

Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for wastewater surveillance

Warish Ahmed ^a, Stuart L. Simpson ^b, Paul M. Bertsch ^a, Kyle Bibby ^c, Aaron Bivins ^c, Linda L. Blackall ^d, Sílvia Bofill-Mas ^e, Albert Bosch ^f, João Brandão ^g, Phil M. Choi ^{h,i}, Mark Ciesielski ^j, Erica Donner ^k, Nishita D'Souza ^l, Andreas H. Farnleitner ^{m,n}, Daniel Gerrity ^o, Raul Gonzalez ^p, John F. Griffith ^q, Pradip Gyawali ^r, Charles N. Haas ^s, Kerry A. Hamilton ^t, Hapuarachchige Chanditha Hapuarachchi ^u, Valerie J. Harwood ^v, Rehnuma Haque ^w, Greg Jackson ^h, Stuart J. Khan ^x, Wesaal Khan ^y, Masaaki Kitajima ^z, Asja Korajkic ^{aa}, Giuseppina La Rosa ^{ab}, Blythe A. Layton ^{ac}, Erin Lipp ^{ad}, Sandra L. McLellan ^{ae}, Brian McMinn ^{aa}, Gertjan Medema ^{af}, Suzanne Metcalfe ^a, Wim G. Meijer ^{ag}, Jochen F. Mueller ⁱ, Heather Murphy ^{ah}, Coleen C. Naughton ^{ai}, Rachel T. Noble ⁱ, Sudhi Payyappat ^{aj}, Susan Petterson ^{ak,al}, Tarja Pitkänen ^{am,an}, Veronica B. Rajal ^{ao}, Brandon Reyneke ^y, Fernando A. Roman Jr ^{ai}, Joan B. Rose ^l, Marta Rusiñol ^{ap}, Michael J. Sadowsky ^{aq}, Laura Sala-Comorera ^{ag}, Yin Xiang Setoh ^u, Samendra P. Sherchan ^{ar}, Kwanrawee Sirikanchana ^{as}, Wendy Smith ^a, Joshua A. Steele ^q, Rosalie Sabburg ^{at}, Erin M. Symonds ^{au}, Phong Thai ⁱ, Kevin V. Thomas ⁱ, Josh Tynan ⁱ, Simon Toze ^a, Janelle Thompson ^{av,aw}, Andy S. Whiteley ^{ax}, Judith Chui Ching Wong ^u, Daisuke Sano ^{ay}, Stefan Wuertz ^{aw,az}, Irene Xagorarakis ^{ba}, Qian Zhang ^{aq}, Amity G. Zimmer-Faust ^q, Orin C. Shanks ^{aa}

^a CSIRO Land and Water, Ecosciences Precinct, Queensland, Australia

^b CSIRO Land and Water, Lucas Heights, New South Wales, Australia

^c Department of Civil & Environmental Engineering & Earth Science, University of Notre Dame, Notre Dame, IN

^d School of BioSciences, The University of Melbourne, Melbourne, Australia

^e Laboratory of Virus Contaminants of Water and Food, Department of Genetics, Microbiology and Statistics, Faculty of Biology, University of Barcelona, Barcelona, Spain

^f Enteric Virus Laboratory, Department of Genetics, Microbiology and Statistics, University of Barcelona, Barcelona, Spain

^g Department of Environmental Health, National Institute of Health Dr. Ricardo Jorge, Lisboa, Portugal

^h Water Unit, Health Protection Branch, Prevention Division, Queensland Health, Queensland, Australia

ⁱ The University of Queensland, Queensland Alliance for Environmental Health Sciences, Queensland, Australia

^j University of North Carolina at Chapel Hill, Institute of Marine Sciences, Morehead City, NC

^k Future Industries Institute, University of South Australia, University Boulevard, Mawson Lakes, Australia

^l Department of Fisheries and Wildlife, Michigan State University, E. Lansing, MI

^m Institute of Chemical, Environmental & Bioscience Engineering, Research Group Environmental Microbiology and Molecular Diagnostic, Technische Universität Wien, Vienna, Austria

ⁿ Research Division Water Quality and Health, Department Pharmacology, Physiology and Microbiology, Karl Landsteiner University of Health Sciences, Krems an der Donau, Austria

^o Southern Nevada Water Authority, Las Vegas, NV

^p Hampton Roads Sanitation District, Virginia Beach, VA

^q Southern California Coastal Water Research Project, Costa Mesa, CA

^r Institute of Environmental Science and Research Ltd (ESR), Porirua, New Zealand

^s Drexel University, Philadelphia, PA

^t School of Sustainable Engineering and the Built Environment and The Biodesign Institute Center for Environmental Health Engineering, Arizona State University, Tempe, AZ

