Supporting Information for
Measles detection in wastewater solids during an outbreak

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Additional details on molecular analysis of wastewater samples. Briefly, solids were isolated from wastewater samples as described in the main text and re-suspended in a buffer at a low enough concentration so as to minimize inhibition (75 mg/ml) and homogenized.[1] Six aliquots of supernatant from each homogenized sample were then subjected to nucleic-acid extraction and purification (Chemagic Viral DNA/RNA 300 Kit H96, PerkinElmer, Shelton, CT), and inhibitor removal (Zymo OneStep PCR Inhibitor Removal Kit, Irvine, CA). Dry weight was determined using another aliquot of solids and drying in an oven.1

For retrospective testing, the nucleic acids from the 6 aliquots of each sample were used as template in 6 replicate 1-step RT-ddPCR reaction wells to measure measles RNA using the wild-type measles assay (Table 1). The measles assay (probe labeled using FAM) was run in duplex with an assay for SARS-CoV-2 N gene (probe labeled with hexachlorofluorescein, HEX).[1] For prospective testing, the measles assay was run in multiplex with assays for Dengue virus RNA types 1, 2, 3 and 4, the results of which are not reported herein.

For the prospective testing, nucleic-acids from the 6 aliquots of each sample were each used used neat as template in 10 replicate droplet digital 1-step RT-PCR (RT-ddPCR) reaction wells to measure measles RNA using measles assay (Table 1). Nucleic acids from 4 of the aliquots were run in duplicate to obtain the 10 replicate wells. During pilot testing, the measles assay was run in multiplex with assays for Dengue virus RNA types 1, 2, 3 and 4, the results of which are not reported herein. During national testing, the measles assay was run in multiplex with assays for parvovirus, human adenovirus, and rotavirus, the results of which are not reported herein. The measles assay was run using a probe labeled with the fluorescent molecule fluorescein amidite (FAM).

For both pilot and national testing, negative and positive RT-PCR controls were included on each plate (n=2 and 1 wells, respectively). Synthetic gene blocks (IDT, Coralville, IA) controls

were used as positive controls for B3 and D8 (Table S1) and negative controls used molecular grade water.

Table S1. Gene block sequences for wild type measles genotypes D8 and B3.

D8 gene block	ATCTGGCCTCACCTTCGCATCAAGAGGTACCAACATGGAGGATGAGG CGGACCAATACTTTTCACATGATGATCCAAGTAGTAGTGATCAATCCA GGTTCGGATGGTTCGAGAACAAGGAAATCTCAGATATCGAAGTGCAA GACCCTGAGGGATTTAACATGATTCTGGGTACCATTCTAGCCCAAATT TGGGTCTTGCTCGCAAAGGCGGTTACGGCCCCAGACACGGCAGCTGA TTCGGAGCTAAGAAGGTGGATAAAGTACACCCAACAAAGAAGGGTAG TTGGTGAATTTAGATTGGAGAGAAAATG
B3 gene block	GCCTTACCTTCGCATCGAGAGGTACTAATATGGAGGATGAGGCGGAC CAGTACTTTTCACATGATGATCCAAGTAGTAGTGATCAATCCAGGTTC GGGTGGTTTGAGAACAAGGAAATCTCAGATATTGAAGTGCAAGACCCT GAGGGCTTCAACATGATTCTGGGTACCATCTTAGCTCAAATTTGGGTC TTGCTCGCAAAGGCGGTTACGGCTCCAGACACAGCAGCTGATTCAGA GCTAAGAAGGTGGATCAAATACACCCAACAAAGAAGAGTAGTTGGTGA ATT

Negative extraction controls and viral RNA recovery controls were also run on the samples. The buffer used for suspending the wastewater solids prior to nucleic-acid extraction was spiked with bovine coronavirus (BCoV) vaccine during the sample processing steps, and nucleic-acid extracts from each sample were assayed for BCoV RNA following methods described elsewhere to calculate BCoV RNA recovery (measured BCoV divided by added BCoV).[1] Nucleic-acid extracts from the buffer were also used as template for negative extraction controls as they are not expected to contain measles or SARS-CoV-2 RNA targets. The negative and positive controls were run in separate plates that assayed a wide variety of pathogen biomarkers measured prospectively (within 48 h of sample receipt in the lab), as described by Boehm et al.[1]

Digital droplet RT-PCR was performed on 20 µl samples from a 22 µl reaction volume, prepared using 5.5 µl template mixed with 5.5 µl of One-Step RT-ddPCR Advanced kit for Probes (catalog no. 1863021; Bio-Rad), 2.2 µl reverse transcriptase, 1.1 µl dithiothreitol (DTT), and primers and probes at a final concentration of 900 nM and 250 nM, respectively. Droplets were generated using the AutoDG Automated Droplet Generator (Bio-Rad).

PCR was performed using Mastercycler Pro (Eppendorf, Enfield, CT) thermocycler with the following protocol: reverse transcription at 50 °C for 60 min, enzyme activation at 95°C for 5 min, 40 cycles with 1 cycle consisting of denaturation at 95°C for 30 s and annealing and extension at 59°C for 30 s, enzyme deactivation at 98 °C for 10 min, and then an indefinite hold at 4°C. The ramp rate for temperature changes was set at 2°C/s, and the final hold at 4°C was performed for a minimum of 30 min to allow the droplets to stabilize. Droplets were analyzed

using the QX200 (retrospective analyses) or QX600 (prospective analyses) Droplet Reader (Bio-Rad). All liquid transfers were performed using the Agilent Bravo (Agilent Technologies).

A well had to have over 10,000 droplets for inclusion in the analysis. Results from replicate wells were merged for analysis. Concentrations of the targets in wastewater samples are presented as copies per gram dry weight. For a sample to be scored as a positive, there had to be at least 3 positive droplets across the merged wells. The lowest measurable concentration is approximately 1000 copies/g dry weight (corresponds to three positive droplets across 6 to 10 merged wells). Errors are reported as standard deviations of the measurements as obtained from vendor's software.

