

Wastewater surveillance for SARS-CoV-2: Potential, Progress, Problems

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Much interest in wastewater surveillance for SARS-CoV-2

> 50 teams in the US, and hundreds worldwide, are working on this

March 30

First proof-of-concept report
KWR Institute, the Netherlands

Presence of SARS-Coronavirus-2 in sewage

 Gertjan Medema, Leo Heijnen, Goffe Elsinga, Ronald Italiaander, Anke Brouwer

doi: <https://doi.org/10.1101/2020.03.29.20045880>

April 7, MIT/BioBot, Massachusetts
reports concentrations
estimate # infected persons

SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases

Fuqing Wu, Amy Xiao, Jianbo Zhang, Xiaoqiong Gu, Wei Lin Lee, Kathryn Kauffman, William Hanage, Mariana Matus, Newsha Ghaeli, Noriko Endo, Claire Duvallet, Katya Moniz, Timothy Erickson, Peter Chai, Janelle Thompson, Eric Alm

May 1, New York Times

MATTER

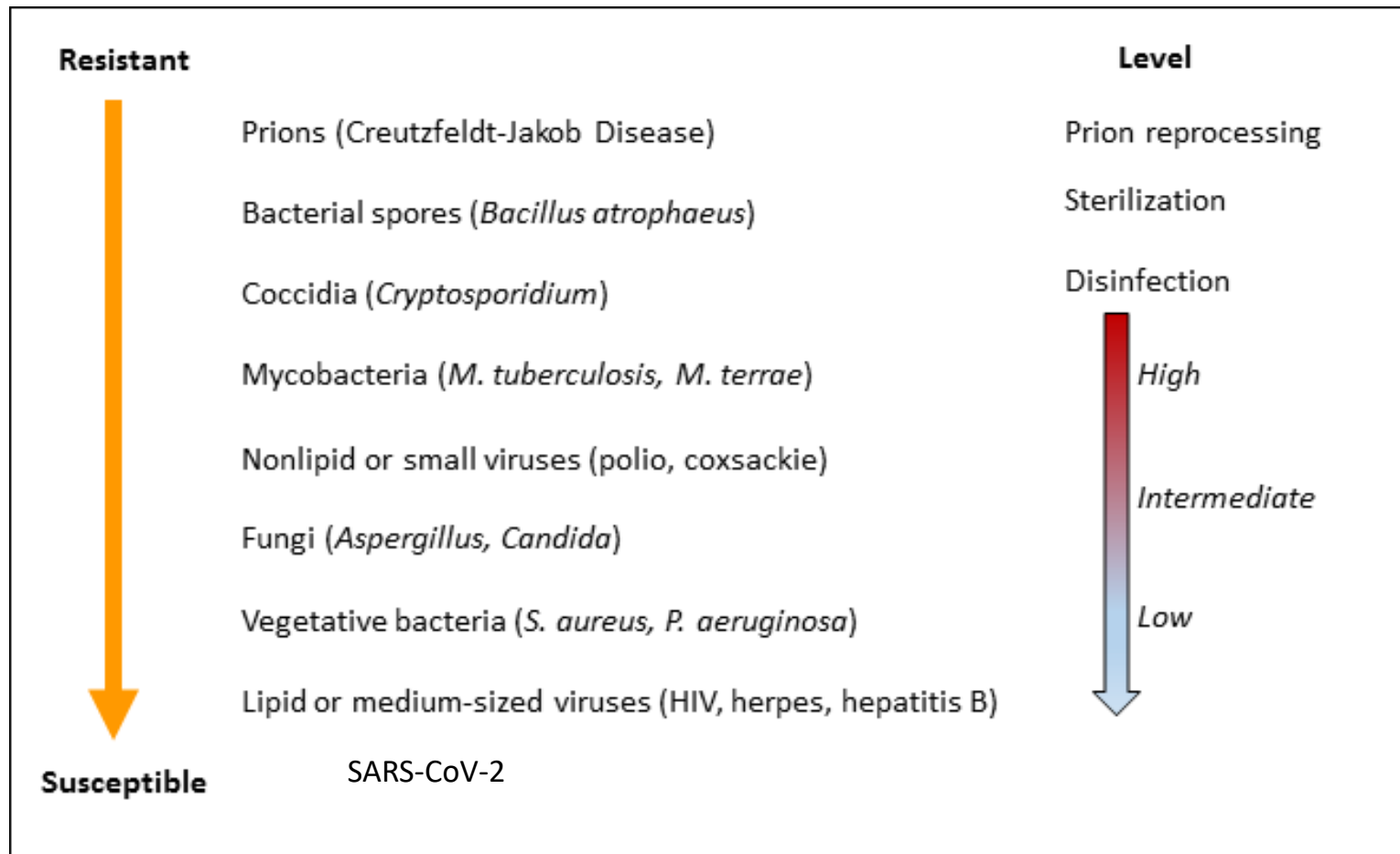
Is It Safe to Come Out of Lockdown? Check the Sewer

Wastewater could provide early, painless and localized data about the rise or fall of coronavirus levels.

Is SARS-CoV-2 in feces?

- **Fecal-oral transmission is still considered unlikely, no evidence to date**
- SARS-CoV-2 is accompanied by persistent shedding in stool (27%- 89% of patients)
- Densities of 0.8 to 7.5 log gene copies/gram
- Viral RNA is shed even after oral swabs are negative, even with asymptomatic patients
- Infectious virus? MOST LIKELY NO-
presence of genetic material doesn't imply risk

SARS-CoV-2 is easier to inactivate than other viruses or bacteria – CDC hierarchy of resistance



What do we mean by wastewater surveillance for SARS-CoV-2?

