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National Microbiology Laboratory

Community-Led Wastewater Testing Startup Guide

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Contents

Purpose of this Guide

This guide provides general information useful in considering, planning, and operating a community-led wastewater testing program in support of public health. Wastewater testing for pathogens can be a useful tool that provides a measure of community health that may be used to guide public health action or support community outreach. A wastewater testing program can be set up differently based on each community's unique infrastructure and the goals of their program.

Although each wastewater program is unique, there are several steps that are generally followed when starting routine wastewater testing in a community. This guide outlines these steps. The guide's appendices provide additional information such as basic safety practices, training documents, and protocols for wastewater collection, shipping, and testing.

The main document of this guide covers the following topics:

1. An introduction to wastewater testing.
2. Assembling an Implementation Committee.
3. Making a project plan.
4. Developing a wastewater collection plan.
5. Developing a wastewater testing plan.
6. Confirming results externally.

Part 1 – An Introduction to Wastewater Testing

1.1 What is Wastewater Testing?

Routine wastewater testing can provide information on overall community health and may allow communities to take a more proactive approach to public health. Wastewater testing may detect parts of viruses and/or bacteria found in urine or stool. For example, wastewater testing is used across Canada to detect the spread of Flu, RSV (Respiratory Syncytial Virus) and COVID-19. As nearly everyone contributes to wastewater, this is a fair way to monitor community health, even if residents may have trouble accessing timely clinical testing.

Previously wastewater testing programs require highly specialized technicians and expensive equipment. Because of these requirements, many remote communities ship their wastewater to federal or provincial labs for testing. Although this option is still possible, it may not be right for some communities as it may delay results as shipping may be a challenge. To overcome these challenges, a community may choose to perform wastewater testing using a rapid test device that automates part of the process.

1.2 Steps Towards Starting a Community-Led Wastewater Testing Program

- Community-led wastewater testing programs begin with discussions driven by Community Leadership/Residents to determine if wastewater testing is in the community's best interest.
- Next, if the community proceeds with wastewater testing, representatives from the community should be selected that will lead the program by making major decisions and guiding program development. An Implementation Committee should be assembled that includes community representatives, wastewater operators and/or engineers, technical advisors/specialists, and public health representatives.
- Then, a wastewater sampling strategy will be developed for the community (Part 4) outlining how and when samples will be collected.
- Following this, a plan for wastewater testing needs to be developed. This can be accomplished through in-community testing (Part 5) or off-community testing. For in-community testing, it is recommended that samples initially be cross-checked at an established laboratory for 1-2 months to ensure test accuracy. For off-community testing, samples are shipped to another facility for testing (e.g., Provincial or Federal laboratory).
- A strategy should be developed for how the testing results will be communicated back to the community and/or used to guide public health action. Clear procedures should be formed for data usage, ownership, and security.
- Once implemented, the program should be re-evaluated regularly to identify areas for improvement.

