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SARS CoV-2 Wastewater Monitoring: Linking Research and Application To Meet Immediate Needs

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USEPA Office of Research & Development
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Research Team

- ***EPA-ORD*** Nichole Brinkman, Scott Keely, Michael Jahne, Emily Wheaton, Eunice Varughese, Maitreyi Nagarkar
- ***Cincinnati Metropolitan Sewer District*** Bruce Smith, John Barton, Mary Lynn Loder
- ***Hamilton County Public Health Department*** Chris Griffith
- **State of Ohio**
  - **Ohio Department of Health** Rebecca Fugitt
  - **Ohio EPA** Brian Hall, Tiffani Kavalec
  - Other collaborators at end

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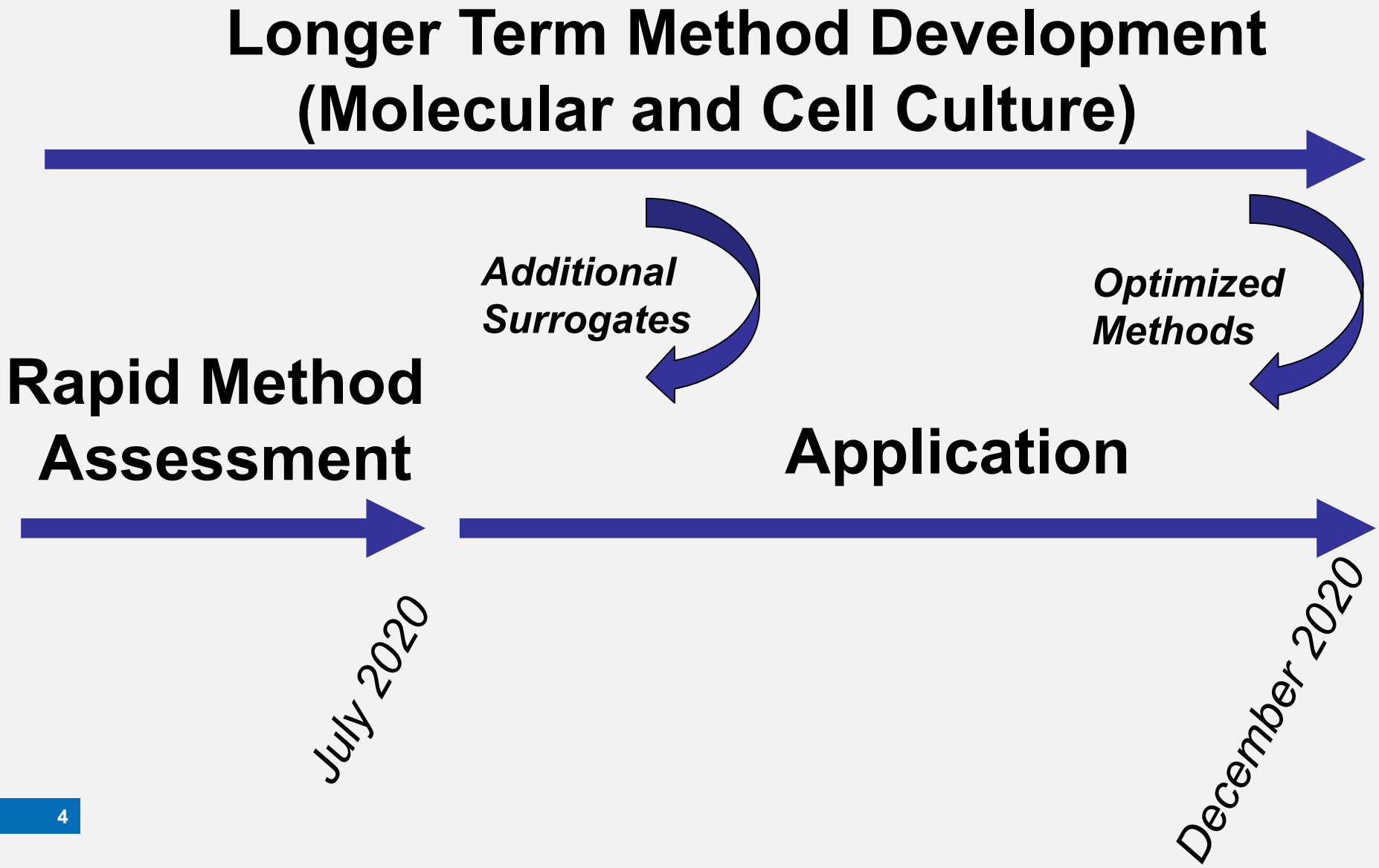
Three Major "Knobs"

- ***Optimizing the analytical methods***

- **Accounting for sewer dynamics**
  - *Decay, degradation, and dilution*

- **Looking into the community**
  - *Relating the sewer signal to imperfect estimates of infection/shedding*