Infected persons (asymptomatic and symptomatic) shed the virus in feces

Feces enters the sewer network

Wastewater at the head of the plant is aggregate or pooled sample

Analyzing for indicators of SARS-CoV-2

- Target genetic material (a short stretch of the viral genome)

- Use molecular assays developed for clinical tests (e.g., N1, N2)

- Genetic sequencing

Results

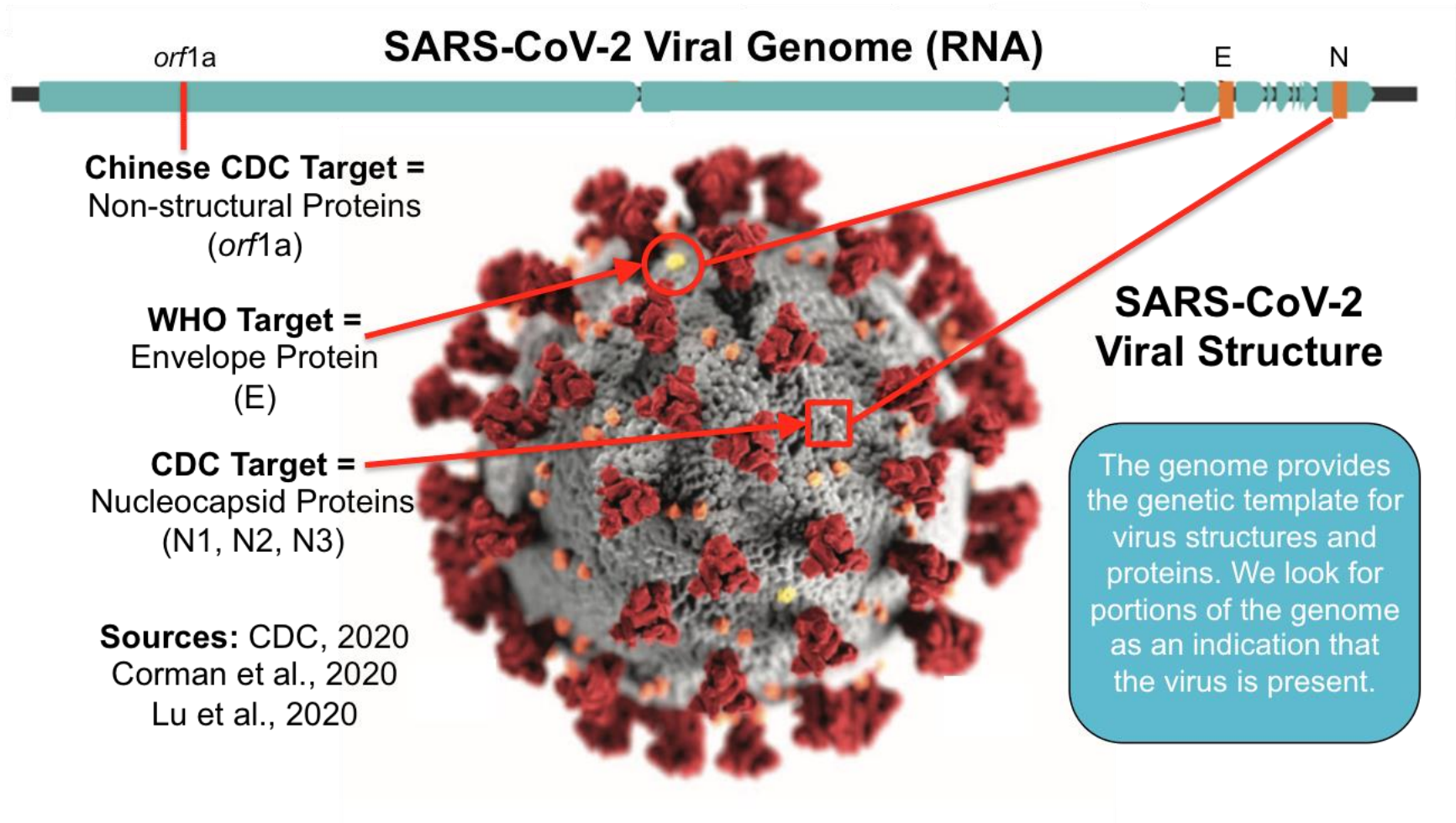
- Qualitative (positive or negative)

- Quantitative (viral genomes/L wastewater)

- Genetic sequence comparisons

These results would not indicate infectivity

Targets for molecular detection



What wastewater surveillance can (and cannot) do

CAN provide trend data to complement other surveillance data for public health decision making

CANNOT be used to reliably and accurately predict the number of infected individuals in a community

Don't fully understand yet...

- Smallest number of people shedding the virus in stool that can be detected by wastewater surveillance methods
- Losses through the sewershed network

Highlight results from three studies

Hart et al., 2020, Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges

Randazzo et al., 2020, Metropolitan Wastewater Analysis for COVID-19 Epidemiological Surveillance (Valencia, Spain)

Nemudryi et al., 2020, Temporal detection and phylogenetic assessment of SARS-CoV-2 in municipal wastewater (Bozeman, Montana)

Wastewater surveillance more cost-effective than clinical screening for population health assessment