Additional details on sites included in national prospective monitoring.
The 147 sites included in prospective national monitoring, including sample collection methods, have been described in detail elsewhere.1 Table S2 describes all sites, the counties they serve, and adjacent counties.

Table S2. Sites included in the national prospective monitoring program May - November, 2025.

Plant	Population	County	Adjacent Counties
Union Beach, NJ	1.00E+05	Middlesex (34023), Monmouth (34025)	Union (34039), Burlington (34005), Richmond (36085), Somerset (34035), Ocean (34029), Queens (36081), Mercer (34021)
Redwood City, CA	199000	San Mateo (06081)	San Francisco (06075), Santa Clara (06085), Alameda (06001), Santa Cruz (06087)
Napa, CA	83300	Napa (06055)	Sonoma (06097), Solano (06095), Yolo (06113), Lake (06033)
Hollywood, MD	55000	St. Mary's (24037)	Northumberland (51133), Calvert (24009), Dorchester (24019), Somerset (24039), Accomack (51001), Westmoreland (51193), Charles (24017)
Bloomington, IN	56090	Monroe (18105)	Greene (18055), Owen (18119), Brown (18013), Morgan (18109), Jackson (18071), Lawrence (18093)
Snohomish, WA	10150	Snohomish (53061)	Island (53029), Chelan (53007), King (53033), Kitsap (53035), Skagit (53057)
Ann Arbor, MI	125000	Washtenaw (26161)	Wayne (26163), Jackson (26075), Ingham (26065), Livingston (26093), Monroe (26115), Oakland (26125), Lenawee (26091)
Mt. Pleasant, MI	21690	Isabella (26073)	Clare (26035), Gratiot (26057), Mecosta (26107), Montcalm (26117), Gladwin (26051), Midland (26111), Osceola (26133)
Johns Creek, Roswell, GA	84486	Fulton (13121)	Clayton (13063), DeKalb (13089), Carroll (13045), Fayette (13113), Coweta (13077), Douglas (13097), Gwinnett (13135), Cobb (13067), Cherokee (13057), Forsyth (13117)
South, Laredo, TX	120000	Webb (48479)	Jim Hogg (48247), Dimmit (48127), McMullen (48311), Duval (48131), Maverick (48323), La Salle (48283), Zapata (48505)
Memphis, TN	3.00E+05	Shelby (47157)	Mississippi (05093), Marshall (28093), Crittenden (05035), Tipton (47167), DeSoto (28033), Fayette (47047)
Wolcott, Kansas City, KS	15000	Wyandotte (20209)	Platte (29165), Johnson (20091), Jackson (29095), Clay (29047), Leavenworth (20103)
East Bank, New Orleans, LA	333406	Orleans (22071)	St. Bernard (22087), Plaquemines (22075), Jefferson (22051), St. Tammany (22103)
Columbus, GA	278000	Muscogee (13215)	Harris (13145), Lee (01081), Chattahoochee (13053), Talbot (13263), Russell (01113)

Jenison, MI	75000	Ottawa (26139)	Milwaukee (55079), Racine (55101), Kent (26081), Allegan (26005), Muskegon (26121)
North Miami, FL	776150	Miami-Dade (12086)	Broward (12011), Monroe (12087), Collier (12021)
Stamford, CT	140000	Fairfield (09001)	Suffolk (36103), Dutchess (36027), Nassau (36059), Westchester (36119), New Haven (09009), Putnam (36079), Litchfield (09005)
Portland, ME	65000	Cumberland (23005)	Androscoggin (23001), Sagadahoc (23023), York (23031), Oxford (23017)
Winston-Salem, NC	92000	Forsyth (37067)	Davidson (37057), Stokes (37169), Guilford (37081), Yadkin (37197), Davie (37059), Rockingham (37157), Surry (37171)
Big Creek, Roswell, GA	189593	Fulton (13121)	Clayton (13063), DeKalb (13089), Carroll (13045), Fayette (13113), Coweta (13077), Douglas (13097), Gwinnett (13135), Cobb (13067), Cherokee (13057), Forsyth (13117)
Muscatine, IA	24400	Louisa (19115), Muscatine (19139)	Cedar (19031), Mercer (17131), Rock Island (17161), Johnson (19103), Des Moines (19057), Scott (19163), Henry (19087), Henderson (17071), Washington (19183)
Northeast, Lincoln, NE	60000	Lancaster (31109)	Saline (31151), Otoe (31131), Cass (31025), Gage (31067), Johnson (31097), Saunders (31155), Seward (31159), Butler (31023)
Warren, MI	140000	Macomb (26099)	Wayne (26163), Lapeer (26087), St. Clair (26147), Oakland (26125)
Bridgewater, NJ	130000	Somerset (34035)	Union (34039), Middlesex (34023), Morris (34027), Hunterdon (34019), Mercer (34021)
Carmel, IN	86000	Hamilton (18057)	Clinton (18023), Hancock (18059), Tipton (18159), Boone (18011), Madison (18095), Marion (18097)
Jupiter, FL	90000	Martin (12085), Palm Beach (12099)	Hendry (12051), Broward (12011), Okeechobee (12093), Glades (12043), St. Lucie (12111)
Lewiston, ME	60000	Androscoggin (23001)	Sagadahoc (23023), Kennebec (23011), Oxford (23017), Cumberland (23005), Franklin (23007)
Traverse City, MI	30623	Grand Traverse (26055)	Leelanau (26089), Antrim (26009), Kalkaska (26079), Benzie (26019), Wexford (26165), Manistee (26101), Missaukee (26113)
Chester, PA	220000	Delaware (42045)	New Castle (10003), Philadelphia (42101), Chester (42029), Gloucester (34015), Montgomery (42091), Salem (34033)
Rochester, MN	120000	Olmsted (27109)	Fillmore (27045), Dodge (27039), Winona (27169), Goodhue (27049), Mower (27099), Wabasha (27157)