EPA-ORD Approach

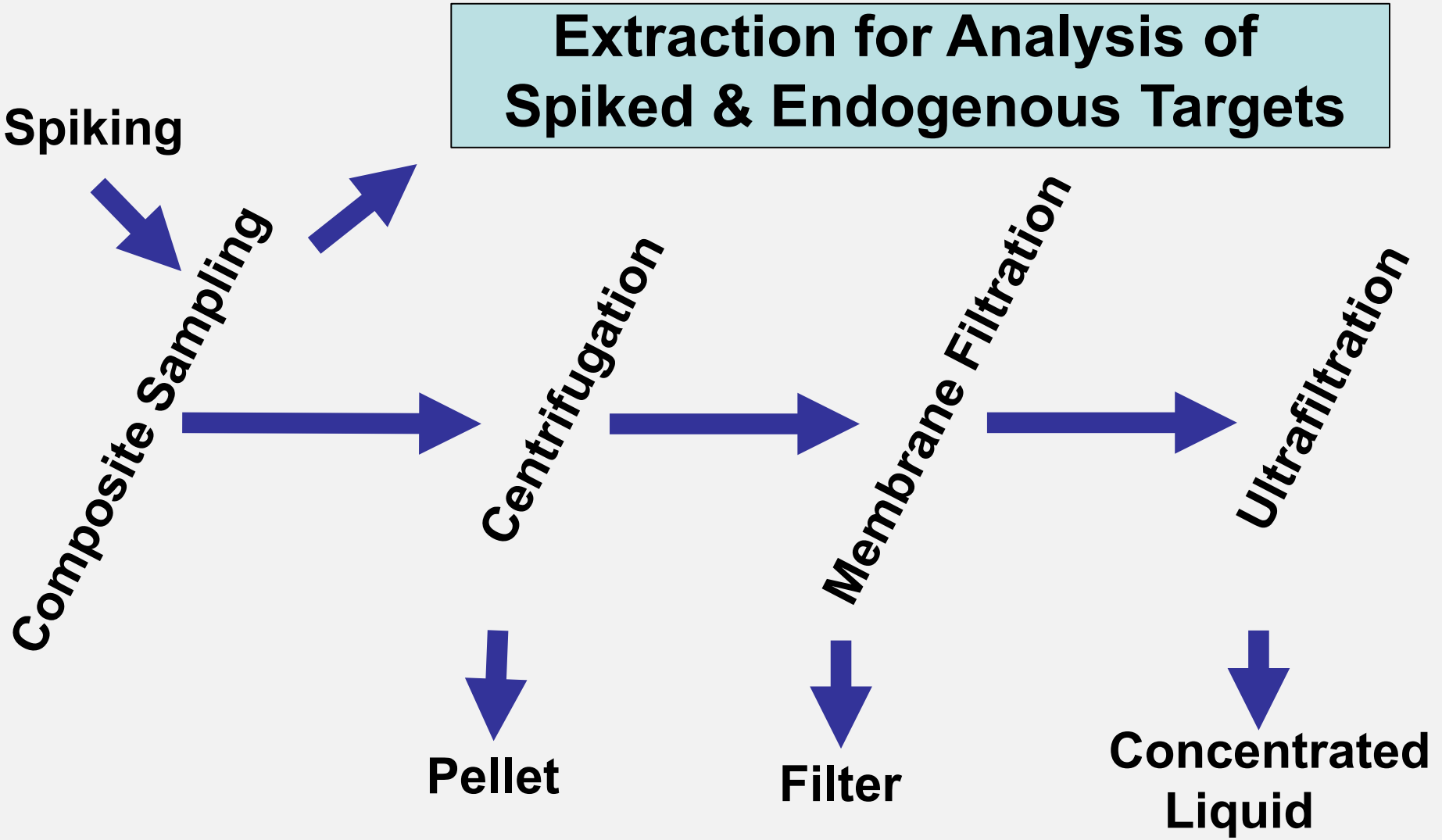


Rapid Method Assessment

- ***Effects of sample processing***
  - *Heat kill, freezing, refrigerated hold time*
  - *Evaluating different fractions*
    - *Solids vs liquid*

- ***Recovery efficiency***
  - *Spiking vs endogenous controls*

Rapid Method Assessment



All Fractions Extracted for Analysis of SARS-CoV-2, Spiked & Endogenous targets

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Recovery Efficiency

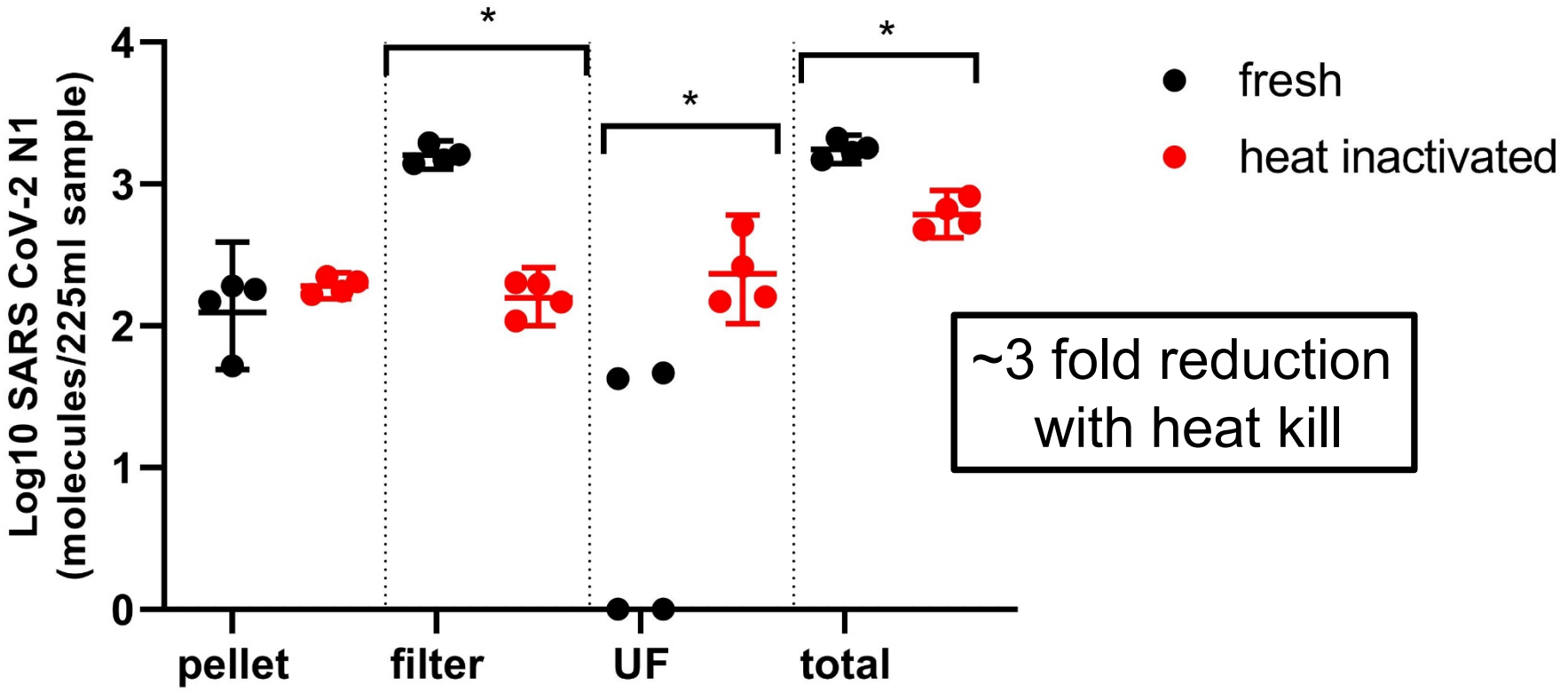
- **Spiking control**
  - Coronaviruses (human coronavirus OC43)

- **Endogenous markers**
  - ***CrAsspahge***(a non-enveloped DNA bacteriophage)
  - Pepper mild mottled virus (***PMMoV***) non-enveloped RNA plant virus (does not infect humans, but found in feces)
  - At high enough levels to compare direct extracts with recovered fractions
  - Allow additional evaluation of method performance
  - Also can be used to normalized data to fecal content rather than volume of wastewater

Initial Methods Assessment *(Results)*

- **Heat Kill**
  - Reduces signal by ~ 3 fold
  - Avoid, particularly given relatively low levels of SARS-Cov-2 gene copes in wastewater
- **Freezing**
  - Reduces signal by ~2.5 fold
  - Avoid, but archived samples are still of value given the interest in defining trends
- **Cold Storage**
  - No significant loss of signal over 2-3 days at 4^{o}C
  - Overnight shipping or next day sampling is sound