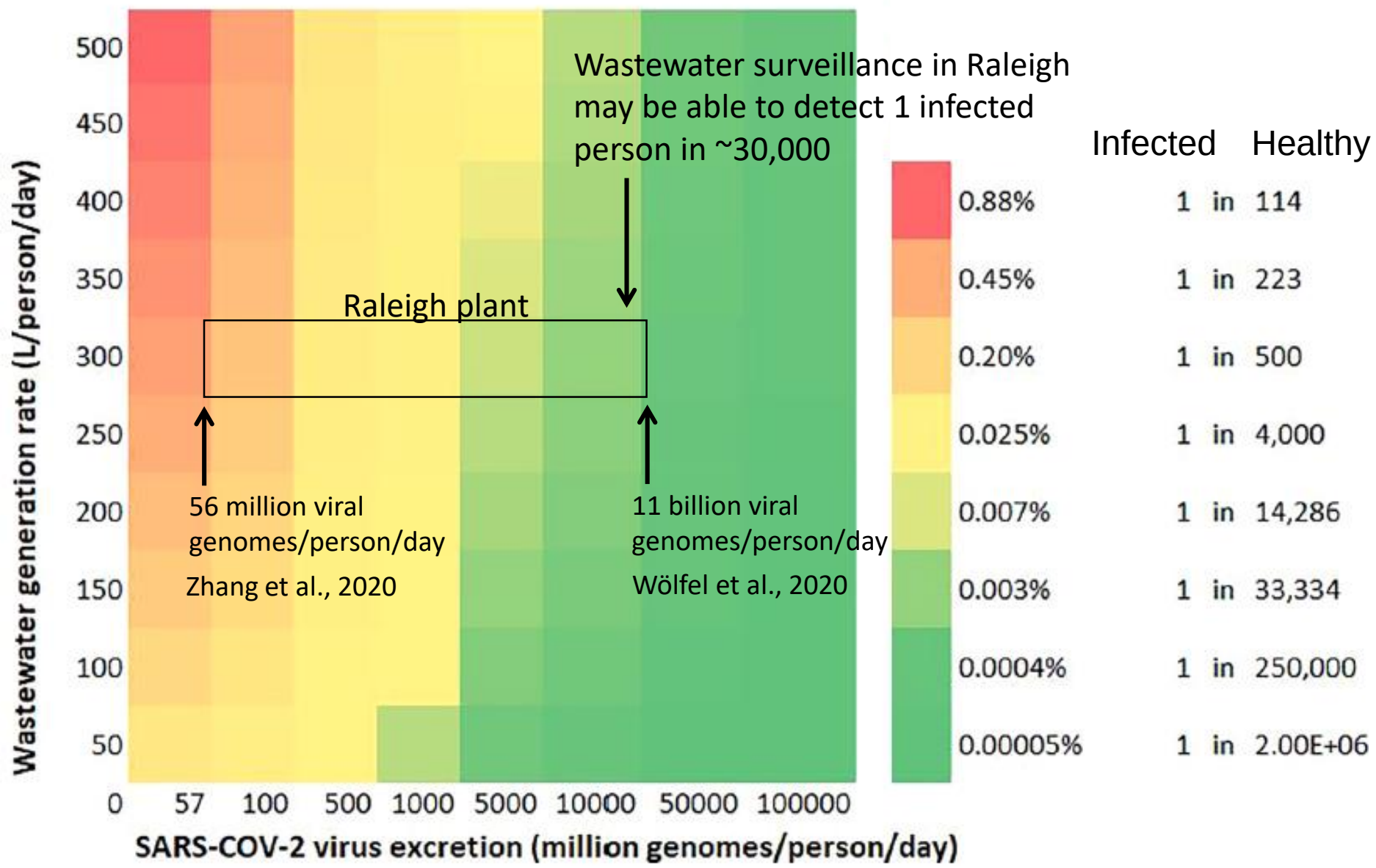
Hart et al., 2020

70% of the US population could be screened for SARS-CoV-2 community spread by analyzing all ~15,000 wastewater treatment plants at an estimate reagent cost of \$225,000 USD

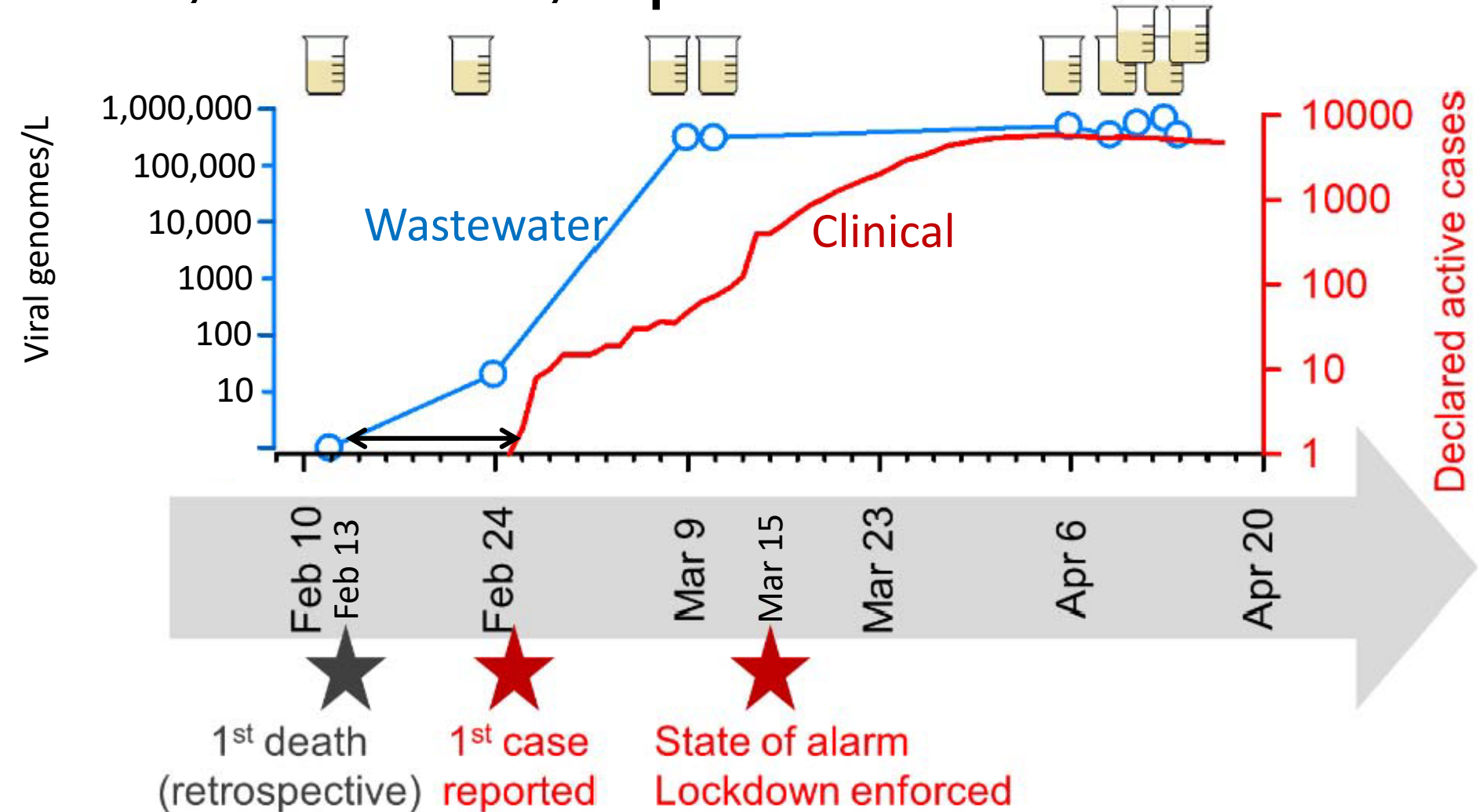
Wastewater based surveillance reagents would constitute ~0.014% of those required for clinical testing of individuals

