

EXHIBIT A – SCOPE OF WORK
Regional COVID-19 Wastewater Surveillance Research

Program Background

The City of Sparks will develop and administer a contract with the University of Nevada Reno to conduct a regional investigation of SARS-CoV-2 presence in wastewater and community prevalence monitoring due to the COVID-19 pandemic.

The purpose of this research is to conduct environmental surveillance of wastewater at the Truckee Meadows Water Reclamation Facility (TMWRF) and in the collection systems by monitoring of SARS-COV2 Marker during the pandemic in the Truckee Meadows region. The monitoring data is used to develop models that can indicate community prevalence of COVID19 in the population. The modeling tools will include the COVID19 epidemiological testing data from local health agencies to develop a predictive ability for incidences of increase or decrease in COVID19. These tools will help decision makers in development of management actions to deal with the pandemic in the region.

Study Administrator

The City of Sparks will negotiate and enter into an agreement with the University of Nevada Reno to carry out a regional investigation of SARS-CoV-2 presence in wastewater and community prevalence monitoring due to the COVID-19 pandemic.

The research team from the Nevada Water Innovation Institute (NWII) at the University of Nevada, Reno has previously developed a comprehensive strategy to monitor wastewater for early outbreak detection and monitoring of COVID-19 disease prevalence. This next phase of the project aims to continue COVID-19 monitoring through wastewater surveillance of SARS-CoV-2 levels with increased granularity and improved methods, focus on variants of concern detection, and developing effective modeling tools to predict trends in community prevalence. Co-occurrence of other pathogens including viruses and bacteria that have implications for COVID-19 will be monitored under the new scope.

Previous efforts concentrated on the Truckee Meadows Water Reclamation Facility (TMWRF) including sewershed monitoring for neighborhood level wastewater surveillance. Influent grab samples were collected from three regional WRFs including TMWRF, South Truckee Meadows WRF (STMWRF) and Reno-Stead WRF (RSWRF). These efforts were funded by the City of Sparks in collaboration with the City of Reno and Washoe County utilizing the CARES Act funds and other fund sources. The future efforts will continue wastewater surveillance at TMWRF and its sewersheds.

Furthermore, expanded methods include composite, flow-proportional sampling of WRF influent and two main interceptors to better estimate the viral loads in the wastewater. The sampling frequency for sewershed monitoring will be increased each quarter for a period of time (to be determined) to improve the predictive models of disease prevalence. UNR will also identify hotspots within the sewershed as civil society returns to normal activities through monitoring and surveillance of wastewater. The preliminary work shows that it is possible that wastewater surveillance can be a useful tool to track variants of concern using RNA sequencing of the viral

fragments recovered from wastewater. Future sampling of wastewater at the WRF and in sewersheds will be tested for variants of concern using RNA sequencing methods and quantitative digital droplet PCR (ddPCR) in addition to the reverse transcriptase quantitative PCR (RT-qPCR). UNR envisions the following tasks for the proposed effort over the 3 years of monitoring and modeling. The cornerstone of all future monitoring and wastewater surveillance will be to coordinate with health authorities and sponsors to disseminate information rapidly for actionable pandemic management for public health protection.

Task 1: Continued monitoring of wastewater at TMWRF (influent and within WRF process streams)

Task 2: Quarterly monitoring of sewershed sub-catchments and hotspots

Task 3: Determining variants of concern through RNA sequencing of positive samples

Task 4: Predictive modeling and monitoring of disease burden for public health implications

Task 1. Continued monitoring of TMWRF wastewater - The monitoring at the influent to the TMWRF will be continued by collecting samples at the combined influent and composite samples of both North and South Interceptors. Samples will be collected daily on Monday through Friday and grab samples for quality control and inter-laboratory comparison. The monitoring will also be expanded to include quarterly testing of WRF effluent and in-process streams. In addition to continued surveillance of SARS-CoV-2, this task will develop standardized methods to quantitatively monitor other pathogens and pathogen indicators in wastewater, including studies of pathogen recovery, efficiency of concentration methods, and the application of method blank and replicates for quality control.

Task 2. Sewershed sub-catchment surveillance – Initially, six sewershed sub-catchments will be selected for neighborhood-level virus surveillance. Testing will be conducted on a quarterly basis, but also be ready to measure more frequently or at short notice based on the pandemic development. This task will focus on surveillance at residential neighborhoods to minimize impacts from transient virus shedders, such as tourists, and workplace-related disease clusters, thereby providing a high granularity estimate of disease distribution across the metropolitan area. In areas where the virus levels are increasing during a sampling campaign such as potential hotspots regions influenced by large gatherings, medical facilities, and schools, intensive sampling and monitoring will be conducted to determine the role of hotspots in overall viral loads to the WRFs and in the sewershed. This aim will also require coordination with City of Sparks Public Works staff and local public health agencies to schedule sampling including field safety protocols.

Task 3. Determining variants of concern through RNA sequencing – Current wastewater surveillance by UNR has demonstrated that variants of the wild SARS-CoV-2 strain were detected in wastewater months before they were identified from clinical monitoring. This task will continue RNA sequencing of wastewater samples on a monthly basis to identify the arrival of new variants in the metropolitan area. This task will include coordination with the Washoe County Health District to develop a framework that proposes how the identification of variants of concern can be utilized for public health outreach and interventions.

Task 4. Development of Models for Prediction and Risk Evaluation - Develop a framework in

coordination with Washoe County District Health to develop a conceptual framework for making wastewater surveillance data actionable through integration into public health planning, reporting, and interventions. This task will analyze the relationship between pathogen surveillance and clinical cases to develop threshold risk levels. Geospatial analysis will be conducted to determine the geographical spread and trends in disease prevalence. The risk level classifications and surveillance data can then be implemented in existing public health monitoring programs such as the COVID Risk Meter.