1.3 Wastewater Testing Overview

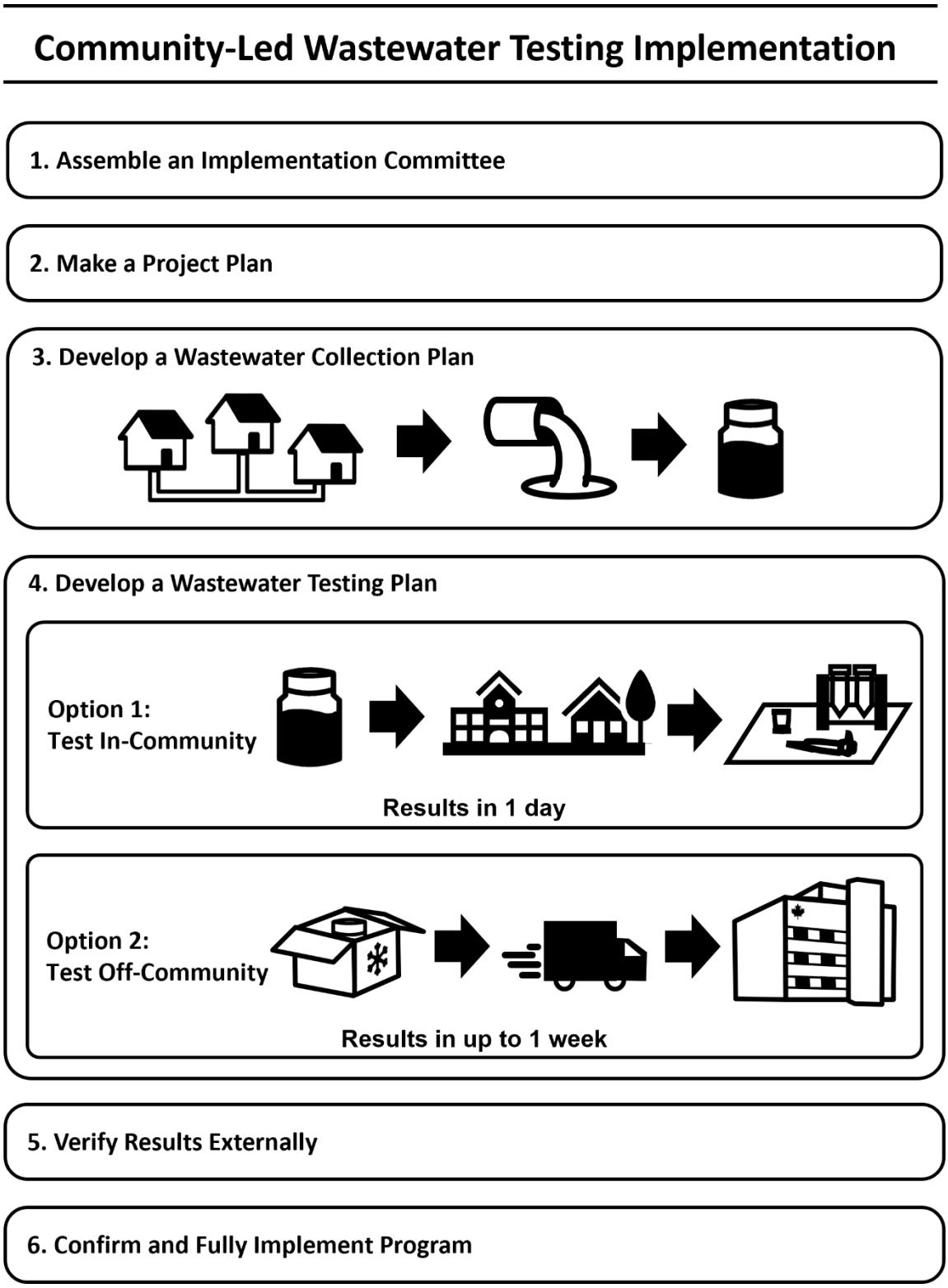

Figure 1. Overview of steps in implementing a wastewater testing program

Part 2 – Assemble an Implementation Committee

This section provides guidance on the first tasks in planning a wastewater testing program. It is composed of the following three steps:

- ❑ 2.1 Form an Implementation Committee.
- ❑ 2.2 Assign Responsibilities.
- ❑ 2.3 Make a Communication Plan to Engage with the Community.

2.1 Form an Implementation Committee

To guide a wastewater testing program, an Implementation Committee will need to be assembled to plan, operate, monitor, and maintain the program. Ideally, the Implementation Committee should include individuals from the following groups:

Members of an Implementation Committee
Community Representatives may be elected community leaders or assigned designates who provide approval for major program decisions and serve as a liaison with the community.
Public Health Experts may include epidemiologists or public health representatives who can help develop strategies for the use of wastewater results.
Wastewater Experts may be treatment plant operators, engineers, or contractors that are familiar with community infrastructure and/or Testing Staff who have access to wastewater infrastructure and sample wastewater.
Technical Advisors/Specialists can provide support and guidance related to laboratory testing, information storage and transport, and logistics.

2.2 Assign Responsibilities

After an Implementation Committee is formed, the responsibilities of members will be assigned. The following is what each group may be responsible for:

Responsibilities of Implementation Committee Members
All Committee Members • Co-develop a project plan. • Have a working understanding of the entire program and maintain lines of communication. • Consider who will own, control, and access the community's data.
Community Representatives • Lead the Implementation Committee. • Identify resources and mobilize infrastructure for sampling and testing. • Assist in the development of a communication plan with the community. • Approve a project plan that aligns with the interests of the community.
Public Health Experts • Can assist with the development of action plans that use both community wastewater and clinical testing data. • May receive and interpret the wastewater testing results. • Implement the communication plan.
Wastewater Experts • Provide working knowledge of community wastewater infrastructure. • Guide on best practices for sample collection and develop a sampling plan. • Help troubleshoot any issues with sample collection.
Technical Advisors/Specialists • Provide technical advice on testing, along with standardized protocols and training materials. • Assist in the development of community wastewater testing plan. • Assist in the development of solutions for transfer and storage of community wastewater. • Help troubleshoot issues with wastewater testing.