More rapidly and repeatedly obtain a health profile of a community

Sensitivity of wastewater surveillance



Wastewater data and clinical case data, Valencia, Spain

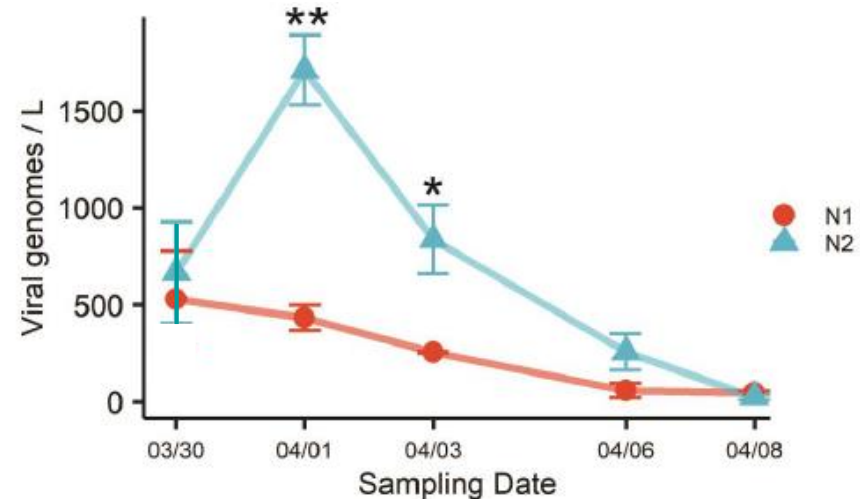
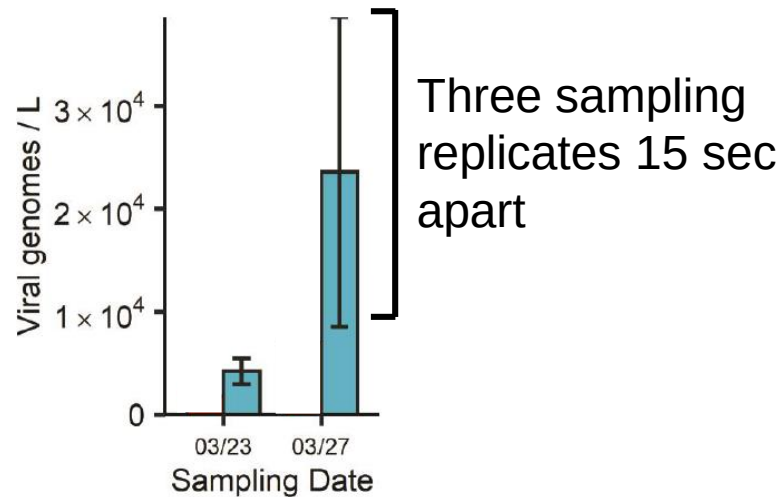


Study design/methodological choices must be driven by the intended use of the data

Grab sampling

vs.

24-hr composite sampling



Viral concentrations grab samples > Composite samples

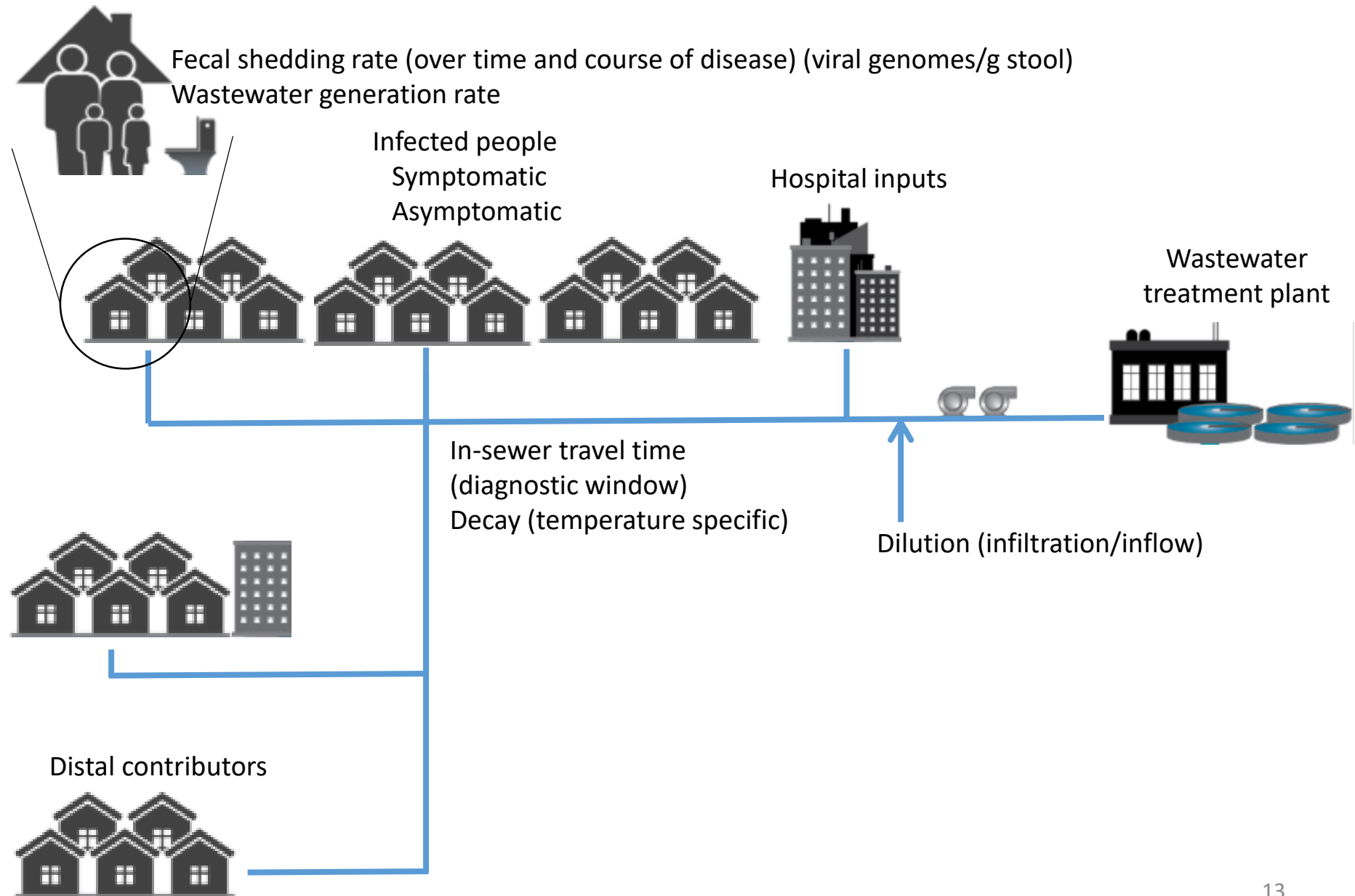
If estimating infection prevalence, may want multiple grab samples

