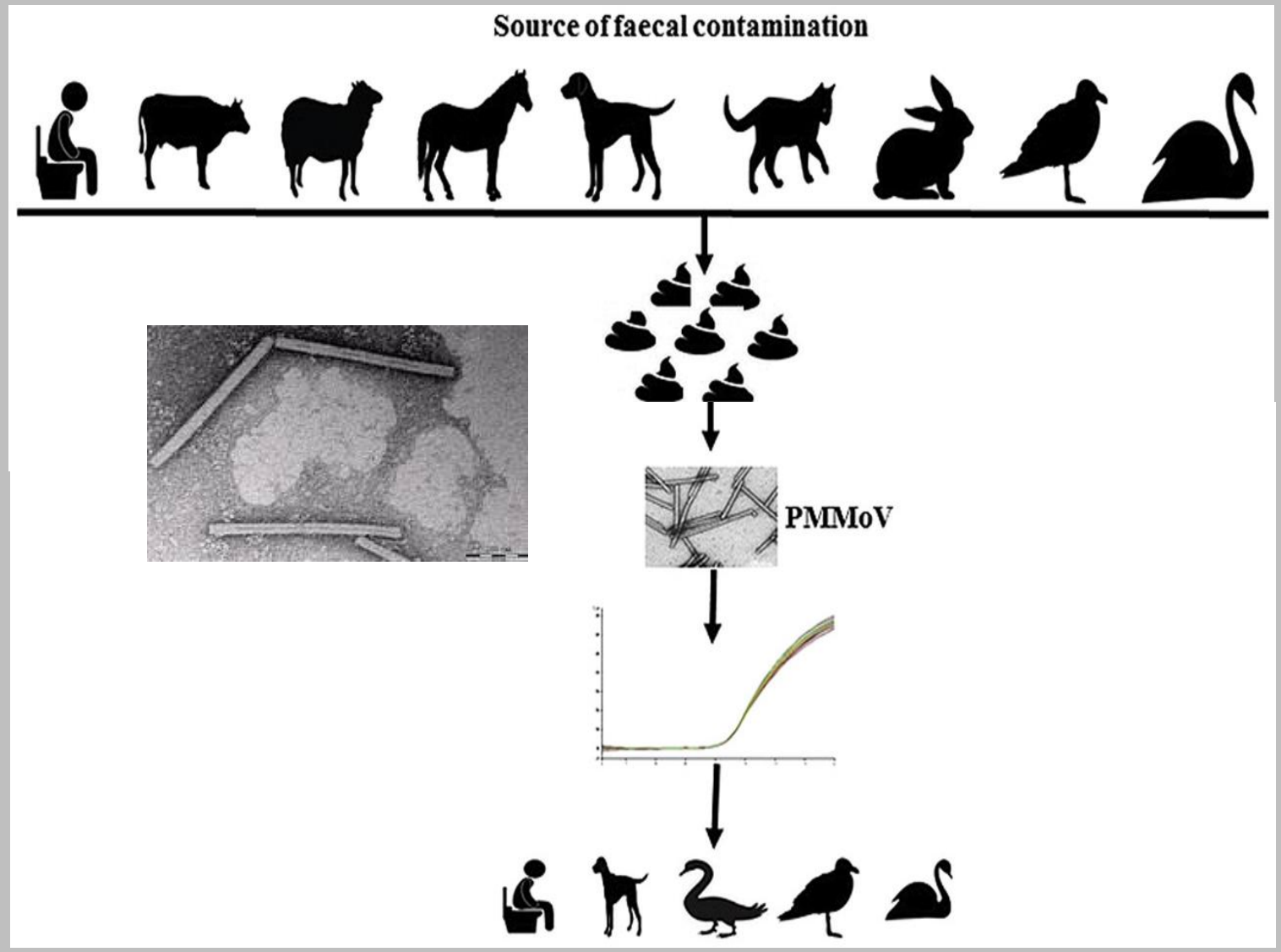
Optimizing the assay: normalization, flow, stability

qRT PCR Analysis: standardization/normalization





WW Analysis: standardization/normalization





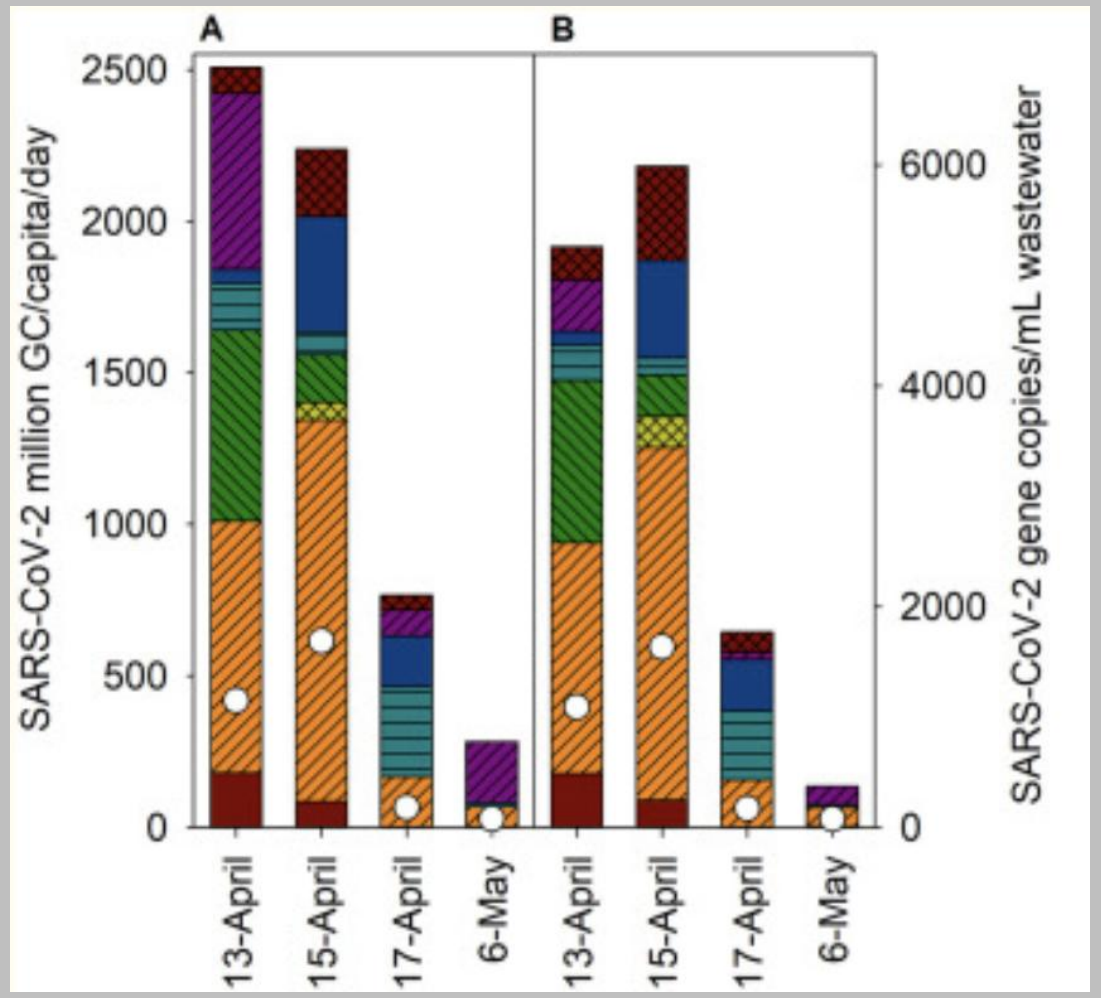
plant pathogen Pepper mild mottle virus (PMMoV) is widespread and abundant in wastewater from the United States, suggesting the utility of this virus as an indicator of human fecal pollution/presence

https://www.sciencedirect.com/science/article/pii/S0043135419301137

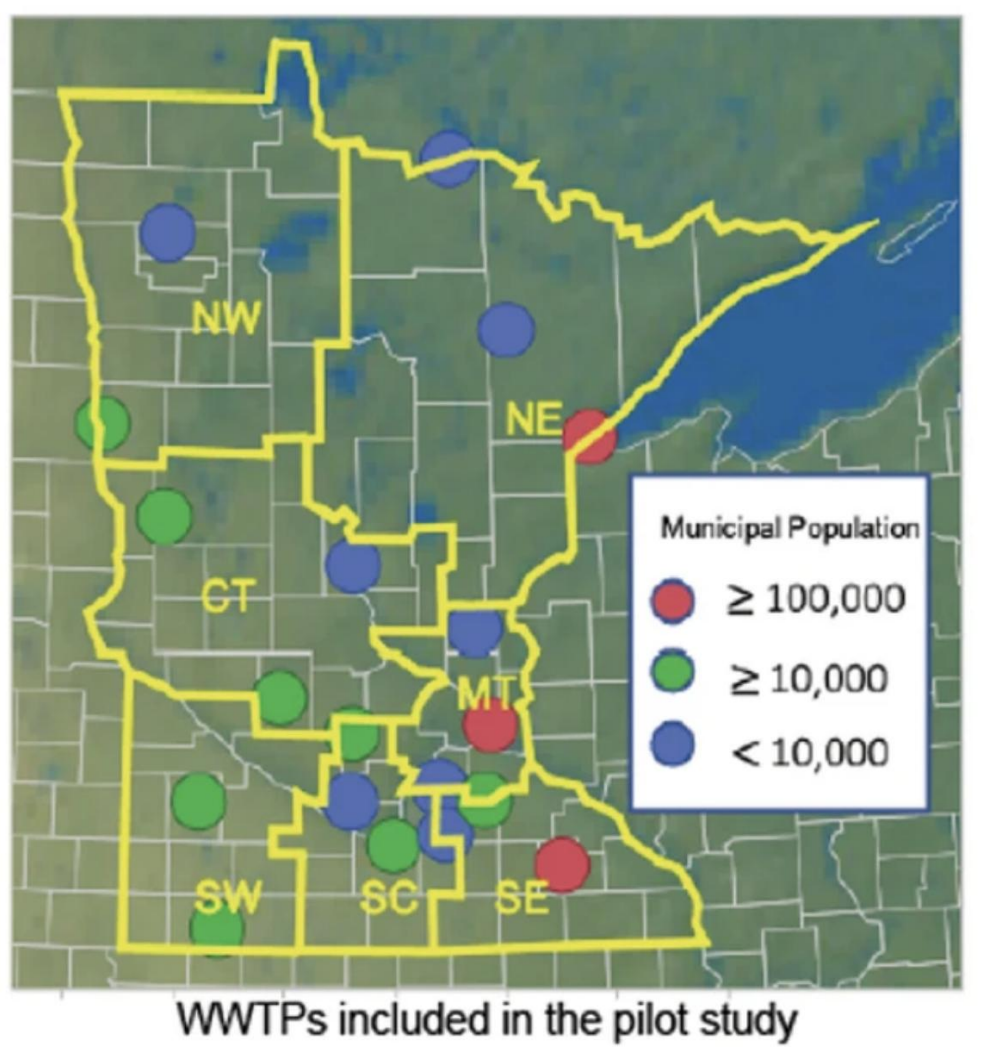
Continuous Methodological/Statistical Assessment

- Accounting for wastewater sewer travel time, flow rate, and other sewer-specific factors will be important in the data interpretation of a widespread wastewater epidemiology effort.

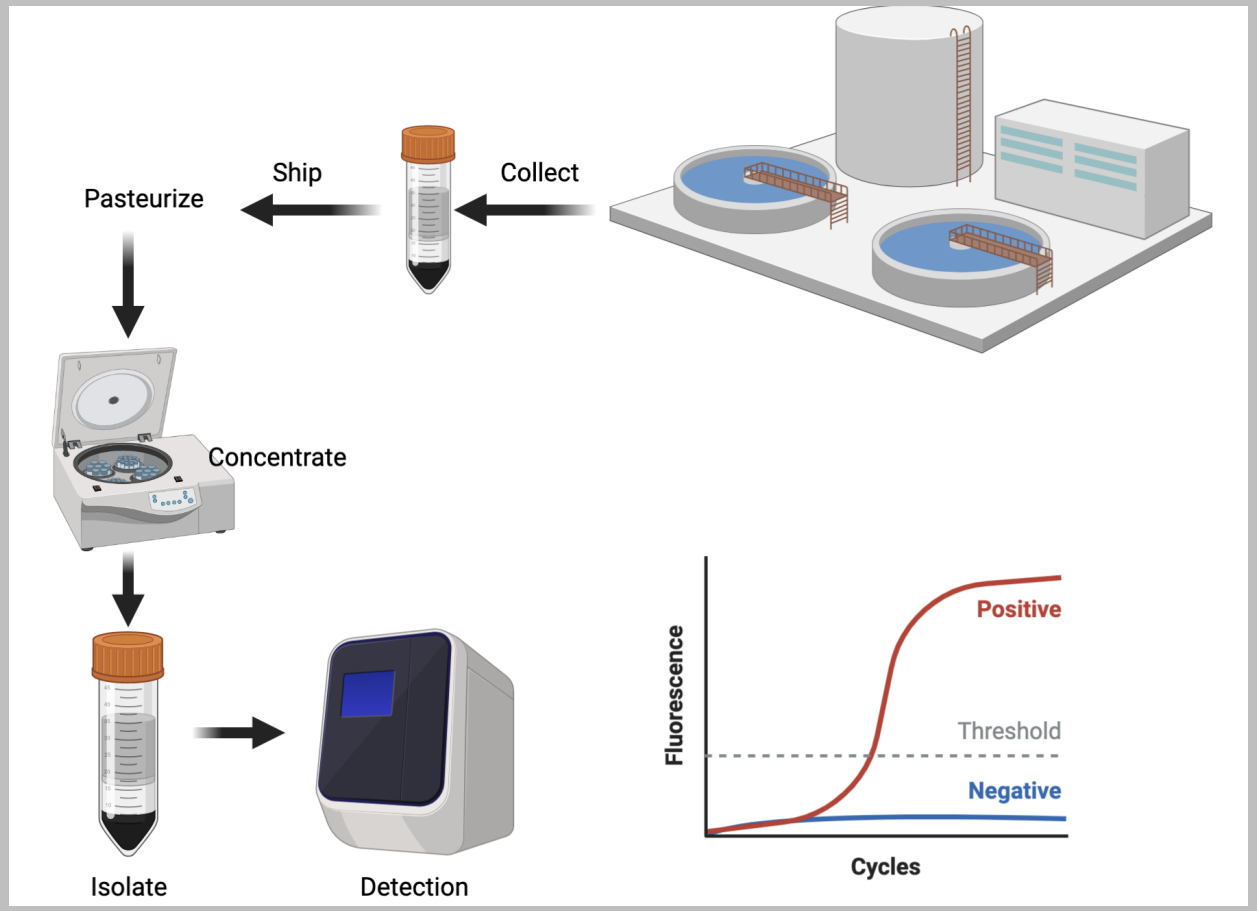




Sampling and Prediction

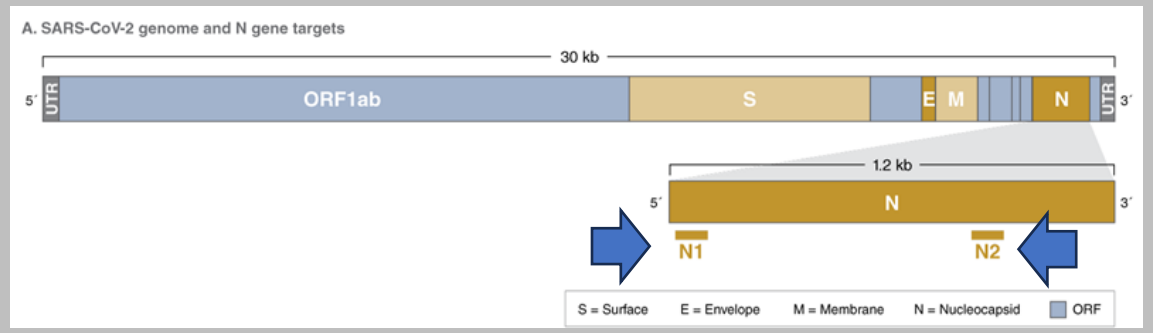


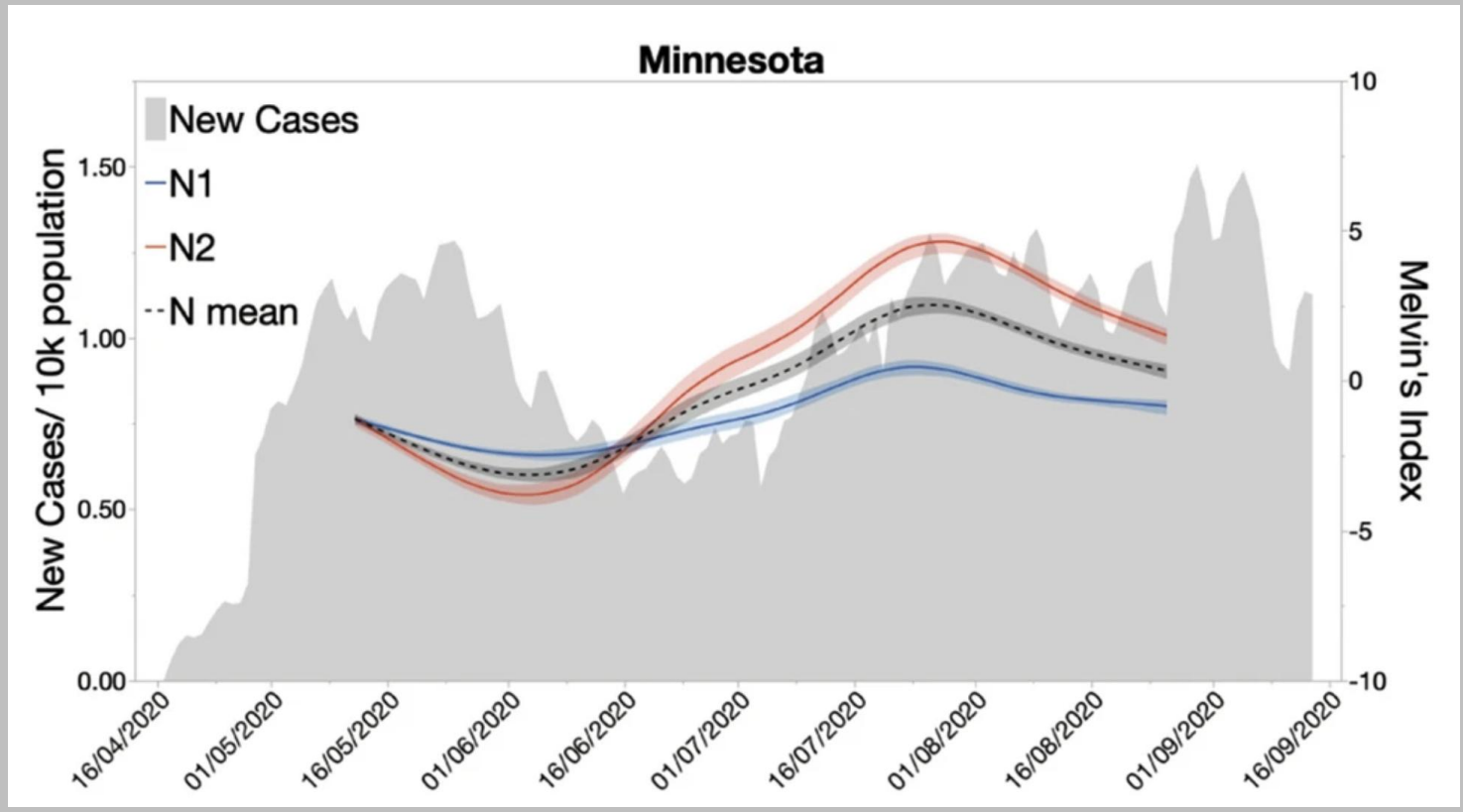
WWTPs included in the pilot study



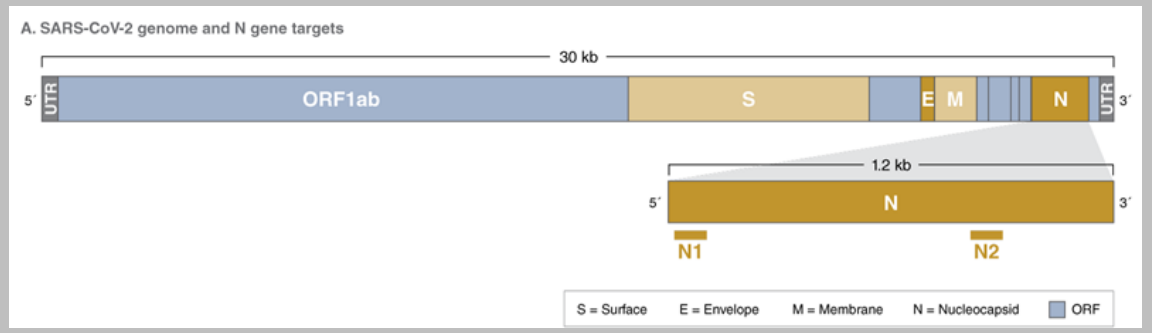
Broad geographic area with 2 samples/week