Oakhurst, NJ	50000	Monmouth (34025)	Burlington (34005), Middlesex (34023), Richmond (36085), Ocean (34029), Queens (36081), Mercer (34021)
Marina, CA	262000	Monterey (06053)	Kings (06031), San Benito (06069), Fresno (06019), Santa Cruz (06087), San Luis Obispo (06079)
Woodlands SJRA WWTF No. 1, TX	65000	Montgomery (48339)	San Jacinto (48407), Waller (48473), Walker (48471), Harris (48201), Grimes (48185), Liberty (48291)
Mankato, MN	70000	Blue Earth (27013)	Waseca (27161), Watonwan (27165), Le Sueur (27079), Faribault (27043), Martin (27091), Brown (27015), Nicollet (27103)
Theresa Street, Lincoln, NE	240000	Lancaster (31109)	Saline (31151), Otoe (31131), Cass (31025), Gage (31067), Johnson (31097), Saunders (31155), Seward (31159), Butler (31023)
Zacate Creek, Laredo, TX	140000	Webb (48479)	Jim Hogg (48247), Dimmit (48127), McMullen (48311), Duval (48131), Maverick (48323), La Salle (48283), Zapata (48505)
Chattanooga, TN	4.00E+05	Marion (47115), Hamilton (47065)	Grundy (47061), Jackson (01071), Sequatchie (47153), Catoosa (13047), Rhea (47143), Bradley (47011), Meigs (47121), Walker (13295), Bledsoe (47007), Franklin (47051), Whitfield (13313), Dade (13083)
San Diego, CA	2200000	San Diego (06073)	Riverside (06065), Orange (06059), Imperial (06025)
Coralville, IA	23000	Johnson (19103)	Cedar (19031), Benton (19011), Linn (19113), Iowa (19095), Louisa (19115), Muscatine (19139), Washington (19183)
Southwest, Saint Petersburg, FL	47790	Pinellas (12103)	Pasco (12101), Hillsborough (12057)
Northwest, Orange County, FL	66690	Orange (12095)	Volusia (12127), Lake (12069), Seminole (12117), Polk (12105), Osceola (12097), Brevard (12009)
Youngstown, OH	174000	Mahoning (39099)	Portage (39133), Trumbull (39155), Lawrence (42073), Mercer (42085), Stark (39151), Columbiana (39029)
Vallejo, CA	121000	Solano (06095)	Sacramento (06067), Sonoma (06097), Marin (06041), Napa (06055), Contra Costa (06013), Yolo (06113)
Anchorage, AK	220000	Anchorage (02020)	Kenai Peninsula (02122), Matanuska-Susitna (02170), Chugach (02063)
Jackson, MI	90000	Jackson (26075)	Ingham (26065), Livingston (26093), Washtenaw (26161), Hillsdale (26059), Calhoun (26025), Eaton (26045), Lenawee (26091)
West Bank, New Orleans, LA	50591	Orleans (22071)	St. Bernard (22087), Plaquemines (22075), Jefferson (22051), St. Tammany (22103)

Montpelier, VT	10100	Washington (50023)	Orange (50017), Lamoille (50015), Caledonia (50005), Chittenden (50007), Addison (50001)
Sacramento, CA	1480000	Yolo (06113), Sacramento (06067)	Placer (06061), Napa (06055), Solano (06095), Contra Costa (06013), El Dorado (06017), Colusa (06011), San Joaquin (06077), Sutter (06101), Amador (06005), Lake (06033)
Lander Street, Boise, ID	97237	Gem (16045), Ada (16001)	Elmore (16039), Adams (16003), Valley (16085), Boise (16015), Payette (16075), Owyhee (16073), Washington (16087), Canyon (16027)
South Burlington, VT	16000	Chittenden (50007)	Franklin (50011), Essex (36031), Clinton (36019), Lamoille (50015), Grand Isle (50013), Washington (50023), Addison (50001)
Cahaba River, Birmingham, AL	95000	Jefferson (01073)	Tuscaloosa (01125), Blount (01009), St. Clair (01115), Shelby (01117), Walker (01127), Bibb (01007)
Little River, Roswell, GA	12818	Cherokee (13057), Fulton (13121)	Clayton (13063), DeKalb (13089), Pickens (13227), Carroll (13045), Gordon (13129), Fayette (13113), Bartow (13015), Coweta (13077), Dawson (13085), Douglas (13097), Gwinnett (13135), Cobb (13067), Forsyth (13117)
Wheaton, IL	63000	DuPage (17043)	Kendall (17093), Cook (17031), Kane (17089), Will (17197)
North, Parker, CO	35000	Douglas (08035)	Park (08093), Jefferson (08059), El Paso (08041), Teller (08119), Elbert (08039), Arapahoe (08005)
Salina, KS	47000	Saline (20169)	McPherson (20113), Marion (20115), Lincoln (20105), Ottawa (20143), Dickinson (20041), Ellsworth (20053)
White Rock Central, Dallas, TX	630000	Dallas (48113)	Rockwall (48397), Kaufman (48257), Tarrant (48439), Ellis (48139), Collin (48085), Denton (48121)
Yankton, SD	20000	Yankton (46135)	Hutchinson (46067), Clay (46027), Bon Homme (46009), Turner (46125), Cedar (31027), Knox (31107)
Glen Ellyn, IL	86000	DuPage (17043)	Kendall (17093), Cook (17031), Kane (17089), Will (17197)
Oswego, NY	30000	Oswego (36075)	Cayuga (36011), Jefferson (36045), Madison (36053), Lewis (36049), Oneida (36065), Onondaga (36067)
River Road, Amarillo, TX	140000	Randall (48381), Potter (48375)	Armstrong (48011), Oldham (48359), Carson (48065), Swisher (48437), Deaf Smith (48117), Hutchinson (48233), Castro (48069), Moore (48341), Hartley (48205)
Paso Robles, CA	31037	San Luis Obispo (06079)	Santa Barbara (06083), Kings (06031), Monterey (06053), Kern (06029)
Kaw Point, Kansas City, KS	90000	Wyandotte (20209)	Platte (29165), Johnson (20091), Jackson (29095), Clay (29047), Leavenworth (20103)