For trends tracking, may want composite samples with better precision

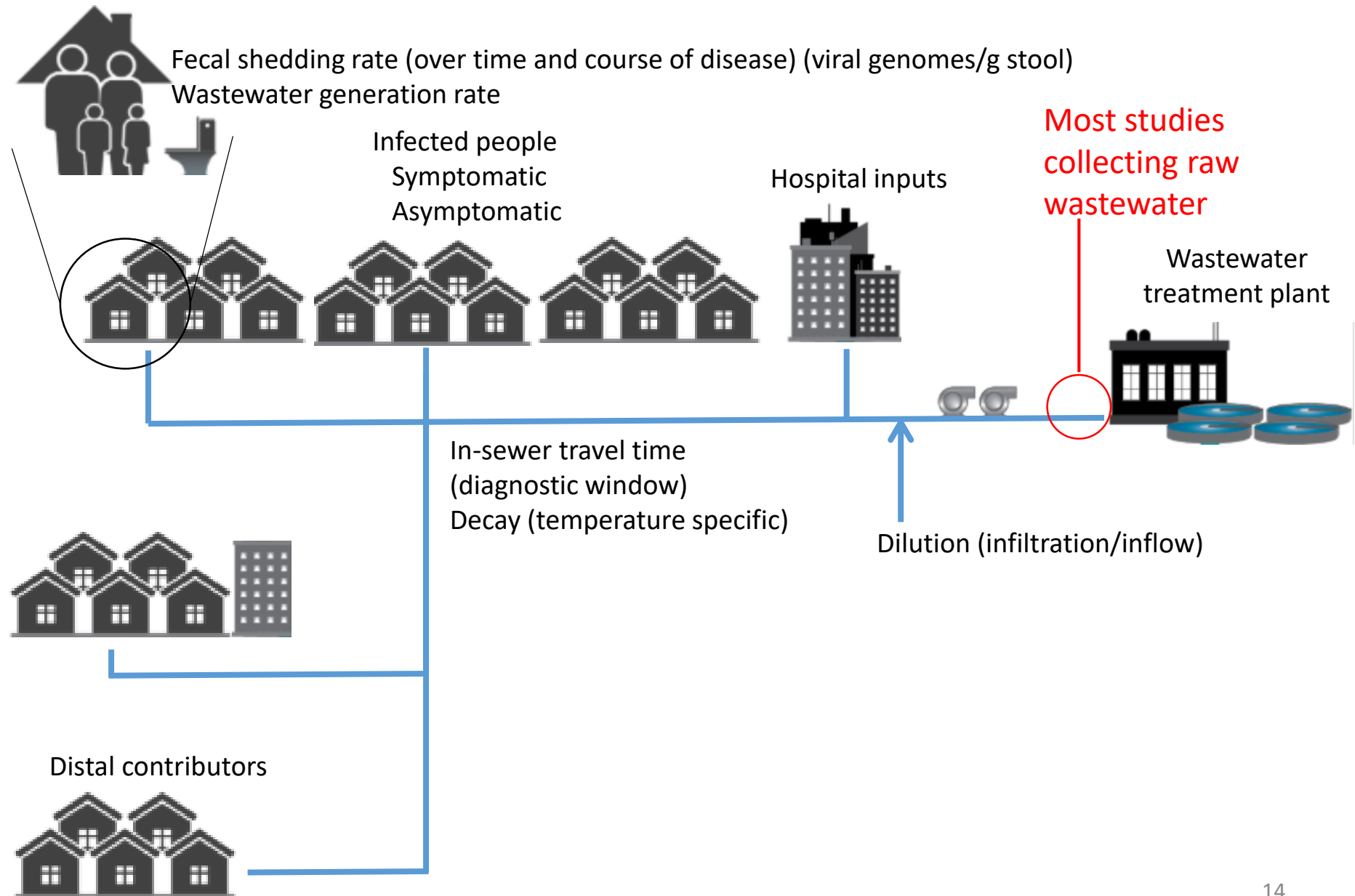
Need multiple molecular targets

Reported finding one predominant SARS-CoV-2 strain in the wastewater