Wastewater predicted new cases by 15 days for N1 and by 17 days for N2





Higher Sensitivity and Preserving/Restoring Predictions





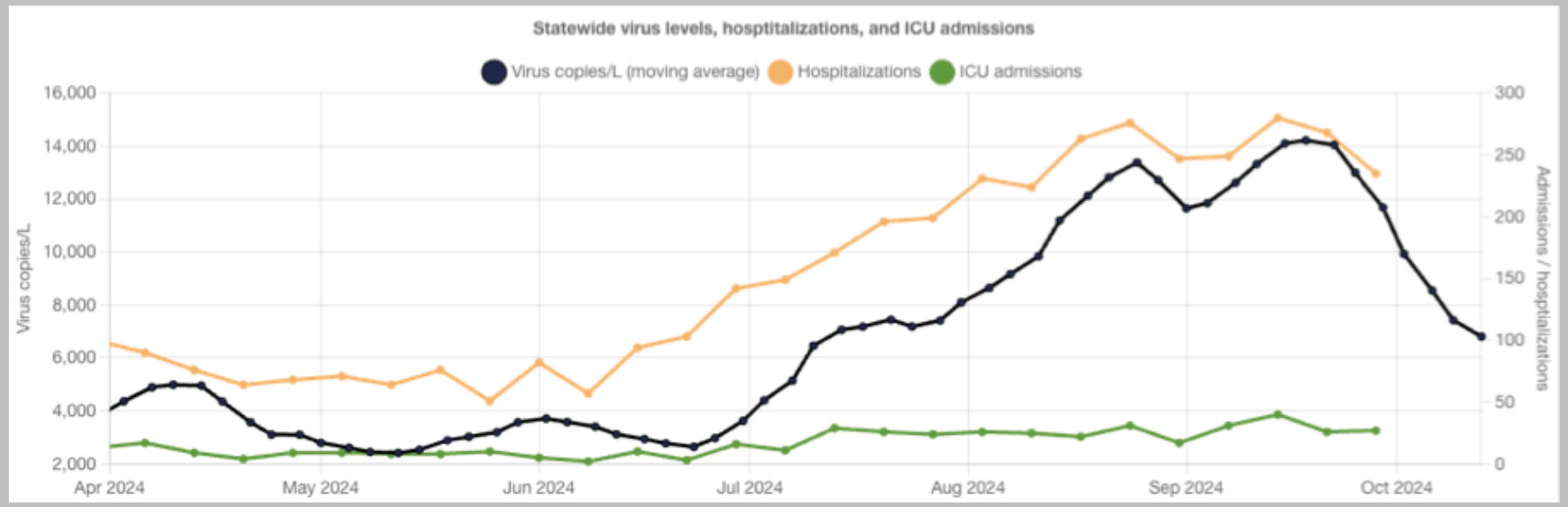
- TaqPath Assay: 3 gene assay

- Statistical methodology development and application for healthcare worker population evaluation

Reporting metrics: State Data

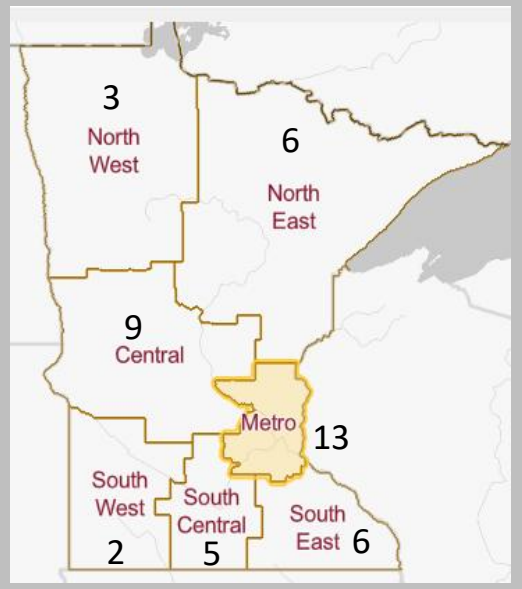
Virus copies/l
hospitalization
ICU admits





Each site is reported individually and then averaged for a statewide/regional assessment

Reporting metrics: regional/site data

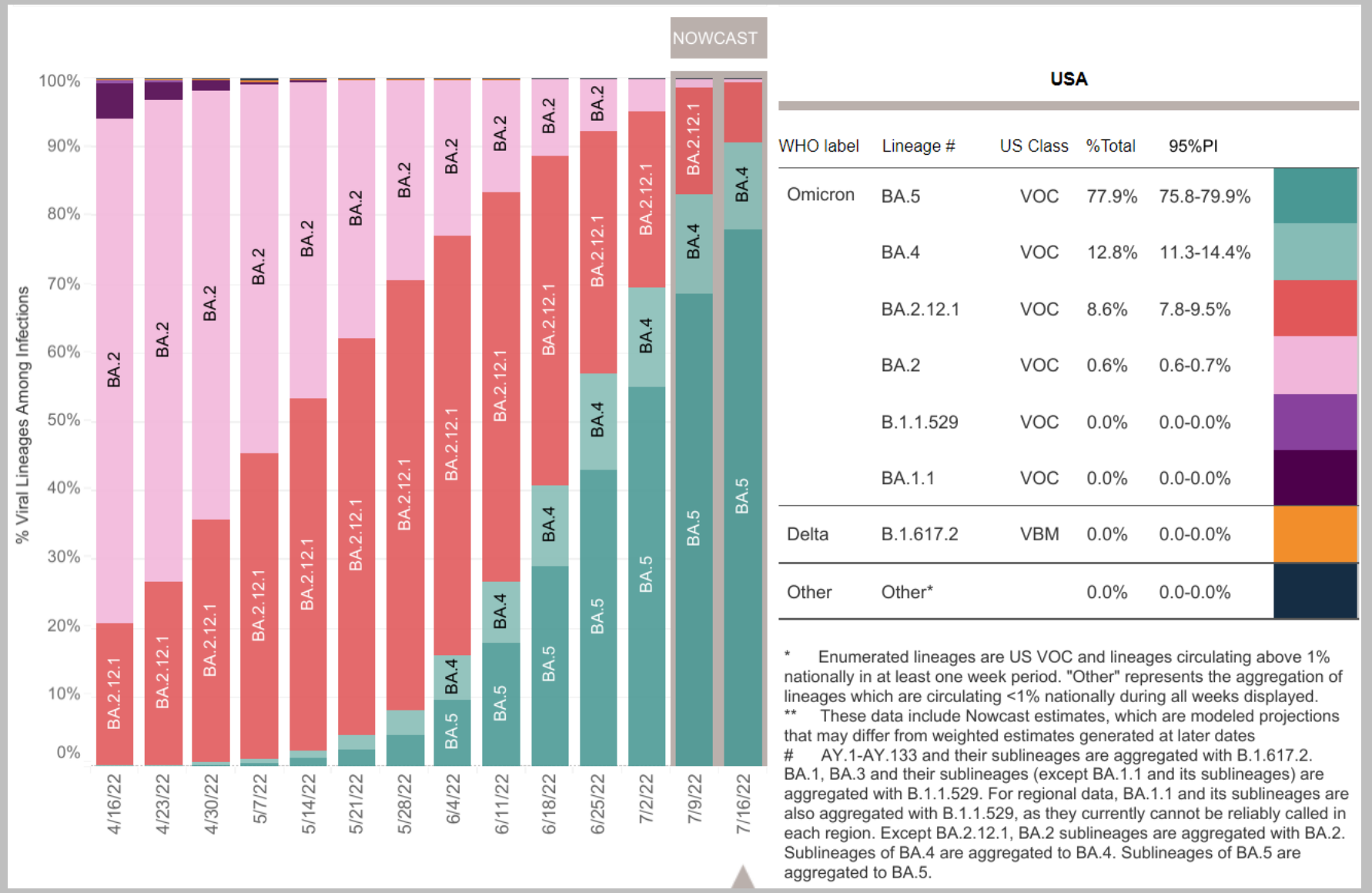




Virus copies/l (region)
Virus copies/l (site)
regional hospitalizations



Integrating with other programs: CDC Variant monitoring Report

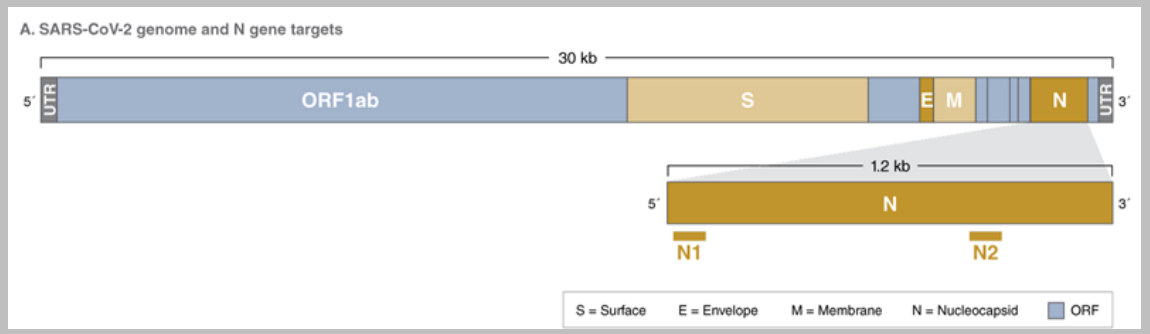


USA

WHO label	Lineage #	US Class	%Total	95%PI
Omicron	BA.5	VOC	77.9%	75.8-79.9%
	BA.4	VOC	12.8%	11.3-14.4%
	BA.2.12.1	VOC	8.6%	7.8-9.5%
	BA.2	VOC	0.6%	0.6-0.7%
	B.1.1.529	VOC	0.0%	0.0-0.0%
	BA.1.1	VOC	0.0%	0.0-0.0%
Delta	B.1.617.2	VBM	0.0%	0.0-0.0%
Other	Other*		0.0%	0.0-0.0%

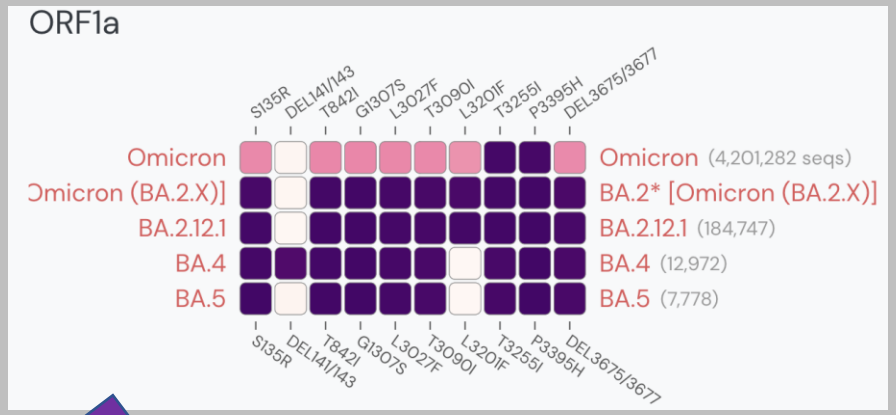
* Enumerated lineages are US VOC and lineages circulating above 1% nationally in at least one week period. "Other" represents the aggregation of lineages which are circulating <1% nationally during all weeks displayed.
** These data include Nowcast estimates, which are modeled projections that may differ from weighted estimates generated at later dates
# AY.1-AY.133 and their sublineages are aggregated with B.1.617.2. BA.1, BA.3 and their sublineages (except BA.1.1 and its sublineages) are aggregated with B.1.1.529. For regional data, BA.1.1 and its sublineages are also aggregated with B.1.1.529, as they currently cannot be reliably called in each region. Except BA.2.12.1, BA.2 sublineages are aggregated with BA.2. Sublineages of BA.4 are aggregated to BA.4. Sublineages of BA.5 are aggregated to BA.5.

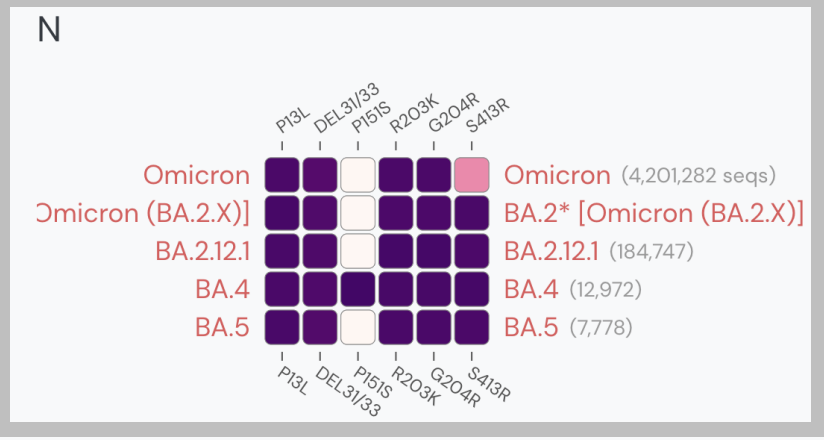
Taqpath qRT-PCR: from 1 to 3 and Variant Monitoring



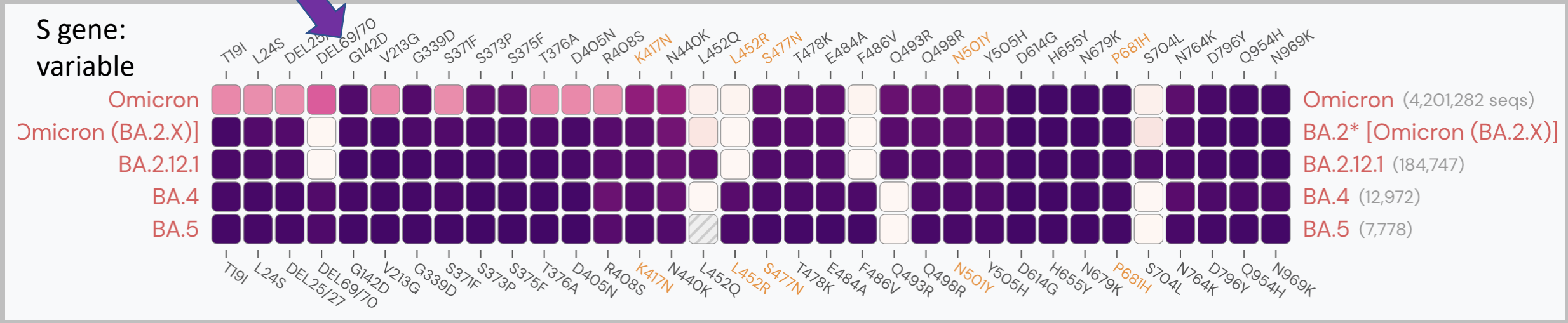


Orf1a Constant (relatively)

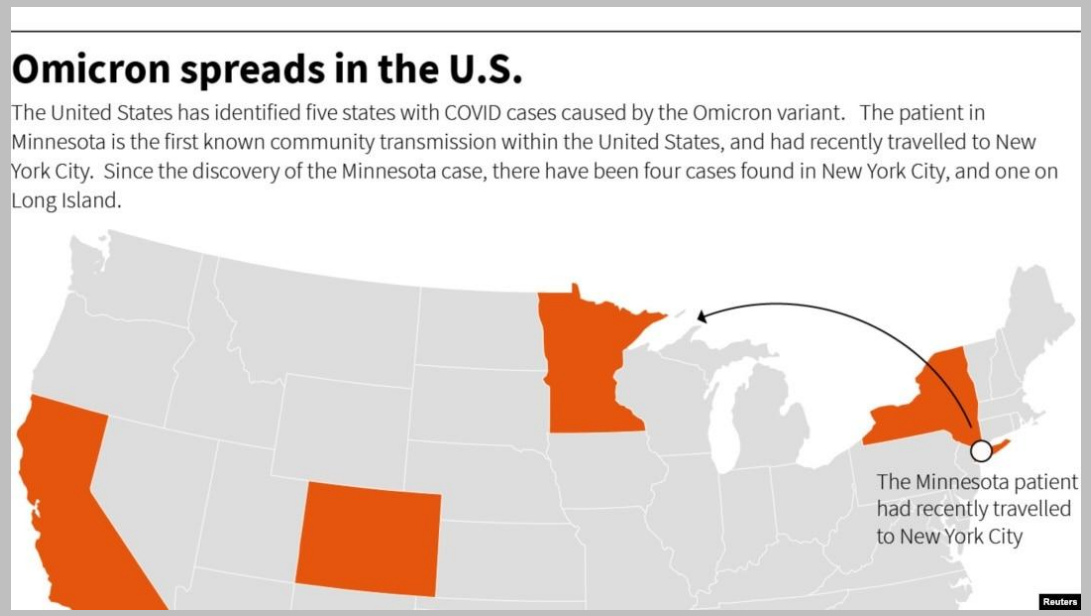


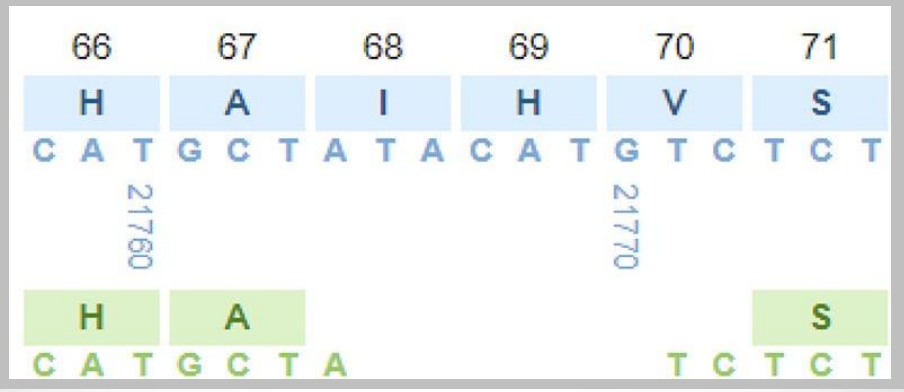


N Constant (relatively)