Palo Alto, CA	236000	Santa Clara (06085)	San Benito (06069), San Joaquin (06077), Alameda (06001), Merced (06047), Stanislaus (06099), San Mateo (06081), Santa Cruz (06087)
Sand Island, Honolulu, HI	390000	Honolulu (15003)	NA
South Bend, IN	130000	St. Joseph (18141)	Marshall (18099), LaPorte (18091), Berrien (26021), Elkhart (18039), Starke (18149), Cass (26027)
Sunnyvale, CA	153000	Santa Clara (06085)	San Benito (06069), San Joaquin (06077), Alameda (06001), Merced (06047), Stanislaus (06099), San Mateo (06081), Santa Cruz (06087)
Honouliuli, Honolulu, HI	3.00E+05	Honolulu (15003)	NA
Hollywood Road, Amarillo, TX	60000	Potter (48375), Randall (48381)	Armstrong (48011), Oldham (48359), Carson (48065), Swisher (48437), Deaf Smith (48117), Hutchinson (48233), Castro (48069), Moore (48341), Hartley (48205)
Northwest, Saint Petersburg, FL	94218	Pinellas (12103)	Pasco (12101), Hillsborough (12057)
Aquia, Stafford, VA	1.00E+05	Stafford (51179)	Fauquier (51061), Prince William (51153), Charles (24017), Fredericksburg (51630), King George (51099), Culpeper (51047), Spotsylvania (51177), Caroline (51033)
Lawrence, KS	80000	Douglas (20045)	Osage (20139), Franklin (20059), Johnson (20091), Shawnee (20177), Miami (20121), Jefferson (20087), Leavenworth (20103)
Akron, OH	365000	Summit (39153)	Portage (39133), Wayne (39169), Stark (39151), Geauga (39055), Cuyahoga (39035), Medina (39103)
Davis, CA	68000	Yolo (06113)	Sacramento (06067), Napa (06055), Solano (06095), Colusa (06011), Sutter (06101), Lake (06033)
Louisville, KY	423913	Jefferson (21111)	Floyd (18043), Harrison (18061), Clark (18019), Bullitt (21029), Shelby (21211), Hardin (21093), Oldham (21185), Spencer (21215)
Southside, Dallas, TX	421700	Dallas (48113)	Rockwall (48397), Kaufman (48257), Tarrant (48439), Ellis (48139), Collin (48085), Denton (48121)
College Park, GA	73821	Fulton (13121)	Clayton (13063), DeKalb (13089), Carroll (13045), Fayette (13113), Coweta (13077), Douglas (13097), Gwinnett (13135), Cobb (13067), Cherokee (13057), Forsyth (13117)
Santa Rosa, CA	230000	Sonoma (06097)	Marin (06041), Napa (06055), Mendocino (06045), Solano (06095), Contra Costa (06013), Lake (06033)
Woodlands SJRA WWTF No. 2, TX	70000	Montgomery (48339)	San Jacinto (48407), Waller (48473), Walker (48471), Harris (48201), Grimes (48185), Liberty (48291)

Southeast San Francisco, CA	750000	San Francisco (06075)	Marin (06041), Contra Costa (06013), Alameda (06001), San Mateo (06081)
Provo, UT	115000	Utah (49049)	Duchesne (49013), Salt Lake (49035), Juab (49023), Carbon (49007), Sanpete (49039), Wasatch (49051), Tooele (49045)
Sunnyvale, TX	186000	Dallas (48113)	Rockwall (48397), Kaufman (48257), Tarrant (48439), Ellis (48139), Collin (48085), Denton (48121)
Wheeling, WV	1.00E+05	Marshall (54051), Ohio (54069)	Wetzel (54103), Greene (42059), Monroe (39111), Jefferson (39081), Belmont (39013), Brooke (54009), Washington (42125)
Ontario, CA	890000	San Bernardino (06071)	Clark (32003), Los Angeles (06037), Inyo (06027), Riverside (06065), Kern (06029), Mohave (04015), La Paz (04012), Orange (06059)
Little Falls Run, Stafford, VA	50000	Stafford (51179)	Fauquier (51061), Prince William (51153), Charles (24017), Fredericksburg (51630), King George (51099), Culpeper (51047), Spotsylvania (51177), Caroline (51033)
Pinson, AL	30000	Jefferson (01073)	Tuscaloosa (01125), Blount (01009), St. Clair (01115), Shelby (01117), Walker (01127), Bibb (01007)
Gilroy, CA	110338	Santa Clara (06085)	San Benito (06069), San Joaquin (06077), Alameda (06001), Merced (06047), Stanislaus (06099), San Mateo (06081), Santa Cruz (06087)
Harrison, AR	15000	Newton (05101), Boone (05009)	Carroll (05015), Johnson (05071), Searcy (05129), Taney (29213), Madison (05087), Pope (05115), Marion (05089)
Woodland, CA	59000	Yolo (06113)	Sacramento (06067), Napa (06055), Solano (06095), Colusa (06011), Sutter (06101), Lake (06033)
Gautier, MS	19008	Jackson (28059)	Mobile (01097), Stone (28131), George (28039), Harrison (28047)
Las Vegas, NV	1250000	Clark (32003)	Nye (32023), Inyo (06027), San Bernardino (06071), Mohave (04015), Lincoln (32017)
Bessemer, AL	225000	Jefferson (01073)	Tuscaloosa (01125), Blount (01009), St. Clair (01115), Shelby (01117), Walker (01127), Bibb (01007)
South, Orange County, FL	183009	Orange (12095)	Volusia (12127), Lake (12069), Seminole (12117), Polk (12105), Osceola (12097), Brevard (12009)
Wichita Falls, TX	90000	Archer (48009), Wichita (48485), Clay (48077)	Jack (48237), Cotton (40033), Tillman (40141), Montague (48337), Young (48503), Baylor (48023), Jefferson (40067), Throckmorton (48447), Wilbarger (48487)
Los Angeles, CA	4.00E+06	Los Angeles (06037)	Ventura (06111), San Bernardino (06071), Kern (06029), Orange (06059)