^u Environmental Health Institute, National Environment Agency, Singapore

^v Department of Integrative Biology, University of South Florida, Tampa, FL

^w Environmental Interventions Unit, Dhaka, Bangladesh

- ^x *Water Research Centre, School of Civil and Environmental Engineering, University of New South Wales, Australia*
- ^y *Department of Microbiology, Faculty of Science, Stellenbosch University, Stellenbosch, South Africa*
- ^z *Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, Hokkaido, Japan*
- ^{aa} *United States Environmental Protection Agency, Office of Research and Development, Cincinnati, OH*
- ^{ab} *Department of Environment and Health, Istituto Superiore di Sanità, Rome, Italy*
- ^{ac} *Department of Research & Innovation, Clean Water Services, Hillsboro, OR*
- ^{ad} *Environmental Health Sciences Department, University of Georgia, Athens, GA*
- ^{ae} *School of Freshwater Sciences, University of Wisconsin-Milwaukee, WI*
- ^{af} *KWR Water Research Institute, Nieuwegein, the Netherlands*
- ^{ag} *UCD School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland*
- ^{ah} *Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada*
- ^{ai} *University of California Merced, Department of Civil and Environmental Engineering, Merced, CA*
- ^{aj} *Sydney Water, Parramatta, Australia*
- ^{ak} *Water and Health Pty Ltd., North Sydney, Australia*
- ^{al} *School of Medicine, Griffith University, Parklands Drive, Gold Coast, Australia*
- ^{am} *Finnish Institute for Health and Welfare, Expert Microbiology Unit, Kuopio, Finland*
- ^{an} *University of Helsinki, Faculty of Veterinary Medicine, Department of Food Hygiene and Environmental Health, Finland*
- ^{ao} *Facultad de Ingeniería and Instituto de Investigaciones para la Industria Química (INIQUI) – CONICET and Universidad Nacional de Salta, Salta, Argentina*
- ^{ap} *Institute of Environmental Assessment & Water Research (IDAEA), CSIC, Barcelona, Spain*
- ^{aq} *Biotechnology Institute and Department of Soil, Water, and Climate, University of Minnesota, St. Paul, MN*
- ^{ar} *Department of Environmental Health Sciences, Tulane University, New Orleans, LA*
- ^{as} *Research Laboratory of Biotechnology, Chulabhorn Research Institute, Bangkok, Thailand*
- ^{at} *CSIRO Agriculture and Food, Bioscience Precinct, St Lucia, Australia*
- ^{au} *College of Marine Science, University of South Florida, St. Petersburg, FL*
- ^{av} *Asian School of the Environment, Nanyang Technological University, Singapore*
- ^{aw} *Singapore Centre for Environmental Life Sciences Engineering (SCELSSE), Singapore*
- ^{ax} *CSIRO Land and Water, Floreat, Australia*
- ^{ay} *Department of Civil and Environmental Engineering, Graduate School of Engineering, Tohoku University, Miyagi, Japan*
- ^{az} *School of Civil and Environmental Engineering, Nanyang Technological University, Singapore*
- ^{ba} *Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI*

ABSTRACT

Wastewater surveillance for pathogens using reverse transcription-polymerase chain reaction (RT-PCR) is an effective and resource-efficient tool for gathering community-level public health information, including the incidence of coronavirus disease-19 (COVID-19). Surveillance of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) in wastewater can potentially provide an early warning signal of COVID-19 infections in a community. The capacity of the world's environmental microbiology and virology laboratories for SARS-CoV-2 RNA characterization in wastewater is increasing rapidly. However, there are no standardized protocols or harmonized quality assurance and quality control (QA/QC) procedures for SARS-CoV-2 wastewater surveillance. This paper is a technical review of factors that can cause false-positive and false-negative errors in the surveillance of SARS-CoV-2 RNA in wastewater, culminating in recommended strategies that can be implemented to identify and mitigate some of these errors. Recommendations include stringent QA/QC measures, representative sampling approaches, effective virus concentration and efficient RNA extraction, PCR inhibition assessment, inclusion of sample processing controls, and considerations for RT-PCR assay selection and data interpretation. Clear data interpretation guidelines (e.g., determination of positive and negative samples) are critical, particularly when the incidence of SARS-CoV-2 in wastewater is low. Corrective and confirmatory actions must be in place for inconclusive results or results diverging from current trends (e.g., initial onset or reemergence of

COVID-19 in a community). It is also prudent to perform interlaboratory comparisons to ensure results' reliability and interpretability for prospective and retrospective analyses. The strategies that are recommended in this review aim to improve SARS-CoV-2 characterization and detection for wastewater surveillance applications. A silver lining of the COVID-19 pandemic is that the efficacy of wastewater surveillance continues to be demonstrated during this global crisis. In the future, wastewater should also play an important role in the surveillance of a range of other communicable diseases.

Full text:

https://ftp.sccwrp.org/pub/download/DOCUMENTS/JournalArticles/1225_SARS-CoV-2Surveillance.pdf