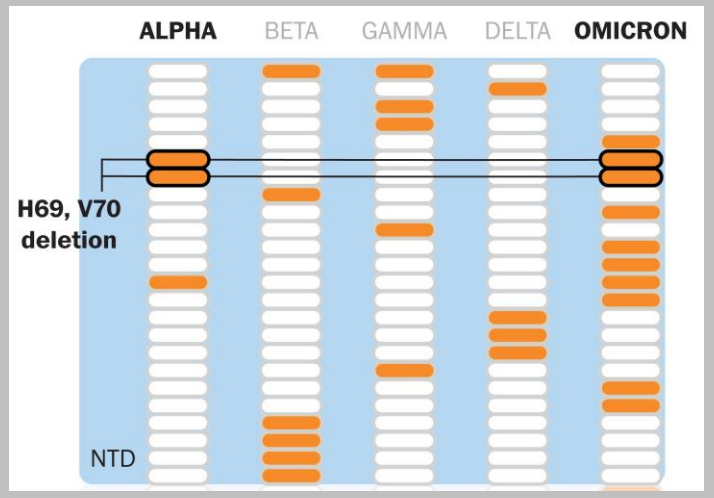


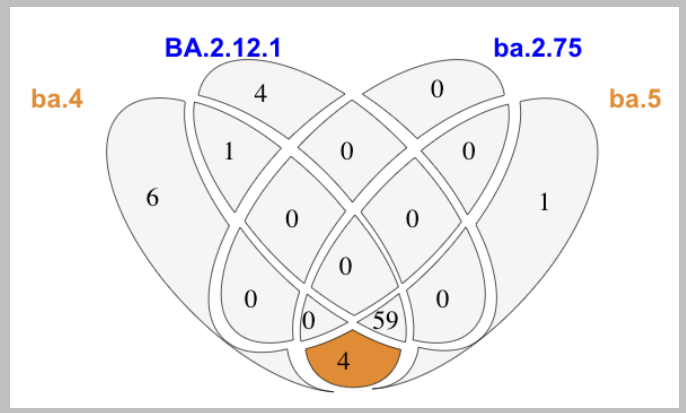
Variant Detection: Delta to Omicron



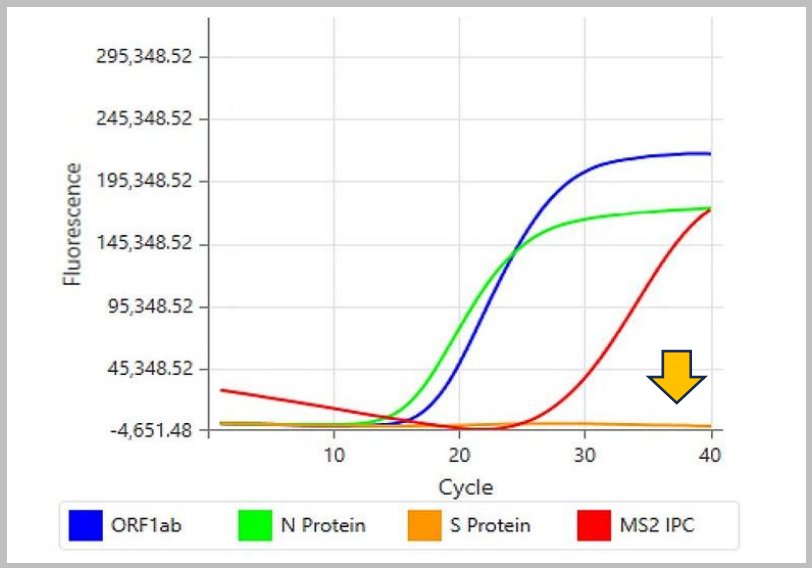






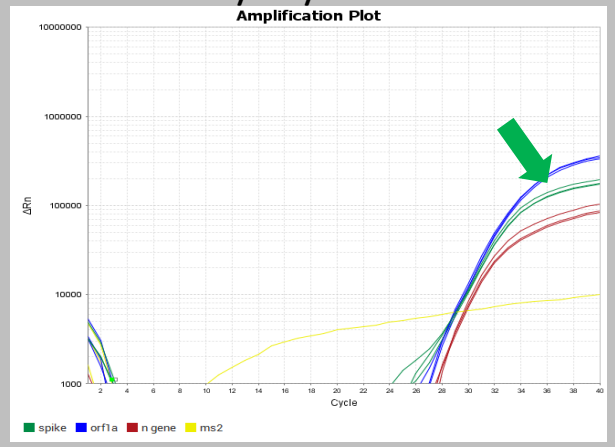


Loss of signal tracking

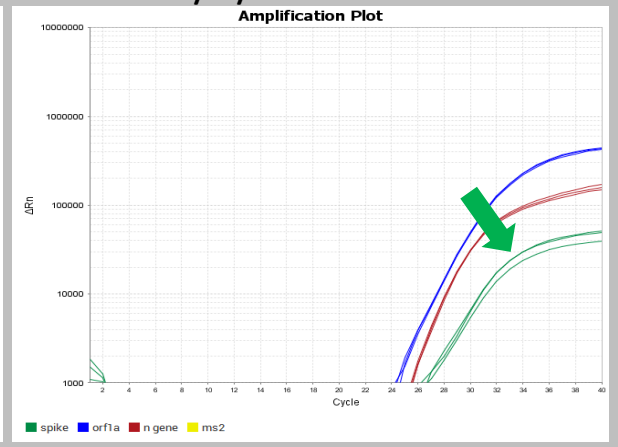


S gene target failure: Omicron BA1

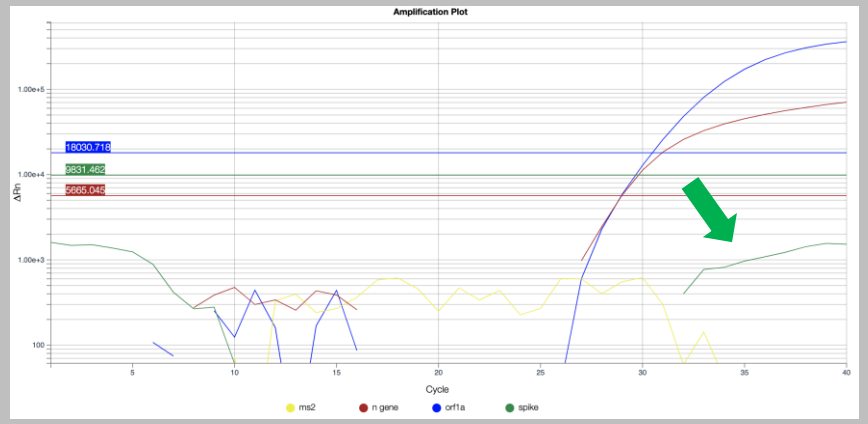
12/18/21



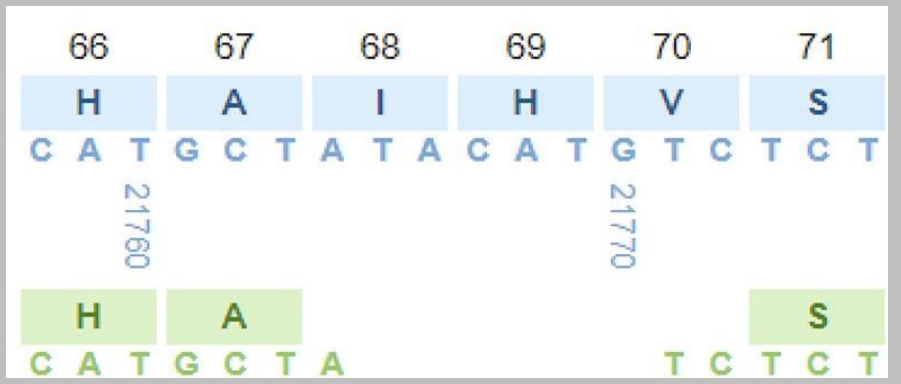
1/5/22: decline



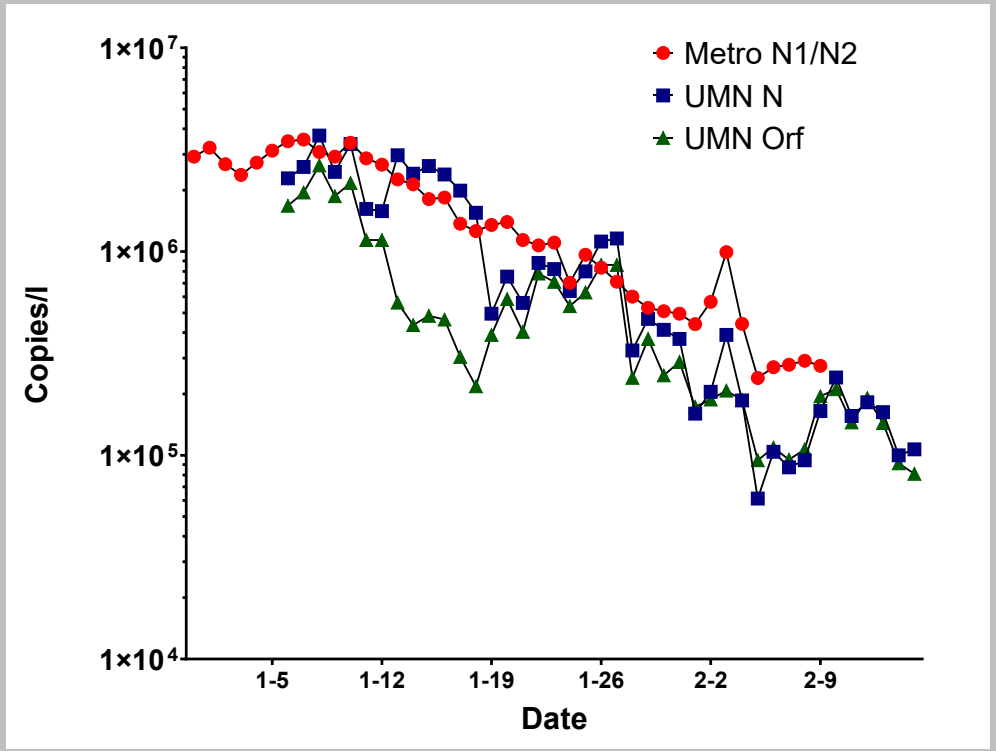
2/8/22:
Absent



Delta

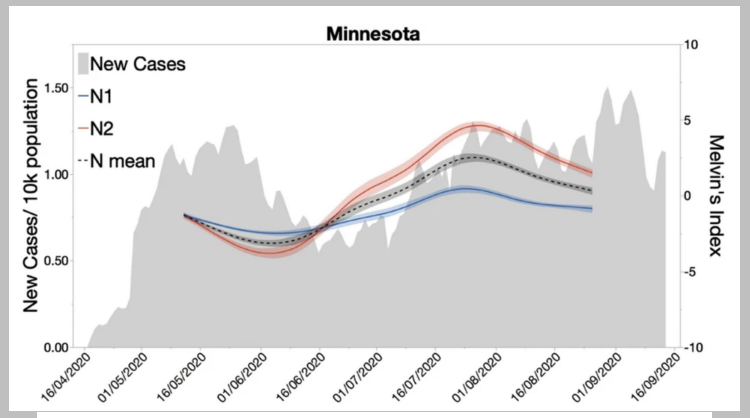


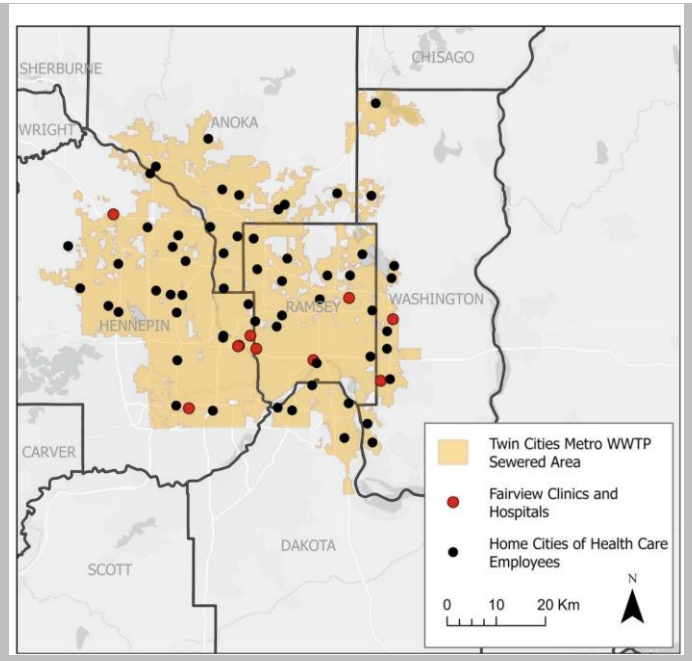
Omicron (BA1)

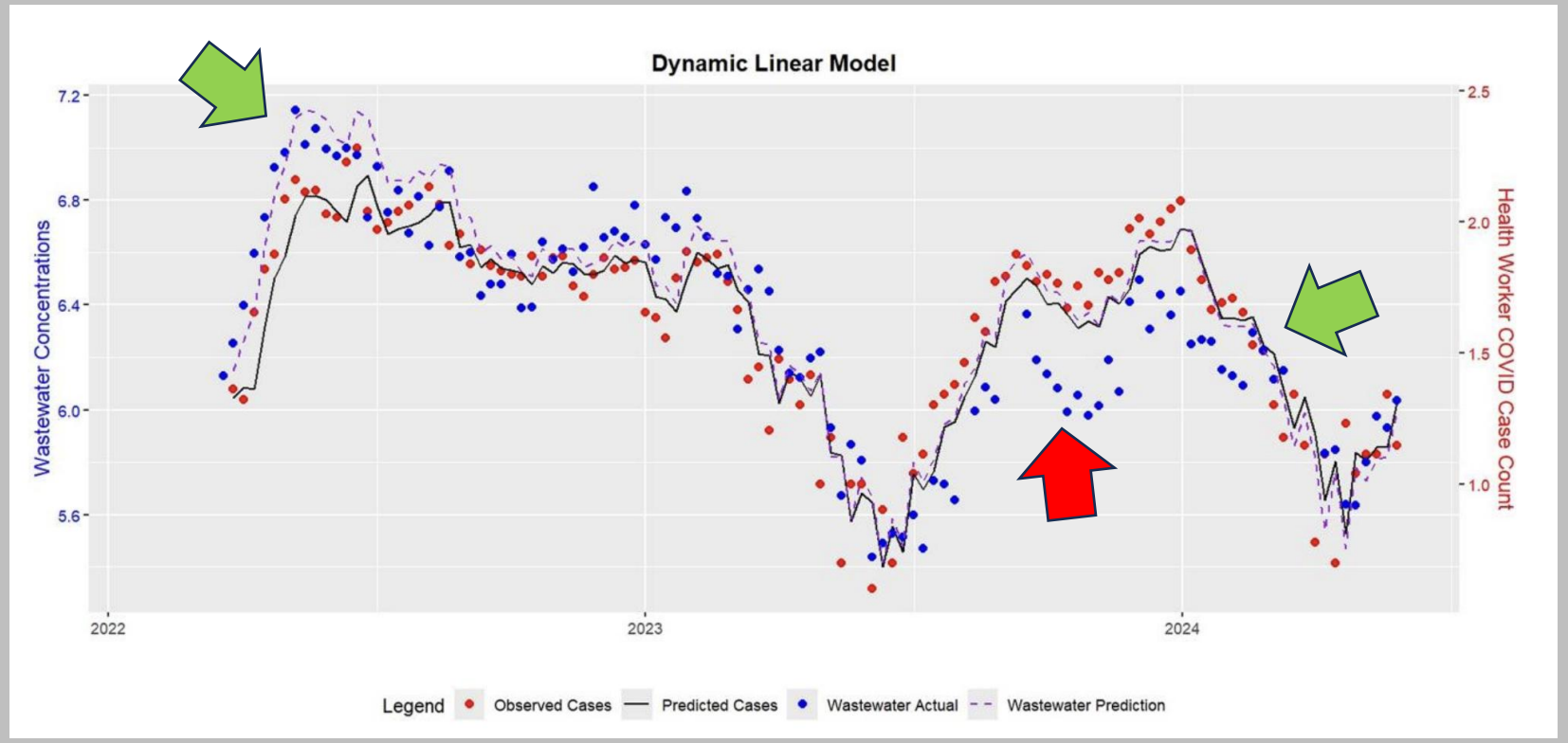


Predictions

- Predictive values degraded as the pandemic proceeded







Conclusions:

- What have we learned
  - community level assessment
  - predicts lead in case counts*
    - focus on healthcare workers (HCW) **restored** predictive properties
    - enabled mitigation/prevention efforts that preserved staffing levels
  - variants can be tracked
- Where are we?
- Where do we want to go?

The Journal of Infectious Diseases

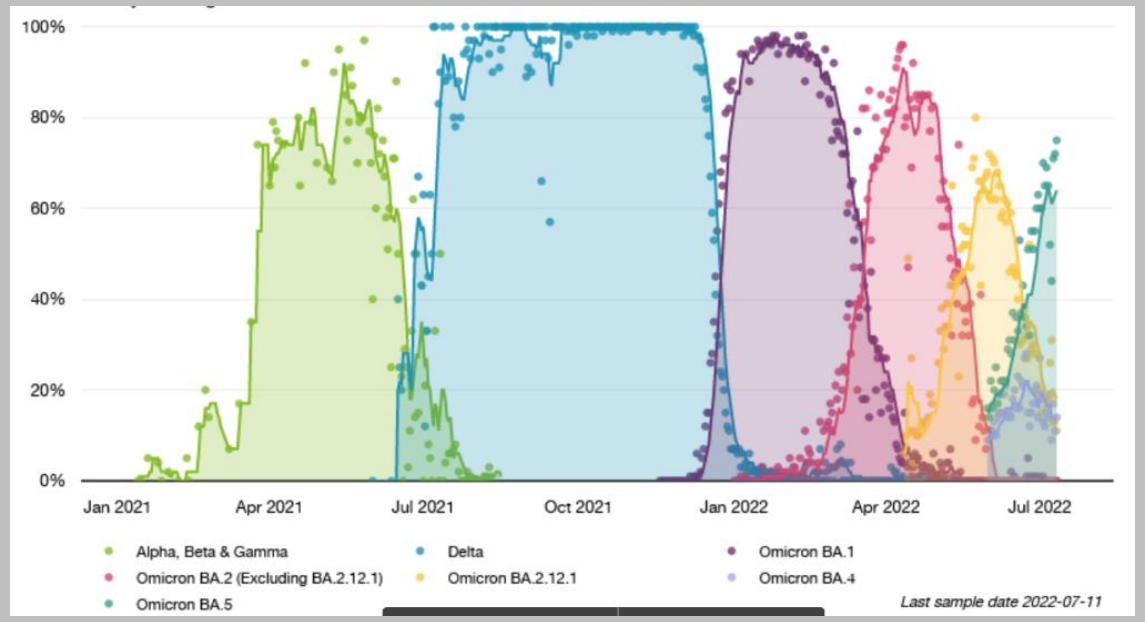
MAJOR ARTICLE

IDSA OXFORD

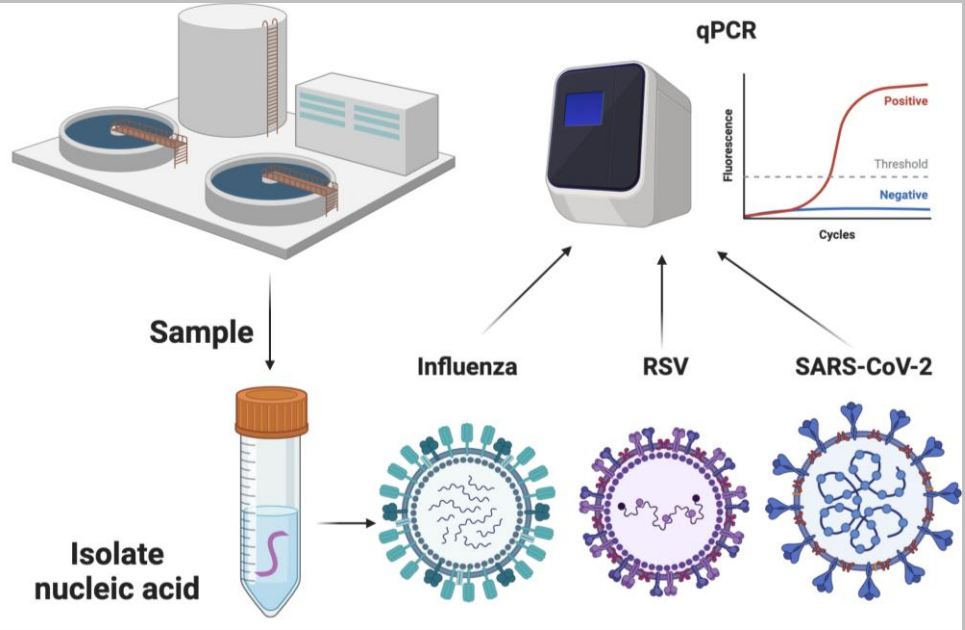
Wastewater Measures of SARS-CoV-2 Accurately Predict Frequency of Symptomatic Infections in the Community