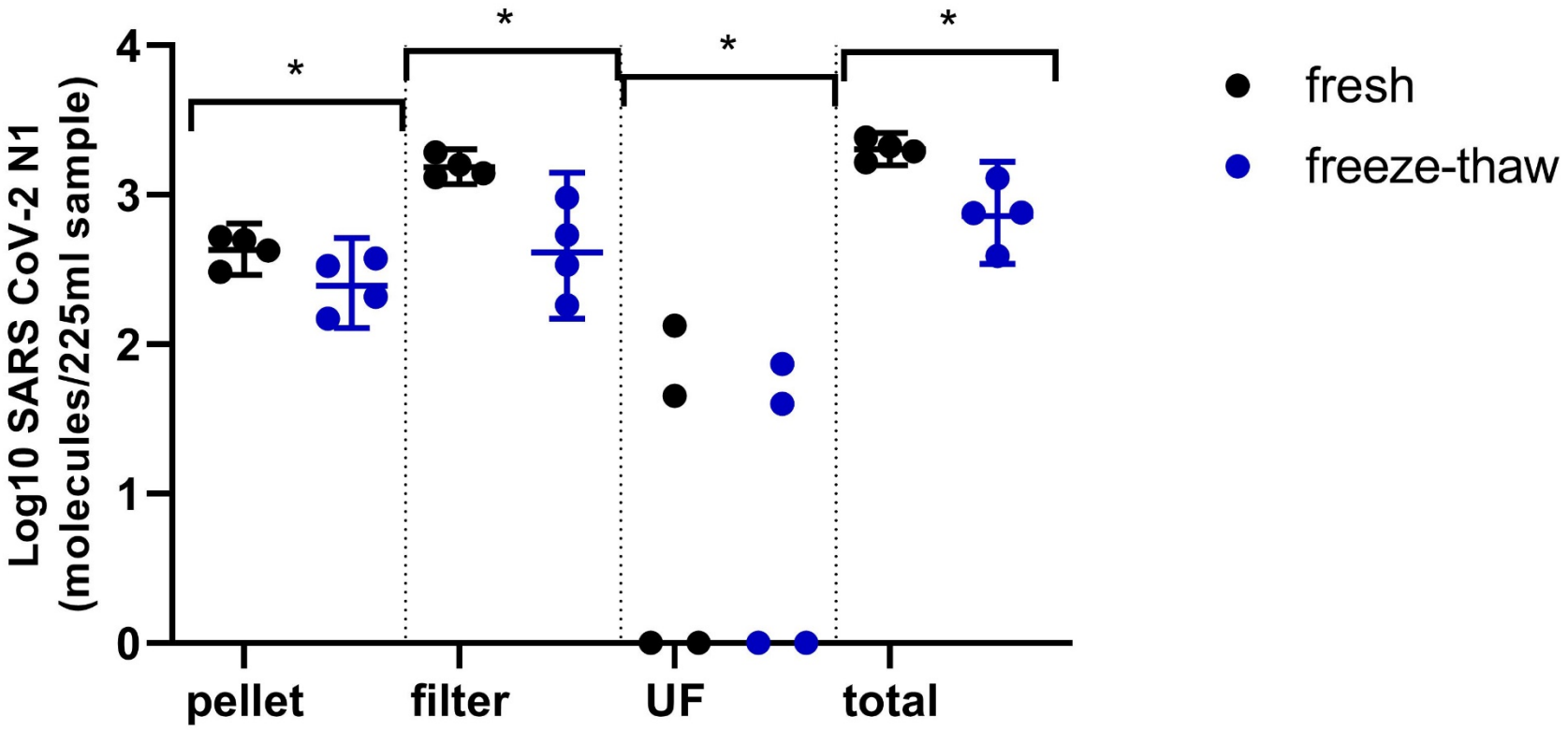
Effects of Heat Kill (60°C for 90 min)



Lick Run Sample (June 18)

- Dots represent 2 biological reps, with 2 RT-ddPCRs each)
- Geometric mean and 95% confidence interval
- Bayesian T-tests indicating credible differences

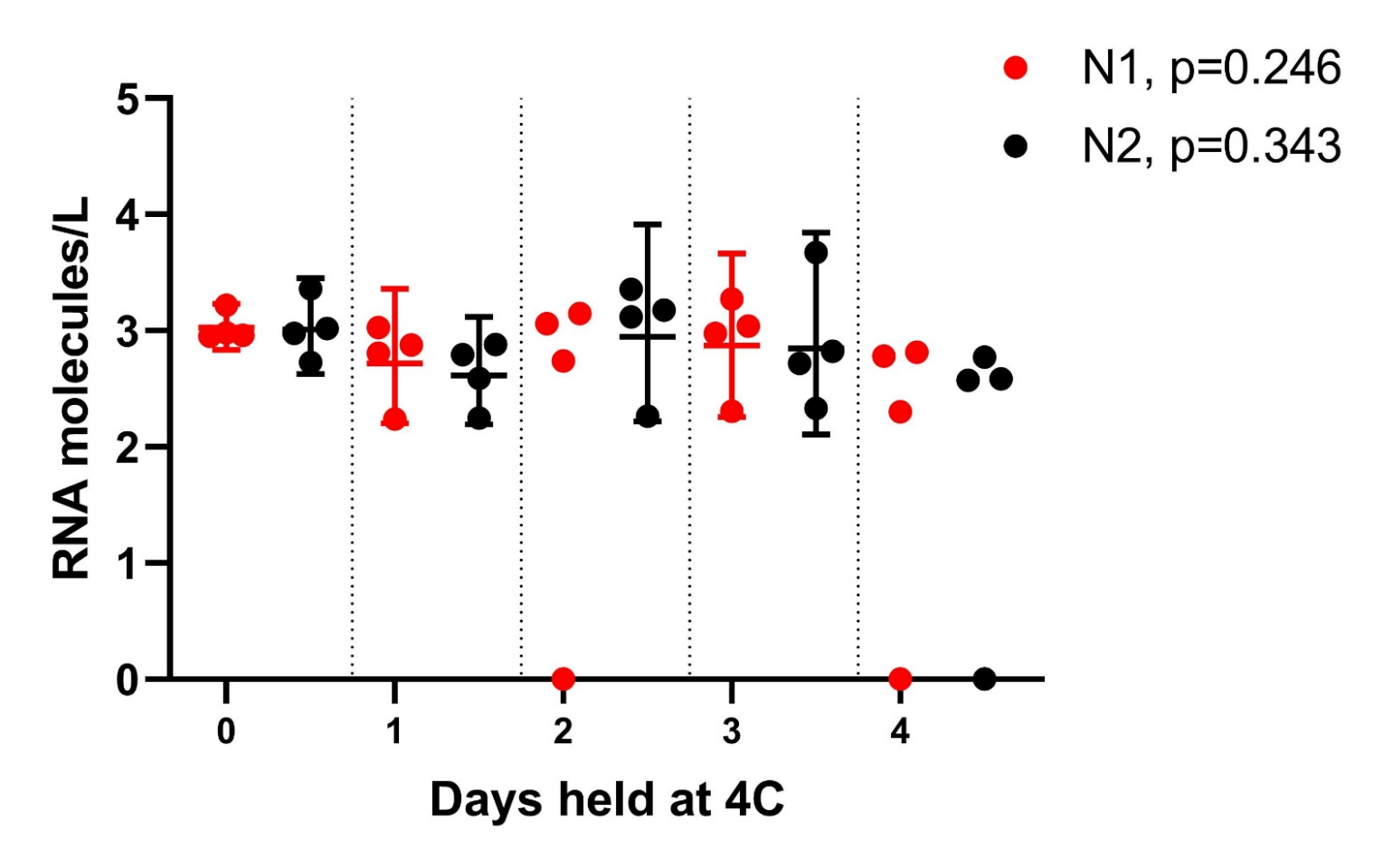
Effects of Freezing at -70°C



Lick Run Sample (June 11)

