No 73, Al Mutanabi Street
4th Circle, Amman Jordan
Tel: +962 6 464 2720
Fax: +962 6 464 2721

Ref: 3029-119930/1.2/389
April 18, 2021

Via email: Ahmed_Uleimat@mwi.gov.jo
His Excellency
Eng. Ahmad Uleimat
Acting Secretary General
Water Authority of Jordan
Amman, Jordan

Subject: **USAID Jordan Water Infrastructure**
USAID Contract No. AID- OAA-I-15-00047, Order: 72027818F00002
Task 8-Technical Assistance to Water Utilities
Final Report of the Planning and Capacity Building Related to Covid-19 Monitoring Using Wastewater Surveillance in Amman, Jordan

Dear Your Excellency:

CDM Smith completed the Planning and Capacity Building Related to Covid-19 Monitoring using Wastewater Surveillance in Amman, Jordan under Task 8 to the USAID Jordan Water Infrastructure. The Final Report Text is attached to this letter. The 4 hard copies and 1 CD (includes the Report Text and Appendices) of the Final Report – Capacity Building Related to Covid-19 Monitoring Using Wastewater Surveillance, dated April 2021.

In this regard, we have collaborated with the Water Authority of Jordan (WAJ) Central Laboratory on the wastewater testing program designed to build WAJ capacity for the various components of SARs-CoV-2 testing, from sample collection to concentration to analysis. The activities undertaken in collaboration with WAJ are provided in the report including identification of resources and equipment required to perform the sampling and testing program, and the results of the workshop held on March 10, 2021.

Should you have any questions or comments on the report, please forward them to our attention.

Sincerely,

Richard Minkwitz
Project Manager
CDM International Inc.

Attachment: Final Report– Planning and Capacity Building Related to Covid-19 Monitoring Using Wastewater Surveillance, dated April 2021, 4 hard copies and 1 CD, Report Text and Appendices

cc: H.E. Eng. Mohammad Ershaid, MWI Secretary General, w/Report Text attached, by email
Eng. Akram AlQhaiwi, USAID COR, w/Report Text attached, by email
Eng. Jomanah Al Btoush, USAID Activity Manager, w/Report Text attached, by email
Eng. Mohammad Al Dwairi, Acting Secretary General Assistant for Strategic Planning, w/Report Text attached, by email
Anna Mehrotra, PhD PE, CDM Smith, w/Report Text attached, by email

TASK 8: TECHNICAL ASSISTANCE TO WATER UTILITIES

PLANNING AND CAPACITY BUILDING RELATED TO COVID-19 MONITORING USING WASTEWATER SURVEILLANCE

USAID Jordan Water Infrastructure

Prepared by CDM International
for United States Agency for
International Development under
Global Architect – Engineer Services II IDIQ
USAID Contract No. AID-OAA-I-15-00047
Order: 72027818F00002

The Hashemite Kingdom of Jordan
Ministry of Water and Irrigation
Water Authority of Jordan (WAJ)

This publication was produced for review by the United States Agency for International Development. It was prepared by CDM Smith.

April 2021

Project: USAID Jordan Water Infrastructure

Contract: Global Architect – Engineer Services II IDIQ
USAID Contract No. AID-OAA-I-15-00047
Order: 72027818F00002

Task 8: Technical Assistance to Water Utilities

Planning and Capacity Building Related to COVID-19 Monitoring Using Wastewater Surveillance

Prepared by	Anna Mehrotra, Ayman Halaseh, Bashar Khalil, Rawan Abu Eitah	Date	January 2021
Reviewed by	Anna Mehrotra	Date	January 2021
Approved by	Rick Minkwitz	Date	January 2021
Reviewed by	USAID	Date	February 2021
Revised by	Anna Mehrotra, Rawan Abu Eitah, Ayman Halaseh	Date	April 2021
Approved by	Rick Minkwitz	Date	April 2021

Table of Contents

List of Figures

List of Tables

List of Abbreviations

AGTP	Ain Ghazal Pretreatment Plant
CDM	CDM International, Inc.
COVID-19	coronavirus disease 2019
DOS	Department of Statistics
GIS	Geographic Information Systems
ICM	Integrated Catchment Model
MWI	Ministry of Water and Irrigation
PCR	polymerase chain reaction
QA/QC	quality assurance/quality control
RNA	ribonucleic acid
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
TAT	turn-around time
WAJ	Water Authority of Jordan
WIP	Wastewater Infrastructure Project
WWTP	wastewater treatment plant

Section 1
Introduction

1.1 Background

This activity focused on capacity building related to wastewater surveillance for coronavirus disease 2019 (COVID-19) and was part of Task 8 of the USAID Jordan Water Infrastructure project. The project provides architecture, engineering and construction management services to improve water and wastewater infrastructure facilities in Jordan. These services include assessments, feasibility studies, designs, tender documents, capacity building, operations and maintenance, customer service, and financial support. The project partners include: the Ministry of Water and Irrigation (MWI), the Water Authority of Jordan (WAJ), the Jordan Valley Authority, the Ministry of the Environment, and CDM International, Inc (CDM). Task 8 (Technical Assistance to Water Utilities) is focused on providing support for capacity building and training for Jordan's water sector organizations.

COVID-19 is a devastating infectious disease caused by the recently discovered severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of March 2021, more than 2.7 million deaths have been attributed to COVID-19. Many public health tools have been used to attempt to manage the spread of the disease, with perhaps one of the more novel tools being wastewater surveillance. Wastewater surveillance has been used around the world during the COVID-19 pandemic (Ahmed *et al.* 2020; Miyani *et al*. 2020; Randazzo *et al.* 2020) to:

- Confirm trends in clinical data
- Provide early warning for new outbreaks
- Identify hotspots
- Estimate disease prevalence
- Evaluate presence of variants
- Confirm absence of disease

As of March 2021, at least 50 countries and 1,570 sites are monitoring SARS-CoV-2 in wastewater.[1] Wastewater surveillance programs have been implemented by a wide range of institution types, from city, local, or national governments to colleges and universities to private entities.

[1] See dashboard available at: https://ucmerced.maps.arcgis.com/apps/opsdashboard/index.html#/c778145ea5bb4daeb58d31afee389082

The goal of this activity, conducted between August 2020 and March 2021, was to develop the capacity for COVID-19 wastewater surveillance in Jordan to be able to use this tool for mitigating the impact of COVID-19. Because many other human viral pathogens can be measured in wastewater, development of wastewater surveillance capacity in Jordan will also be helpful for management of outbreaks of diseases – both known and unknown – other than COVID-19.

1.2 Purpose

The activity consisted of three tasks, each with a specific objective, as follows:

1. Task 1: Develop a wastewater monitoring plan for COVID-19 in the Ain Ghazal sewer service area in Amman, including actions to take when an increasing trend in the wastewater SARS-CoV-2 is observed.
2. Task 2: Build MWI/WAJ capacity for sensitive SARS-CoV-2 testing in wastewater, including all aspects of sample collection, preparation and analysis.
3. Task 3: Document step-by-step approach for implementing wastewater surveillance for COVID-19 throughout Jordan, using lessons learned from the Ain Ghazal surveillance pilot.

1.3 Approach

The key stakeholder in this activity was the WAJ Laboratories and Quality Directorate (WAJ Lab), part of the MWI. WAJ's Wastewater Monitoring and Environmental Surveillance Department had initiated wastewater sampling for COVID-19 in June 2020. They did not, however, have a specific monitoring plan for widespread implementation of wastewater surveillance for COVID-19, nor a sufficiently high throughput capacity for processing wastewater samples for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) analysis. Note that the CDM International, Inc. (CDM) team, at the request of USAID, contacted the Ministry of Health's Environmental Health Department (EHD) and offered to have them participate in this capacity-building activity. EHD has a water quality testing laboratory and is responsible for monitoring domestic water quality. EHD declined to participate in the project due to the fact that wastewater testing is not within their mandate and they are therefore not equipped or staffed to do such testing.

This activity was conducted at the WAJ Lab in collaboration with the Wastewater Monitoring and Environmental Surveillance Department, as well as the Microbiology Department. The activity also involved field work performed in collaboration with the Water Company Jordan Miyahuna (Miyahuna), the Greater Amman Municipality, and the Amman Traffic Department.

Because the focus of this activity was on capacity building, the following was performed:

1. General assessment of WAJ Lab capacity related to:
    a. Health and safety practices
    b. Wastewater sampling process
    c. Wastewater sample preparation and testing steps
    d. Quality assurance/quality control (QA/QC) practices

    e. Lab resources

2. Health and safety training for all WAJ Lab staff

3. Practical and hands-on training for:

    a. Wastewater sampling and handling provided for the Wastewater Monitoring and Environmental Surveillance Department engineers in charge of wastewater sampling.

    b. SARS-CoV-2 testing at the WAJ Lab for the lab technologists including wastewater handling, pasteurization, virus concentration, viral RNA extraction, PCR testing and analysis. Note that five lab technologists were trained, including three with biological sciences background, one with a biotechnology background, and one with a biotechnology and environmental engineering background.

The capacity building activity utilized the Ain Ghazal Pretreatment Plant (AGTP) sewershed as a pilot area. The AGTP currently serves more than 2 million people in Amman in the following districts: Basman; part of An-Nasir; Al-Madeena; Al-Yamouk; parts of Al-Qweismah; Abu Alanda; and Al Jwaydah; Al-Abdali, part of Al-Jubeiha; Ras Al Ein; Badir; Zahran; and part of Sweileh.

1.4 Organization of Report

Each section of the report is focused on one of the activity tasks:

- Section 2: Wastewater Monitoring Plan
- Section 3: Testing Capacity Building
- Section 4: Roadmap for Expanding Beyond Ain Ghazal

Important supporting information is included in the report appendices.

Section 2

Wastewater Monitoring Plan

2.1 Task Objective

The goal of Task 2 was to develop a targeted plan for ongoing monitoring of SARS-CoV-2 in wastewater within the AGTP sewershed, including actions to be implemented once SARS-CoV-2 is detected at one of the ongoing monitoring locations.

AGTP serves around 2 million Amman citizens as of 2020. The served areas include the districts of: Basman; part of An-Nasir; Al-Madeena; Al-Yamouk; parts of Al-Qweismah; Abu Alanda; and Al Jwaydah; Al-Abdali, part of Al-Jubeiha; Ras Al Ein; Badir; Zahran; part of Sweileh. The wastewater flows generated from these areas are collected and finally conveyed to AGTP through three trunk sewers:

1) 1200 mm serving the east/northern part of the sewershed

2) 1200 mm pipe serving the east/southern part of the sewershed

3) 1200 mm to 1500 mm serving the western part of the sewershed

Figure 1 shows the served districts within AGTP sewershed. The population within each district was estimated for 2020 using the population analysis presented in Section 2.2.1.2. The served population is labeled under the district name label. For example, there are around 32,322 people within the Marj Al Hamam district served by AGTP as of 2020.