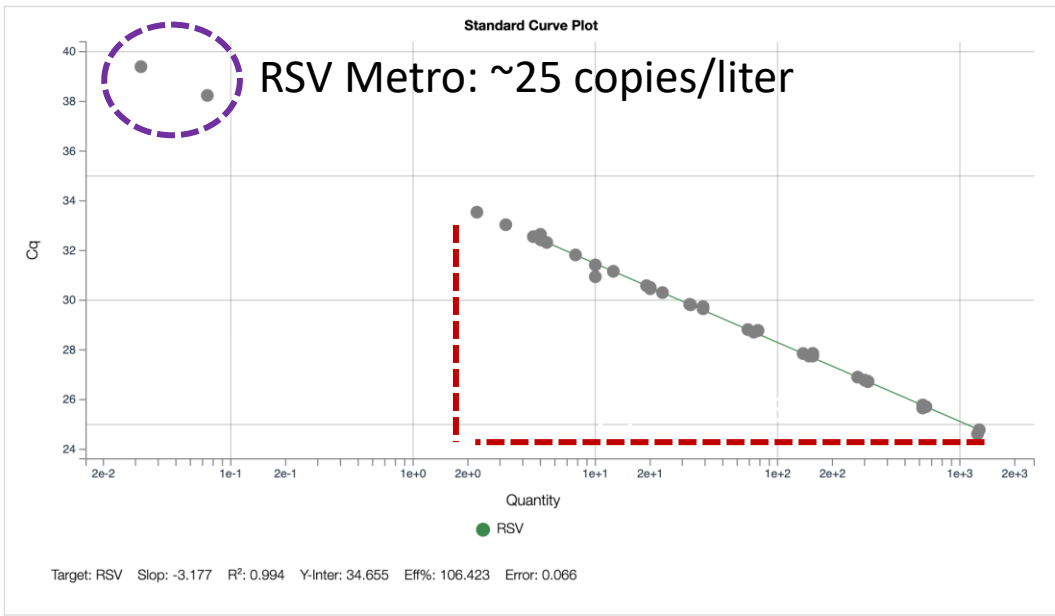
Charles R. Doss,[1,a] Mark J. Osborn,[2,a] Stacey Stark,[3] Joshua Rhein,[4] Jacalynn Donkersgoed,[5] Donna Budde,[5] Shannon Champeau,[2] Carolyn Meyer,[2] Mason Hayden,[2] Laura Landini,[2] Difan Ouyang,[1] Lappui Chung,[1] Yi Tang,[6] Sara Vetter,[7] and Timothy W. Schacker[4]

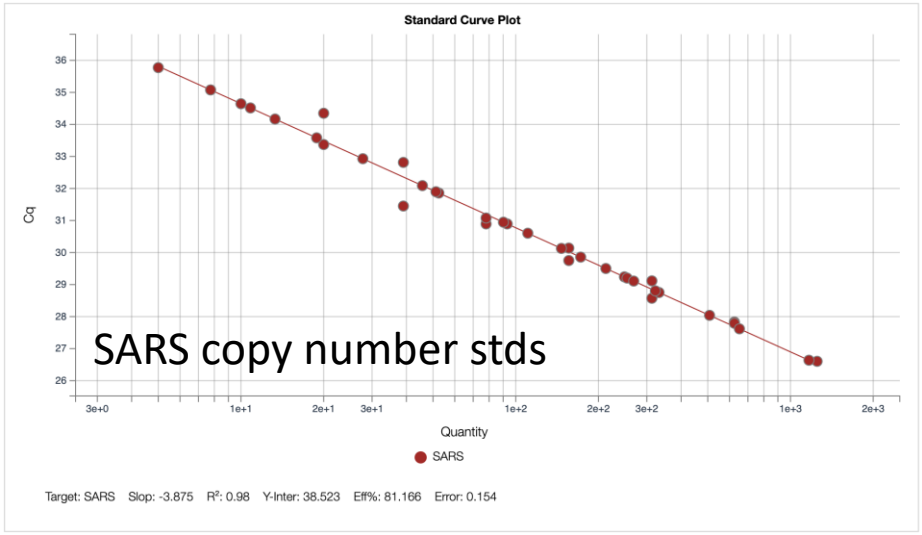
[1]School of Statistics, University of Minnesota, Minneapolis, Minnesota; [2]Department of Pediatrics, University of Minnesota, Minneapolis, Minnesota; [3]U-Spatial, Research Computing, University of Minnesota, Duluth, Minnesota; [4]Department of Medicine, University of Minnesota, Minneapolis, Minnesota; [5]Office of Employee Occupational Health and Safety, Fairview Health Services, Minneapolis, Minnesota; [6]School of Public Administration, University of New Mexico, Albuquerque, New Mexico; and [7]Minnesota Department of Public Health, Saint Paul, Minnesota

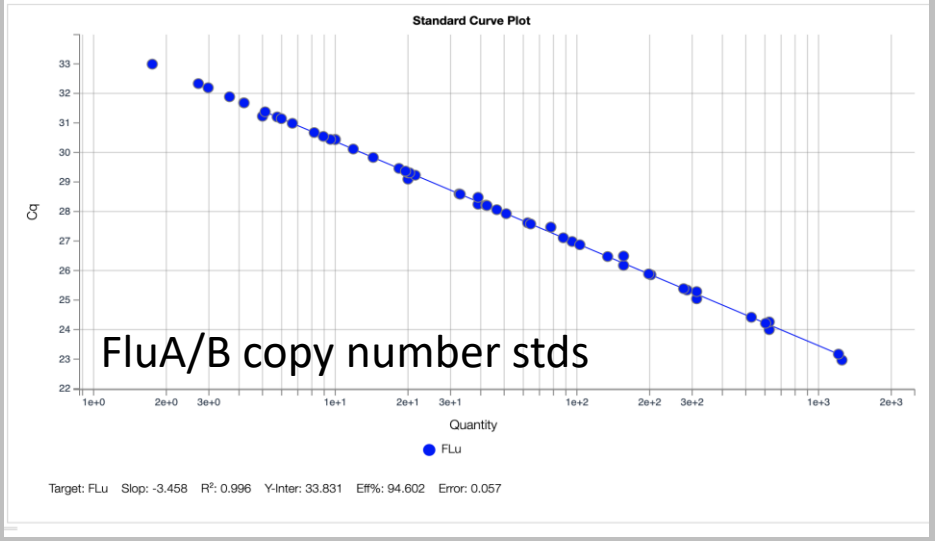


BROADER RESPIRATORY PATHOGEN SURVEILLANCE

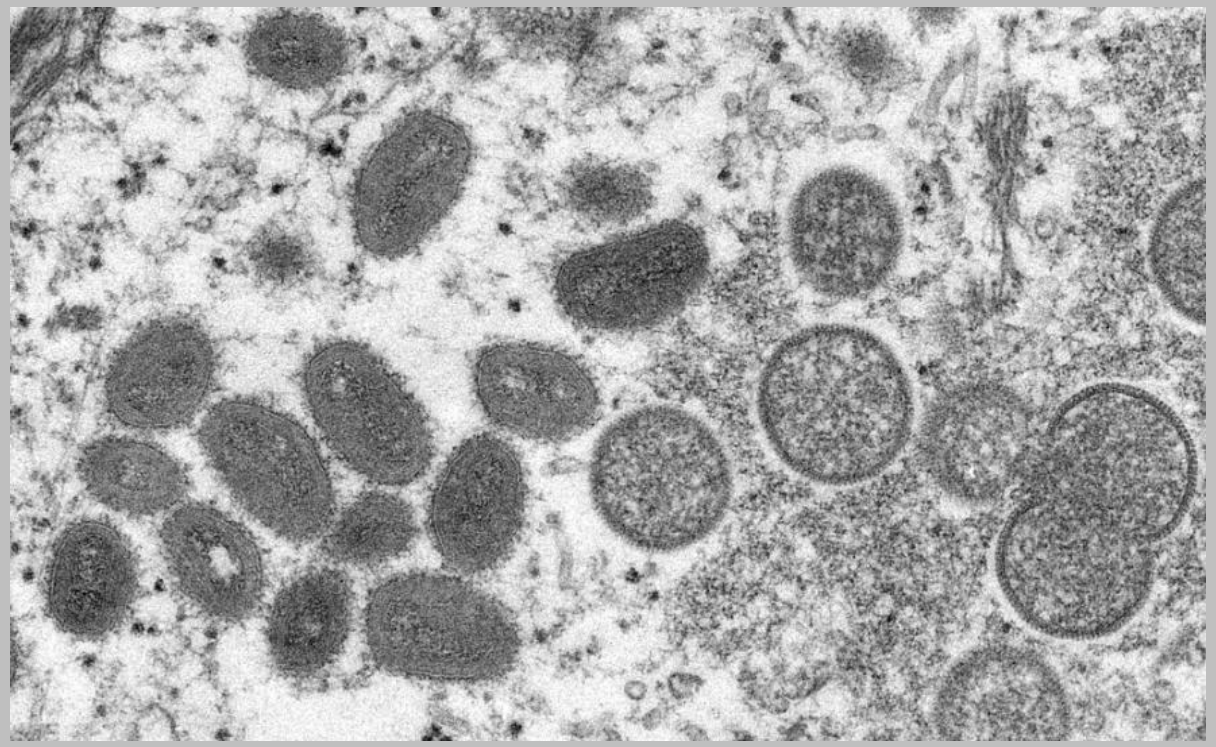


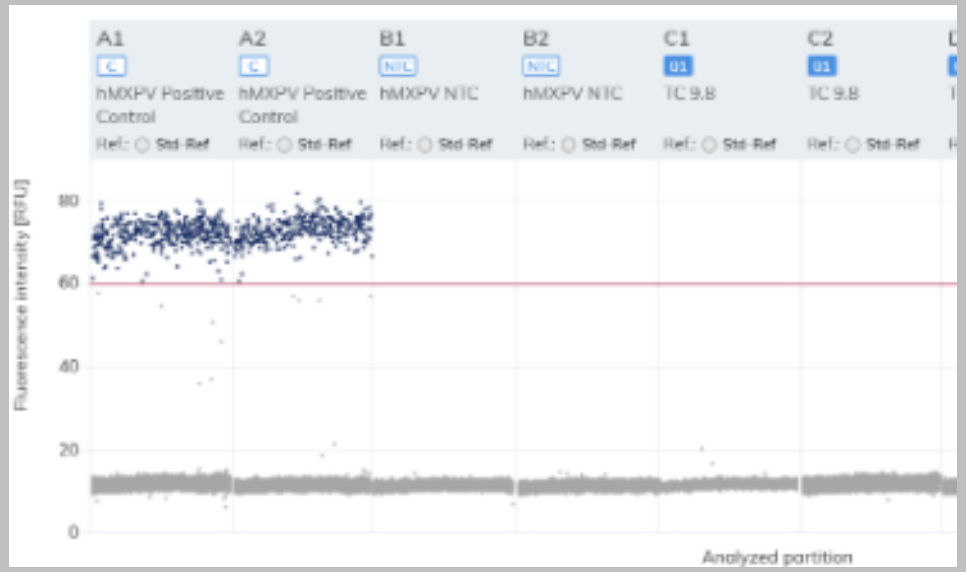




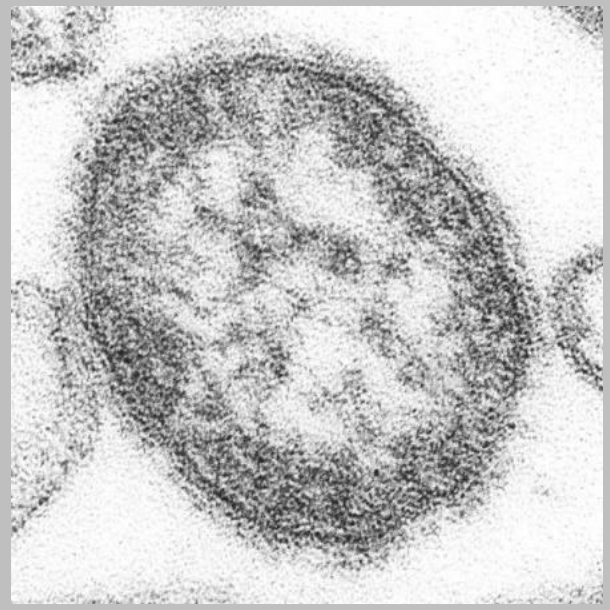


Wastewater testing helpful in tracking mpox outbreaks





Rochester, MN MeV

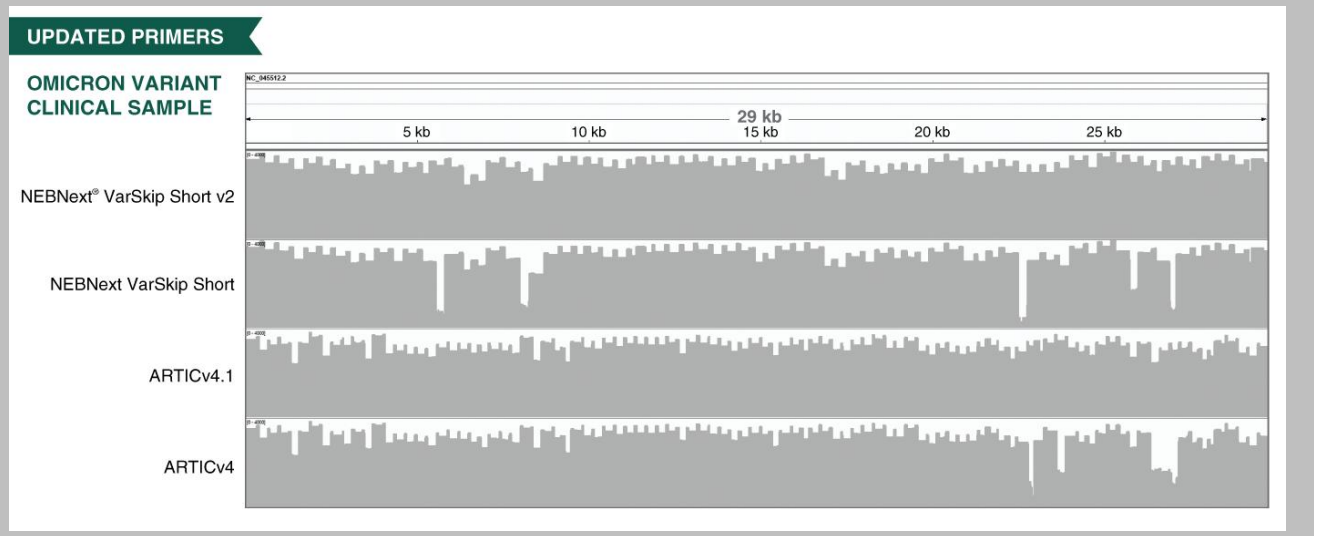


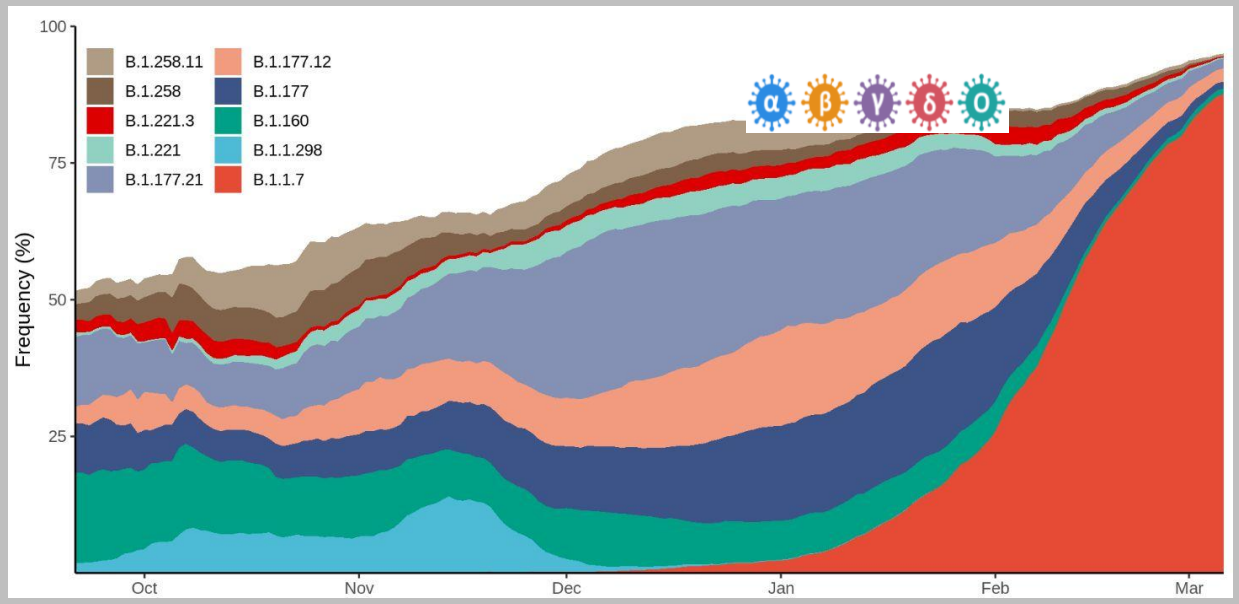
Wastewater is helping detect measles cases, and it comes at a critical time

Meyer says MDH is working with the University of Minnesota to expand on its groundbreaking wastewater surveillance network that helped detect spikes in COVID transmission before hospitals saw a surge in patients. In recent years, the same sampling has also helped monitor flu and RSV transmission.

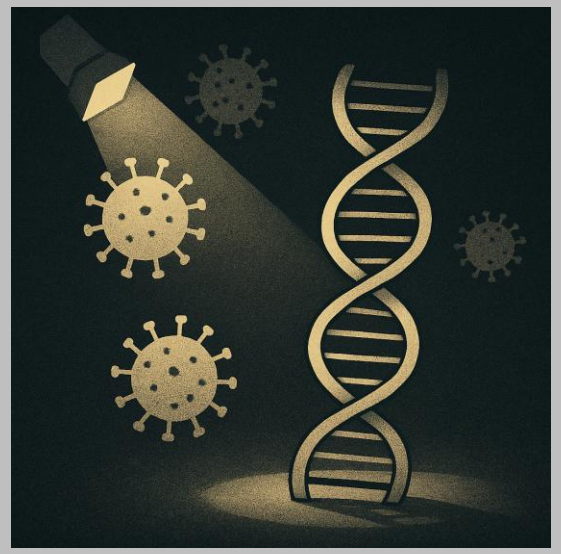
Stephanie: "What we found in Rochester was a positive (measles) finding in wastewater and we did identify one case shortly after that finding in wastewater. The peak of the amount of virus that we were finding in the (wastewater) samples went right along with that person's course of illness."