Village Creek, Birmingham, AL	2.00E+05	Jefferson (01073)	Tuscaloosa (01125), Blount (01009), St. Clair (01115), Shelby (01117), Walker (01127), Bibb (01007)
Bridgeton, NJ	50000	Salem (34033), Cumberland (34011)	New Castle (10003), Cape May (34009), Gloucester (34015), Delaware (42045), Kent (10001), Atlantic (34001)
Dallas Central, Dallas, TX	270000	Dallas (48113)	Rockwall (48397), Kaufman (48257), Tarrant (48439), Ellis (48139), Collin (48085), Denton (48121)
Garland, TX	2.00E+05	Dallas (48113)	Rockwall (48397), Kaufman (48257), Tarrant (48439), Ellis (48139), Collin (48085), Denton (48121)
Los Angeles County, CA	3500000	Los Angeles (06037)	Ventura (06111), San Bernardino (06071), Kern (06029), Orange (06059)
San Rafael, CA	104250	Marin (06041)	Sonoma (06097), San Francisco (06075), Solano (06095), Contra Costa (06013)
Hagerstown, MD	90000	Washington (24043)	Allegany (24001), Fulton (42057), Frederick (24021), Jefferson (54037), Berkeley (54003), Adams (42001), Franklin (42055), Loudoun (51107), Morgan (54065)
Merced, CA	91000	Merced (06047)	Mariposa (06043), Tuolumne (06109), San Benito (06069), Fresno (06019), Madera (06039), Santa Clara (06085), Stanislaus (06099)
Novato, CA	53000	Marin (06041)	Sonoma (06097), San Francisco (06075), Solano (06095), Contra Costa (06013)
Red Wing, MN	16000	Goodhue (27049)	Pierce (55093), Dodge (27039), Olmsted (27109), Dakota (27037), Wabasha (27157), Rice (27131), Pepin (55091), Steele (27147)
Utoy Creek, Atlanta, GA	70887	Fulton (13121)	Clayton (13063), DeKalb (13089), Carroll (13045), Fayette (13113), Coweta (13077), Douglas (13097), Gwinnett (13135), Cobb (13067), Cherokee (13057), Forsyth (13117)
Boston, MA	2400000	Suffolk (25025), Norfolk (25021), Middlesex (25017)	Worcester (25027), Bristol (25005), Providence (44007), Hillsborough (33011), Essex (25009), Plymouth (25023)
Turlock, CA	86000	Stanislaus (06099)	Calaveras (06009), Mariposa (06043), Tuolumne (06109), Santa Clara (06085), San Joaquin (06077), Alameda (06001), Merced (06047)
RM Clayton, Atlanta, GA	294660	Fulton (13121), DeKalb (13089)	Clayton (13063), Carroll (13045), Fayette (13113), Coweta (13077), Henry (13151), Douglas (13097), Gwinnett (13135), Rockdale (13247), Cobb (13067), Cherokee (13057), Forsyth (13117)
West Boise, ID	206629	Boise (16015), Ada (16001)	Gem (16045), Custer (16037), Elmore (16039), Valley (16085), Owyhee (16073), Canyon (16027)
San Jose, CA	1500000	Santa Clara (06085)	San Benito (06069), San Joaquin (06077), Alameda (06001), Merced (06047), Stanislaus (06099), San Mateo (06081), Santa Cruz (06087)

Millbury, MA	250000	Worcester (25027)	Hampshire (25015), Cheshire (33005), Hampden (25013), Tolland (09013), Middlesex (25017), Windham (09015), Providence (44007), Hillsborough (33011), Norfolk (25021), Franklin (25011)
South, Parker, CO	25000	Douglas (08035)	Park (08093), Jefferson (08059), El Paso (08041), Teller (08119), Elbert (08039), Arapahoe (08005)
Kinston, NC	25000	Jones (37103), Lenoir (37107)	Carteret (37031), Craven (37049), Duplin (37061), Greene (37079), Pitt (37147), Onslow (37133), Wayne (37191)
Lompoc, CA	69290	Santa Barbara (06083)	Ventura (06111), Kern (06029), San Luis Obispo (06079)
Downtown, Jeffersonville, IN	25000	Clark (18019)	Scott (18143), Jefferson (18077), Floyd (18043), Jefferson (21111), Oldham (21185), Trimble (21223), Washington (18175)
North, Jeffersonville, IN	25000	Clark (18019)	Scott (18143), Jefferson (18077), Floyd (18043), Jefferson (21111), Oldham (21185), Trimble (21223), Washington (18175)
Santa Cruz, CA	160000	Santa Cruz (06087)	Monterey (06053), San Benito (06069), Santa Clara (06085), San Mateo (06081)
Riverside, CA	350000	Riverside (06065)	San Diego (06073), San Bernardino (06071), La Paz (04012), Orange (06059), Imperial (06025)
Coeur d'Alene, ID	50540	Kootenai (16055)	Benewah (16009), Spokane (53063), Bonner (16017), Shoshone (16079)
Oceanside, San Francisco, CA	250000	San Francisco (06075)	Marin (06041), Contra Costa (06013), Alameda (06001), San Mateo (06081)
Pascagoula Moss Point, MS	34333	Jackson (28059)	Mobile (01097), Stone (28131), George (28039), Harrison (28047)
Essex Junction, VT	30000	Chittenden (50007)	Franklin (50011), Essex (36031), Clinton (36019), Lamoille (50015), Grand Isle (50013), Washington (50023), Addison (50001)
Ithaca, NY	90000	Tompkins (36109)	Schuyler (36097), Cayuga (36011), Cortland (36023), Tioga (36107), Seneca (36099), Chemung (36015)
Fultondale, AL	77000	Jefferson (01073)	Tuscaloosa (01125), Blount (01009), St. Clair (01115), Shelby (01117), Walker (01127), Bibb (01007)
Ottumwa, IA	25529	Wapello (19179)	Jefferson (19101), Mahaska (19123), Appanoose (19007), Van Buren (19177), Keokuk (19107), Davis (19051), Monroe (19135)
Eastern, Orange County, FL	195299	Orange (12095)	Volusia (12127), Lake (12069), Seminole (12117), Polk (12105), Osceola (12097), Brevard (12009)