Symbols, lines and t-tests same as previous slide

Effects of Cold Storage (4°C)



Lick Run Sample (7/13)

Symbols, lines as in previous slides

p values for repeated measures ANOVA with time

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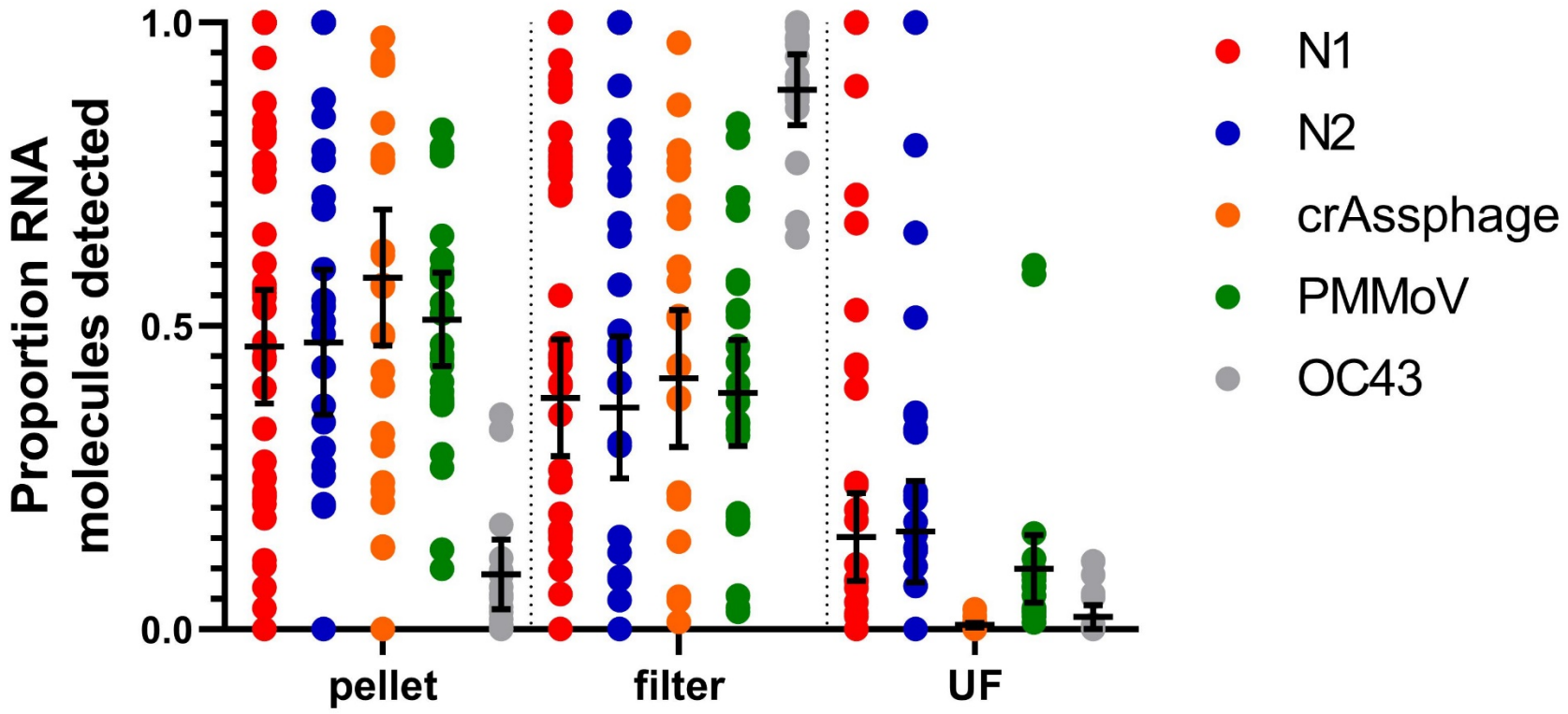
Initial Methods Assessment *(Results, cont'd)*

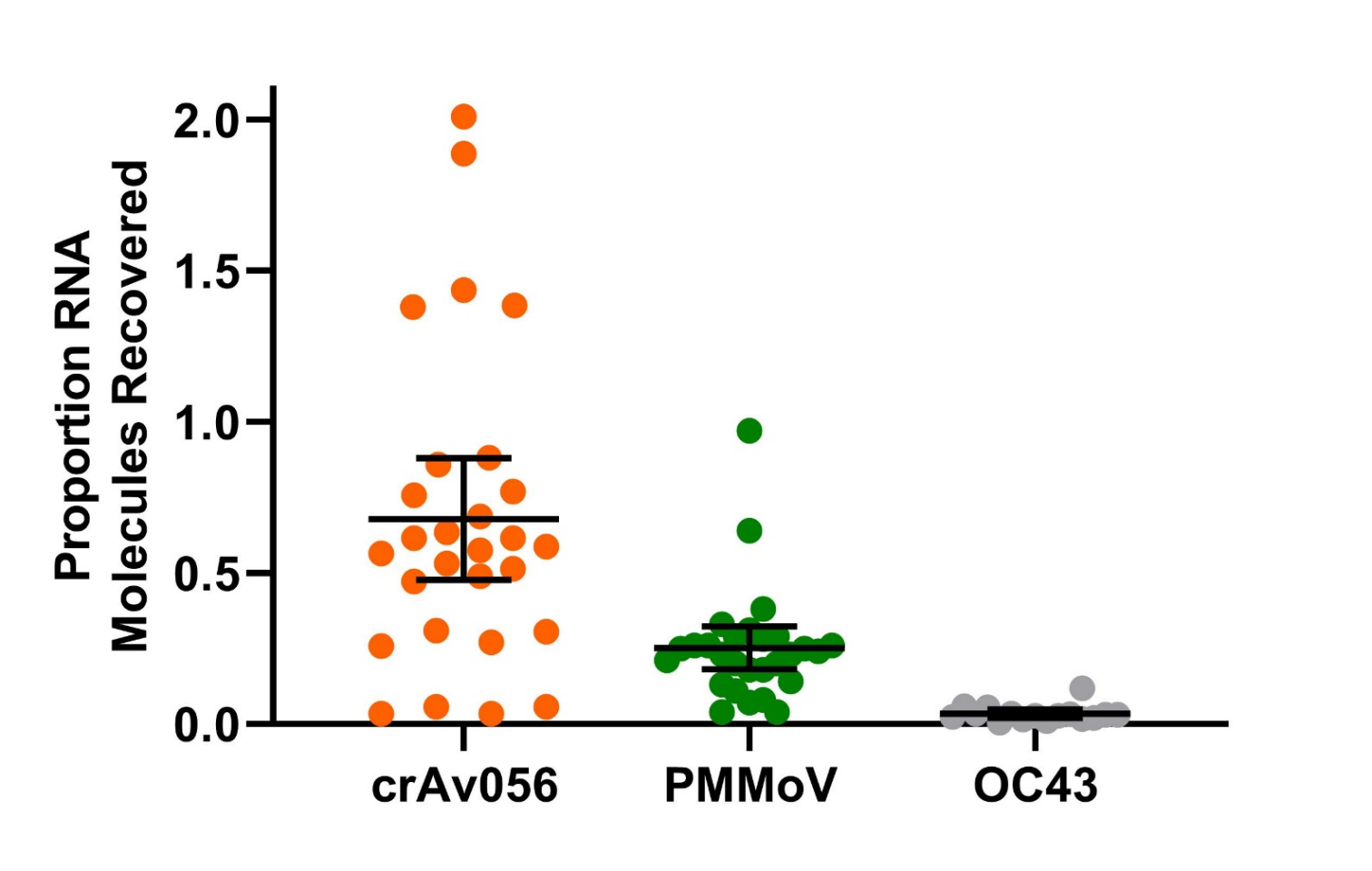
- **Occurrence in Different Fractions**
  - ~80% of gene copies associated with solids (pellet, membrane filter)
  - While low on average, the gene copies in liquid fraction can be the dominant fraction in some sewage samples
- **Percent Recovery**
  - Spiked coronavirus surrogate (OC43) was less than ~3%
  - Endogenous viral surrogate recovery ranged from 25 (PMMoV) to 60% (CrAssphage)