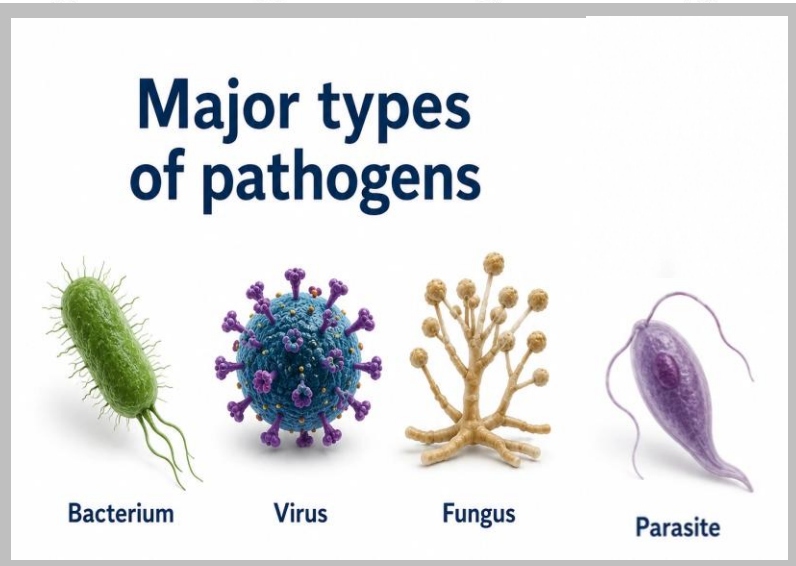
Where we are going: Surveillance through sequencing







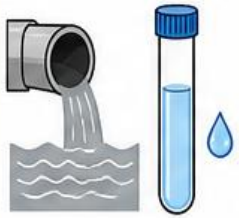




Finding viruses and bacteria (and their antibiotic-resistance genes) in a wastewater sample

PHASE 1 • START

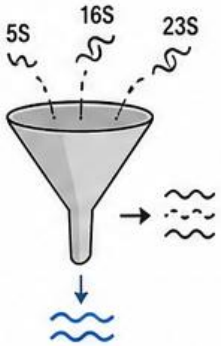
1 Collect the wastewater sample



Take RNA or DNA from the microbes in a wastewater sample.

PHASE 2 • PREP

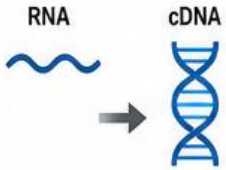
2 Filter out the noise



Remove bacterial ribosomal RNA (5S, 16S, 23S) — the boring stuff that would waste most of your reads.

QIAseq FastSelect –5S/16S/23S (Cat# 335927)

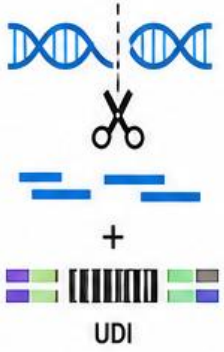
3 Copy RNA into DNA



If starting from RNA, use reverse transcriptase to make a stable DNA copy (cDNA) of every microbial gene.

PHASE 3 • BUILD LIBRARY

4 Fragment and barcode the DNA

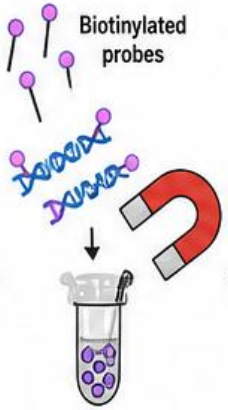


Fragment the DNA, polish the ends, and attach Illumina adapters + unique barcode tags (UDI) so each sample is identifiable.

QIAseq xHYB Hyb&Lib Kit A (Cat# 334525)

PHASE 4 • ENRICH

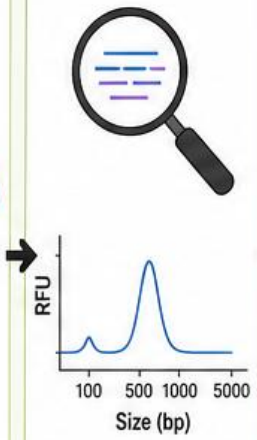
5 Enrich the targets



Mix with biotin-tagged probes that match viral & bacterial genes. Probes grab their targets; magnetic beads pull them out. Hybridize overnight.

QIAseq xHYB Microbial Panel (Cat# 334565)

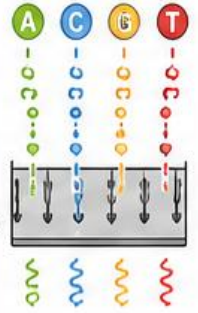
6 Check the library



Measure how much DNA you have (Qubit) and check fragment sizes (Bioanalyzer/ TapeStation).

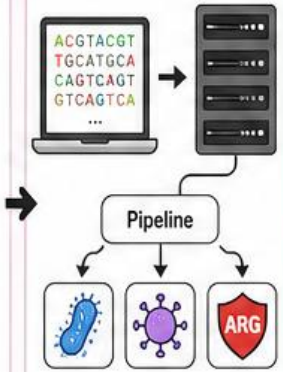
PHASE 5 • SEQUENCE

7 Read the code



Load onto the Illumina NextSeq flow cell. The machine reads each DNA fragment letter-by-letter (A, C, G, T), millions at a time.

8 Submit to UMN/MSI bioinformatics pipeline for analysis



Send the sequencing data to the UMN/MSI bioinformatics pipeline, which sorts reads by barcode, matches them to known microbes, and flags antibiotic-resistance genes.

PREP | BUILD LIBRARY | ENRICH | SEQUENCE | ANALYZE

Analysis

1. Raw DNA Reads



Millions of short DNA fragments sequenced from a wastewater sample.

input →

2. Kraken Database



A lookup library of known DNA sequences from thousands of microbes, built in advance.

compare →

3. Kraken Classification



Each read is matched to the database and assigned to the microbe it most likely came from.

tally →

4. Bracken Abundance



Reads are re-counted at each biological level (species, genus) to estimate how common each microbe is.

report →

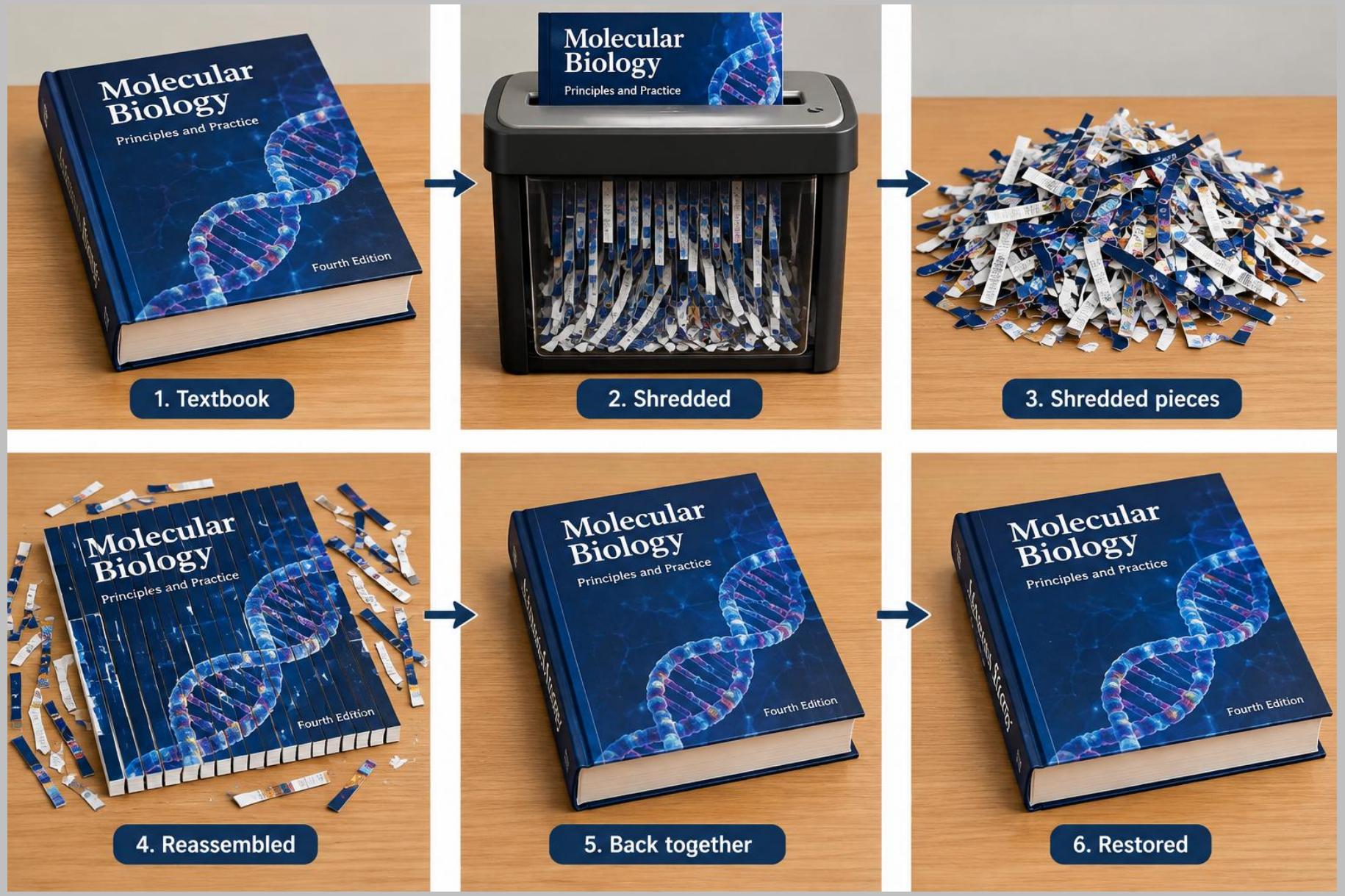
5. Microbe Report



A table of which microbes are present and how abundant each one is in the sample.

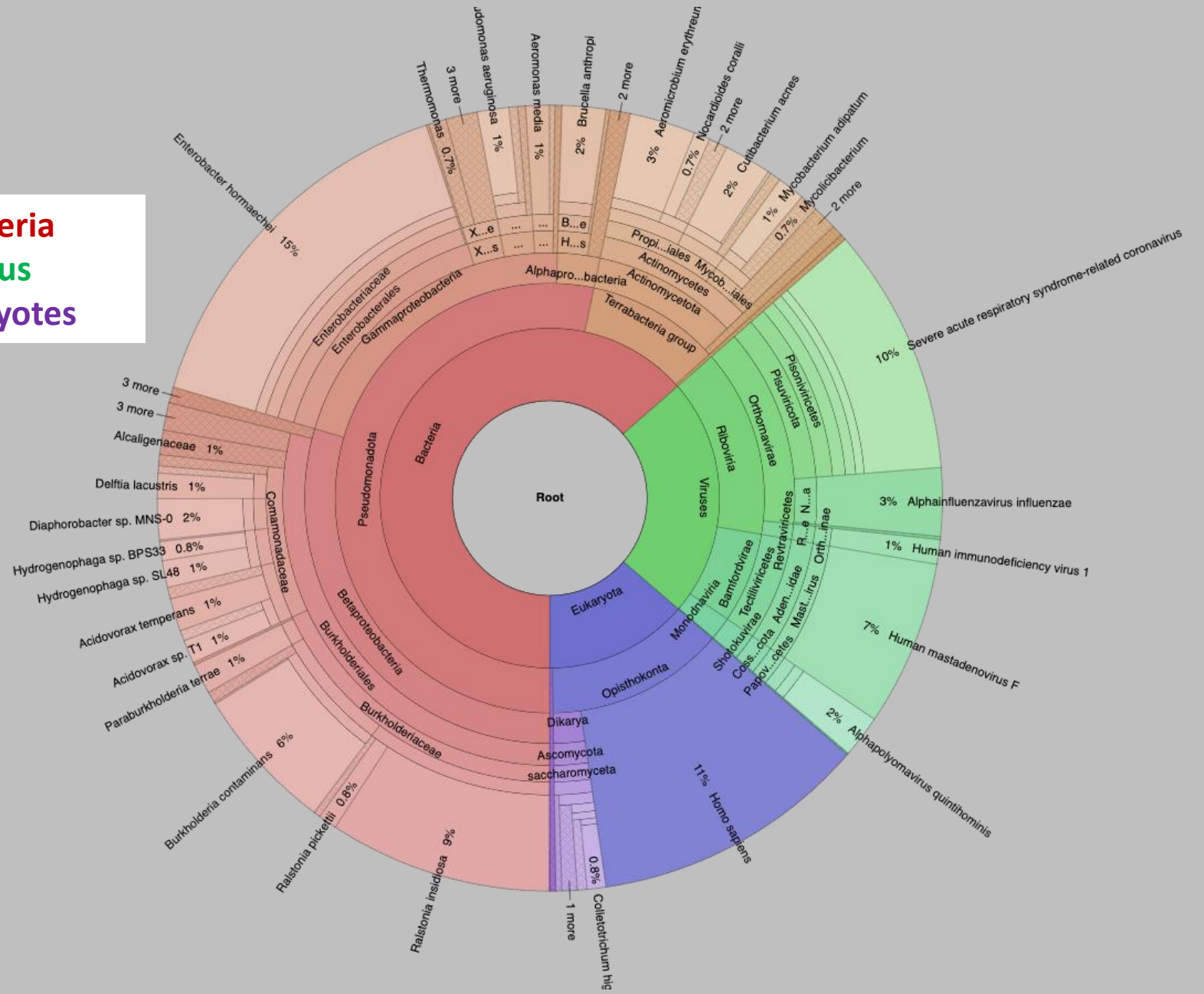
Kraken = names the microbes. Bracken = counts them.
Together they turn raw DNA into a census of the microbial community.

What it amounts to:



Interactive Output: point and click

Bacteria
Virus
Eukaryotes



Searchable database

Species of interest editable table

Show 10 entries

Search:

	sample	site	date	replicate	QC	control_QC	Human.adenovirus.F	JC.polyomavirus
1	MK_02182025_1_S2	MK	02182025	1	11.62	0.08	434.697	3125.482
2	MK_02182025_2_S7	MK	02182025	2	11.2	0.08	400.998	3449.612
3	MK_03192025_1_S12	MK	03192025	1	9.92	0	125.723	1765.742
4	MK_03192025_2_S17	MK	03192025	2	8.45	0	146.737	2157.079
5	MK_04232025_1_S20	MK	04232025	1	10.95	0.03	52.586	2826
6	MK_04232025_2_S25	MK	04232025	2	7.52	0.03	52.722	2038.568
7	MK_05212025_1_S30	MK	05212025	1	13.46	0.01	0	883.12
8	MK_05212025_2_S35	MK	05212025	2	12.26	0.01	0	803.744
9	MK_08202025_1_S40	MK	08202025	1	5.53	0.2	0	759.233
10	MK_08202025_2_S45	MK	08202025	2	6.89	0.2	0	961.479

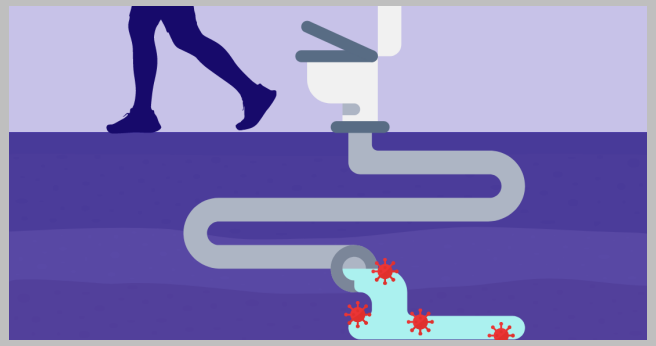
Showing 1 to 10 of 48 entries

Previous 1 2 3 4 5 Next

Where do we want to go?



- Current timelines are days (PCR) to weeks (sequencing)
- Applying the principles of at home testing to WW