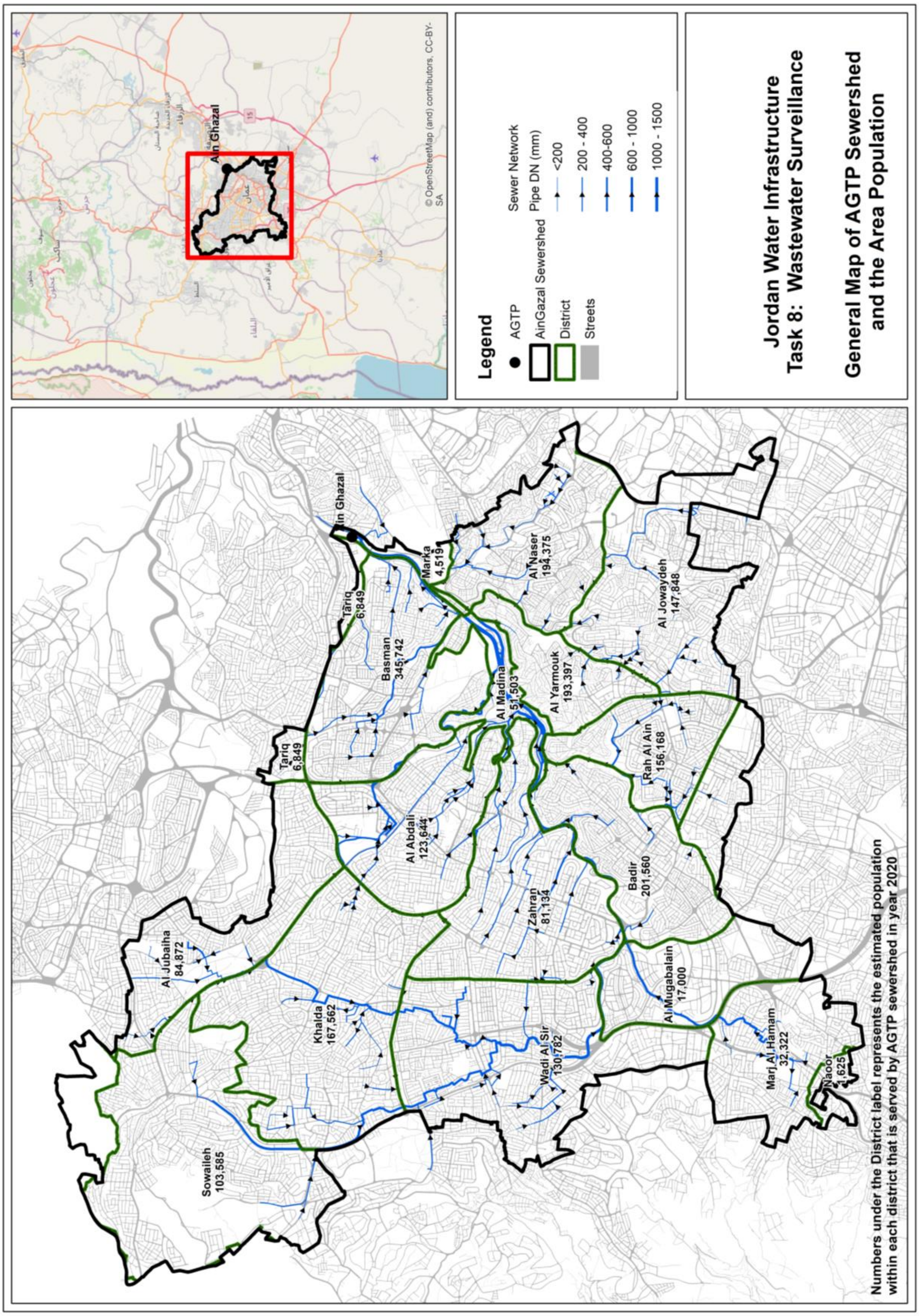


Figure 1 General Map for AGTP Sewershed and 2020 Estimated Population for the Districts Served

2.2 Approach

Figure 2 shows a workflow for developing a wastewater surveillance plan. The following sections discuss each step.

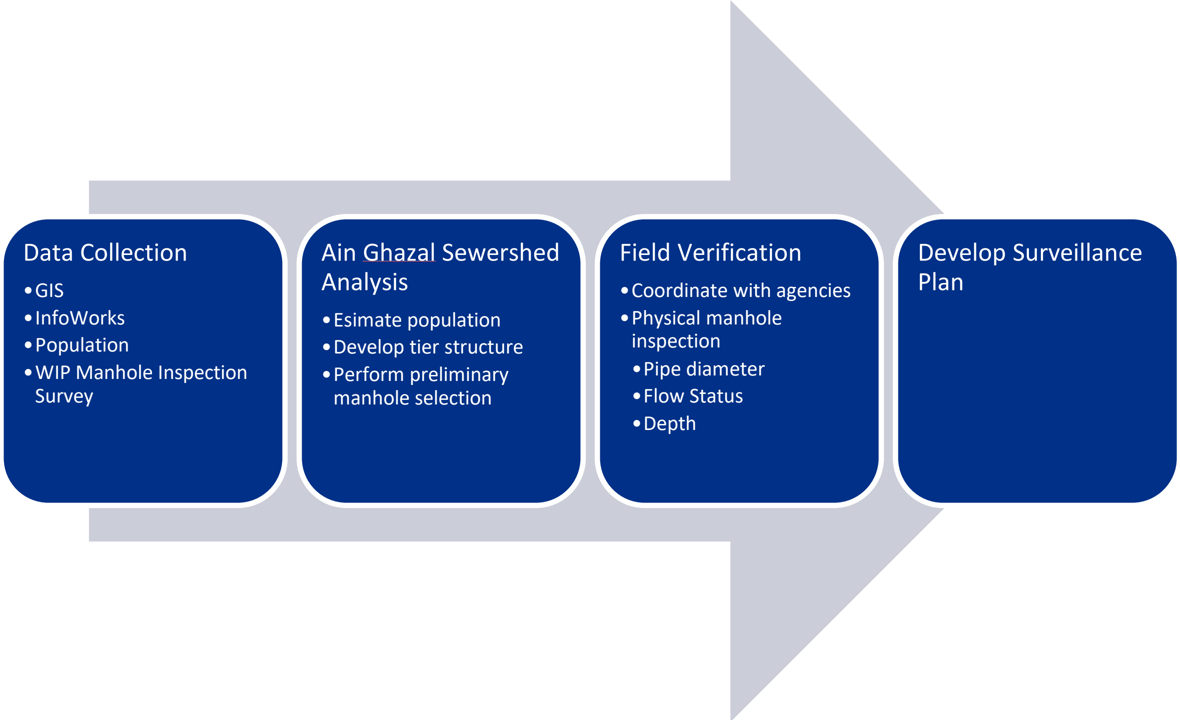


Figure 2: Workflow for Developing AGTP Wastewater Surveillance Plan

2.2.1 Data Collection

Most data for this analysis were obtained from USAID Water/Wastewater Infrastructure Project (WIP), in addition to the updated GIS Geodatabase that were obtained from WAJ. Principle data sources include:

2.2.1.1 Sewer Hydraulic Model

An InfoWorks Integrated Catchment Modeling (ICM) model for the AGTP collection system was developed between 2010 and 2012 under the WIP. The model is made up of nodes, links and polygons. Nodes represent manholes, outfalls and storage facilities. Links simulate pipes while polygons represent drainage sub catchments where wastewater is generated.

In general, collection system hydraulic models integrate and cross-check different data sources including record drawings, flow metering data, survey data, field verification data and the physical representation of the system. Therefore, well-developed collection system hydraulic models have the most representative system hydraulic connectivity and wastewater flows generated in different parts of the sewershed.

The AGTP InfoWorks ICM model was instrumental for dividing AGTP sewershed into sub-sewersheds (i.e., different "tiers"), since it contains information on the population and area of each sub-sewershed, as well as how the sub-sewershed is hydraulically connected to the rest of the sewer network. **Figure 3** shows a screen shot of the InfoWorks hydraulic model, with the AGTP network highlighted in red.

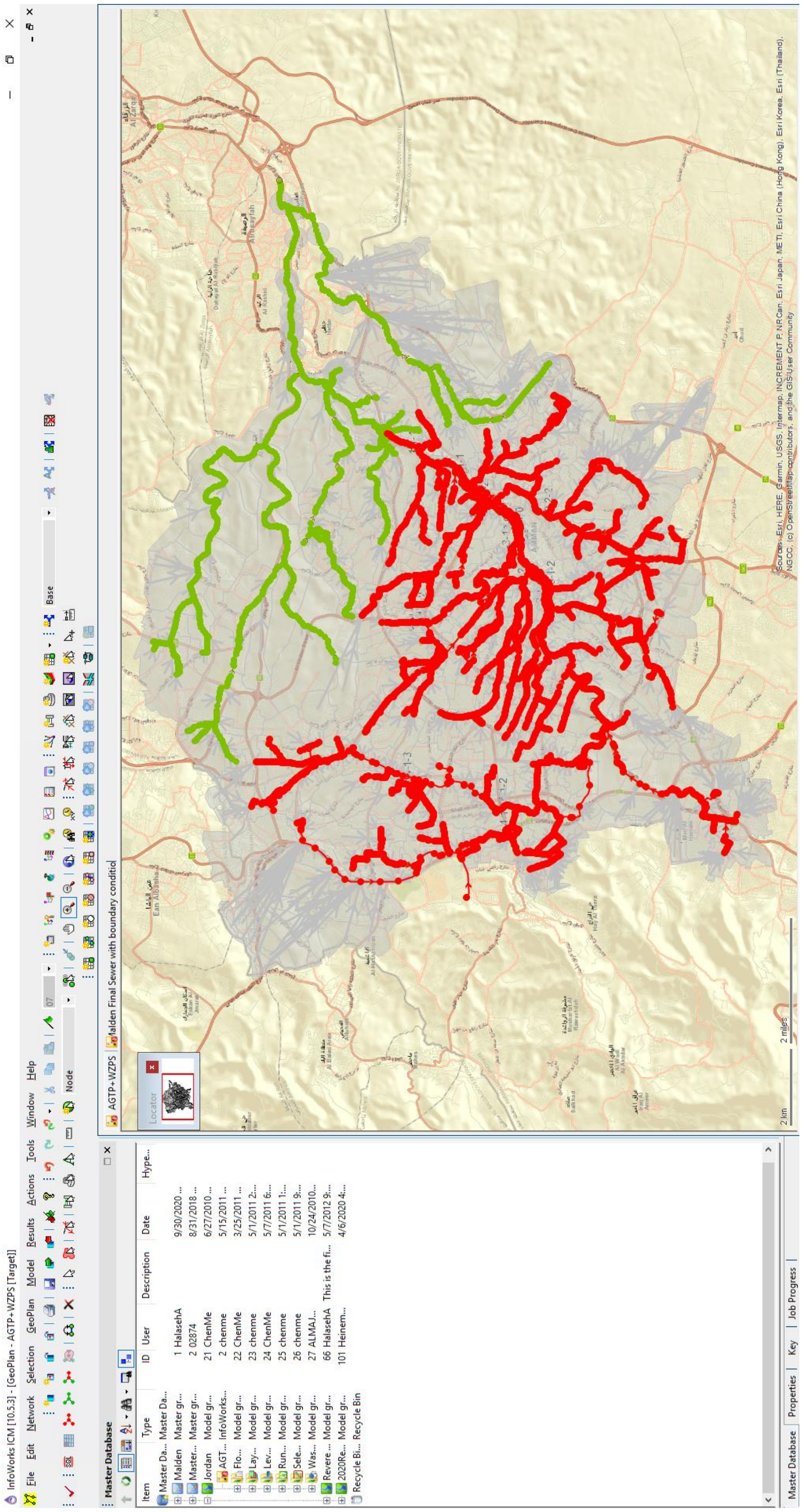


Figure 3: Screenshot of InfoWorks Collection System Hydraulic Model

2.2.1.2 Population Data

Population forecast data were obtained from a previous project (CDM International Inc., 2010). Under WIP, population forecasts were prepared using the 2010 national census results for the Department of Statistics (DOS) for 5,500 census blocks within Amman Governorates. Population data was projected using three different growth rates based on each neighborhood density. **Table 1** shows the estimated population for the entire AGTP sewershed area. **Figure 4** shows the projected population density within the AGTP sewershed in 2020.

Table 1: Population Projections for AGTP Sewershed

Year	AGTP Sewershed Population
2020	1,886,403
2025	2,106,761
2030	2,339,056
2035	2,436,849
2040	2,507,209
2045	2,583,658
2050	2,646,923

2.2.1.3 WIP Manhole Inspection Data

In 2013, under USAID Water/Wastewater Project (WIP), 3,561 manholes were inspected within the Amman and Zarqa wastewater network; of these, 2,706 manholes are within the AGTP sewershed. During the manhole inspection, the inflow and outflow pipe diameters and pipe invert depth were recorded, and a picture was taken. This information was important for identifying suitable wastewater monitoring locations for the current project. **Figure 5** shows a sample of the location of the surveyed manholes. The complete WIP manhole inspection data is available upon request.