Recovery in Different Fractions



Lines and symbols as in previous slides
N1 (N=18), N2 (N=13)
CrAv056 (N=8), PMMov (N=7), OC43 (N=4)

Percent Recovery
(Endogenous and Spiked Markers)



Symbols, lines, and number of samples same as previous

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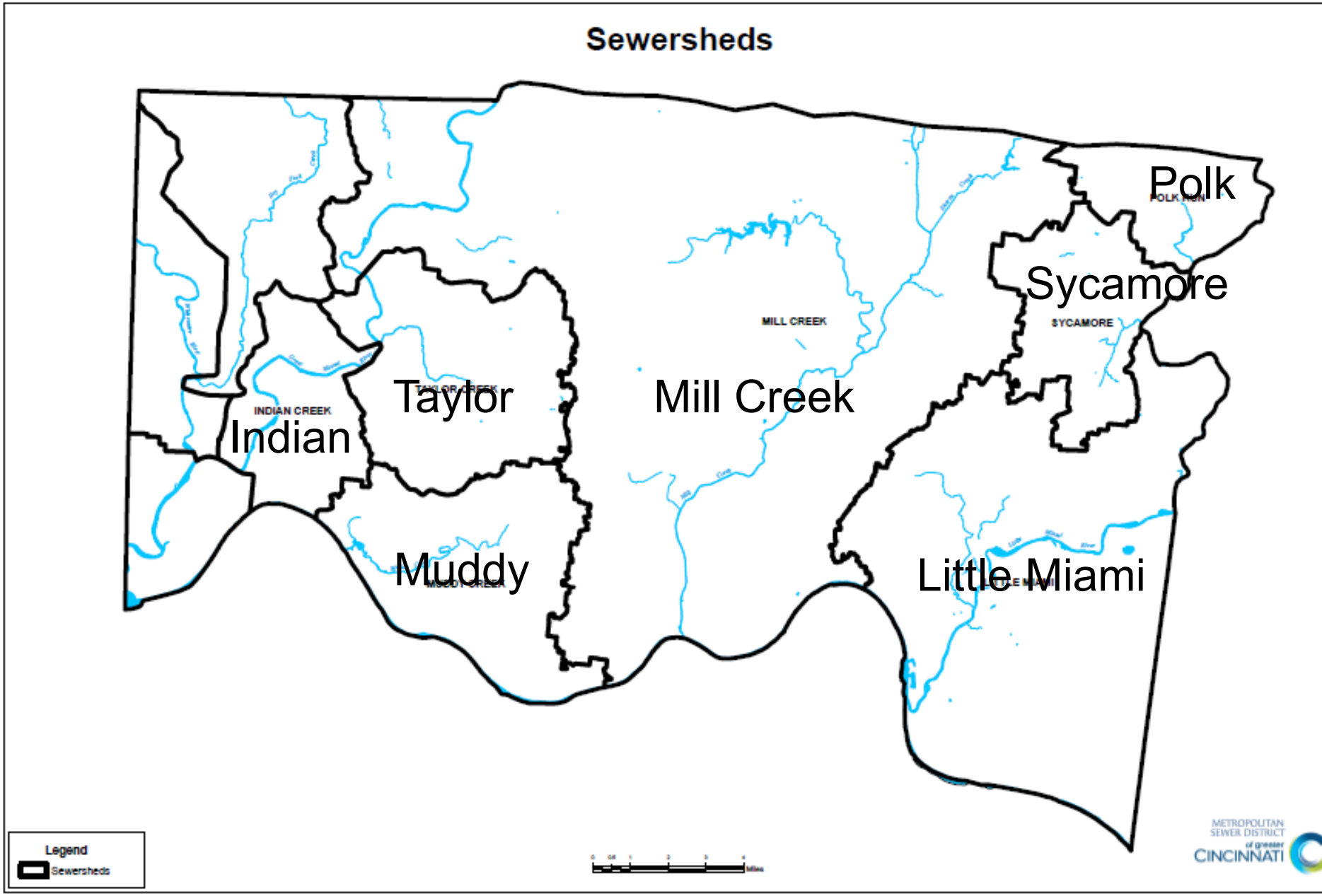
Sewer Dynamics