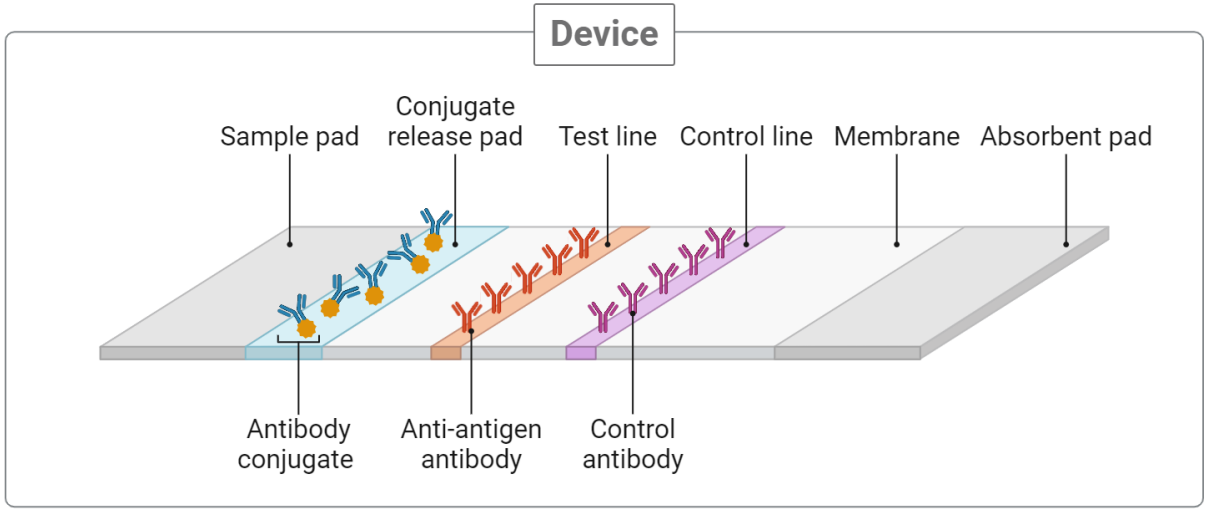


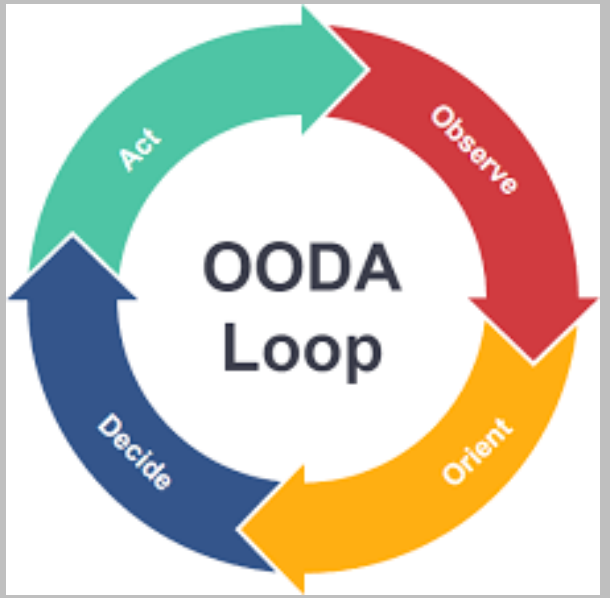
Toward decreasing turn around times



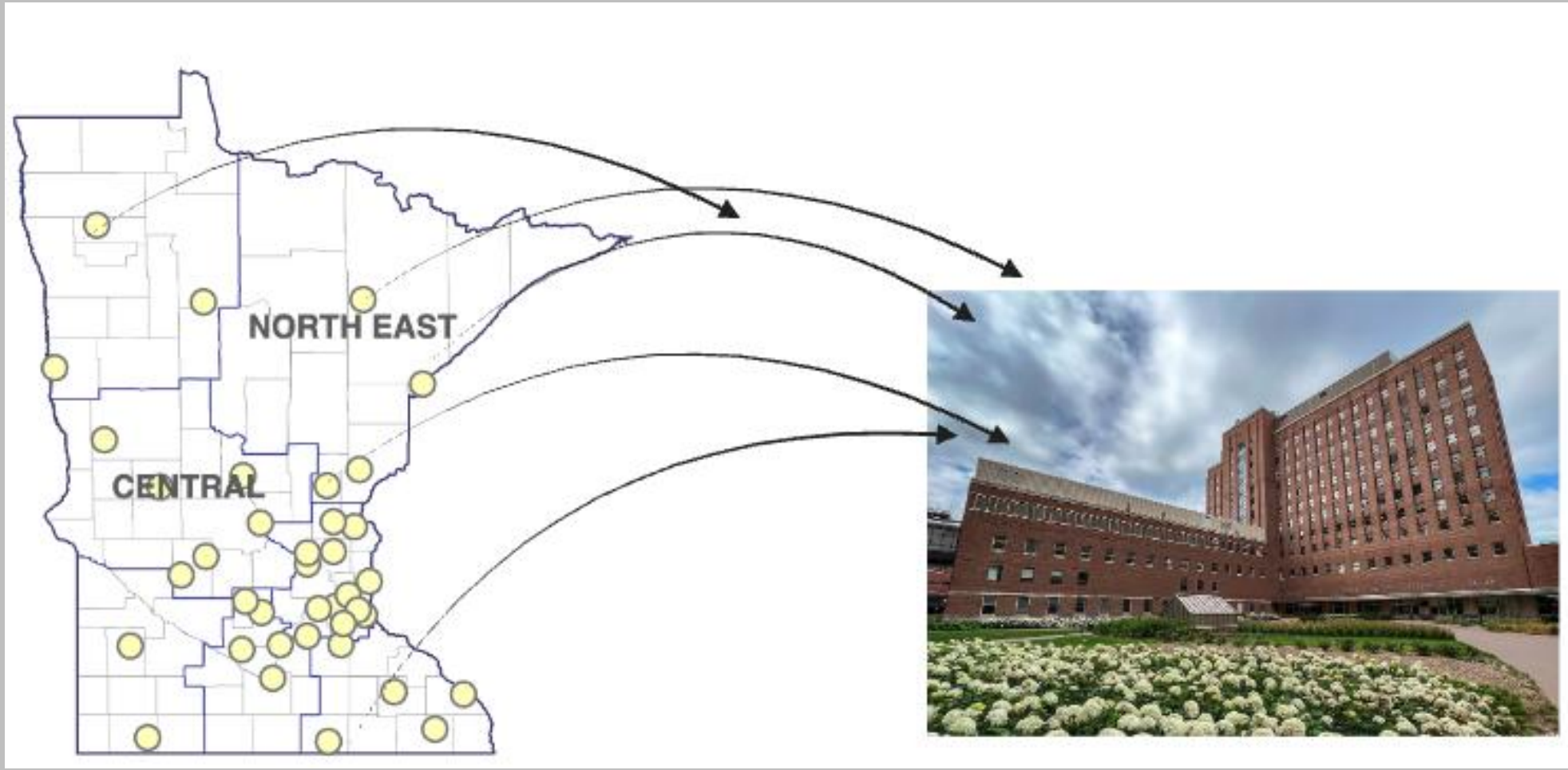
Wastewater is helping detect measles cases, and it comes at a critical time

Stephanie: "What we found in Rochester was a positive (measles) finding in wastewater and we did identify one case shortly after that finding in wastewater. The peak of the amount of virus that we were finding in the (wastewater) samples went right along with that person's course of illness."





R&D: Point of collection detection: from 2 days to 2 hours



Binding is critical

Lateral Flow Assay (LFA)

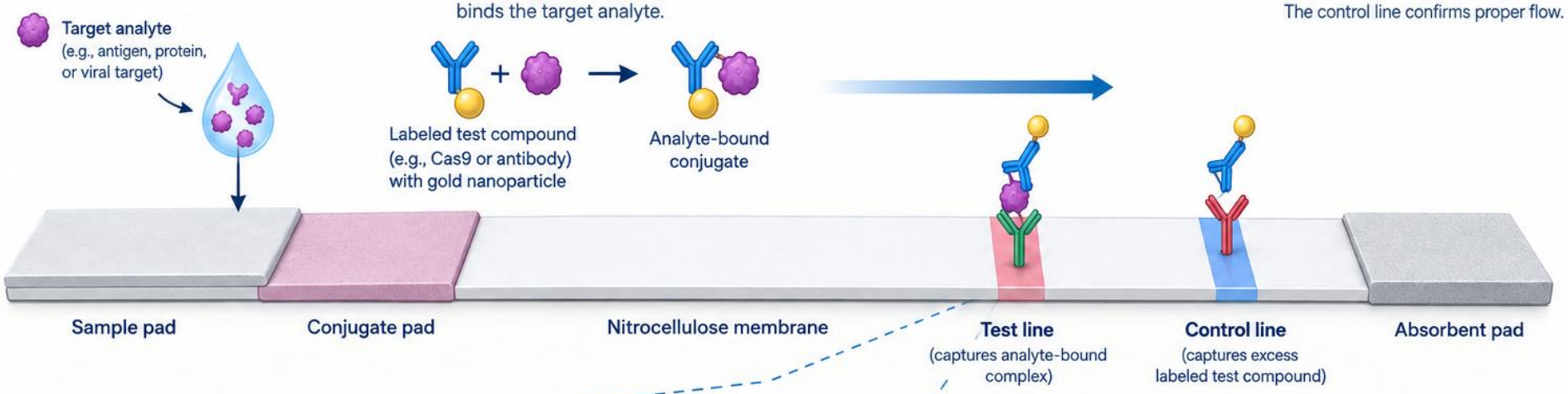
The test compound (e.g., Cas9 or an antibody) must bind the target anaayte te for a signal to be generated.

1 Sample application
Sample containing the target analyte is added to the sample pad.

2 Binding in the conjugate pad
The test compound (e.g., Cas9 or antibody) is labeled (e.g., with color or gold nanoparticles) and binds the target analyte.

3 Migration
The complex migrates by capillary action along the nitrocellulose membrane.

4 Signal generation
The bound complex is captured at the test line by a second immobilized reagent, producing a visible signal. The control line confirms proper flow.



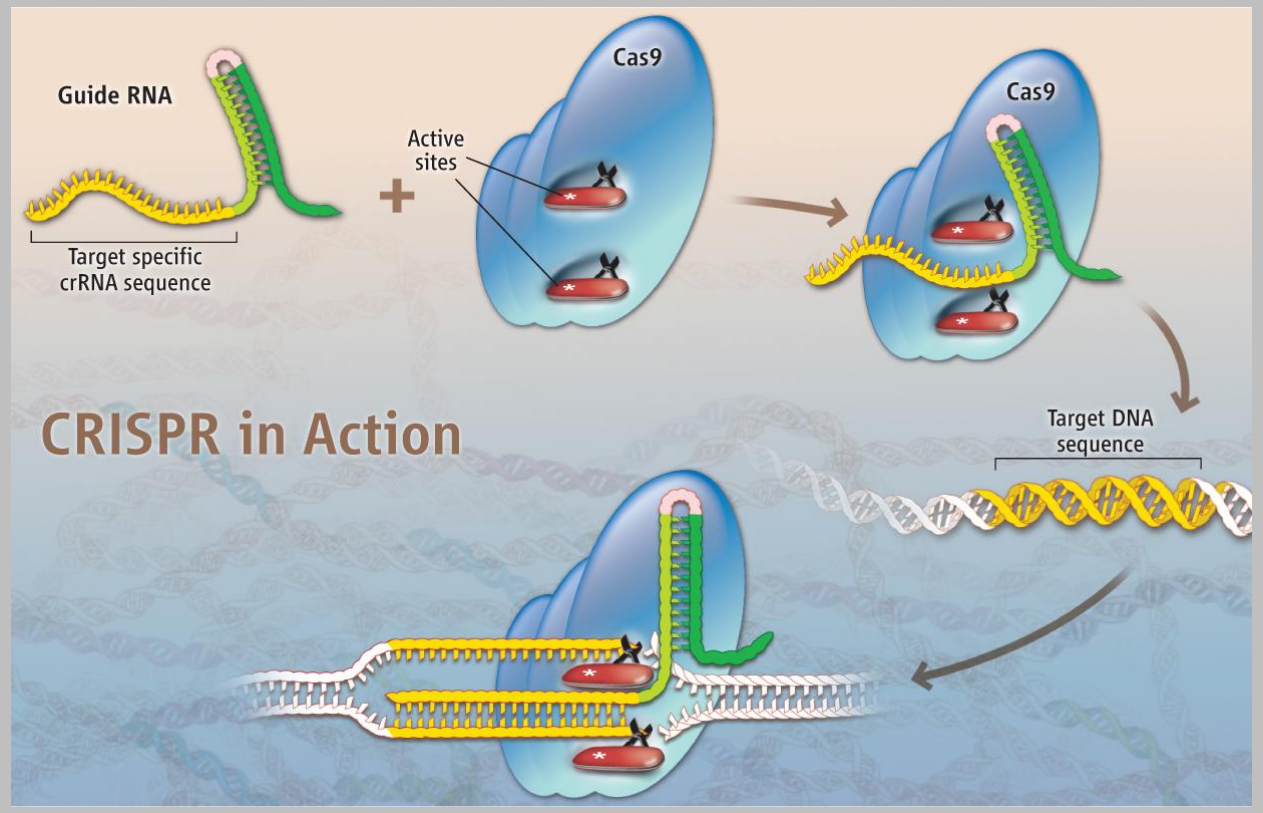
The Importance of Analyte Binding



Why Analyte Binding Matters

- **Enables specific detection** – The test compound (Cas9 or antibody) must bind the target analyte to form a detectable complex.
- **Reduces false positives** – Unbound test compound will not be captured at the test line.
- **Improves sensitivity** – Efficient binding increases signal strength and assay performance.
- **Validates the assay** – A signal at the test line confirms the presence of the target analyte in the sample.

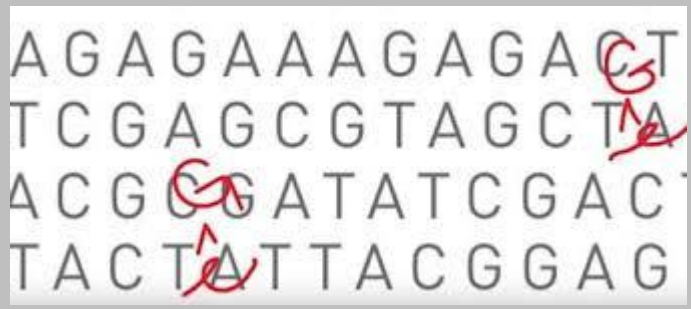
In my other job: Precision genome recognition: CRISPR/Cas9