2.2.1.4 Amman Wastewater Geographic Information Systems Data

Updated sewer network information in geographic information systems (GIS) format was provided by WAJ by the end of 2020. These GIS files contained maps of sewer pipes ranging from 150 mm to 1500 mm, as well as manhole locations.

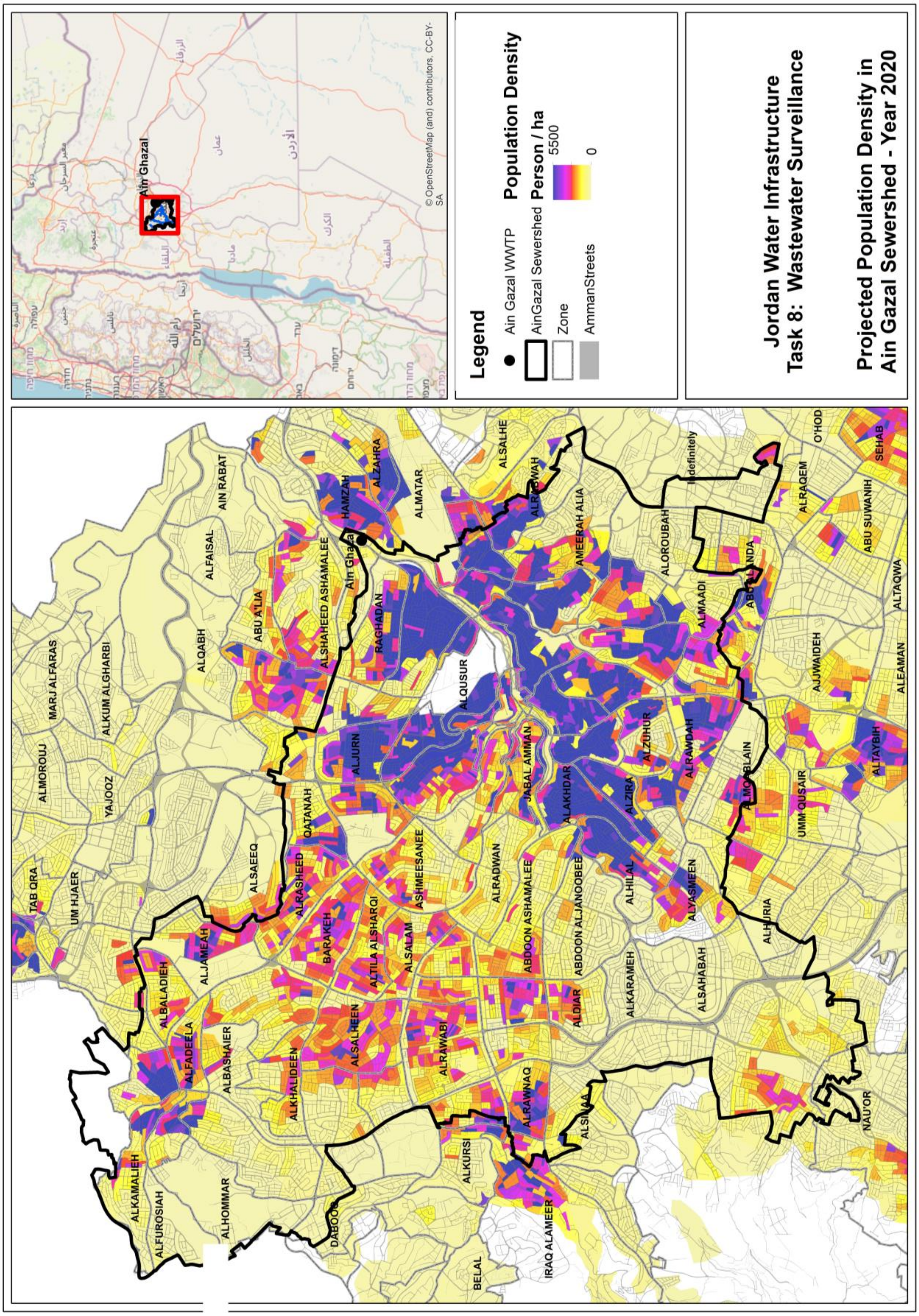


Figure 4: Projected Population Density in AGTP Sewershed

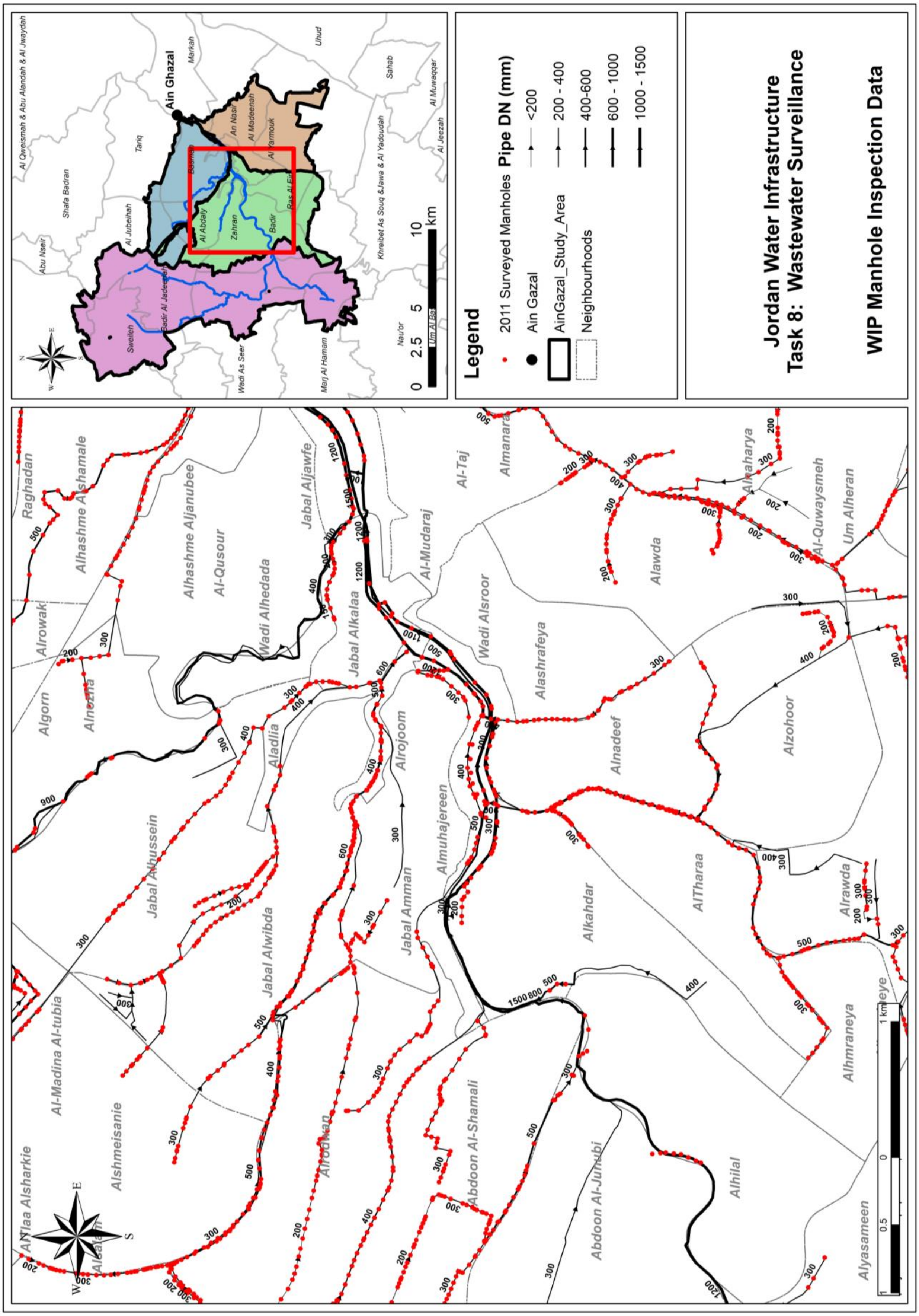


Figure 5: WIP Manhole Inspection Data

2.2.2 AGTP Sewershed Analysis

The objective of this task was to select manholes to divide AGTP sewershed into sub-sewersheds for different monitoring tiers. These manholes will be used as sampling locations for the Ain Ghazal wastewater monitoring plan.

2.2.2.1 Population Estimates

AGTP InfoWorks ICM model was used to quantify the population that each pipe and manhole of the AGTP sewer network serves. **Figure 7** shows the sewer network color coded by the population that each pipeline serves. For example, the orange thick line represents a sewer pipeline that serves 250,000 people. The snapshot on the figure shows an example of the model output and the population that each pipe serves.

2.2.2.2 Sub-Sewershed Dividing

The analysis in the previous section was used to divide the sewershed into sub-sewersheds of four tiers. Each tier has a different resolution of population precision ranging from approximately 5,000 to 500,000 people. Tier 1 divided Ain Gazal sewershed into four sub-sewersheds with population around 500,000 people each. Tier 2 divided each of the Tier 1 sub-sewersheds into two smaller sub-sewersheds with population around 250,000 people. A similar approach was used to divide Tier 2 sub-sewersheds into Tier 3 and then Tier 4 sub-sewersheds as shown in **Figure 6.**

For Ain Ghazal a total of four sampling tiers were identified: four Tier-1 sub-sewersheds, eight Tier-2 sub-sewersheds, 17 Tier-3 sub-sewersheds, and 82 Tier-4 sub-sewersheds. **Figure 8** provides an illustration of how the four tiers are related and the population within each one, and drills down into one set of Tier 4 sets. We identified the downstream manhole for each sub-sewershed to be a potential sampling location in the proposed wastewater monitoring plan. **Appendix A** includes the information for the selected manholes and site maps for each sub-sewershed within the four tiers.

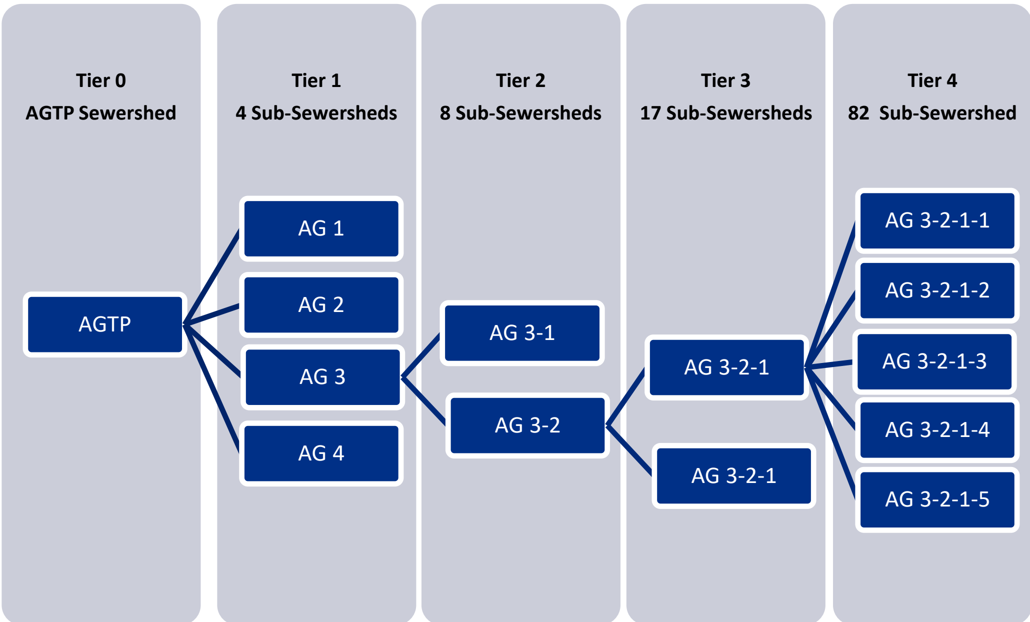


Figure 6: AGTP Sub-Sewershed Structure

For illustration purposes only. Not all sub-sewersheds are shown.