Decay, Degradation, & Dilution

Cincinnati Municipal Sewer District



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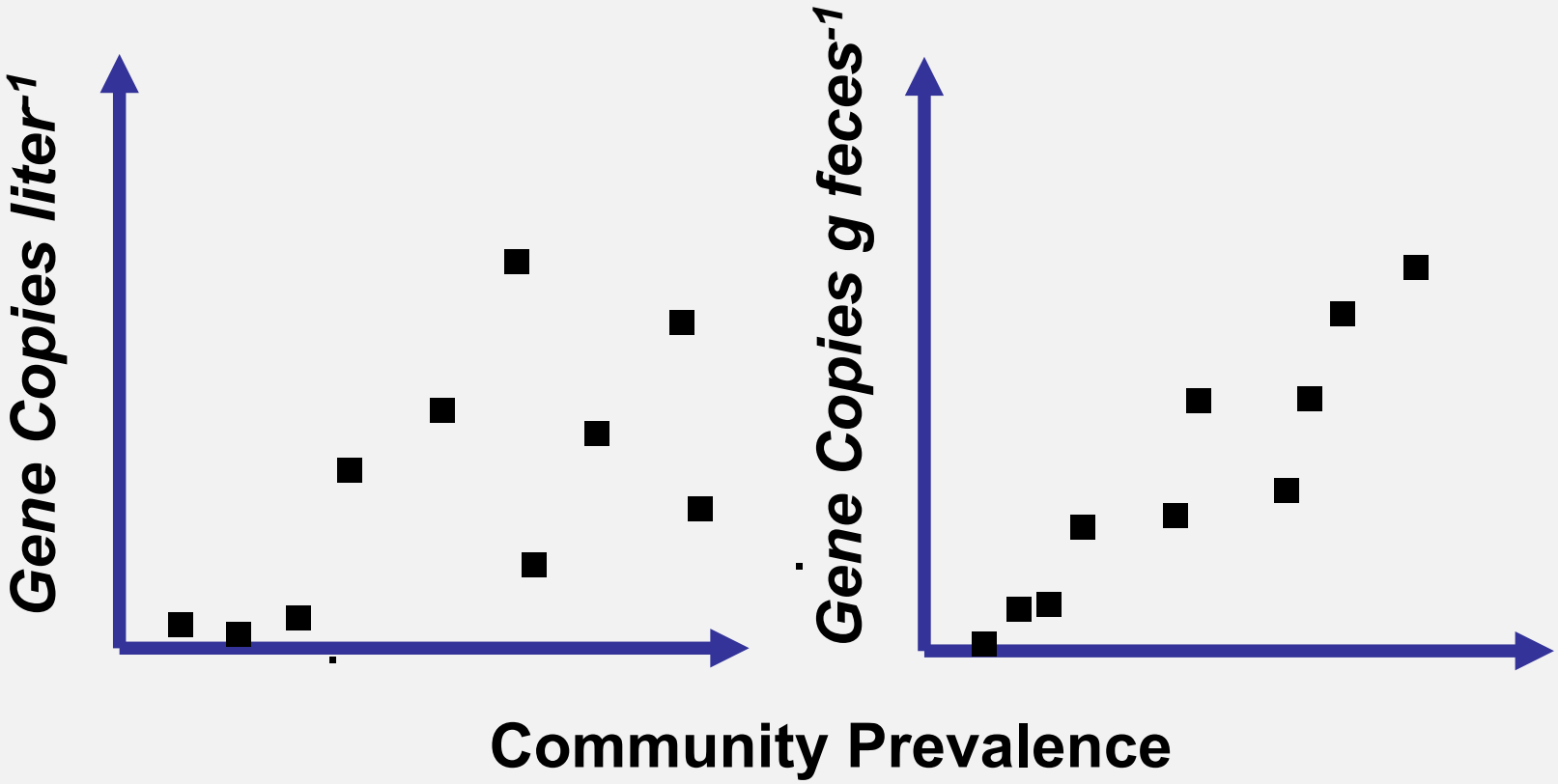
Average Age

	% Industrial	% Combined	Dilution
Mill Creek	5.0	40	0.5:1
Little Miami	4.2	30	0.4:1
Muddy Creek	<0.05	30	0.5:1
Sycamore Creek	1.1	0	0.5:1
Polk Run	<0.1	0	0.8:1
Indian Creek	0	0	1:1
Taylor Creek	0	0	1:8:1



Decrease in Variability Predicted

Accounts for Dilution

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Looking into the Community

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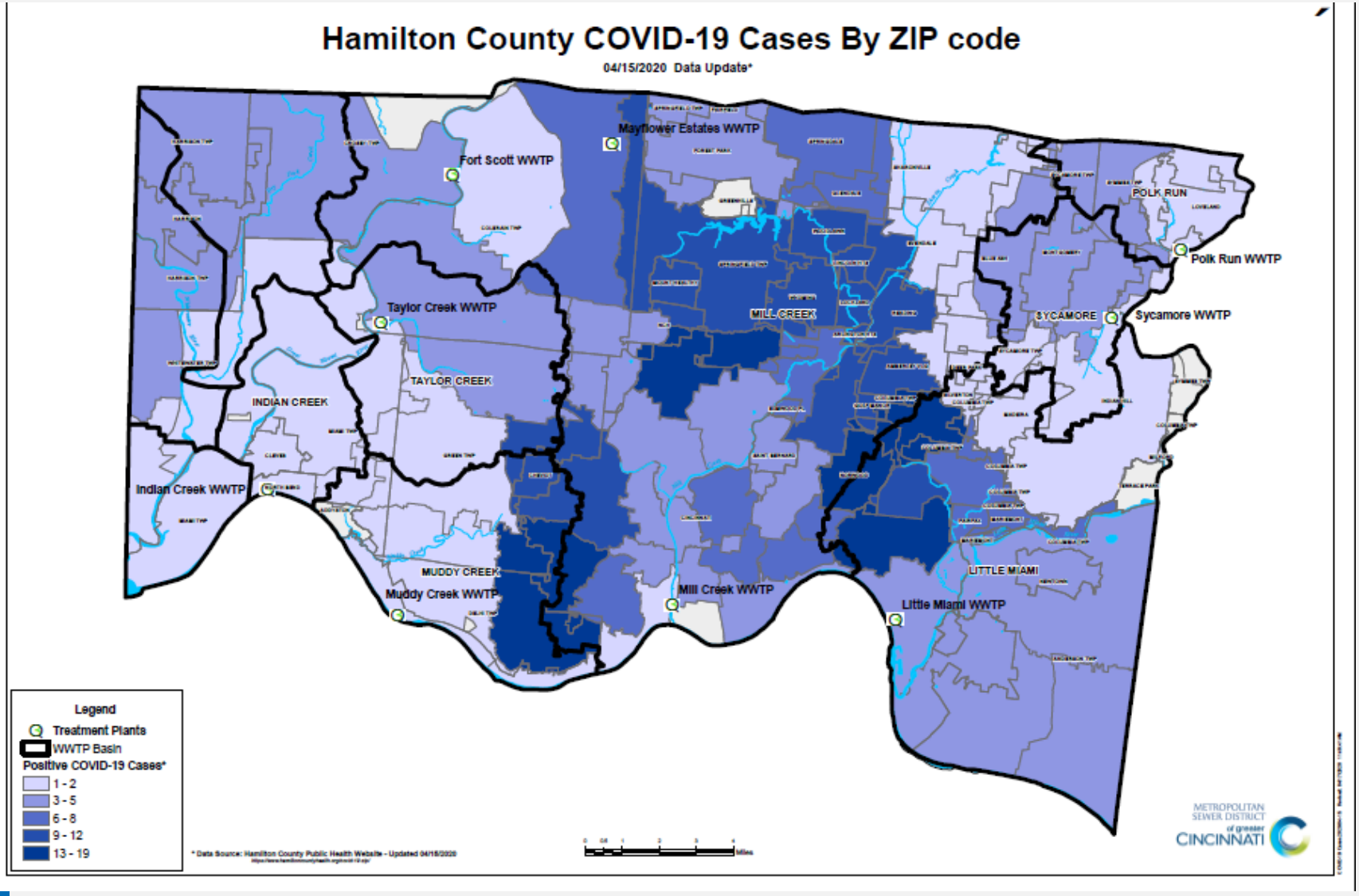