Fremont, CA	229476	Alameda (06001)	San Francisco (06075), Contra Costa (06013), Santa Clara (06085), San Joaquin (06077), Stanislaus (06099), San Mateo (06081)
Tallahassee, FL	212065	Leon (12073)	Wakulla (12129), Jefferson (12065), Thomas (13275), Liberty (12077), Grady (13131), Gadsden (12039)
Belmar, NJ	52672	Monmouth (34025)	Burlington (34005), Middlesex (34023), Richmond (36085), Ocean (34029), Queens (36081), Mercer (34021)
Dover, NH	30000	Strafford (33017)	Merrimack (33013), York (23031), Carroll (33003), Rockingham (33015), Belknap (33001)
Wausau, WI	44000	Marathon (55073)	Waupaca (55135), Portage (55097), Langlade (55067), Taylor (55119), Shawano (55115), Wood (55141), Lincoln (55069), Clark (55019)
Seaford, DE	13172	Sussex (10005)	Worcester (24047), Cape May (34009), Dorchester (24019), Wicomico (24045), Caroline (24011), Kent (10001)
Newark, NJ	1500000	Bergen (34003), Passaic (34031), Hudson (34017), Essex (34013)	Sussex (34037), Union (34039), Westchester (36119), Richmond (36085), Morris (34027), Kings (36047), New York (36061), Rockland (36087), Orange (36071), Bronx (36005)
Key Biscayne, FL	829725	Miami-Dade (12086)	Broward (12011), Monroe (12087), Collier (12021)
Bangor, ME	40000	Penobscot (23019)	Washington (23029), Waldo (23027), Piscataquis (23021), Hancock (23009), Aroostook (23003), Somerset (23025)
Southwest, Orange County, FL	50000	Lake (12069), Orange (12095)	Volusia (12127), Sumter (12119), Seminole (12117), Polk (12105), Osceola (12097), Marion (12083), Brevard (12009)
Indio, CA	91765	Riverside (06065)	San Diego (06073), San Bernardino (06071), La Paz (04012), Orange (06059), Imperial (06025)
Central Salt Lake Valley, UT	6.00E+05	Salt Lake (49035)	Davis (49011), Morgan (49029), Summit (49043), Utah (49049), Wasatch (49051), Tooele (49045)
Marshalltown, IA	27400	Marshall (19127)	Poweshiek (19157), Story (19169), Grundy (19075), Hardin (19083), Jasper (19099), Tama (19171)
St. Cloud, MN	120000	Stearns (27145)	Todd (27153), Pope (27121), Kandiyohi (27067), Wright (27171), Meeker (27093), Benton (27009), Sherburne (27141), Morrison (27097), Douglas (27041)
Northeast, Saint Petersburg, FL	89847	Pinellas (12103)	Pasco (12101), Hillsborough (12057)
Clinton, IA	29300	Clinton (19045)	Cedar (19031), Rock Island (17161), Jackson (19097), Jones (19105), Scott (19163), Whiteside (17195), Carroll (17015)

Harrisburg, PA	125000	Dauphin (42043)	Perry (42099), Schuylkill (42107), Northumberland (42097), York (42133), Juniata (42067), Cumberland (42041), Lebanon (42075), Lancaster (42071)
Wilson, NC	50000	Wilson (37195)	Johnston (37101), Greene (37079), Pitt (37147), Edgecombe (37065), Wayne (37191), Nash (37127)
Gainesville, TX	17300	Cooke (48097)	Grayson (48181), Love (40085), Wise (48497), Montague (48337), Denton (48121)
South River, Atlanta, GA	105160	DeKalb (13089), Clayton (13063), Fulton (13121)	Carroll (13045), Fayette (13113), Coweta (13077), Henry (13151), Douglas (13097), Gwinnett (13135), Rockdale (13247), Cobb (13067), Cherokee (13057), Spalding (13255), Forsyth (13117)
P20, Kansas City, KS	35000	Wyandotte (20209)	Platte (29165), Johnson (20091), Jackson (29095), Clay (29047), Leavenworth (20103)

Codes beside county names represent FIPS codes

Additional details related to EMMI guidelines. The retrospective samples had an average (standard deviation) droplets across the 6 replicate wells of 96,265 (20,913). The volume per droplet, as reported by the manufacturer, is 0.00085 µL. The average (standard deviation) copies per droplet of MeV was 4.5×10^{-5} (2.6×10^{-4}). For the prospective sampling, the average (standard deviation) droplets was 188,187 (17,139). The average (standard deviation) copies per droplet was 1.1×10^{-6} (7.3×10^{-6}).

For an example fluorescence plot, see Boehm *et al*.[1]

Fig S3 includes the EMMI checklist.

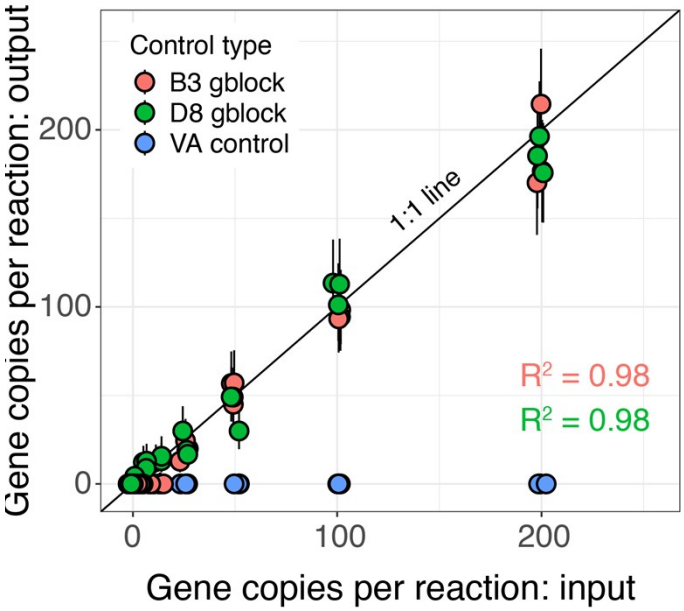

Figure S1. Sensitivity testing using the wild-type specific measles assay. Expected concentrations based on preparation of control material and measured concentrations with and without the RT step for digital PCR. Error bars represent the standard deviation. If error bars cannot be seen, they are smaller than the symbols. VA represents the vaccine sequence.