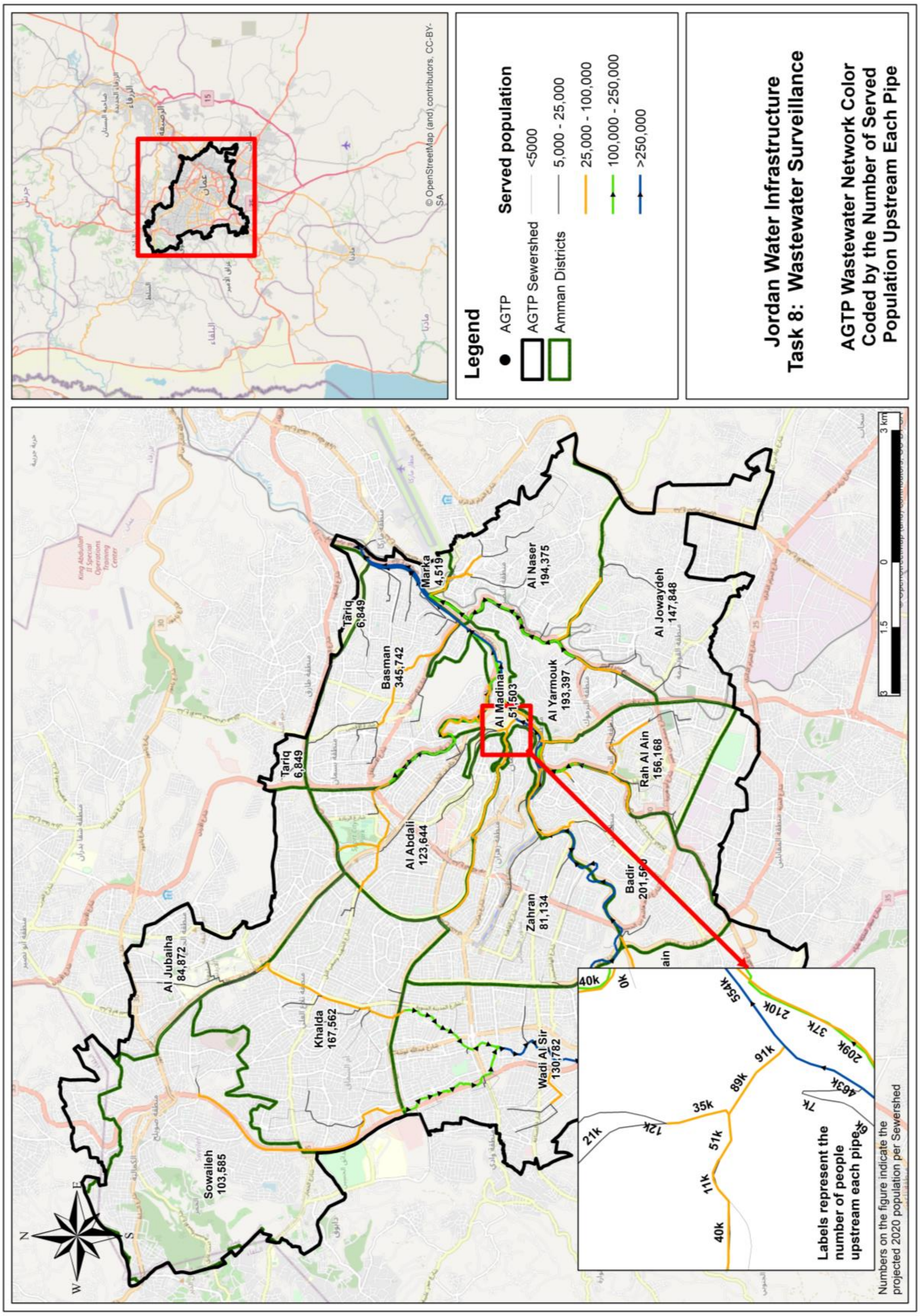


Figure 7: AGTP Wastewater Network Color Coded by the Number of Served Population for Each Pipe

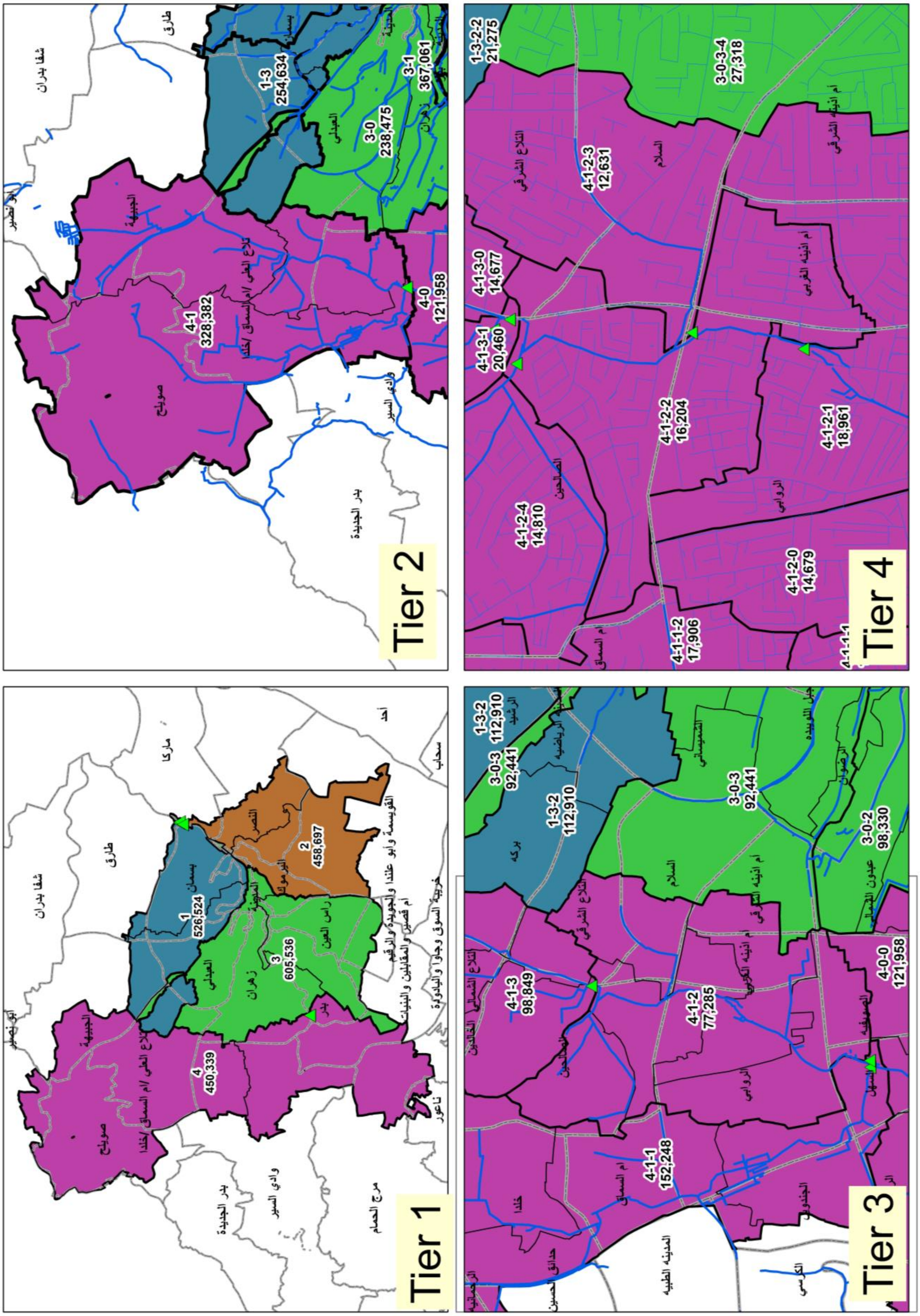


Figure 8: Example of Four Tier Division

2.2.3 Manhole Inspection

Our team, in coordination with WAJ and Miyahuna, visited and opened all selected manholes of the first three tiers (30 manholes total) in November and December 2020 to confirm their locations and hydraulic connectivity. Photos of the manhole verification are provided in Photo Group I. Critical information was captured about inspected manholes such as manhole depth, pipe diameter, flow conditions, site accessibility, cover type and safety concerns. **Appendix A** summarizes the manhole inspection outcome.

WIP manhole inspection data was utilized to select Tier 4 manholes. Therefore, it was found that manhole inspections for Tier 4 manholes was not necessary.

Photo Group I: Field Manhole Verification

2.2.4 Training

CDM Smith conducted a training for the Director of Planning and Evaluation Directorate, Mr. Refaat Bani Khalaf, by illustrating the process of dividing the sewershed into sub-sewersheds using GIS. This included a review of the current data that MWI has, as well as the population estimates.

2.3 Wastewater Monitoring Plan

A proposed wastewater monitoring (or surveillance) plan for the Ain Ghazal area is presented in **Table 2**. This monitoring plan-which applies to Ain Ghazal only—consists of four modes:

- *Maintenance Mode*, to be used when the average number of new cases reported on a daily basis does not exceed one case in a million on average throughout Jordan. At this case rate, the pandemic is under control and the goal of the wastewater monitoring is to anticipate any increase in cases in Ain Ghazal.
- *Baseline Mode*, to be used when the average number of new cases reported on a daily basis is greater than one per million on average throughout Jordan. The goal of this mode is to anticipate increases in cases in Ain Ghazal and identify which quadrant of Ain Ghazal (i.e., which Tier 1 location) is experiencing increasing cases. Baseline Mode is also designed to confirm decreasing trends in disease in the Ain Ghazal sewershed.
- *Increasing Mode*, to be used once the SARS-CoV-2 is detected at a Tier 1 location. In this case, all Tier 3 manholes corresponding to the Tier 1 location at which an increasing trend in wastewater SARS-CoV-2 was observed would be tested.
- *Targeted Mode*, which is an optional mode available if more granular information is desired beyond Tier 3 (100,000 people per sample location): Tier 4 sites correspond to populations of about 25,000 people; Tier 0 and Tier 1 locations should always be sampled unless in *Maintenance Mode*.

Appendix A contains a fact sheet for each manhole to help with easy execution of the monitoring plan. Each fact sheet contains the information shown in **Figure 9**. The manhole sheets are organized in packages for each tier group. For example, all Tier 3 manholes are packaged under the relevant Tier 2 manholes.

Section 4 discusses further application of wastewater surveillance beyond Ain Ghazal.