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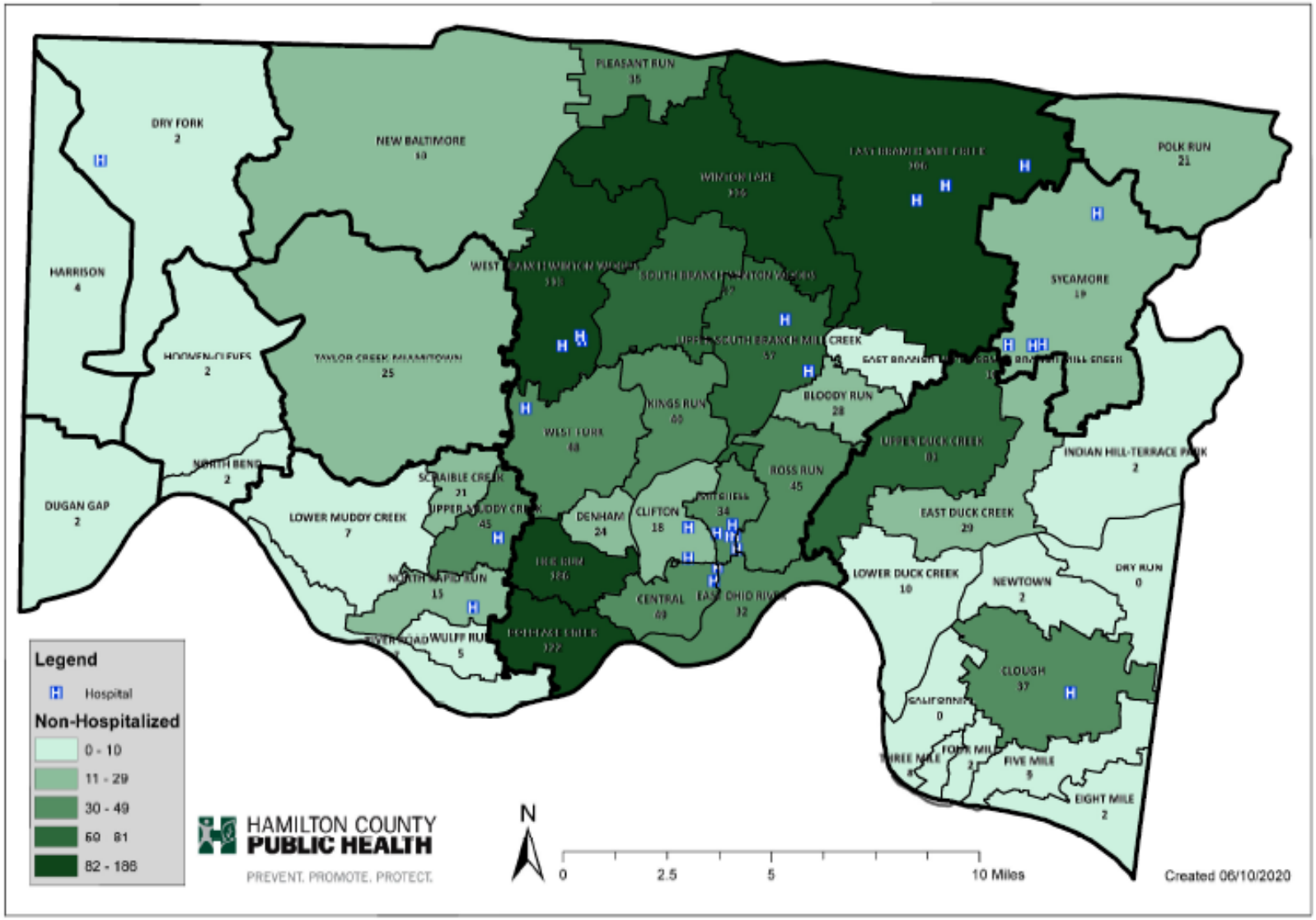
COVID-19 Non-Hospitalized Cases Per Sewershed
Hamilton County, OH



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COVID-19 Hospitalized Cases Per Sewershed
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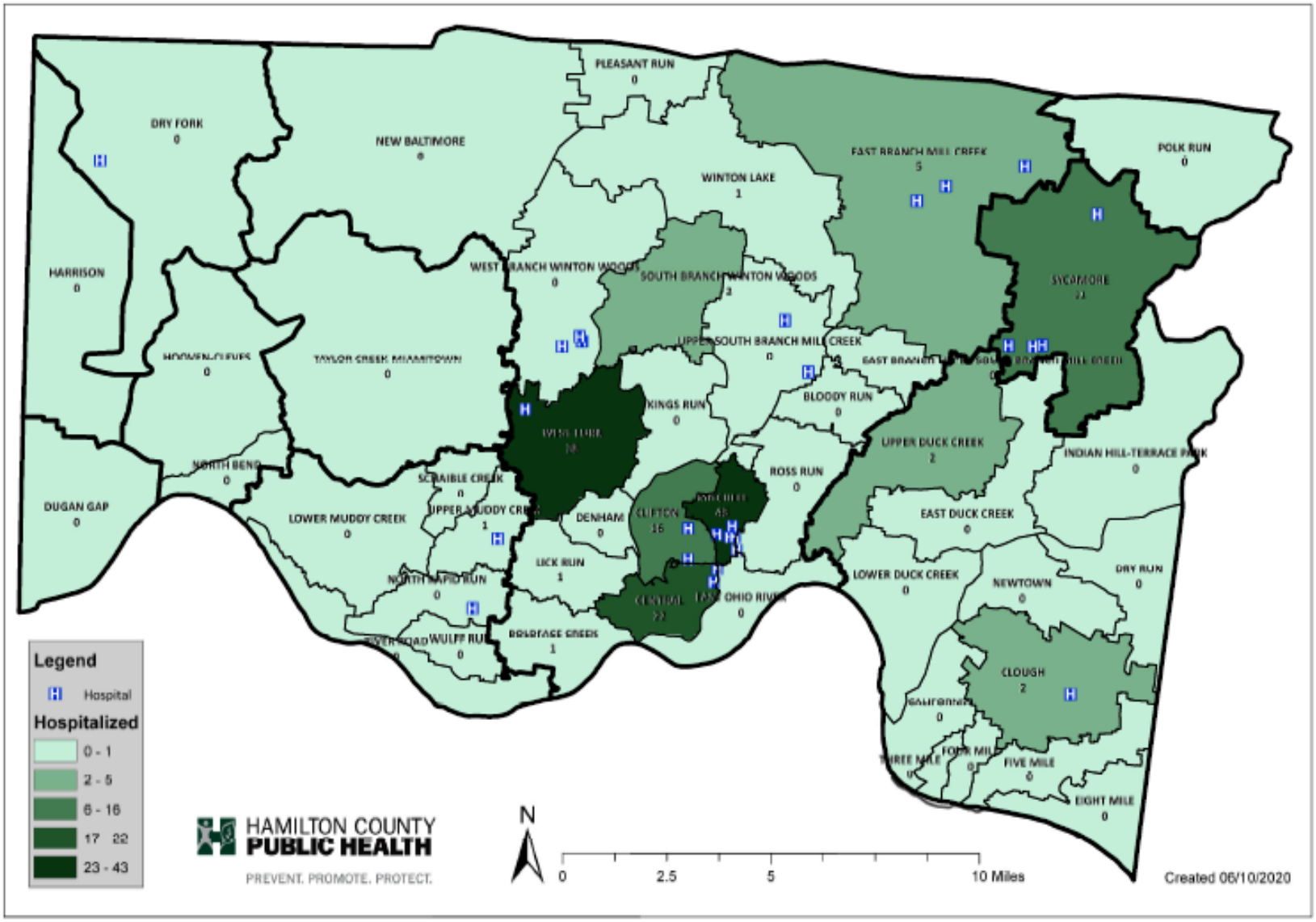