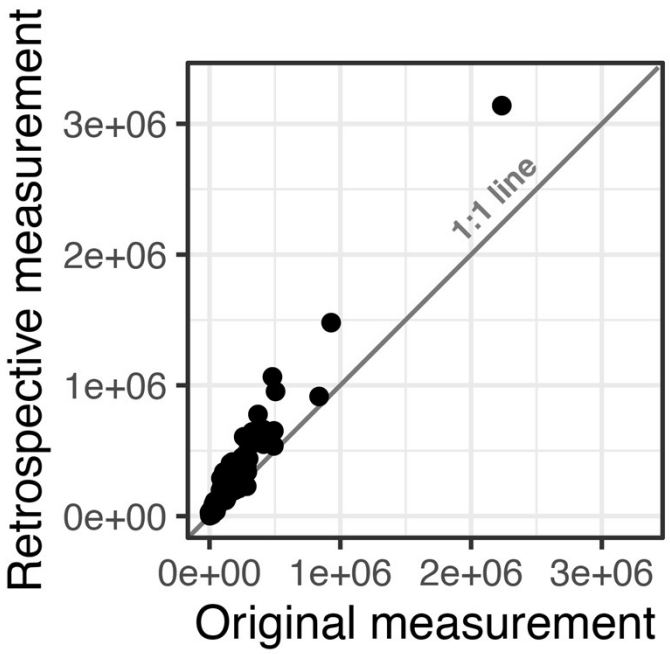

Figure S2. SARS-CoV-2 N gene measurements taken immediately after sample collection and at the time of retrospective testing for pilot samples.

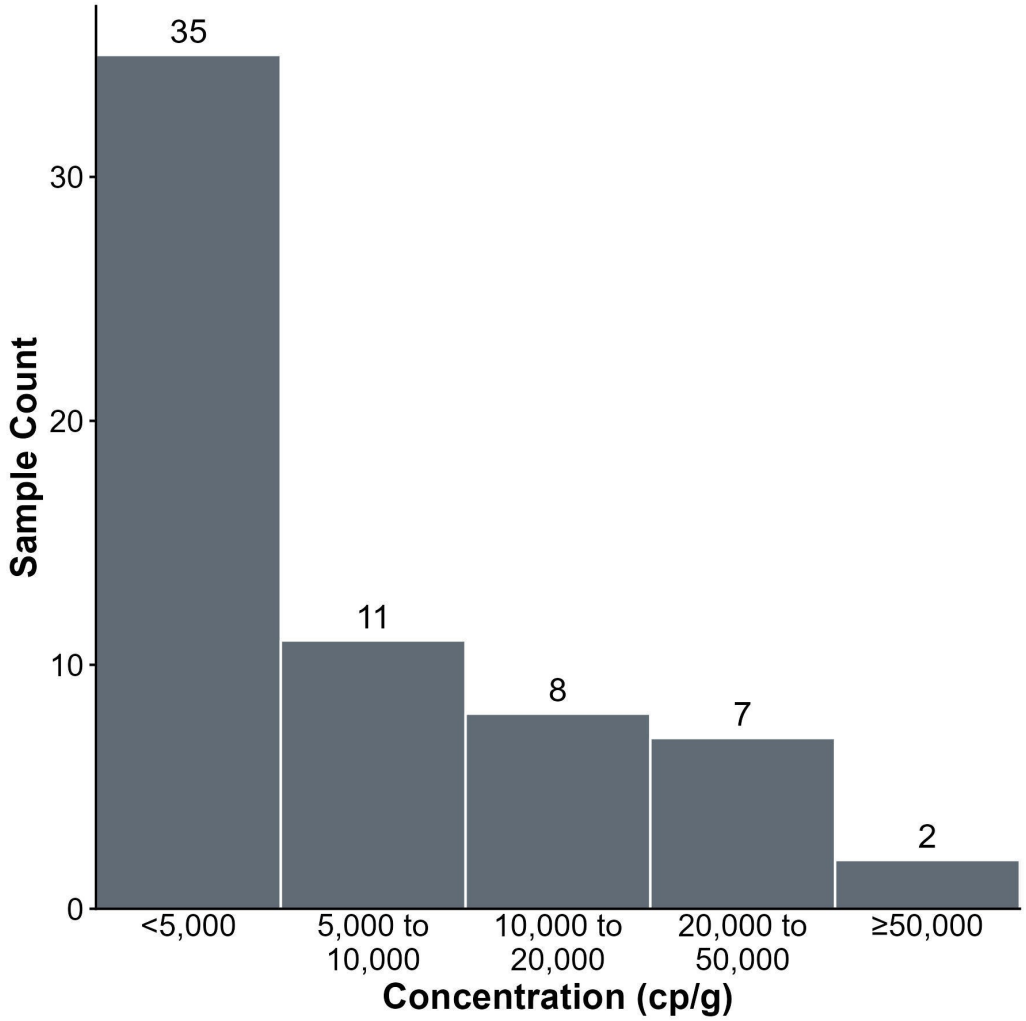

Figure S3. Histogram of measured concentrations of measles virus over the course of the study. The number above the column is the count of samples (out of 11,598) falling within the designated concentration range. Samples not included are those less than the lower detection limit (approximately 1,000 copies per g dry weight (cp/g)).