Table 2: Summary of Suggested Wastewater Surveillance Strategy for Ain Ghazal

Surveillance Component	Surveillance Mode			
	Maintenance	*Baseline*	*Increasing*	*Targeted*
Pandemic Status	Long-term monitoring of entire Ain Ghazal population when there are very low levels of Covid-19 disease reported in Jordan	Long-term monitoring of entire Ain Ghazal population when there is known Covid-19 disease in Jordan	Rapidly identify which areas in Ain Ghazal are the source for new Covid-19 cases by jumping to Tier 3 sites corresponding to any Tier 1 site with an increasing viral signal	This is an optional mode available if more granular information is desired beyond Tier 3 (100,000 people per sample location): Tier 4 sites correspond to populations of about 25,000 people; Tier 0 and Tier 1 locations should always be sampled unless in *Maintenance* mode
Objective	Provide early indication of appearance of disease in Ain Ghazal	Show increases and confirm decreases in Covid-19 cases	Identify hot spots	Further identify hot spots
Number Tiers and of Sampling Locations	Tier 0 (1 location)	Tier 0 (1 location) Tier 1 (4 locations)	Tier 0 (1 location) Tier 1 (4 locations) Tier 3 (4 to 17 locations), corresponding to the Tier 1 location showing an increasing viral trend	Tier 0 (1 location) Tier 1 (4 locations) Tier 4: as needed
Number of Days per Week	Tier 0: 4 →4 samples per week	Tier 0: 4 Tier 1: 2 →12 samples per week	Tier 0: 4 Tier 1: 2 Tier 3: 1 →16 to 28 samples per week	Tier 0: 4 Tier 1: 2 Tier 4: as needed to meet mode objectives
Trigger to Change Mode	Move to *Baseline* when the average number of daily cases reported kingdom-wide in a given week exceeds 1 case per million people	Move to *Increasing* when virus concentration in wastewater at any one Tier 1 site shows two increasing sample results in a row; Move to *Maintenance* once the average number of daily cases reported kingdom-wide in a given week is less than 1 case per million people	Move to *Baseline* when virus concentration in monitored Tier 3 site shows two decreasing sample results in a row	N/A

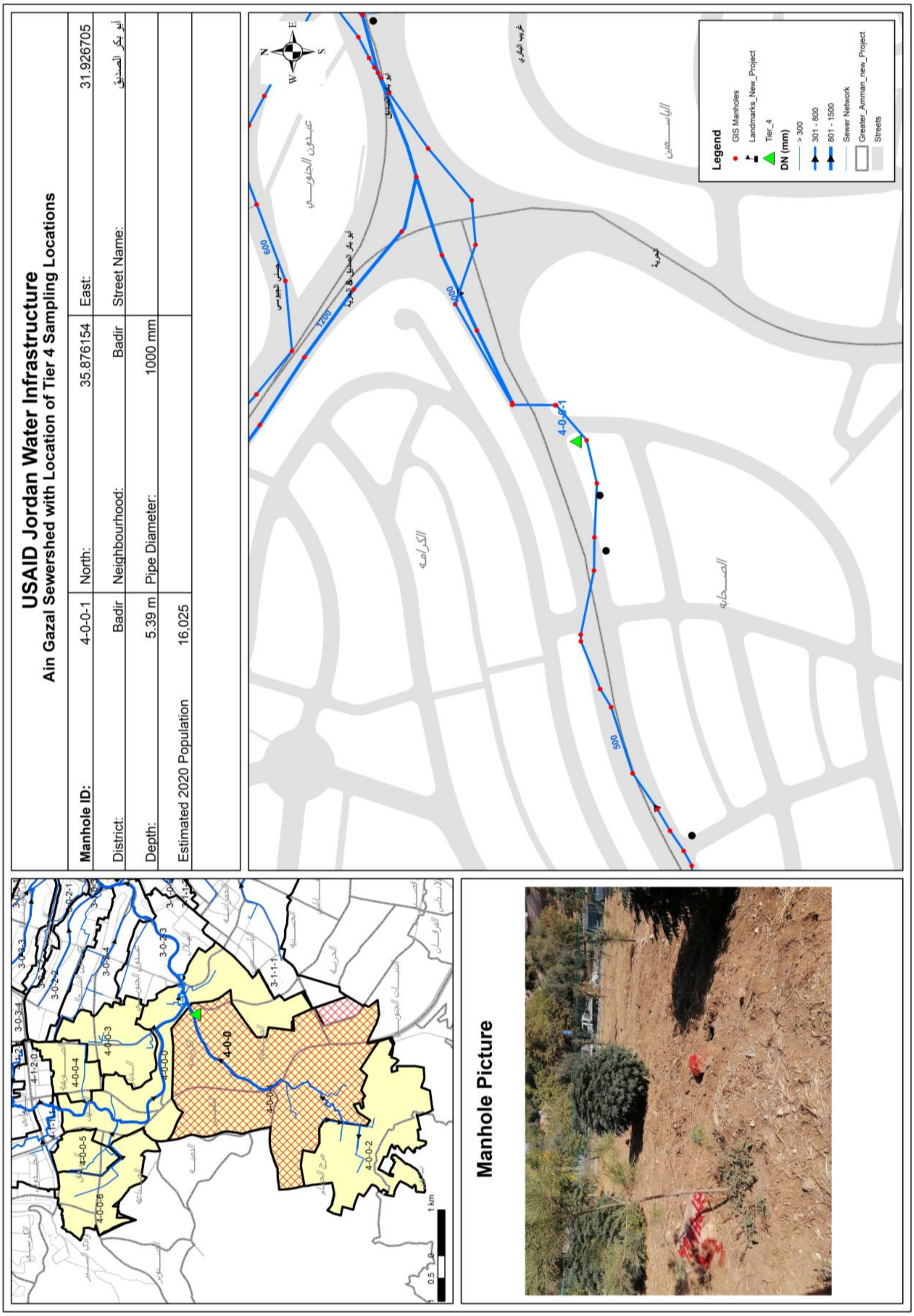


Figure 9: Sample of the Manhole Information Sheets Provided in Appendix A

Section 3

Testing Capacity Building

3.1 Task Objective

The objective of this task 2 was to develop MWI/WAJ laboratory capacity for sensitive SARS-CoV-2 testing in Ain Ghazal wastewater. WAJ capacity for all steps of wastewater testing were considered, including sample collection, sample concentration, RNA extraction and PCR analysis, and reporting.

3.2 Approach

Four steps were used for building WAJ testing capacity:

- Perform a detailed review of the SARS-CoV-2 testing method already in use at the WAJ virology laboratory and identify gaps in equipment and consumables that were preventing WAJ from implementing the optimal testing method possible for SARS-CoV-2 measurement in wastewater ("gap analysis").
- Provide assistance with procurement of the necessary equipment and supplies to improve testing sensitivity, reproducibility, and efficiency.
- Compare three approaches to sample concentration, and provide hands-on assistance related to all sample processing steps from sample collection to analysis during this method comparison.
- Document all recommended testing protocols.

3.3 Gap Analysis

As summarized in **Appendix C** ("Task 8 Current Situation Analysis" memorandum), the key findings from the gap analysis were:

- Re-evaluation of sample concentration approach given that the original approach was initially developed for sludge samples and the method requires a long time to complete.
- Utilization of a different method for sample sterilization than autoclaving (such as pasteurization) to preserve the SARS-CoV-2 viral signal as much as possible.
- Stirring the sample for 1 to 2 hours instead of overnight.
- Investigations of the use of Centricon™ filters to better understand the feasibility of this approach.
- Procurement of missing materials and supplies essential for conducting SARS-CoV-2 analysis.
- Consideration to the potential for filter clogging with high-strength wastewater for any filtration approach.

- Characterization of options for wastewater concentration based on how quickly the supplies required for the EPA method could be obtained and the results of the Centricon filter test. (Note that these options were developed and compared, as described in Section 3.5).
- Use of nucleic extraction kits specific for the wastewater applications with suitable proof on its quality.
- Consideration of suitable and additional staff for sample collection and sample processing at the laboratory, including:
  - One full time sample collection person with a vehicle must be available. The human resources of WAJ laboratory should be dedicated for SARS-CoV-2 for sample collection.
  - In addition to the current PCR laboratory staff, two lab technicians are needed at a minimum. One of the technicians should be responsible for sample concentration and elution. The other technician must have good experience in using PCR machines with practical experience.
  - The sufficiency of the human resources may be evaluated after deciding the planned approach to monitoring and the quantity of samples on a weekly basis.

Subsequent to performing the gap analysis, it was decided that an alternative to the "EPA method" would be identified to streamline the concentration approach. Three methods were evaluated by the CDM Smith/WAJ team, as described in Section 3.5. To support this method evaluation, the items shown in **Table 3** were procured between October and November 2020 for the WAJ laboratory Some of the procured equipment is shown in the photos below the table (Photo II and Photo Group II).

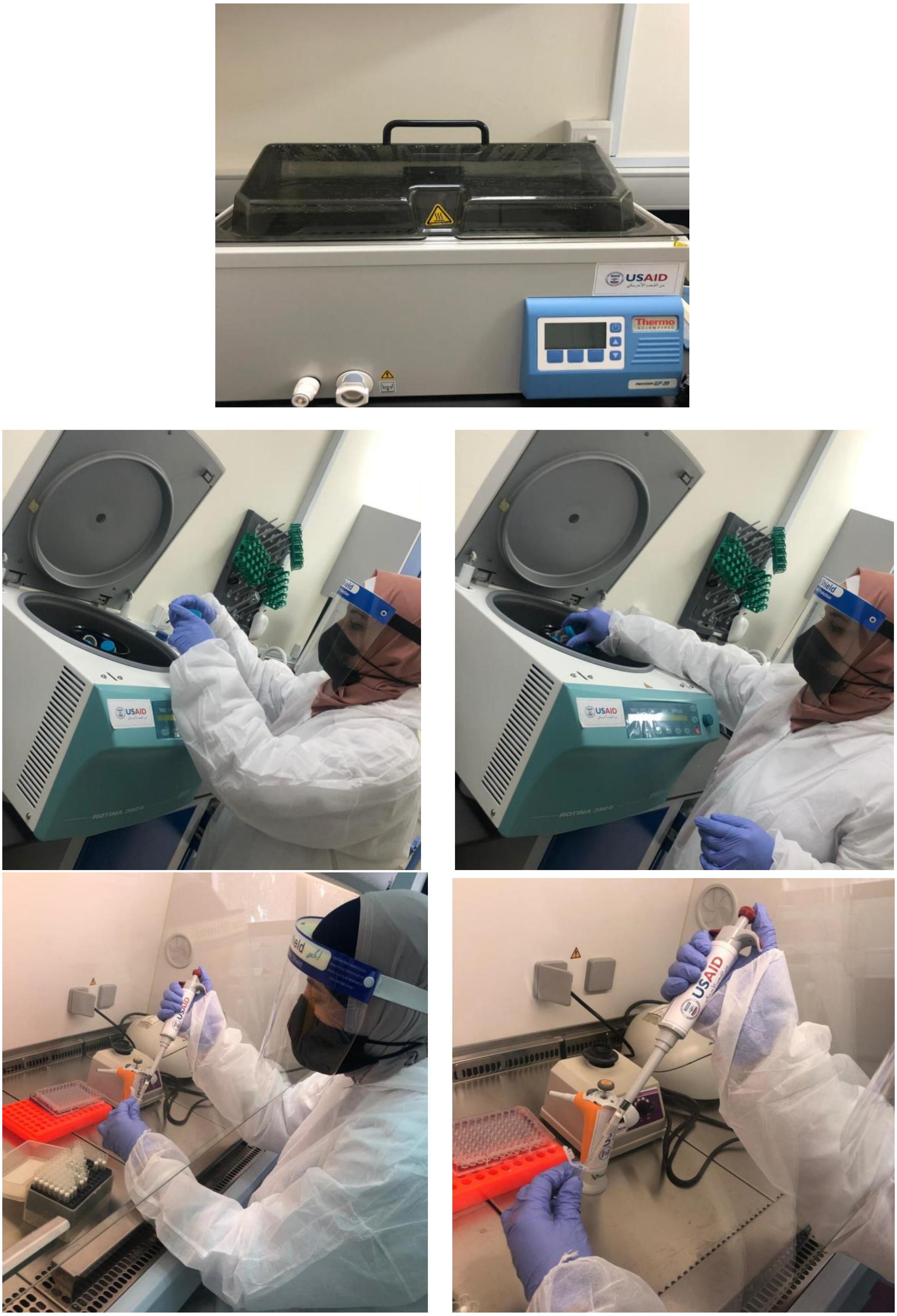
Table 3: Items Procured to Achieve Objectives of Task 2

Category	Equipment or Consumable
Health & Safety	Coveralls
	Facemask
	Goggles
	Gloves
	Face shields
	Shoe cover
	Sanitizing agent - Gel based
	Sanitizing agent - Liquid
Sample Collection	Sterile Containers
	Telescopic Sample Collection
Sample pasteurization & Concentration	Water Bath
	Refrigerated Centrifuge
	250 mL centrifuge bottles
Sample pasteurization & Concentration	PEG 8000
	50 ml tube rack
	2-propanol or isopropanol

Category	Equipment or Consumable
Sample Analysis	Magnetic SARS-CoV-2 Nucleic Acid Extraction kit
	SARS-CoV-2 RT-PCR kit
	Magnetic Separation stand
	SARS-CoV-2 Reference Material Kit
	Absolute Ethanol
	Nuclease free water
	Single Channel Micropipette, variable volumes (1-10 uL)
	Single Channel Micropipette, variable volumes (0.2-2 uL)
	Single Channel Micropipette, variable volumes (10-100 uL)
	Single Channel Micropipette, variable volumes (100-1000 uL)
	ATCC Human enterovirus 71
	Thermofisher Nano drop calibration (Installed at WAJ labs)
	Applied Biosystems 7500 calibration (Installed at WAJ labs)
	Microcentrifuge (Spinner)Vortex for PCR tubes
	Microcentrifuge tubes rack (1.5-2ml)

Photo II: Procured PPE

Photo Group II: Some of the Equipment Procured for Task 2



3.4 Health and Safety Considerations

Health and safety should be a primary consideration during implementation of wastewater surveillance activities. On November 26 and December 8, 2020 CDM conducted health and safety training sessions on “COVID-19 Health and Safety Protocols” at the WAJ Central Laboratory (see Photo Group IV). The purpose of the training was to provide WAJ Lab staff who will be engaged in Task 8 activities with the basic principles of health and safety requirements of handling wastewater samples to minimize the possibility of health hazards on laboratory and field staff. Nine WAJ employees attended the training, including seven from the Microbiology Section and two from the Wastewater Surveillance Section.