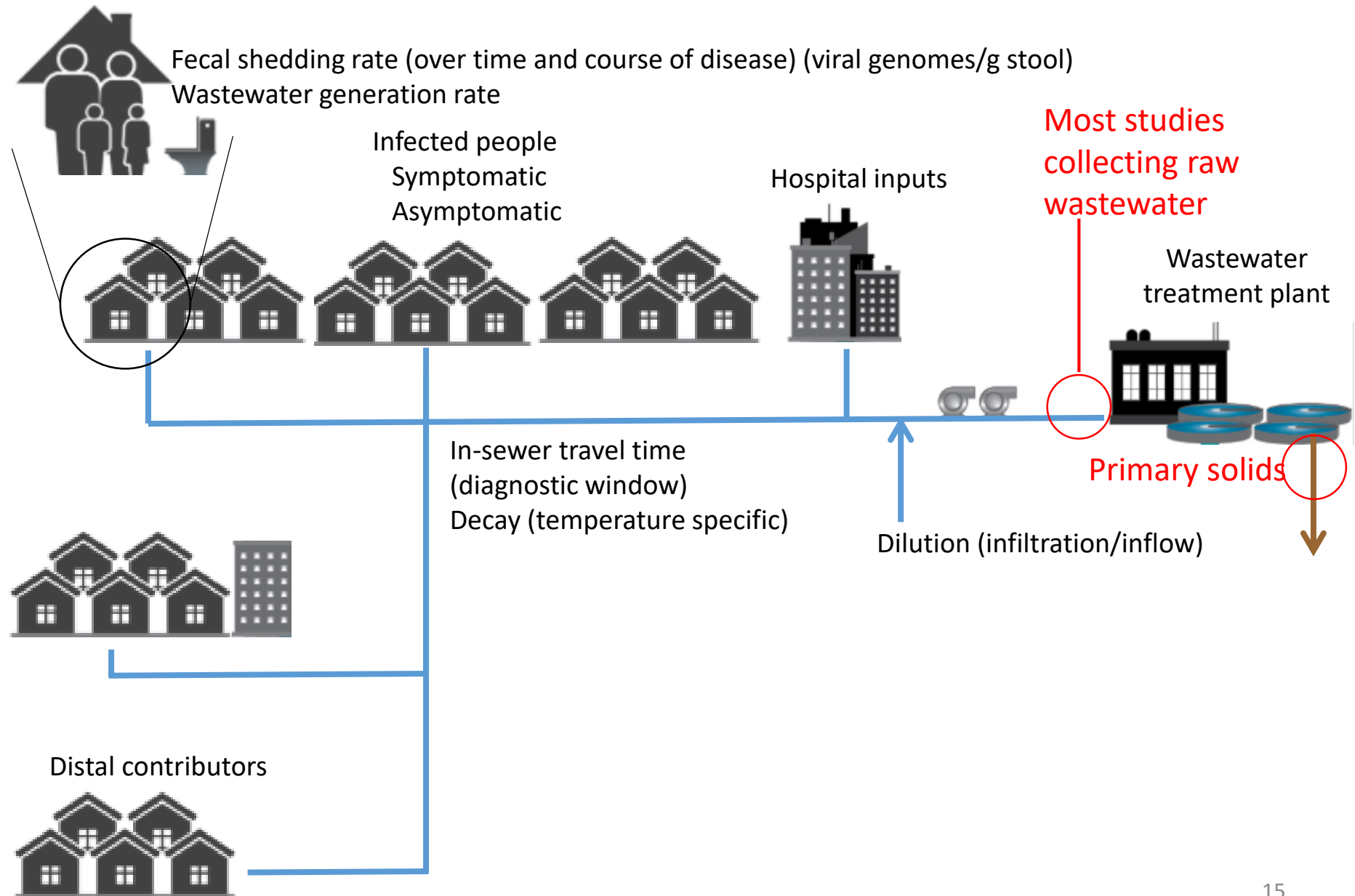
Variables that impact wastewater signal



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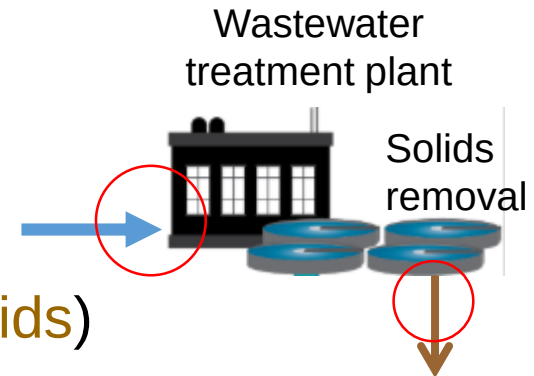