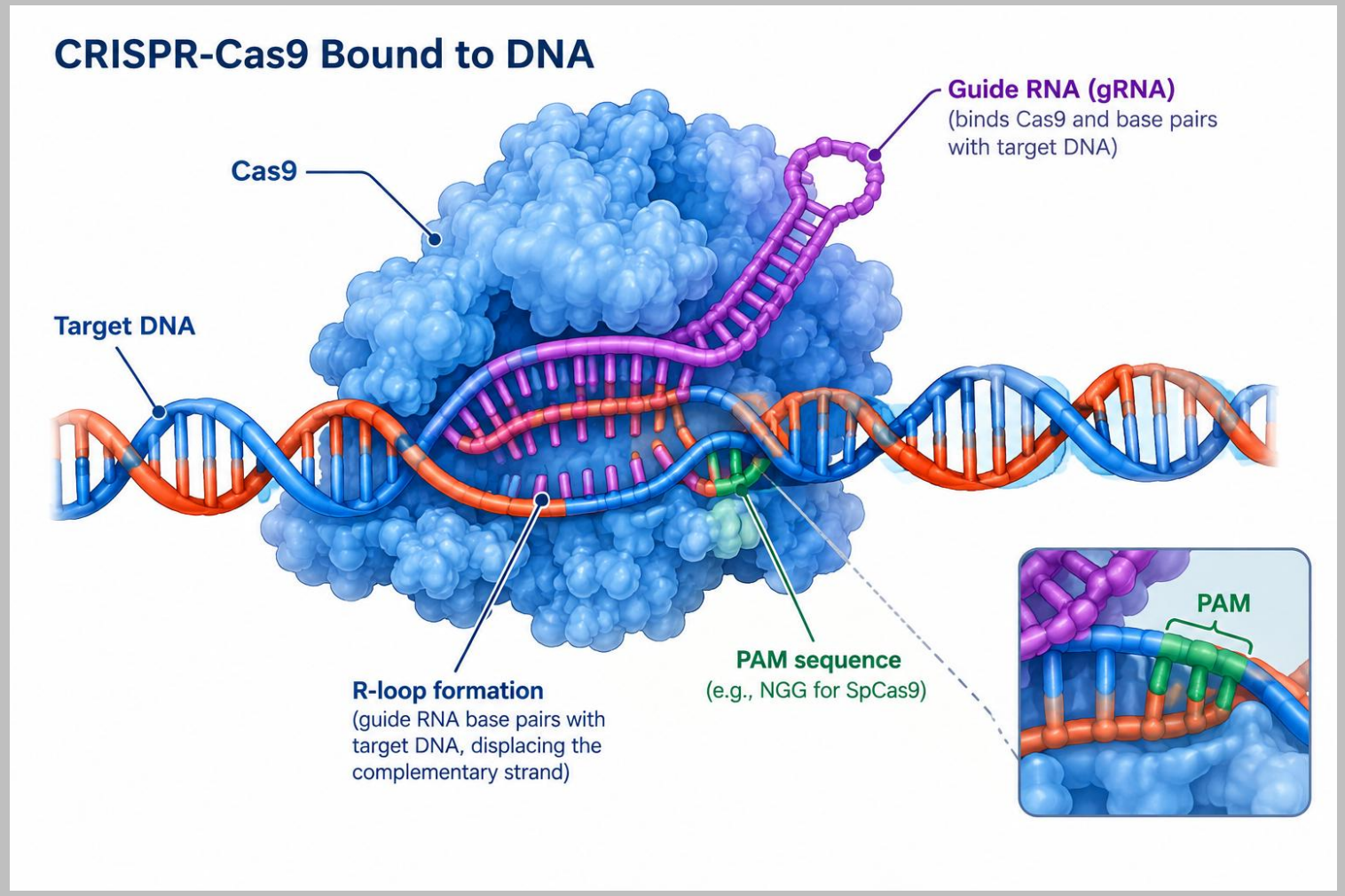




CENTER FOR GENOME ENGINEERING



Programmable binding of nucleic acid

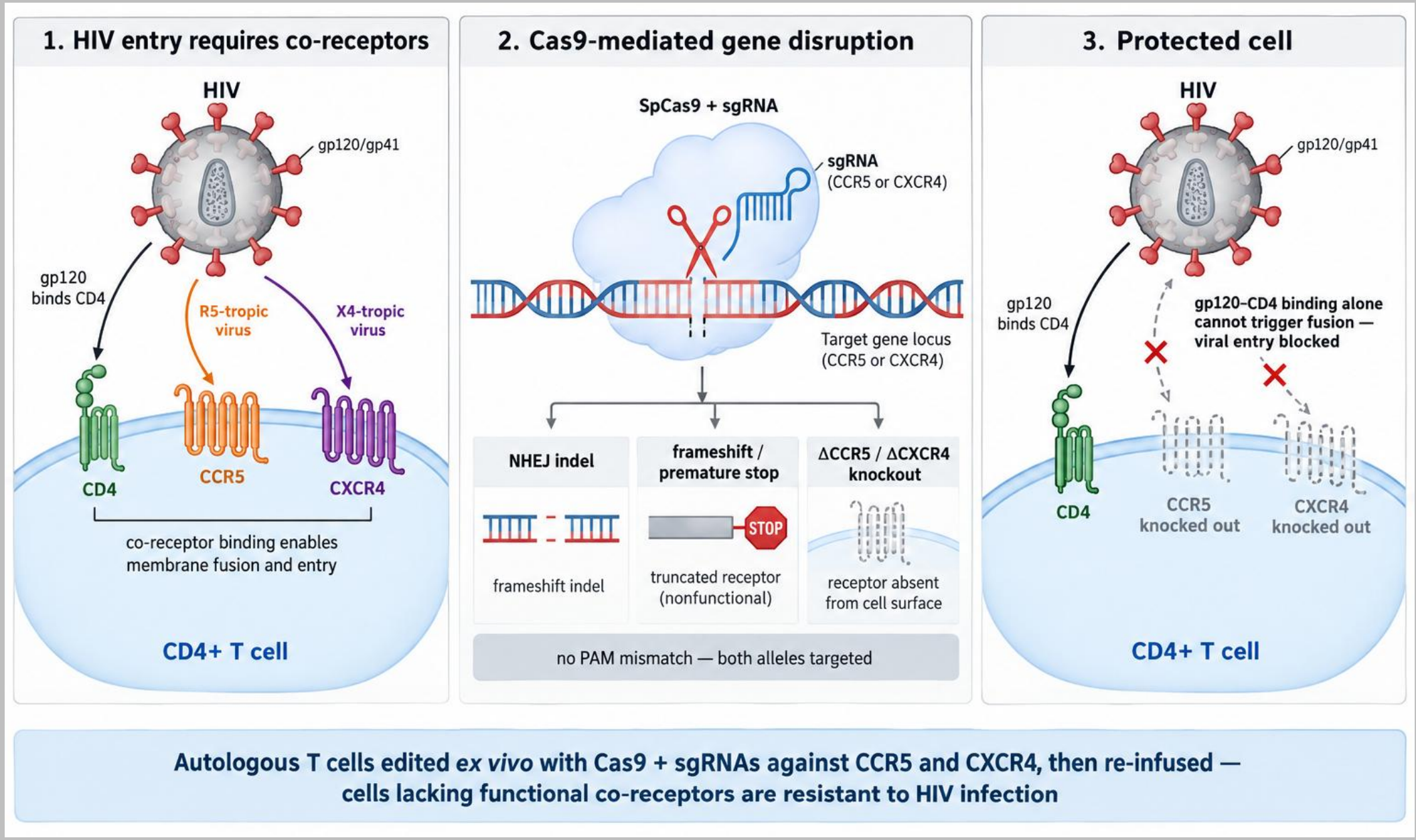


→ Breaking DNA: Better T-cells

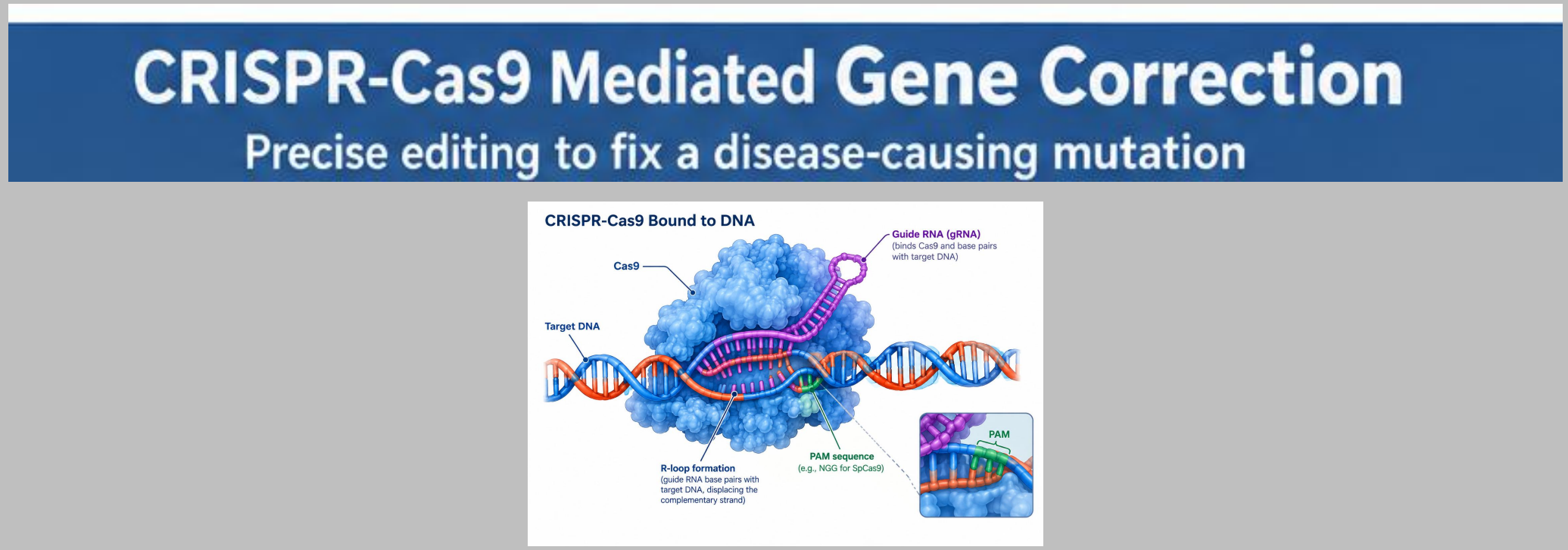
→ Fixing DNA: Genetic disease

→ Binding DNA:
Better diagnostics

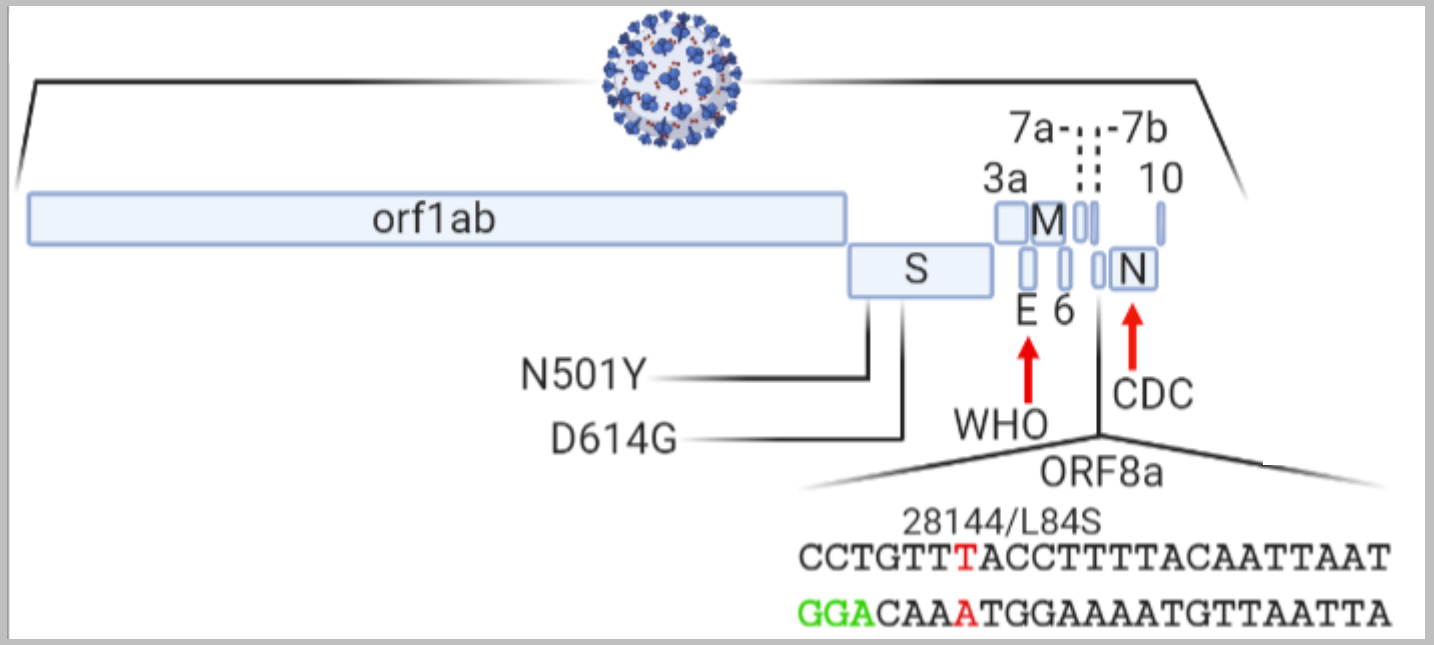
Breaking DNA: Preventing HIV

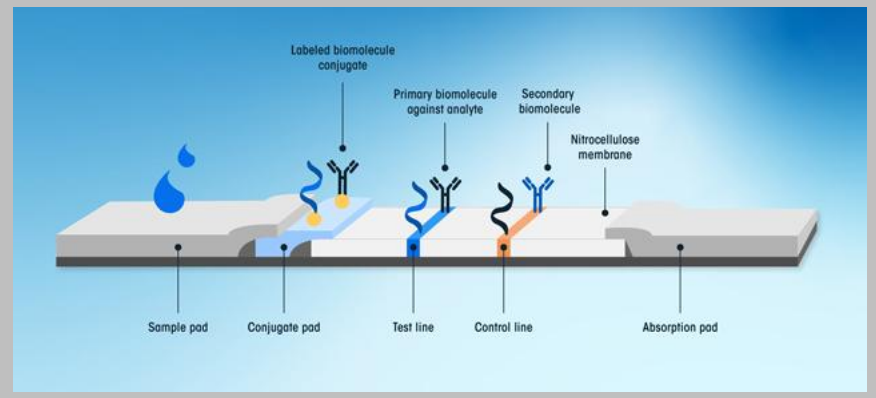


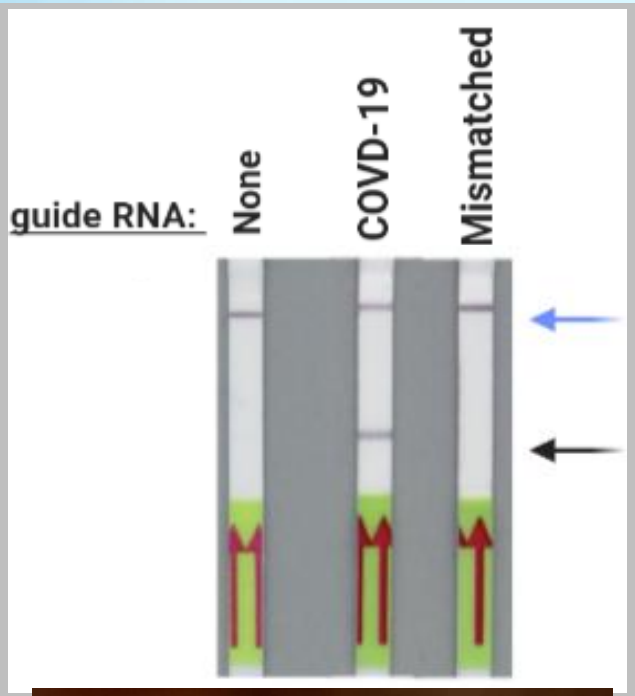
Fixing DNA

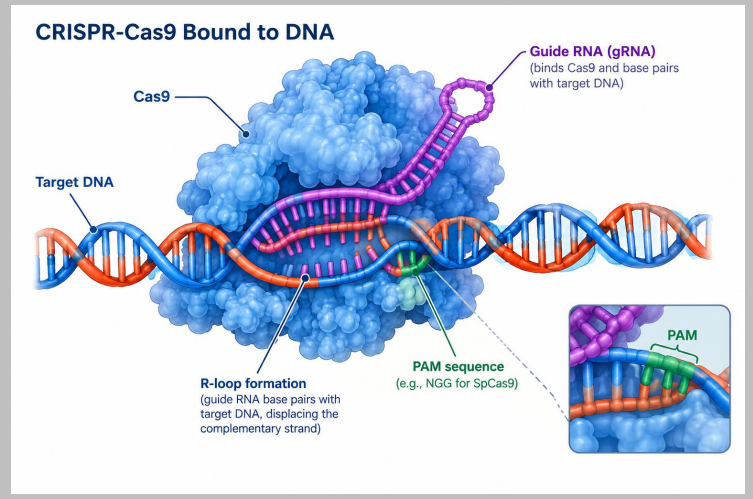

CRISPR-Cas9 Mediated Gene Correction
Precise editing to fix a disease-causing mutation
CRISPR-Cas9 Bound to DNA
Guide RNA (gRNA)
(binds Cas9 and base pairs with target DNA)
Cas9
Target DNA
PAM
PAM sequence
(e.g., NGG for SpCas9)
R-loop formation
(guide RNA base pairs with target DNA, displacing the complementary strand)
Before Correction (Disease Causing Mutation)
... C T A C A G T T C C ...
Mutant nucleotide
Mutant gene
(e.g., A → G)
Abnormal protein
• Loss of function
• or gain of function
• or altered activity
CRISPR-Cas9 Editing
Cas9 + gRNA
+ donor template
After Correction (Healthy)
... C T A C A T T T C C ...
Corrected nucleotide
Corrected gene
(e.g., G → A)
Functional protein
• Restored function
• Normal activity
• Therapeutic benefit

Binding DNA: LFA diagnostics



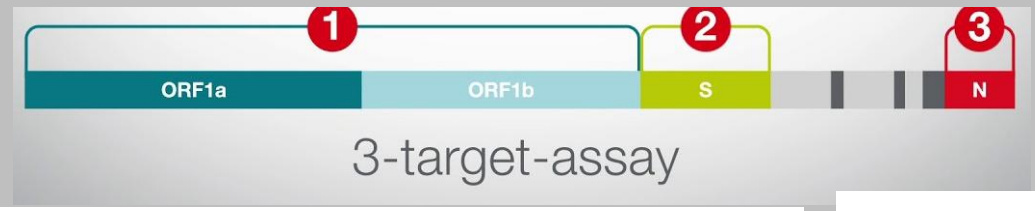


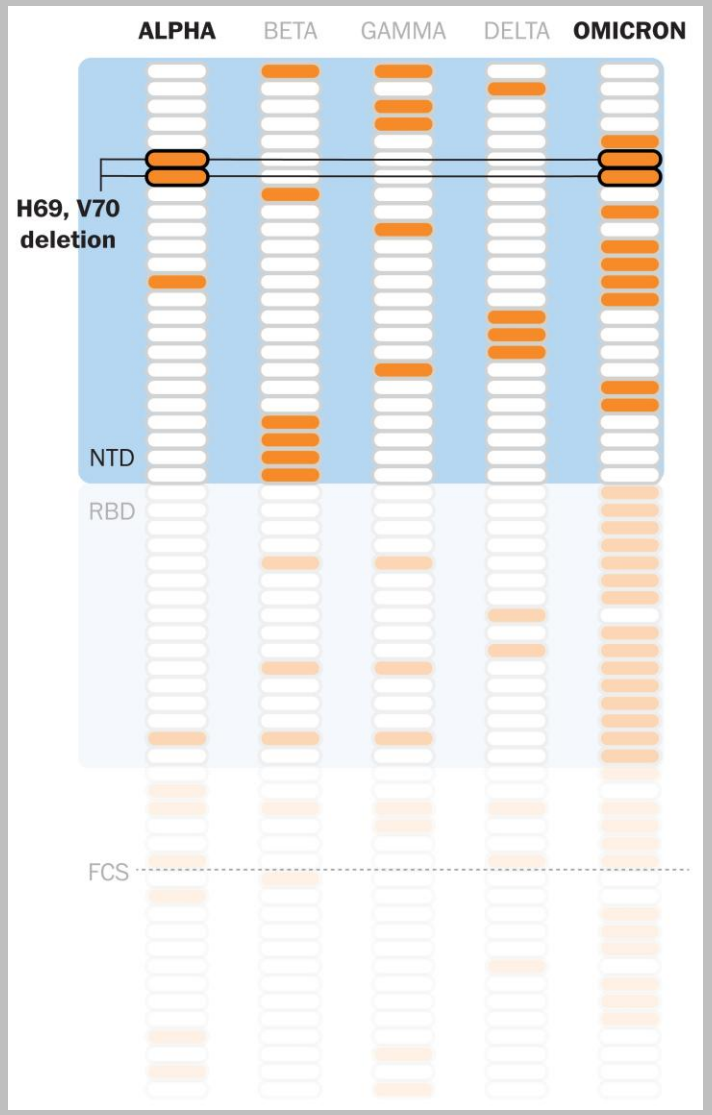


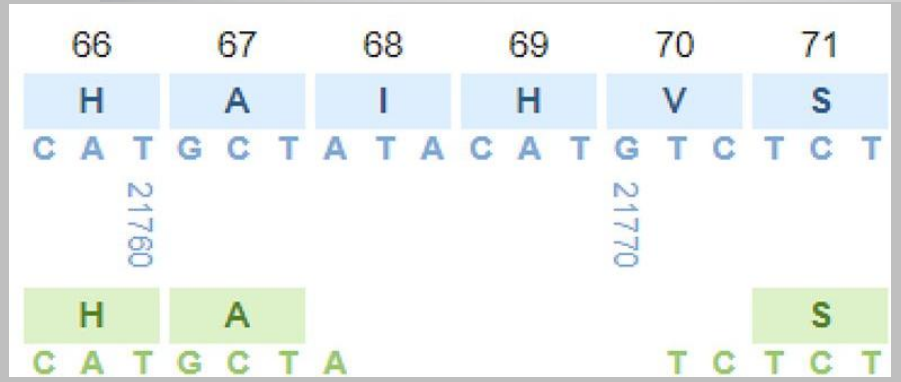




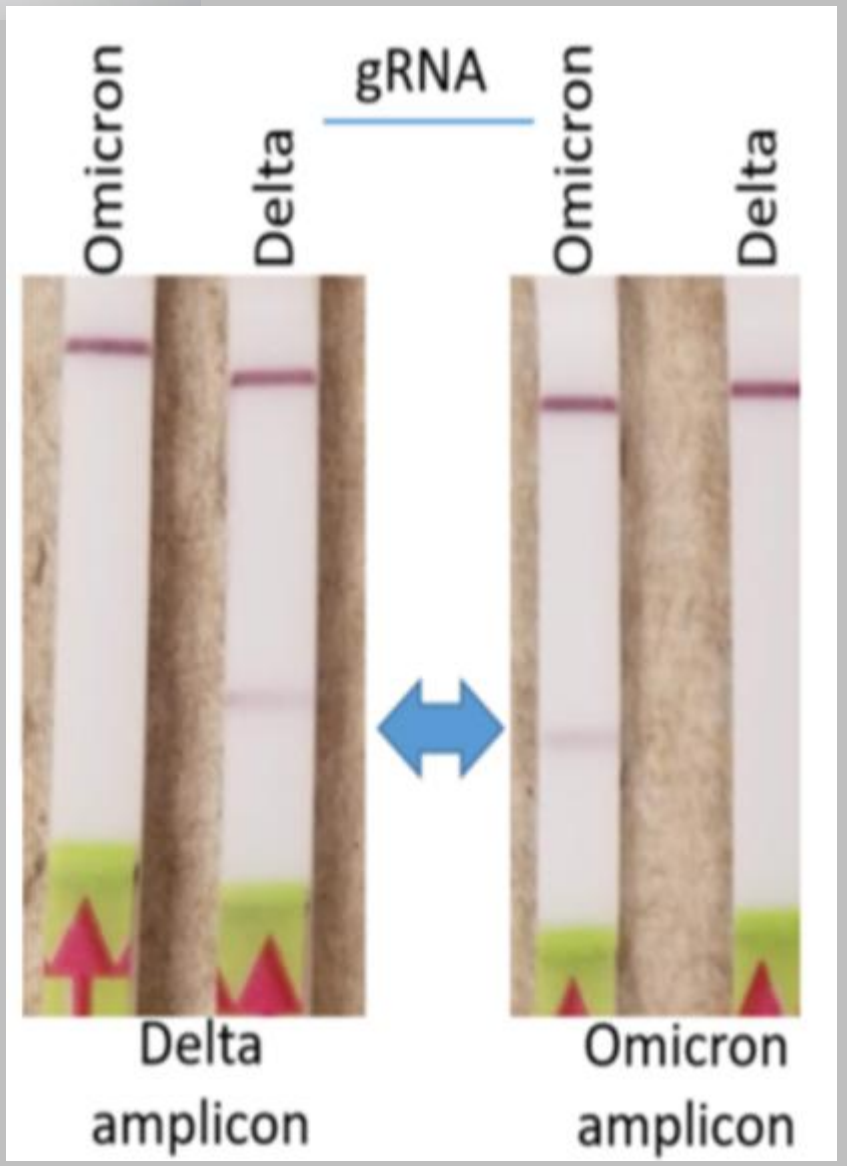
Variant Detection













Interpretation

- **Tests can display lower sensitivity and are interpreted and reliant on the optical acuity of the operator**

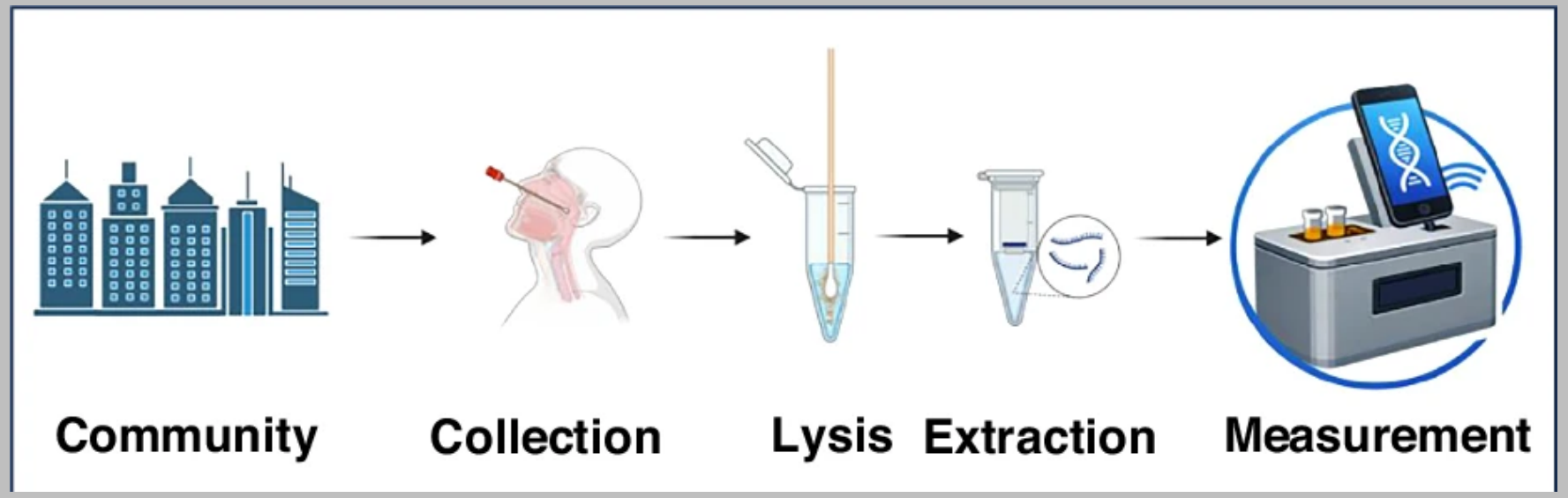
- **Goal: Ultrasensitive methodology using small, low power requirement devices**