Project Plan and Methods

Task 1: *Continued monitoring of TMWRF*

Re-new sampling plan and methods improvements for on-going monitoring of wastewater at TMWRF.

- **Activity 1-1.** Expand the ongoing sampling schedule for monitoring of TMWRF. This includes quarterly monitoring of WRF effluent as well as daily (M-F) sampling of WRF influents. Develop a sampling plan that identifies sampling protocols, sampling locations (including two composite sampling locations of interceptors), and quality assurance measures. We anticipate minimal changes to sampling campaigns, but improved methods will be used based on previous monitoring experience.
- **Activity 1-2.** This activity will conduct a literature review to establish state-of-the-art test methods in terms of gene markers used for virus monitoring, and other resources required to incorporate monitoring of other pathogenic viruses that are shed into wastewater. Microorganisms or indicators of interest for wastewater surveillance include pathogens and indicator organisms such as SARS-CoV-2, enterovirus, norovirus, adenovirus, influenza, pepper mild mottle virus (PMMoV), male-specific bacteriophage (MS2), *E. coli*, *Legionella*, and *Cryptosporidium*.
- **Activity 1-3.** Pathogen recovery and concentration methods will be optimized based on different sources of wastewater. These methods will be used in wastewater surveillance. Improved methods are being developed to enhance accuracy, precision, and quality of virus data collection during the pandemic and latest methods will identified and used.
- **Activity 1-4.** The laboratory will set up the molecular assay methods for each pathogen mentioned above, including evaluation of multiple gene targets in different pathogens, RT-qPCR conditions and RT-ddPCR conditions.
- **Activity 1-6.** Data analysis will be conducted, at a minimum, on a weekly basis to evaluate trends in SARS-CoV-2 and other monitored pathogens over time across the TMWRF sewershed area. Sources of data uncertainty analysis of variability will also be assessed.

Task 1 Deliverables: WRF Surveillance Results, QA/QC Procedures and Data, and Weekly Monitoring Update Report of WRF SARS-CoV-2 Surveillance.

Task 2: *Sewershed sub-catchment surveillance*

- **Activity 2-1.** Identify sampling locations for community aggregated flows for neighborhood sites representative of at least 1,000 persons. A total of six sites will be included, with a minimum of three sites in the TMWRF North and South sewersheds. Geospatial analysis and coordination with City of Sparks will be utilized based on previous efforts to locate the best sampling locations. Increased incidence of COVID-19

will warrant additional sampling and locations in hotspots to address emerging trends and is included in this scope.

- **Activity 2-2.** Evaluate the deployment of autosamplers at candidate sampling sites for manual and remote sampling including water quality data monitoring.
- **Activity 2-3.** Conduct a preliminary evaluation of flow variability at sampling sites including measurement of diurnal variability in flow rates and water chemistry. These characteristics will be used to develop statistical sampling protocols.
- **Activity 2-4.** Quarterly surveillance of sewershed sub-catchments using composite sampling techniques. Additionally, sampling will be conducted at selected sites to capture hotspots or emerging trends in disease.
- **Activity 2-5.** Data will be analyzed and reported to identify trends over time and across the metropolitan area, and to evaluate sources of uncertainty sample variability.

Task 2 Deliverables: Sampling and Testing Plan for Sub-Catchments, QA/QC Data, and Quarterly Monitoring Update Report of Sub-Catchment Surveillance of SARS-CoV-2.

Task 3: Determining variants of concern through RNA sequencing

Sewershed and TMWRF monitoring activities will be targeted for surveillance of microbiological pathogens and potential pathogenic indicator organisms.

- **Activity 3-1.** Pool extracted RNA samples for sequencing on a quarterly basis.
- **Activity 3-2.** Based on sequencing results quantitatively monitor for SARS-CoV-2 variants on a weekly basis using RT-ddPCR.
- **Activity 3-3.** An ongoing literature review will track the reports of new SARS-CoV-2 variants, changes in risks presented (e.g. hospitalization rates, fatality, shedding rates, ro values, and reported prevalence nationally)
- **Activity 3-4.** Variants of SARS-CoV-2 will be identified in order to evaluate ongoing public health risks, including the frequency at which the variants were identified across the regional sewersheds and the date of first detection.

Task 3 Deliverables: Monthly Summary of SARS-CoV-2 Variants Detected in Wastewater Samples.

Task 4: Predictive modelling and monitoring of disease burden for public health implications

Develop a framework in coordination with regional health and planning agencies in Washoe County to develop a conceptual framework for making wastewater surveillance data actionable through integration into public health planning, reporting, and interventions.

- **Activity 4-1.** Coordinate with Washoe County Health District and other agencies to collect data on hospitalizations, reported infections, and ICU admissions.
- **Activity 4-2.** Utilize geospatial information systems to categorize public health data based on sewershed areas and sewershed sub-catchments.
- **Activity 4-3.** Conduct a statistical analysis of the correlations between detected pathogen concentrations, reported symptomatic and asymptomatic cases, hospitalizations, and ICU admissions.

- **Activity 4-4.** Refine the SARS-CoV-2 predictive model developed from regional empirical observations previously to provide more probabilistic estimations of likely community infections from wastewater monitoring.
- **Activity 4-5.** Develop reproducible, evidence-based thresholds of disease risk categorization from observed wastewater surveillance data.
- **Activity 4-6.** Develop a statistical summary of virus concentrations in wastewater influent and effluent at TMWRF with consideration of diurnal and seasonal variability in order to inform future source control programs.

Task 4 Deliverables: Technical Memorandum on the assessment of pathogen monitoring in wastewater for assessing community disease burden. Weekly updates summarizing likely infections and disease risk levels based on wastewater monitoring.