NC State sampling 5 sites in Wake County

Funded by NSF Rapid program to start sample collection on April 10

Funding from NC State Policy Collaboratory

Neuse River Resource Recovery Facility

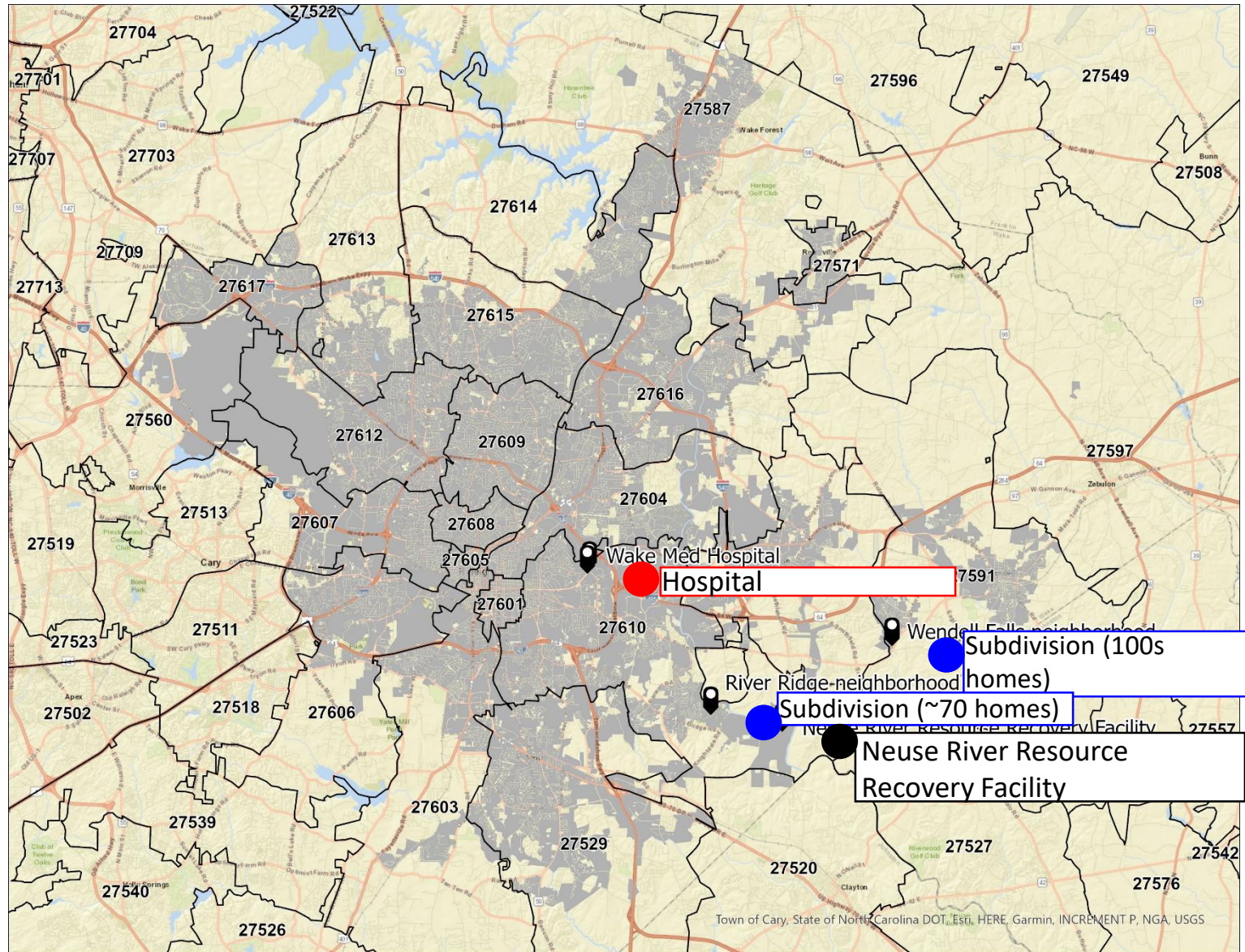


- 1) Head of the plant (**Wastewater** and **solids**)
- 2) Residential pump station serving ~70 homes (**Wastewater**)
- 3) Residential pump station serving ~100s homes (**Wastewater**)
- 4) Hospital (**Wastewater**)

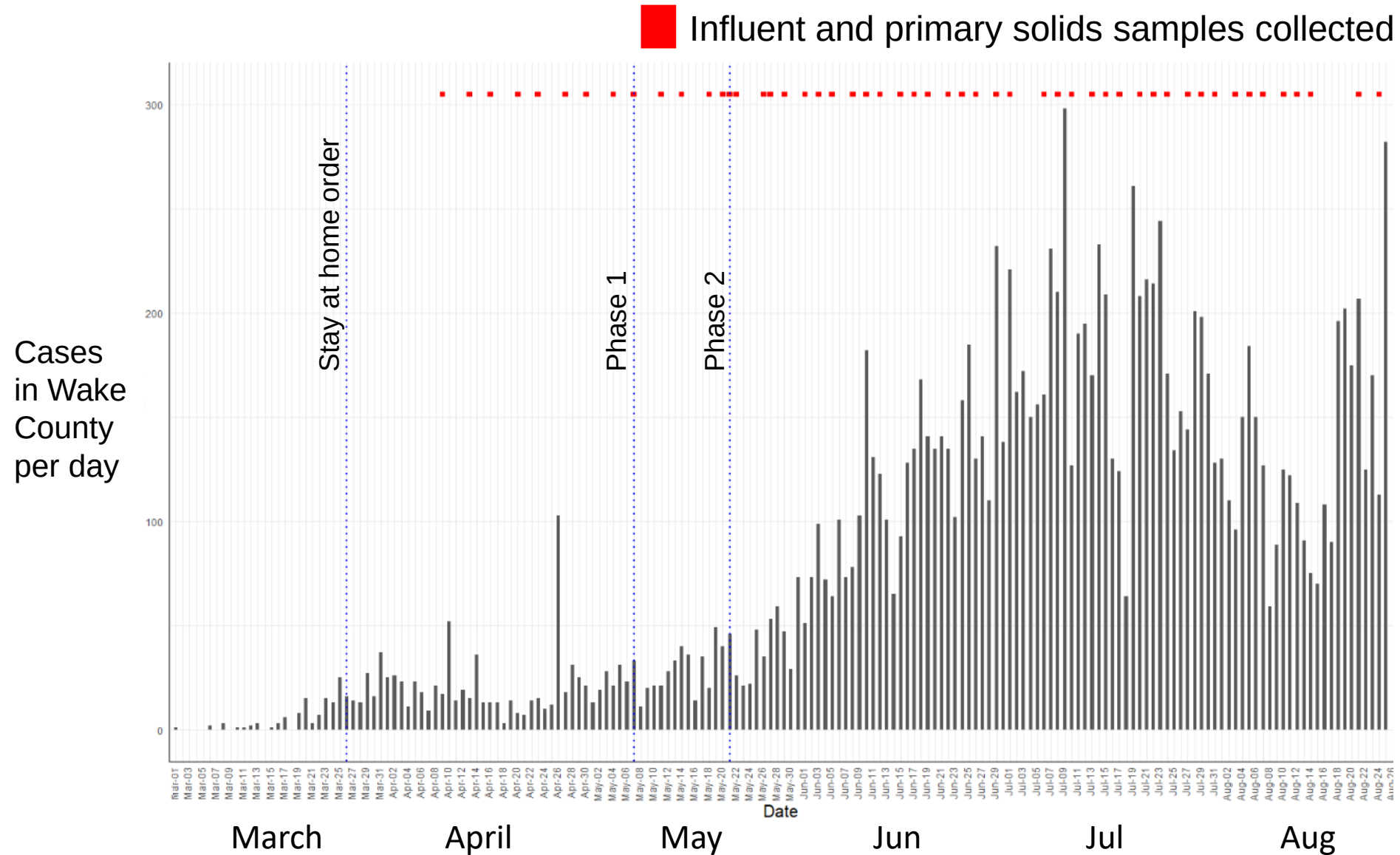
5) Mobile Home Community (**Wastewater**)