2.3 Make a Communication Plan to Engage with the Community

A well-designed communication plan is needed to make sure the community is comfortable with wastewater testing and is benefitting from the program. The results from wastewater testing can improve the uptake of public health messaging such as taking additional health precautions, hand-washing, masking, or testing. Residents are also more likely to support a wastewater testing program if they understand how the information is being used to improve community health.

Below are examples of communication strategies to engage and inform community residents:

Strategy	Description	Location
Online Dashboard	Location to publicly share wastewater results and provide updates on community disease trends.	Online website. Example: https://health-infobase.canada.ca/covid-19/wastewater/.
Information Sheets and/or Reports	A more controlled way to share wastewater results only with community residents or approved stakeholders.	Email newsletters, printed flyers or pamphlets.
Display Boards and Signage	Physical displays in community that may provide information/updates on the wastewater program or provide important updates.	Common spaces in the community or at large community events.
Social Media/News Releases	A strategy to rapidly share key messages and updates with the community.	Social media platforms and news outlets frequented by community residents.

Part 3 – Make a Project Plan

This section sets out to describe the major decisions that will direct the wastewater testing program. It involves the following steps:

- ❑ 3.1 Decide How and Where Samples Will be Collected.
- ❑ 3.2 Decide What Tests to Perform and Where to Test Samples.
- ❑ 3.3 Identify the Resources Needed to Carry Out the Project Plan.

3.1 Decide How and Where Samples Will be Collected

When it comes to wastewater testing, collecting a good wastewater sample is one of the most important steps towards producing reliable results. The location and method to collect the wastewater sample are key. Samples can be collected manually (e.g., grab sample) or automatically using a special device called an autosampler. As an autosampler collects wastewater slowly over a long period of time (called a composite sample) it is highly recommended whenever possible.

Regardless of method chosen, decisions need to be made on the sampling location, frequency, and who will perform the sampling. Further guidance for developing a sampling plan can be found in Part 4.

Option 1 **Collect samples using an autosampler (i.e., composite samples)**	**Option 2** **Collect samples manually (i.e., grab samples)**
Collect samples over a longer period and are thought to be a more representative measure the amount of virus in wastewater.	Collection at one point in time may produce results that vary based on when the sample is collected.
Will require initial setup and maintenance, and may not be possible for all locations. Following set up, sample collection should be quick and easy.	Can quickly and easily change where samples are collected. Considered a “one and done” approach.
Requires a dedicated space and usually requires access to a power outlet.	Requires little equipment and can be suitable for hard-to-reach sampling locations.

3.2 Decide What Tests to Perform and Where Samples Will Be Tested

Wastewater samples collected in community can be tested in-community or off-community. This decision will influence what tests can be performed and what resources will be needed. Further guidance for developing in-community testing can be found in Part 5 – Develop In-Community Wastewater Testing Infrastructure.

Option 1 **Test wastewater in-community in a dedicated location with special equipment**	**Option 2** **Test wastewater off-community at an external laboratory**
Results can be obtained within a day.	Results may be delayed (up to 7-10 days).
Requires Testing Staff and an equipped workspace in the community.	No need for testing staff or workspace in the community.
No shipping of samples out of the community. Although, samples may be shipped for confirmatory testing or additional testing.	Requires routine shipping of samples that must be kept cold. Delays in shipping may impact test results (decrease sensitivity).
Current tests include COVID-19, Influenza (Flu) viruses, and RSV.	Testing for other diseases may be provided in addition to COVID-19, Flu viruses, and RSV.
Results may need to be shared out of community for analysis.	Additional information may be provided such as variant detection.

In-Community Testing using the GeneXpert System

Current in-community wastewater testing uses the Cepheid GeneXpert system, a rapid diagnostic test device commonly used in clinical testing. The GeneXpert is suitable for testing wastewater in-community as it is easy to use, small (size of a microwave), and highly accurate. Currently, the system only supports testing for COVID-19, Influenza (Flu) Viruses, and RSV. It also requires a dedicated space for the GeneXpert system, maintenance of the system, as well as a laboratory instrument to spin samples at high speeds (centrifuge).

3.3 Identify the Resources Needed to Carry Out the Project Plan

Community Representatives in consultation with the Implementation Committee should identify what resources are already available to support the project plan. For the additional resources needed, potential Community, Provincial, and Federal sources of funding/supplies should be identified.