A smartphone-powered decentralized microfluidic platform for sensitive CRISPR-based nucleic acid detection

Peng Zhou, Amber N. McElroy, Yingming Xu, Andrew Decker, Timothy W. Schacker, Terrence W. Simon, Mark J. Osborn ✉ & Tianhong Cui ✉

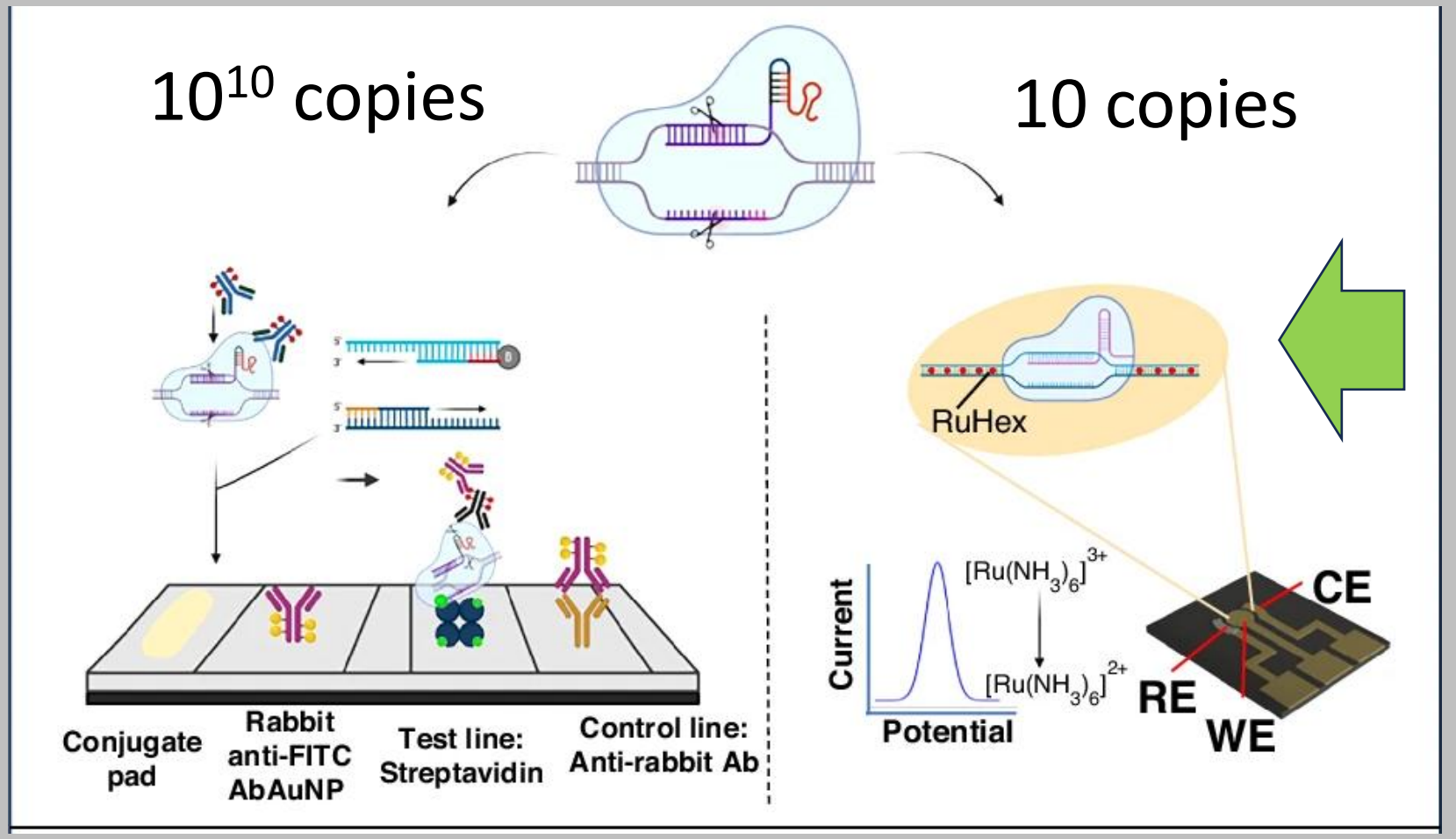
College of Science and Engineering

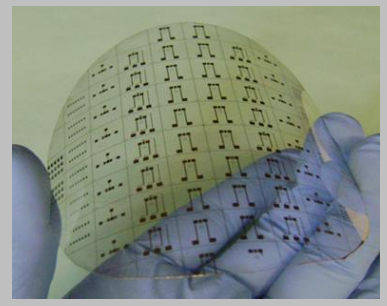
Mechanical Engineering



Small footprint, graphene sensor device shows enhanced sensitivity

Lateral Flow Assay

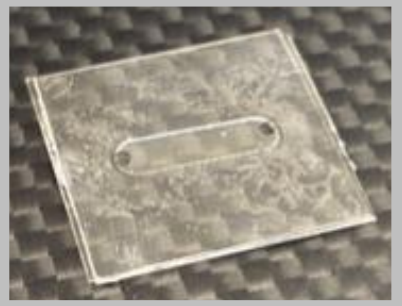




Graphene Engineered Device:
(200x stronger than steel with exceptional conductivity)

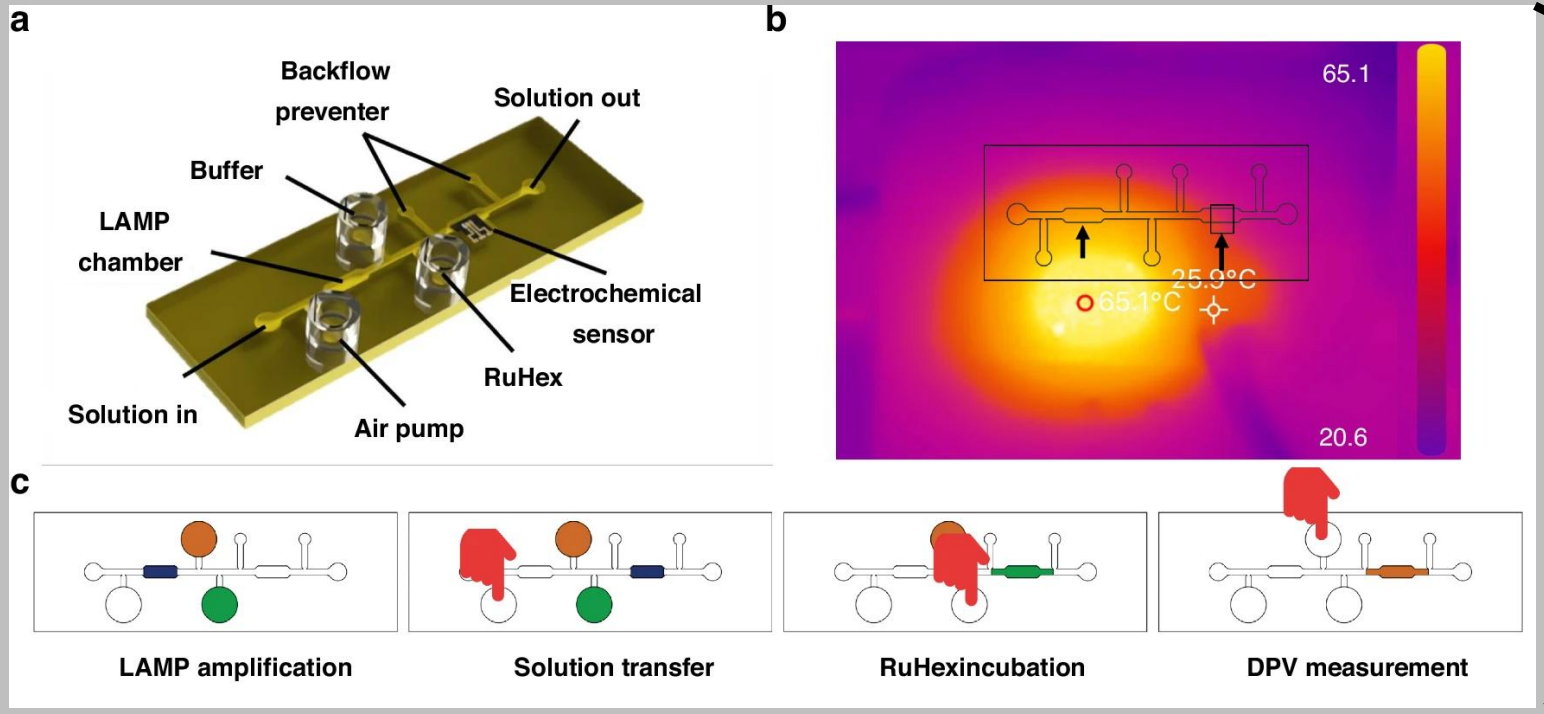
WE: working electrode,
RE: reference electrode;
CE: counter electrode

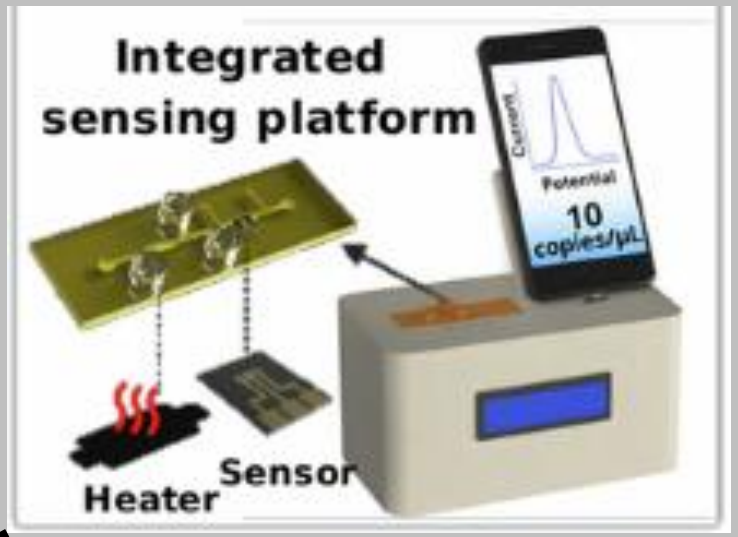
Small footprint and low power usage properties

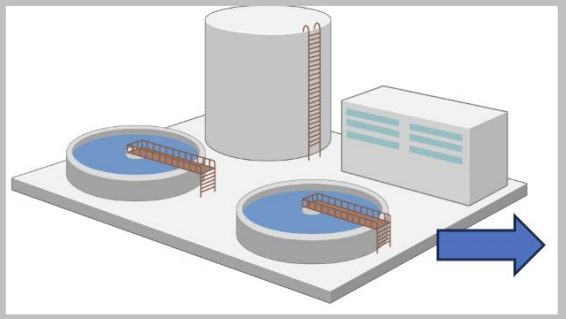


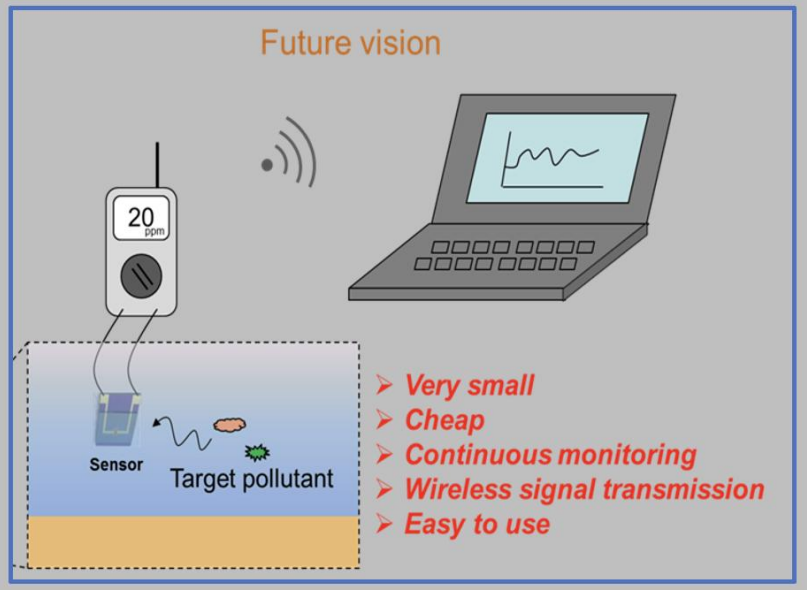


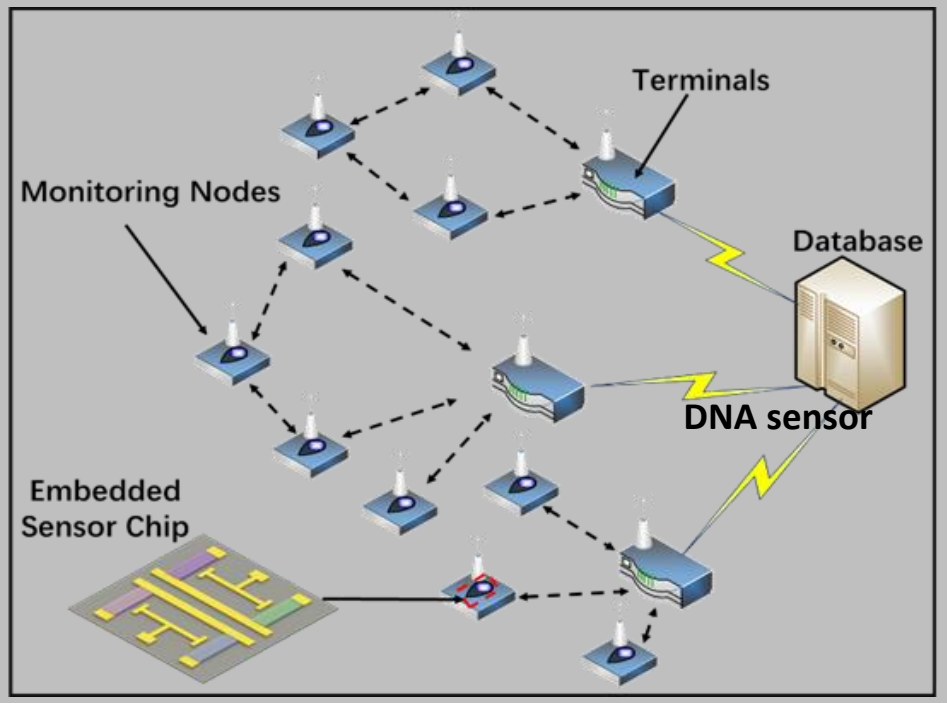
monitoring electrical current changes using Differential Pulse Voltammetry (DPV), allowing molecular recognition events between Cas9 and a specific nucleic acid target to be quantitatively converted into electrical signals.

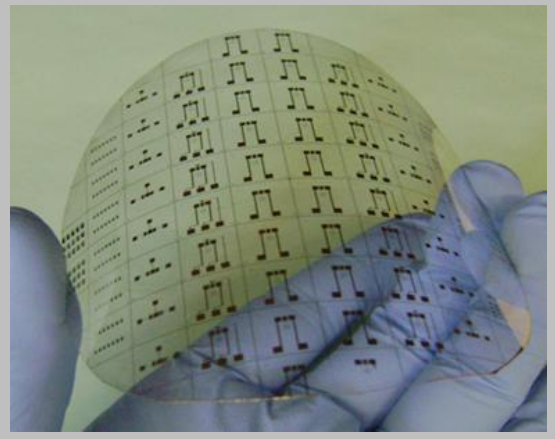




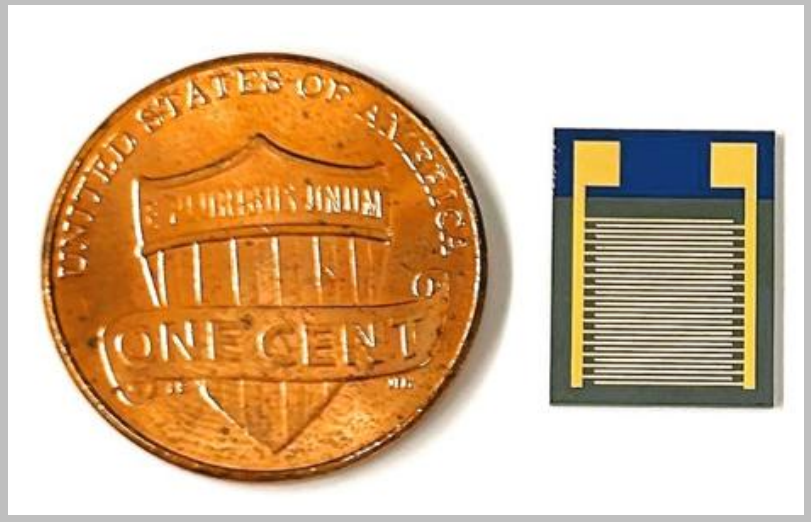








- **Graphene-based micro and nano sensors** for detecting pathogens in water
- **Portable and real-time sensing platforms** for rapid, on-site detection and monitoring
- **AI-enabled sensor networks** for intelligent data analysis, distributed monitoring, and early detection



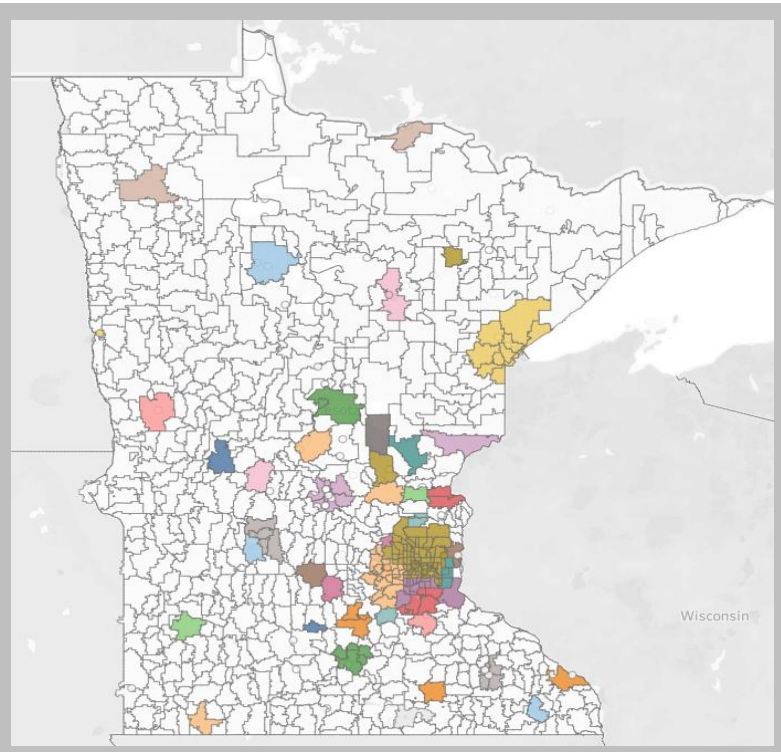
Conclusions: UMN WW Surveillance Program

- Service, innovation, integration, collaboration, adaptation
- Continuous monitoring and reporting
- Broadening pathogen analytics
- Maximizing sensitivity
- Streamlining application for field-based testing

DEPARTMENT OF HEALTH

https://www.health.state.mn.us/diseases/wastewater/stats/index.html

Wastewater Monitoring in Minnesota (Data & Statistics)



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- Jakub Tolar, MD, PhD
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- Tianhong Cui, PhD