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Current Status of Research

- Rapid assessment of method development nearly complete (looking at repeating experiments – heat kill, freezing, hold time – with strongly positive samples)

- Correlate weekly signal at the sewershed level to current cases within the area
  - Archived from beginning of May until present
  - With and without normalization to fecal content

Application – State of Ohio Effort

- Governor Dewine asked what it would take to develop a state based wastewater effort on Memorial Day
- Ohio EPA, ODH, Ohio Water Resource Center (in coordination with state university system researchers), implementing the plan
  - Initial work on 7 major metropolitan areas (Cleveland, Columbus, Cincinnati, Dayton, Toledo, Akron, Youngstown)
    - Multiple plants within some areas
    - Data on dashboard by first week of August
  - Additional medium and small cities (~70 total sampling sites by first of September
    - Goal of expanding coverage to additional counties
    - Selection criteria: current status, vulnerability, infrastructure (e. g. , composite sampling), utility interest

State of Ohio University Involvement

- **Zuzana Bohrerova**, Associate Director and Research Specialist, *Ohio Water Resources Center and OSU CEGE*
- **Linda Weavers**, co-Director and Professor, *Ohio Water Resources Center and OSU Civil, Environmental and Geodetic Engineering*
- **John Lenhart**, co-Director and Professor, *Ohio Water Resources Center and OSU Civil, Environmental and Geodetic Engineering*
- **Mark Weir**, Assistant Professor, *OSU College of Public Health (COPH) – Environmental Health and Safety,*
- **Jiyoung Lee**, Professor, *OSU COPH – Environmental Health and Safety*
- **Stan Lemeshow**, Professor, *OSU COPH - Biostatistics*
- **Dae-Wook Kang**, Assistant Professor, *University of Toledo, Civil and Environmental Engineering*
- **Travis Taylor**, Assistant Professor, *UT, Medical Microbiology and Immunology*
- **Saurabh Chattopadhyay**, Assistant professor, *UT, Medical Microbiology and Immunology*
- **Xiaozhen (Jen) Mou**, Associate Professor, *Kent State University, Biological Sciences*
- **Stephen Duirk**, Associate Professor, *University of Akron, Environmental Engineering*
- **John Senko**, Associate Professor, *University of Akron, Geosciences*

EPA-ORD Involvement

- We have agreed to join the laboratory network
  - Weekly sampling of 3 major plants in Cincinnati and Dayton areas
  - Additional cities in southwestern Ohio
- Facilitating the effort where needed
  - Providing info on method performance as described earlier
  - Coordinating initial laboratory comparison (and participating in on-going monthly comparisons)
  - Helping incorporate improved analytical approaches
    - e.g., fecal normalization
  - Developing standard collection formats
    - Utility information, lab QA/QC

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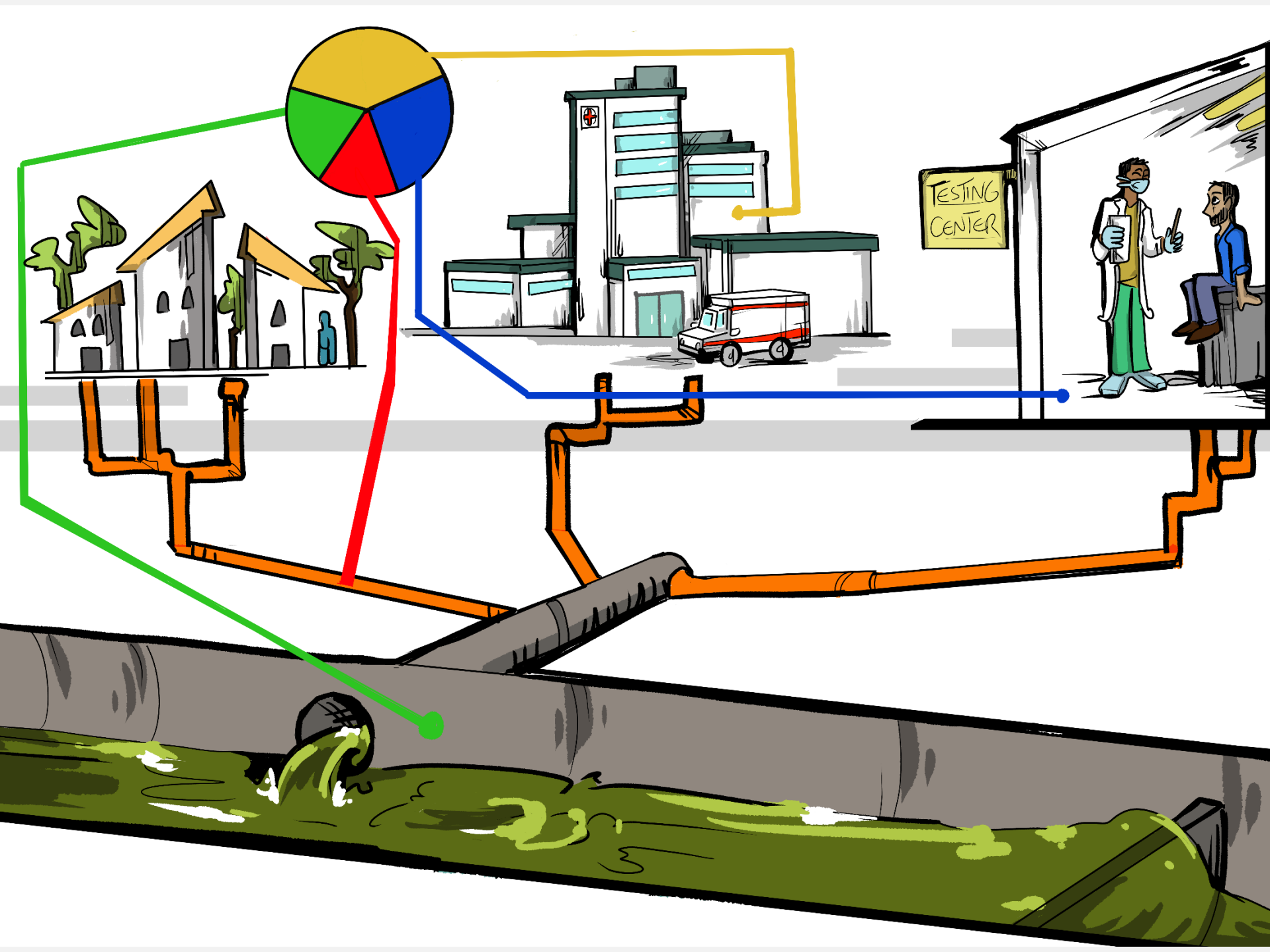
Thoughts On Challenges & Keys to Success

- Complex issue with multiple areas of uncertainty
  - Need to bring together multiple experts environmental analytics / wastewater engineering / public health implementation
  - Don't let specific uncertainties limit overall advancement
- Allow flexibility in analytical methods, but define specific data reporting requirements
- Maintain aggressive timelines; assume that best practices will rapidly evolve


TESTING
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