Required Resources for a Wastewater Testing Program

The following is **required**:
❑ Equipment to collect wastewater samples (e.g., such as an autosampler or grab sampling supplies). (See Part 4)
❑ Dedicated refrigerator to store wastewater samples.
❑ Supplies for shipping wastewater samples and funds to support shipping costs.
❑ Staff or contractors to carry out sample collection, shipping, and possibly testing.

The following is only **required if testing in-community**: (See Part 5)
❑ Workspace to test wastewater samples.
❑ Equipment to test wastewater, such as a GeneXpert device and centrifuge.

A list of all sampling and testing materials can be found in Appendix A. For Northern, Remote and Isolated Communities the Public Health Agency National Microbiology Laboratory may be able to provide support in a case-by-case basis (Contact: **The NML Wastewater Team** **nmlwastewater.nmlwastewater@hc-sc.gc.ca**). Specific item checklists and considerations can be found in the later parts of this guide.

3.4 Develop a Strategy to Interpret and Use Wastewater Results

It is important to first acknowledge the limitations of wastewater testing. Results from wastewater testing represent an entire community. Individuals can only be diagnosed in a clinical setting and wastewater testing cannot replace routine clinical testing. Wastewater results can be impacted by several factors including the rate of wastewater flow, presence of interfering chemicals, and the handling and storage of samples.

When possible, information gathered from wastewater testing should be used along other community health indicators, such as clinical testing data. Some considerations are highlighted below and can be used for initial discussions:

- Who will have rights to the produced data? It is recommended that the community maintains ownership of data and that this is clearly defined in initial discussions.
- Who will be responsible for interpreting the health data for the community and providing public health recommendations?
- Will information from the wastewater program be combined with clinical test data or other community health indicators?
- How might these results be used to inform public health action? Possible responses may include community outreach, mask mandates, event testing, etc.
- How will the results be communicated with the community? A communication plan should be developed as outlined in Part 2.

Part 4 – Develop a Wastewater Sample Collection Plan

This section provides guidance for wastewater sampling from developing a sampling protocol to shipping samples for testing. It is composed of the following steps:

- ❑ 4.1 Identify Who Will Collect Wastewater Samples.
- ❑ 4.2 Select the Sampling Point and Method.
- ❑ 4.3 Develop a Sampling Plan.
- ❑ 4.4 Store and Transport of Samples for Testing.

4.1 Identify Who Will Collect Wastewater Samples

Wastewater sampling exposes individuals to raw sewage which poses an infection risk. Samplers should be trained personnel who are experienced in handling wastewater and have access to the sampling locations. For example, these individuals could be wastewater treatment plant operators or engineers, contractors, or other properly trained community members.

Training and Safety of Wastewater Samplers

Wastewater Samplers should be properly trained in collecting and handling wastewater samples safely. Standard safety practices should be followed according to the Canadian Occupational Safety and Health (OSH) and/or Canadian Biosafety Standard, which can include site-specific safe work procedures, handwashing, and wearing personal protective equipment (PPE). General safety guidelines when sampling wastewater can be found in Appendix B. Wastewater samples should be handled according to the best practices established in your region and in consultation with local experts.

4.2 Select the Sampling Point and Method

Sampling Point

The main goal of wastewater sampling is to collect a sample that covers as much of the community as possible, so choosing the sampling point is critical. Central collection points, such as lift stations, influent wet wells, or influent splitter boxes, are best since these locations have continuous wastewater flow and may include more of the community. It is also important to consider the safety and ease of access of the sampling point. Alternatively, in certain situations, communities may also choose to collect a sample to receive more targeted information from specific location like a long-term care facility or school.

Ideally, collection from stagnant wastewater (e.g., septic tanks, sewage trucks) is not recommended as they may only cover part of the community or be continually positive due to lack of continuous wastewater flow. If trucked sewage empties into a central location, it is preferrable to sample from the central location.

Sampling Method

There are two main types of wastewater samples:

1. A **grab sample** is a taken at a specific time each day – typically in the morning during periods of peak wastewater flow. This is the simplest wastewater sample to collect as it is a 'one and done' approach that may only require a bucket and rod. These samples can only provide a snapshot of the wastewater at the specific time and place the sample was taken.
2. A **composite sample** is collected slowly over a longer period of time (e.g., 24 hours), and can be thought of as wastewater 'sipping'. These samples are generally collected using an autosampler, but it can also be done by manually combining grab samples taken over a set period of time (e.g., 1 hour).

The recommended sampling method for routine wastewater testing is **using an autosampler to collect a 24-hour (or longer) composite sample.** This method offers for the most accurate overview of the community and prevents issues in results due to daily fluctuations in the wastewater flow. If this is not possible, it is recommended to instead collect **1-hour composite sample made by manually combining multiple grab samples**.