Feedback from the attendees based on responses to open-ended evaluation questions included:

- **Pre-Evaluation Feedback**
    - **In simple words what are your expectations from attending this course?**
        - *I expect to improve my knowledge about COVID-19 in WW and wish to get rid of the virus as soon as possible.*
        - *More knowledge about COVID-19 such as study case and the newest information come out about this field*
        - *To refresh my knowledge about SARS-CoV-2 testing and handling safety and sampling.*
- **Post Evaluation Feedback**
    - **What do you like most about the training?**
        - *The lecturers are very confident, they can provide the information very easy and smoothly*
        - *I believe that taking the precaution make me not afraid of the virus, but what about disposal of gloves and other PPEs in the field.*
        - *Safety and handling of samples*
    - **What are the things you like to change in the course?**
        - *Addition of risk assessment procedure in the case of accidents*

Documents that can be used by WAJ during future health and safety trainings are provided in **Appendix C**, and include a health and safety plan, a set of slides that can be used for health and safety training, and relevant guidance from the World Health Organization.

Photo Group IV: Health and Safety Training at WAJ Central Lab

3.5 Summary of Method Comparison

The focus of the method comparison performed by CDM Smith and WAJ staff was on optimizing both the sample concentration and nucleic acid extraction steps. Two alternative sample concentration methods (the "PEG precipitation + IDEXX" and "KWR" methods) were compared with the original concentration method followed by WAJ (also a PEG precipitation method, but using a different nucleic acid extraction approach) to identify the method with the:

- ✓ Highest virus recovery
- ✓ Best sensitivity and efficiency
- ✓ Shortest duration
- ✓ Lowest required effort

The details and results of the method comparison are provided in **Appendix D**. Both the IDEXX and KWR performed better for SARS-CoV-2 virus measurement than the original WAJ method. Either would be suitable for ongoing wastewater surveillance.

3.6 Recommended Testing Approach

Wastewater testing protocols are provided in **Appendix E** (for sample collection) and **Appendix F** (for sample concentration and analysis) and described briefly below.

3.6.1 Sample Collection

Wastewater samples for SARS-CoV-2 analysis should be untreated samples taken from the influent to a wastewater treatment plant (WWTP) or upstream in the wastewater collection network. 24-hour time-based composite samples (i.e., samples that are comprised of small aliquots taken periodically over a 24-hour period with the use of a piece of equipment known as an autosampler) are preferred and considered more sensitive over grab samples (representative sample collected on a one-time basis); however, grab samples are acceptable when composite sampling is challenging and not practical.

The volume of sample concentrated will determine the lowest amount of SARS-CoV-2 RNA that can be detected. The minimum recommended sample volume is 200 mL for grab samples, consistency in sample collection time is very important to guarantee valid comparable results. Grab sampling between early morning to mid-day is recommended. Timing of sample collection is less critical for composite samples because these represent the average conditions over the entire composite period.

All wastewater samples must be handled, treated and comply with WAJ "Quality Assurance Work Instruction for Health and Safety" and with health and safety considerations in **Appendix C**. Samples should be refrigerated during transport to the laboratory, and once received by the laboratory should be stored at 4°C or processed directly. Samples should be concentrated as soon as possible, within 24 hours from the time received by the laboratory to reduce SARS-CoV-2 RNA degradation. Additionally, concentrated samples should be processed with the extraction immediately, or stored overnight at -25°C to -15°C, or for several days at -80°C.

3.6.2 Sample Concentration

Concentration methods used in this project were developed and studied by many scientific groups to concentrate different viruses in wastewater: ultrafiltration using Centricon® Plus-70 centrifugal ultrafilters with a cut-off of 100 kDa and polyethylene glycol (PEG) precipitation. The PEG concentration method as described in Hjelmsø e*t al.* 2017 had already been in use by the WAJ lab before this project and was found to be time consuming and tedious. Further, it was associated with a lower virus recovery when compared with the PEG precipitation concentration method used with the IDEXX kit, as described in Wu *et al.* 2020. For reliable virus recovery it is recommended to use either ultrafiltration or the PEG concentration method following Wu *et al.* 2020.

3.6.3 Sample RNA Extraction

RNA extraction from concentrated samples should be performed using an RNA extraction protocol or commercial kit designed specifically to:

- Purify nucleic acid from environmental samples.
- Purify RNA and that includes RNase denaturants (Like Proteinase K) prior to lysis.
- Deliver the highest RNA yields and consistency in purity.

Degradation of extracted RNA from multiple freeze-thaw cycles by aliquoting extracts into separate tubes and storing them at -70°C or below. This will allow thawing of a single aliquot for qPCR analysis, while retaining other aliquots for future analysis.

3.6.4 Recommended Quality Assurance/Quality Control

The testing procedure should be designed to minimize the potential for false positives, false negatives, and contamination during all steps by using the following quality control (QC) procedures:

- During concentration, use a matrix spike biologically similar to SARS-CoV-2 and calculate the percent recovery of the matrix spike to:
  - Compare virus recovery from different concentration methods.
  - Ensure matrix of sample is not preventing detection of the COVID-19 virus if present.
- During RNA extraction, use the following controls:
  - Extraction Positive Control: extracted and tested with each set of samples to demonstrate successful recovery of RNA during the extraction process and should test positive for the SARS-CoV-2 target during PCR.
  - Extraction Negative Control: To ensure cleanliness during extraction
- During RT-qPCR, use the following controls:
  - SARS-CoV-2 Positive Control: Positive Control for both reverse transcription step and PCR.
  - No Template Control (NTC): To ensure cleanliness during RT-PCR.

3.6.5 Reporting

Reporting of wastewater SARS-CoV-2 testing results should provide an indication of virus circulation in Jordan and even an early warning of the location of new cases. The wastewater testing results obtained in the WAJ laboratory are cycle threshold (Ct) values, or the number of PCR cycles that were needed to amplify viral RNA to reach a detectable level. These can be translated into a SARS-CoV-2 concentration in wastewater (in units of RNA copies per volume) with the use of a standard curve, although Ct values alone are sufficient for tracking trends. It should be kept in mind that a higher Ct value corresponds to a lower viral concentration; Ct values greater than 37 should be considered to correspond to nondetectable levels of SARS-CoV-2 virus in wastewater.

To maximize the usefulness of SARS-CoV-2 data, we recommend including the following in addition to the Ct values themselves:

- Important metadata when reporting SARS-CoV-2 wastewater results, such as:
  - WWTP or manhole information: ID number, location, population served.
  - Sampling details related to sample collection time, date, type of sample, volume.
  - Testing: information about concentration, extraction, and detection methods used. Report to provide Ct value result of the RT-PCR testing and listing all the Ct values measured overtime for comparative analysis.
- Any available information on reported COVID-19 cases in the same populations for which wastewater was sampled.
- Clear explanations of technical terms (e.g., “grab” samples, Ct values etc.)

3.7 Opportunities for Increasing Capacity Further

Through the gap analysis, method comparison, and protocol documentation activities, this project resulted in a doubling of WAJ’s capacity to process wastewater samples for SARS-CoV-2 measurement. The turnaround time (TAT) on sample results—that is, the time from when the sample is received at the laboratory to when the Ct value is reported—is greater than three days.

If WAJ is to implement wastewater surveillance on a national scale (e.g., a National Wastewater Surveillance System), it will become important to both decrease the TAT to two days and increase their wastewater sample concentration capacity to at least 10 to 12 samples per day. This would allow surveillance at all 34 WWTPs throughout Jordan, as well as targeted sampling at sub-sewersheds as needed. To improve both the TAT and the sample concentration capacity would require:

- Larger laboratory space that will accommodate additional procured equipment.
- High throughput and automated laboratory equipment related to sample concentration and RNA extraction, and PCR preparation.
- Hire dedicated persons for wastewater sampling and PCR lab specialist.

Additional considerations related to expanding the analytical capacity for testing SARS-CoV-2 in wastewater are discussed in Section 4.

Section 4

Roadmap for Expanding Beyond Ain Ghazal

4.1 Lessons Learned from the Ain Ghazal Pilot

The following lessons were learned during development of the wastewater monitoring plan for Ain Ghazal and building the wastewater testing capacity at WAJ:

- Field verification for manholes is necessary to confirm that the selected manhole exists and is accessible.
- Coordination is likely needed with other agencies to open manholes.
- Manholes may be within major streets and require coordination to manage safety considerations.
- USAID 2013 hydraulic model was instrumental to divide Ain Ghazal sewershed into four tiers in addition to the updated WAJ GIS information.
- The number of available WAJ staff available for sample collection is insufficient for implementation of wastewater surveillance during the pandemic.
- WAJ testing capacity can be scaled up to 10 to 12 samples per day by using additional consumables, and greater number of samples can be run by introducing automated and specialized instruments.
- WAJ staffing resources must be considered during the development of the wastewater surveillance program.
- Either the IDEXX or KWR method for sample concentration are preferred over the method previously used by WAJ.
- To implement COVID-19 monitoring using wastewater surveillance in Jordan, full-time experienced PCR lab technicians are recommended as well as additional laboratory space.

4.2 Surveillance Outside Ain Ghazal

Because 68% of Jordan's population is connected to the sewer system, wastewater surveillance can be an effective means to track COVID-19 in Jordan. If implemented at a national scale, the wastewater testing data would provide complementary information to clinical testing data and help allocate critical public health resources to manage the spread of COVID-19. This program will help identify hotspots and estimate disease prevalence, provide early warning for new outbreaks, confirm trends in clinical data, evaluate presence of variants and confirm absence of disease.

The steps involved in implementing a national program would include:

1. Establishment of a national wastewater surveillance system (WSS) framework

2. Definition of the program objectives and specific public health actions to be linked to wastewater data

3. Creation of an implementation plan that expands both spatial and temporal coverage of wastewater testing beyond period testing in selected locations in Ain Ghazal

4. Expansion of analytical capacity for quantification of SARS-CoV-2 in wastewater

5. Ongoing institutional cooperation to optimize the use of the data

Each of these steps is discussed briefly below.

4.2.1 National Wastewater Surveillance System Framework

Although there are many potential approaches to developing a national wastewater surveillance system (NWSS), the framework should contain two types of entities at a minimum:

1. Core and partner surveillance agencies responsible for (a) generating viral load information in wastewater via sample collection, concentration and PCR analysis and (b) communicating these data to public health partners

2. A health advisory board responsible for (a) linking the wastewater data with clinical testing information and (b) directing public health actions

These entities are represented schematically in **Figure 10** and their roles are described further in the following sections. An important initial step to be completed upon establishment of the wastewater surveillance framework is definition of the program objectives and specific public health actions to be linked to the wastewater data.