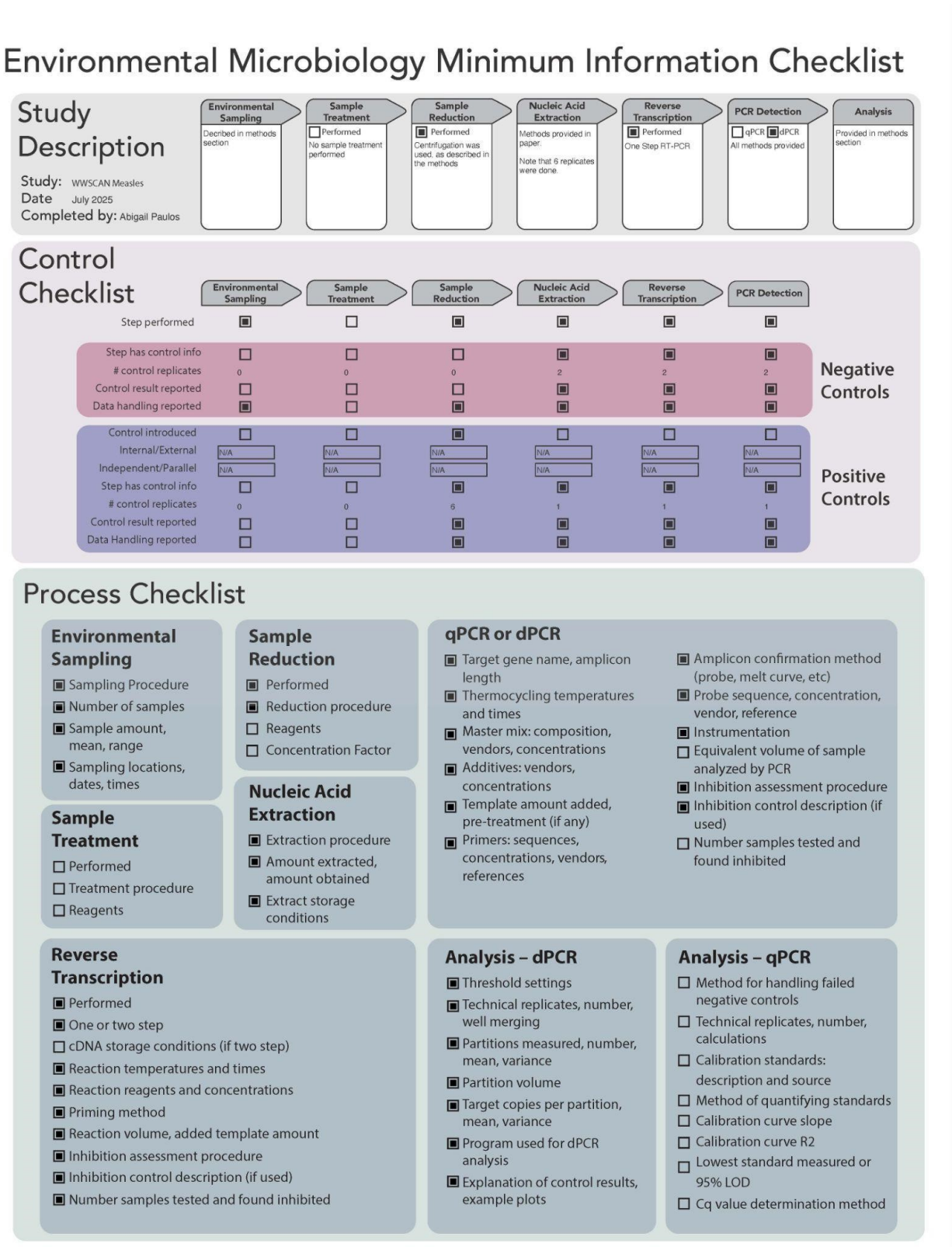

Figure S4. EMMI Checklist for study.

Adjacent county analysis.
In addition to the primary analysis using cases reported in the WWTP counties, we also identified counties adjacent to each WWTP's sewershed, which was defined as any county within 1000 m of a sewershed boundary not directly served by the WWTP. 153 cases were reported in counties served by or adjacent to WWSCAN plants with a measles detection, and 67 cases in counties served by or adjacent to WWSCAN plants without a measles detection. 26 (41%) of detections were within 7 days of a case in the same or adjacent county and 49 (78%) within 30 days. Wastewater detections were associated with clinical cases in the WWTP and adjacent counties, although with lower odds ratios than when considering cases in the WWTP county alone (Table S3).

Table S3. Odds ratios of clinical cases in the WWTP county or adjacent counties.

county	Time period	Odds ratio	5% lower bound	95% upper bound	p	Adjusted p value
WWTP county	Following 7 days	13.77	7.37	24.32	4.30E-18	4.30E-17
WWTP county	Within 7 days	14.71	8.60	24.57	5.60E-24	5.60E-23
WWTP county	Following 30 days	8.91	5.29	14.75	4.10E-17	4.10E-16
WWTP county	Within 30 days	15.95	9.57	27.30	2.20E-25	2.20E-24
WWTP county	Number of cases in surrounding 30 days	2.00	1.76	2.27	1.00E-27	1.00E-26
WWTP and adjacent counties	Following 7 days	7.10	3.99	12.13	3.60E-12	3.60E-11
WWTP and adjacent counties	Within 7 days	7.85	4.69	12.96	1.50E-15	1.50E-14
WWTP and adjacent counties	Following 30 days	5.05	3.05	8.30	1.90E-10	1.90E-09
WWTP and adjacent counties	Within 30 days	13.41	7.60	25.27	1.30E-17	1.30E-16
WWTP and adjacent counties	Number of cases in surrounding 30 days	1.02	0.99	1.03	0.15	1

References

1. Boehm AB, Wolfe MK, Bidwell AL, Zulli A, White BJ, Shelden B, et al. Pathogen nucleic acids data in wastewater solids from 147 treatment plants in the United States: 2024–2025. Data Brief. 2026 Apr 1;65:112503. doi:10.1016/j.dib.2026.112503