Started sampling July 14

The map displays the Cary, North Carolina area, highlighting several key locations and subdivisions. A red dot marks the location of Wake Med Hospital. Two blue dots indicate the locations of subdivisions: one labeled 'Subdivision (100s homes)' and another labeled 'Subdivision (~70 homes)'. A black dot marks the location of the Neuse River Resource Recovery Facility. The map also shows various zip codes (e.g., 27501, 27502, 27503, 27504, 27506, 27507, 27508, 27509, 27510, 27511, 27512, 27513, 27514, 27515, 27516, 27517, 27518, 27519, 27520, 27521, 27522, 27523, 27524, 27525, 27526, 27527, 27528, 27529, 27530, 27531, 27532, 27533, 27534, 27535, 27536, 27537, 27538, 27539, 27540, 27541, 27542, 27543, 27544, 27545, 27546, 27547, 27548, 27549, 27550, 27551, 27552, 27553, 27554, 27555, 27556, 27557, 27558, 27559, 27560, 27561, 27562, 27563, 27564, 27565, 27566, 27567, 27568, 27569, 27570, 27571, 27572, 27573, 27574, 27575, 27576, 27577, 27578, 27579, 27580, 27581, 27582, 27583, 27584, 27585, 27586, 27587, 27588, 27589, 27590, 27591, 27592, 27593, 27594, 27595, 27596, 27597, 27598, 27599, 27600, 27601, 27602, 27603, 27604, 27605, 27606, 27607, 27608, 27609, 27610, 27611, 27612, 27613, 27614, 27615, 27616, 27617, 27618, 27619, 27620, 27621, 27622, 27623, 27624, 27625, 27626, 27627, 27628, 27629, 27630, 27631, 27632, 27633, 27634, 27635, 27636, 27637, 27638, 27639, 27640, 27641, 27642, 27643, 27644, 27645, 27646, 27647, 27648, 27649, 27650, 27651, 27652, 27653, 27654, 27655, 27656, 27657, 27658, 27659, 27660, 27661, 27662, 27663, 27664, 27665, 27666, 27667, 27668, 27669, 27670, 27671, 27672, 27673, 27674, 27675, 27676, 27677, 27678, 27679, 27680, 27681, 27682, 27683, 27684, 27685, 27686, 27687, 27688, 27689, 27690, 27691, 27692, 27693, 27694, 27695, 27696, 27697, 27698, 27699, 27700, 27701, 27702, 27703, 27704, 27705, 27706, 27707, 27708, 27709, 27710, 27711, 27712, 27713, 27714, 27715, 27716, 27717, 27718, 27719, 27720, 27721, 27722, 27723, 27724, 27725, 27726, 27727, 27728, 27729, 27730, 27731, 27732, 27733, 27734, 27735, 27736, 27737, 27738, 27739, 27740, 27741, 27742, 27743, 27744, 27745, 27746, 27747, 27748, 27749, 27750, 27751, 27752, 27753, 27754, 27755, 27756, 27757, 27758, 27759, 27760, 27761, 27762, 27763, 27764, 27765, 27766, 27767, 27768, 27769, 27770, 27771, 27772, 27773, 27774, 27775, 27776, 27777, 27778, 27779, 27780, 27781, 27782, 27783, 27784, 27785, 27786, 27787, 27788, 27789, 27790, 27791, 27792, 27793, 27794, 27795, 27796, 27797, 27798, 27799, 27800, 27801, 27802, 27803, 27804, 27805, 27806, 27807, 27808, 27809, 27810, 27811, 27812, 27813, 27814, 27815, 27816, 27817, 27818, 27819, 27820, 27821, 27822, 27823, 27824, 27825, 27826, 27827, 27828, 27829, 27830, 27831, 27832, 27833, 27834, 27835, 27836, 27837, 27838, 27839, 27840, 27841, 27842, 27843, 27844, 27845, 27846, 27847, 27848, 27849, 27850, 27851, 27852, 27853, 27854, 27855, 27856, 27857, 27858, 27859, 27860, 27861, 27862, 27863, 27864, 27865, 27866, 27867, 27868, 27869, 27870, 27871, 27872, 27873, 27874, 27875, 27876, 27877, 27878, 27879, 27880, 27881, 27882, 27883, 27884, 27885, 27886, 27887, 27888, 27889, 27890, 27891, 27892, 27893, 27894, 27895, 27896, 27897, 27898, 27899, 27900, 27901, 27902, 27903, 27904, 27905, 27906, 27907, 27908, 27909, 27910, 27911, 27912, 27913, 27914, 27915, 27916, 27917, 27918, 27919, 27920, 27921, 27922, 27923, 27924, 27925, 27926, 27927, 27928, 27929, 27930, 27931, 27932, 27933, 27934, 27935, 27936, 27937, 27938, 27939, 27940, 27941, 27942, 27943, 27944, 27945, 27946, 27947, 27948, 27949, 27950, 27951, 27952, 27953, 27954, 27955, 27956, 27957, 27958, 27959, 27960, 27961, 27962, 27963, 27964, 27965, 27966, 27967, 27968, 27969, 27970, 27971, 27972, 27973, 27974, 27975, 27976, 27977, 27978, 27979, 27980, 27981, 27982, 27983, 27984, 27985, 27986, 27987, 27988, 27989, 27990, 27991, 27992, 27993, 27994, 27995, 27996, 27997, 27998, 27999, 28000, 28001, 28002, 28003, 28004, 28005, 28006, 28007, 28008, 28009, 28010, 28011, 28012, 28013, 28014, 28015, 28016, 28017, 28018, 28019, 28020, 28021, 28022, 28023, 28024, 28025, 28026, 28027, 28028, 28029, 28030, 28031, 28032, 28033, 28034, 28035, 28036, 28037, 28038, 28039, 28040, 28041, 28042, 28043, 28044, 28045, 28046, 28047, 28048, 28049, 28050, 28051, 28052, 28053, 28054, 28055, 28056, 28057, 28058, 28059, 28060, 28061, 28062, 28063, 28064, 28065, 28066



Raleigh sample collection frequency



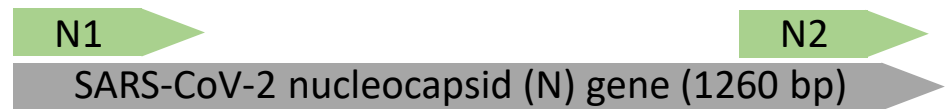
Quantification of SARS-CoV-2 in primary solids

- 1) Sample collection
- 2) Dose internal standard (bovine coronavirus)
- 3) Sample storage at -80 °C
- 4) Thaw sample
- 5) RNA extraction of 2.5 mL
- 6) Digital droplet PCR:

SARS-CoV-2 N1

SARS-CoV-2 N2

Bovine coronavirus



Cost estimate: <\$50/sample
Time estimate: 2-3 days/sample

Preliminary results

- SARS-CoV-2 N2 gene quantifiable in wastewater and primary solids
 - Detectable in every solids sample we have tested (earliest sample tested is from Jun 13, 2020)
- Concentration in solids > conc. in influent
- Positive detections at the neighborhood scale
- Detectable both days at Hospital

How can wastewater surveillance be validated?

Test for relationships with health surveillance data

- 1) COVID like syndromic cases
- 2) Lab confirmed cases
- 3) Hospitalizations
- 4) Deaths

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