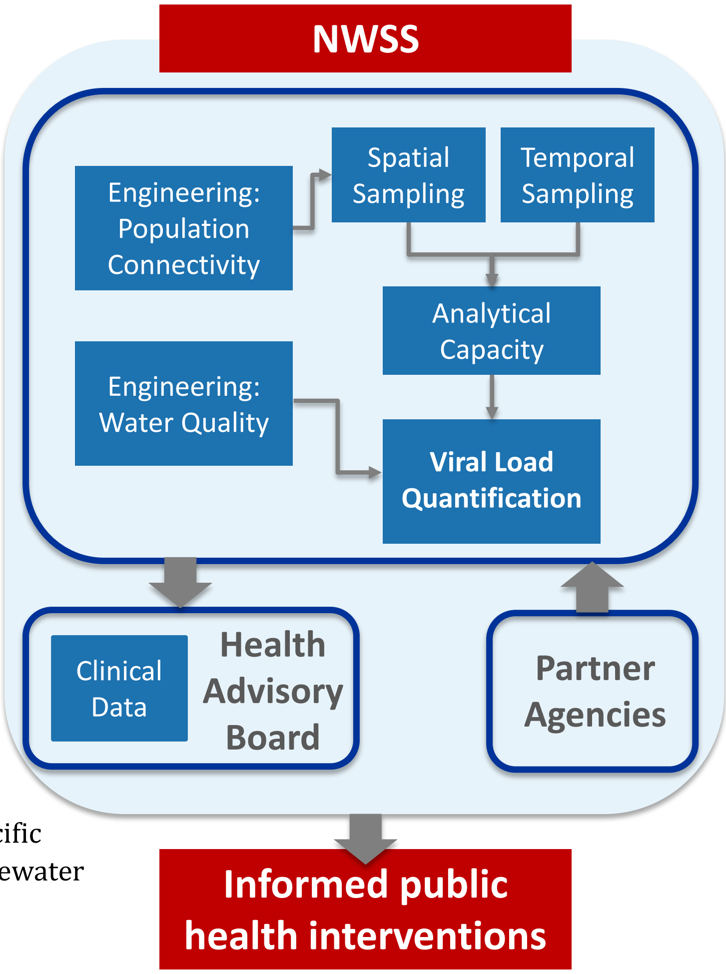


Figure 10: Generic National Wastewater Surveillance System Framework for Jordan
Additional details, including a description of roles, is provided in the text

4.2.2 Implementation Plan for Expanded Spatial Coverage

We recommend that the NWSS cover the entire population in Jordan served by sewers. The wastewater sampling location would include all WWTPs in addition to several manholes, and possibly pump stations, within the sewer network. The location of WWTPs in Jordan are shown in **Figure 11**.

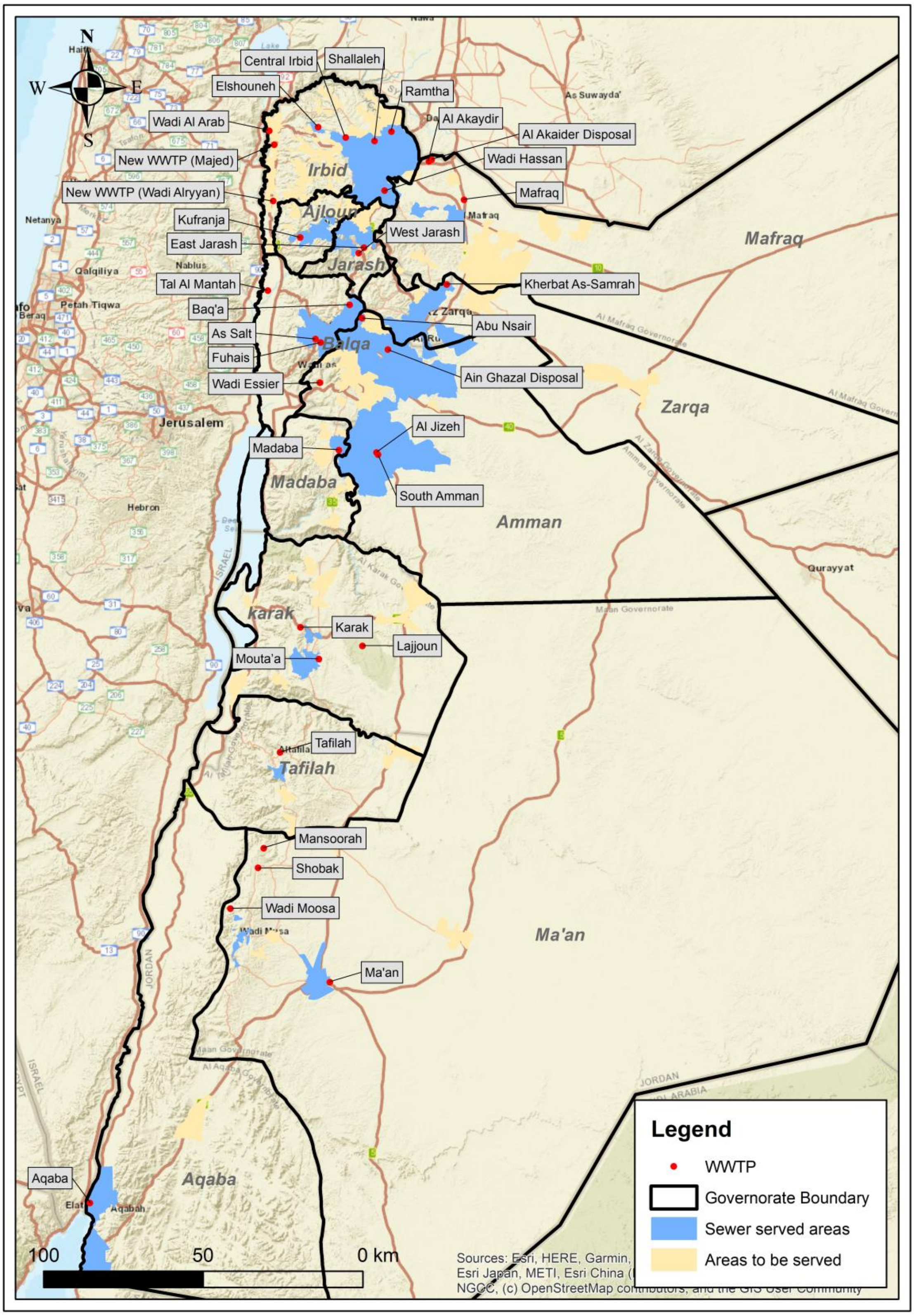


Figure 11: Location of Wastewater Treatment Plants in Jordan

Following the procedure described in Section 2 to divide Ain Ghazal sewershed into four different tiers, we recommend dividing all the WWTP sewersheds into similar tiers. Tier 1 would divide the sewersheds into smaller sub-sewersheds with a maximum population of 500,000 people. For the smaller WWTPs, the Tier 1 population would be substantially lower. Tier 2 would consist of smaller sub-sewersheds with populations up to 250,000 people. Similarly, Tier 3 splits the sewersheds into areas with a maximum population of 100,000 people and Tier 4 splits the sewersheds into areas with a population of about 25,000 people.

Currently, there are 34 WWTPs in Jordan, serving a range of population between 50,000 and 3.75 million each. For simplification, we categorized the WWTP into four categories based on their size and estimated the number of sampling locations needed for each tier. **Table 4** shows the number of WWTPs within each size category (small, medium, large and extra-large) and the number of proposed sampling locations within each tier per WWTP. For example, Khirbet al Samra WWTP serves around 3.75 million people and could be divided into eight Tier 1 sub-sewersheds with a maximum population of 500,000 people each. It also could be divided into 32 Tier 3 sub-sewersheds.

Based on this analysis, we estimate the total number of Tier 1, Tier 2, Tier 3, and Tier 4 sampling locations throughout all of Jordan to be 40, 60, 90, and 250, respectively. These sampling locations can consist of WWTP influents, pumping stations or manholes.

Table 4: Estimated Number of Sampling Locations Within Each Tier for All Wastewater Treatment Plants in Jordan

WWTP Size	Number of WWTPs	Number of Sampling Locations by Tier (per WWTP)			
		Tier 1	*Tier 2*	*Tier 3*	*Tier 4*
Small	14	1	1	1	1
Medium	15	1	1	2	4
Large	4	1	2	3	12
Extra Large	1	8	16	32	128
Total	34	~40	~60	~90	~250
Approximate Population per Tier		Up to 500,000	Up to 250,000	Up to 100,000	Approximately 25,000

Ideally, GIS information would be available for each WWTP sewershed to enable division into different sampling tiers and identify manholes, if needed, for sampling. For the larger WWTPs with complicated sewer networks, GIS information is critical. For smaller WWTPs for which only a few sampling locations need to be identified outside the WWTP itself, GIS information is not necessary. Instead, engineering staff could use as-built drawings coupled with confirmatory field assessments to identify sampling locations. If a hydraulic model for a sewershed exists, it would be a great benefit, and we recommend using it for the population estimate of the sub-sewersheds to the extent possible.

4.2.3 Implementation Plan for Temporal Coverage

The national wastewater surveillance program would require continuous sampling at the WWTPs and the different sampling locations through the end of the pandemic. The sampling frequency could correspond to the pandemic prevalence status, as presented in the suggested wastewater surveillance strategy for Ain Ghazal in Section 2.4.

For example, when the average number of reported new cases doesn't exceed one daily case per million, the pandemic is considered under control. The objective of the surveillance is to confirm the absence of disease and predict future outbreaks. This surveillance mode requires a minimal testing frequency of 1 sample per week at Tier 1 locations.

When the average disease prevalence increases, it is crucial to increase the sampling frequency at each Tier 3 locations to three samples per week. **Table 5** summarizes the national WSS and the sampling frequency within each tier for each surveillance mode.

Table 5: One Possible National Wastewater Surveillance Strategy

Surveillance Component	**Surveillance Mode**			
	Maintenance	*Baseline*	*Increasing*	*Targeted* (optional)
Pandemic Status	Long-term monitoring of the entire country population when there are very low levels of Covid-19 disease reported in Jordan;	Long-term monitoring of the entire country when there is known Covid-19 disease in Jordan to show increases and	Rapidly identify which areas in the country are the source for new Covid-19 cases by jumping to Tier 3 sites corresponding to any Tier 1 site with an increasing viral signal	This is an optional mode available if more granular information is desired beyond Tier 3 (100,000 people per sample location): Tier 4 sites correspond to populations of about 25,000 people;
Objective	Provide early indication of appearance of disease	confirm decreases in Covid-19 cases	Identify hot spots	
Tier Sampled	Tier 1	Tier 2	Tier 3	Tier 4
Number of Sampling Locations By Tier	~40	~60	~90	>90
Samples per Week	1	1-2	2-3	2-3
Total Samples per Week	40	60-120	180-270	>250

4.2.4 Adequate Analytical Capacity

Based on the suggested NWSS plan, we recommend that the laboratories' analytical capacity be sufficient for processing all Tier 3 locations with a testing frequency of three samples per week. This translates into the national wastewater surveillance program needing to be capable of processing 200 to 300 samples per week.

Expansion of laboratories' analytical capacity can be accomplished either by expanding key stakeholder (MWI/WAJ) analytical capacity and/or engaging partner agencies to cooperate in sampling and testing activities.

4.2.4.1 Analytical Capacity Expansion at MWI/WAJ

Having adequate analytical capacity is critical for providing the ability to cover the total number of samples required per week as per pandemic status and the transmission between different wastewater surveillance modes. **Table 6** shows five suggested scenarios for analytical capacity expansion at the WAJ laboratory and the associated total number of samples tested per week, turnaround time (TAT: the interval between a sample's arrival in the laboratory and the time the result is issued), and number of sample collection days per week. These scenarios are as follows:

- Scenario A-1, Enhanced Consumables: Procurement of a different centrifuge tube size (94 mL) would enable the weekly sample processing capacity to increase from 12 to 27.
- Scenario A-2, Enhanced Instruments: In addition to procuring a different centrifuge tube size (as under Scenario A), Scenario B would also involve procurement of one more refrigerated centrifuge. This would increase weekly sample processing capacity to 54.
- Scenario B: Partially Automated: Building on Scenarios A and B, it would be possible to further enhance weekly sample processing capacity to 108 samples per week by implementing automation at the nucleic acid extraction step but staying at the manual level at sample concentration step.
- Scenarios C-1 and C-2: Fully Automated. These scenarios would fully automate sample concentration as well as nucleic acid extraction, with the difference between the two being that Scenario C-2 would include an additional day of sampling each week.

Scenarios C-1 and C-2 would achieve the capacity recommended for a national surveillance effort.

Table 6: Suggested Analytical Capacity Expansion Scenarios Based on NWSS Plan

Scenario	Daily Sample Capacity	Weekly Sample Capacity	Turn-Around-Time (days)	Number of Sample Collection Days per Week
Current WAJ Capacity	4	**12**	5	3
A-1: Enhanced Consumables	9	**27**	5	3
A-2: Enhanced Instruments	18	**54**	5	3
B: Partially Automated	27	**108**	2	4
C-1: Fully Automated	50	**200**	2	4
C-2: Fully Automated	75	**300**	2	4

Specific elements required to implement the expanded lab capacity at WAJ are detailed in **Appendix H** and explained briefly below.

Capital investment

This investment will be required for scenarios that rely on additional equipment (Scenarios A-2, B, and C-1 and C-2). This equipment would include a refrigerated centrifuge, automated virus concentrator, automated nucleic acid extraction equipment, and/or PCR multichannel pipettes.

Consumables

Consumables used for SARS-CoV-2 testing can include PPE, tubes, filtered pipette tips, sample concentration chemicals, nucleic acid extraction kits, and RT-PCR kits, among other items. Scenario B would require the use of nucleic acid extraction kits compatible with the automated instrument instead of manual kits, while Scenario C would depend on using special consumables for the automated concentrator in addition to the nucleic acid extraction kits.

Staffing

Various additional WAJ (or related) staff would be required to increase sample processing capacity each week, including:

- *Drivers*
- *Samplers: Dedicated sampler who can sample from WWTP, and capable to manage safety consideration without the need of coordination with other agencies when sampling from manholes.*
- *Lab technicians: Dedicated PCR specialists.*
- *Senior lab technician: Dedicated PCR specialist who is (familiar / experienced) with wastewater testing and implementation of QA/QC protocols.*

Training is required for all staff so they can fully respond to project's objectives and plans. Staff background, experience, knowledge, and skills are the main factors to be evaluated and fulfilled when training is planned.

Generally, health and safety training should be provided for all staff members. Sampling protocol and procedures training shall be provided for all samplers, while SARS-CoV-2 testing protocols and skills, QA/QC practices must be learned by any newly joined lab technicians.

Space and Location

Considerations to be made when deciding to expand lab capacity include workspace, location, lab organization according to analytical testing sequence, place to install unmovable equipment, frequently used tools, solutions, samples, storage, and waste must be addressed.

Analytical capacity expansion for any alternative WAJ requires enlargement of their current virology testing lab or establishing new testing laboratories based on dividing the kingdom into different geographical regions. This is described further in Section 4.2.4.2.

Budgeting for the WAJ capacity expansion suggested in Table 6 requires considering capital investment for both equipment and additional lab space/new labs, as well as ongoing costs for operation and maintenance of equipment, consumables, staffing (labor), and transportation. Estimates of both the capital costs and the cost per sample for each scenario are provided in **Table 7**. The capital investment includes equipment costs only and does not include any costs associated with WAJ lab spatial expansion, establishing new labs, or purchasing vehicles needed for sampling. The cost per sample includes capital operation and maintenance, consumables, labor, and transportation costs. The cost development is detailed in **Appendix H**.

Table 7: Estimated Cost to Expand WAJ Lab to Achieve Different Capacity Expansion Scenarios

Scenario	Weekly Sample Processing Capacity	Approximate Capital Investment, USD	Approximate Ongoing Cost per Sample, USD[1]	Approximate Total Cost per Week, USD
Current WAJ Capacity	**12**	0	195	2,500
A-1: Enhanced Consumables	**27**	0	175	4,700
A-2: Enhanced Instruments	**54**	25,000	160	8,700
B: Partially Automated	**108**	75,000	145	16,000
C-1: Fully Automated	**200**	95,000	210	42,000
C-2: Fully Automated	**300**	95,000	205	62,000

Note: (1) Total cost per sample includes operations and maintenance costs for capital equipment as well as costs for consumables, labor, and transportation. These costs are detailed in Appendix H.

4.2.4.2 Analytical Capacity Expansion via New Labs

In addition to bolstering capacity at the WAJ Central Lab, it may make sense to divide Jordan into three geographical regions (north, central and south) covering the 12 governorates, and establish two new labs to cover the north and south. The three labs, then, could serve the following governorates:

- North: Irbid, Ajloun, Jerash, Mafraq
- Central (existing): Balqa, Amman, Zarqa, Madaba
- South: Karak, Tafilah, Ma'an, Aqaba

Since WAJ central lab capacity has been already built for sensitive SARS-CoV-2 testing, it can receive any sample to be processed at different level of analytical testing activities like sample concentration, viral RNA extraction, virus detection and quantification by RT-PCR, and result analysis and reporting.

Table 8 provides a suggested distribution of analytical activities (listed in steps 1,2,3,4,5) between the labs, and shows the required equipment and consumables for those activities to be procured for north and south lab. The new testing laboratories would report to the central lab in Amman. Capacity of those labs should be related to how many sewersheds will be covered in the designated geographical region.

Table 8: Example of Potential Testing Coordination Between WAJ Labs

Option / Analytical Activity	Sample collection (Step 1)	Sample Pasteurization (Step 2)	Sample Concentration (Step 3)	Viral RNA Extraction (Step 4)	Quantification by RT-PCR (Step 5)	Logistics	Main Instruments and Consumables Required
A	Central North South	Central North South	Central North South	Central Only	Central Only	Virus concentrated at the lab and to be transported to WAJ central lab for downstream processing	PPE, sample collection tools, water bath, refrigerated centrifuge, refrigerator (+4°C), vortex, micropipettes, and all consumables related to those activities
B	Central North South	Central North South	Central North South	Central North South	Central Only	Extract RNA at the lab and transport extracted RNA to WAJ Central Lab in Amman	PPE, sample collection tools, water bath, refrigerated centrifuge, refrigerator (+4°C), freezer (-20°C), vortex, micropipettes, microcentrifuge, heat block RNA extraction kits and all other consumables related to those activities

4.2.5 Ongoing Institutional Cooperation

Ongoing institutional cooperation between MWI and its partners and the health advisory board is critical for the NWSS success. The cooperation with MWI and partner laboratories is essential to generating viral load information in wastewater via sample collection, concentration and PCR analysis to the health advisory board. The coordination with the health advisory board helps blend the viral load concentration data with the clinical data to guide public health decisions.

The coordination between MWI laboratories and partner surveillance agencies includes adapting one WSS strategy described in Section 4.2.3. The main national WSS body is recommended to direct all testing partners with the frequency and spatial coverage based on the pandemic perveance status.

The analysis testing results are susceptible to many testing details. Therefore, it is essential to develop standard and consistent testing procedures with quality assurance and control protocols to yield consistent results. It is also important to have protocols in place for managing coordination during any national lockdown events.

The coordination between partners expands to the program logistics and geographic coverages of the program. Therefore, the program will benefit from dividing the country into different zones to be distributed among interested partners.

Finally, the testing results should be reported consistently following the same procedures and stored in the same data depository. It will be beneficial to have all the analysis results accessible for all parties to share and compare results to facilitate communicating these data to public health partners. **Figure 12** shows the suggested ongoing agencies cooperation and the flow of information from the surveillance phase to the public health intervention phase.

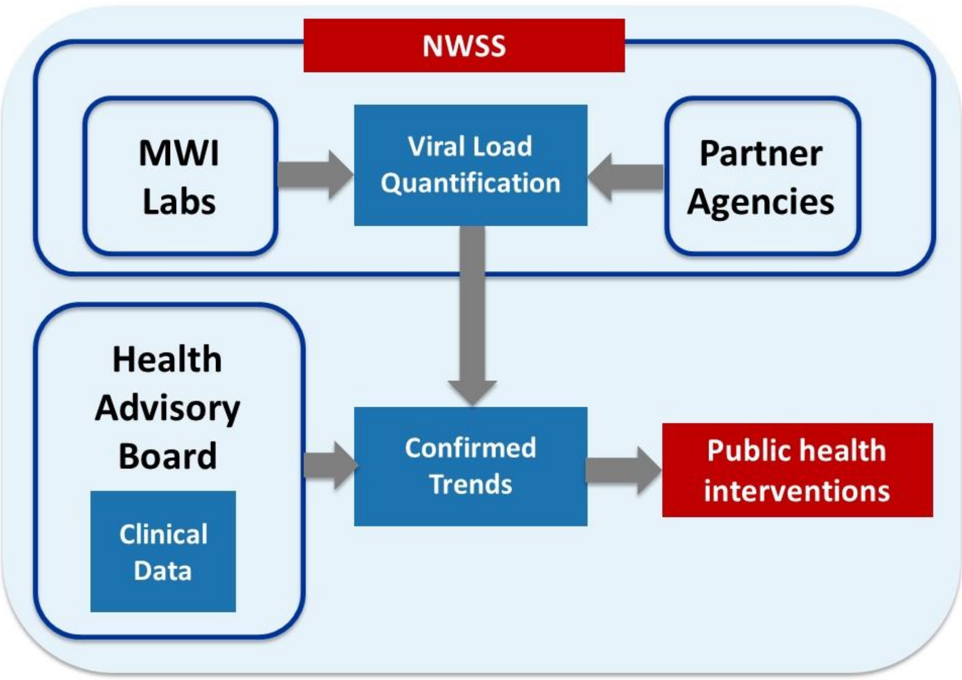


Figure 12: Ongoing Institutional Cooperation Framework

References

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J. and Tscharke, B., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: a proof of concept for the wastewater surveillance of COVID-19 in the community. *Science of the Total Environment*, 728, p.138764.

Bibby, K. and Peccia, J., 2013. Identification of viral pathogen diversity in sewage sludge by metagenome analysis. *Environmental science & technology*, *47*(4), pp.1945-1951.

CDM International Inc., 2010. Water/Wastewater Infrastructure Project, Technical Memorandum Water/Wastewater Forecasts, Task 1-4, December.

Hjelmsø, M.H., Hellmér, M., Fernandez-Cassi, X., Timoneda, N., Lukjancenko, O., Seidel, M., Elsässer, D., Aarestrup, F.M., Löfström, C., Bofill-Mas, S. and Abril, J.F., 2017. Evaluation of methods for the concentration and extraction of viruses from sewage in the context of metagenomic sequencing. *PloS one*, 12(1).

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R. and Brouwer, A., 2020. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. *Environmental Science & Technology Letters*.

Miyani, B., Fonoll, X., Norton, J., Mehrotra, A. and Xagoraraki, I., 2020. SARS-CoV-2 in Detroit wastewater. *Journal of Environmental Engineering*, 146(11), p.06020004.

Mohamed Hamouda, Farah Mustafa, Munjed Maraqa, Tahir Rizivi and Ashraf Hassan, Wastewater surveillance for SARS-Cov-2: Lessons learnt from recent studies to define further application. *Science of Total Environment* 759 (2021) 143493

Randazzo, W., Truchado, P., Cuevas-Ferrando, E., Simón, P., Allende, A. and Sánchez, G., 2020. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. *Water research*, *181*, p.115942.

Wu, F., Zhang, J., Xiao, A., Gu, X., Lee, W.L., Armas, F., Kauffman, K., Hanage, W., Matus, M., Ghaeli, N. and Endo, N., 2020. SARS-CoV-2 titers in wastewater are higher than expected from clinically confirmed cases. *Msystems*, 5(4).

Appendices

A: Detailed Manhole Information
B: Current Situation Analysis Technical Memorandum
C: Health and Safety Materials
D: Method Comparison
E: Wastewater Sample Collection Protocol
F: Recommended Wastewater Concentration and Analysis Protocol
G: Materials from March 10, 2021 Workshop
H: Details Related to MWI/WAJ Laboratory Expansion Scenario