4.3 Develop a Sampling Plan

Sampling Protocol

Standardized sampling protocols for composite and grab sampling can be found in Appendix B. These documents have been authored by members of the Public Health Agency of Canada (PHAC), who can be consulted if there are any questions or concerns regarding these protocols. Note that autosampler setup and maintenance are not detailed here, instead refer to the manufacturer or PHAC for model-specific instructions.

Sampling Frequency

How often wastewater samples are collected and tested is a major factor in the quality and value of the test results. Collecting more often provides more detailed information on trends in the community. For these reasons, wastewater samples are generally collected **two or three times a week**.

Wastewater samples should be collected consistently on the same schedule (i.e., the same times and days). Collection date/time, sample location, and an estimate of total daily flow (if possible) should be recorded for each sample. If drawing a sample from a suction/sewage truck, the collection date and time, total load within the truck, and number of sites collected should be recorded for each sample.

Sampling Equipment Checklist

Equipment	Description
❑ PPE (Personal Protective Equipment)	Protective face mask/shield, proper attire, and disposable gloves. Depending on the site, rubber safety boots, and liquid-repellant coveralls, etc.
❑ Autosampler	For collecting automatic composite samples.
❑ 20L Buckets, Ropes, Rods, and Funnels	For collecting grab samples.
❑ Plastic Sampling Bottles	Bottles may be reused if properly disinfected between uses. If testing off-community, use 500 mL bottles. If testing in-community, smaller bottles may be used.
❑ Insulated Container and Cold Packs	For transportation.
❑ Label for Bottle	To record sample information.
❑ Shipping Supplies	Bottle seal, tape, resealable plastic bags, absorbent pads, packing paper, insulated shipping cold box, and flat ice packs.

4.4 Store and Transport of Samples for Testing

For most tests, **samples must be kept cold and should be tested within 7-10 days of collection**. Samples must be stored in a dedicated refrigerator at 4°C or be transported in an insulated container with cold packs after they are collected. Sample containers/bottles must remain upright to prevent leakage. If samples are being tested off-community, multiple samples may be taken throughout the week and be shipped in batches. A detailed protocol for shipping wastewater samples can be found in Appendix C.

Part 5 – Develop a Wastewater Testing Plan

This section describes the steps needed to begin in-community wastewater testing. This section does not apply to communities that choose to ship their samples elsewhere for testing. In-community wastewater testing requires a dedicated workspace that currently includes a GeneXpert system. Setup of this workspace can be broken down into the following steps:

- ❑ 5.1 Identify Testing Staff.
- ❑ 5.2 Set Up a Wastewater Testing Workspace.
- ❑ 5.3 Receiving Wastewater Samples.
- ❑ 5.4 Testing Wastewater Samples.
- ❑ 5.5 Send Results.

5.1 Identify Testing Staff

Training and Safety of Testing Staff

Although the GeneXpert-based wastewater testing procedure is relatively straightforward, it still requires operator training. Ideally, Testing Staff should have experience working with wastewater and/or basic laboratory equipment. For example, Testing Staff could be wastewater treatment plant operators, contractors, or Wastewater Samplers.

Raw sewage is a potential source of infection. It is essential that all Testing Staff be trained on how to work safety with wastewater samples and how to use basic laboratory equipment. Examples of general safety guidelines to follow when handling wastewater, as well as training materials for equipment required to test wastewater can be found in Appendix D.

5.2 Set Up a Wastewater Testing Workspace

Considerations for Testing Workspace Setup

A wastewater testing workspace is a dedicated space that is used to test wastewater samples. Ideally, the best space to set up this workspace is within an already established wastewater testing facility or laboratory. If such a space does not exist in-community, wastewater testing can be done in an atypical testing workspace. This space must still follow local guidelines and regulations for wastewater testing. This may include meeting sanitation requirements, having safety assessments, and meeting additional building requirements.

The following recommendations represent general good principles for setting up an atypical wastewater testing workspace. However these are guidelines that are always overruled by institutional, local, and regional regulations.

Testing Workspace Design Minimum Recommendations

A wastewater testing workspace must be designed to be a contained and safe space to test wastewater samples. To facilitate workspace setup, consult local authorities and guidelines for specific requirements. Below are some of the minimal requirements for an atypical workspace and an example workspace layout is shown in Figure 2.

Minimal Testing Workspace Checklist

Characteristic
❑ Space meets local guidelines and regulations.
❑ Floor that is non-porous and easily cleaned.
❑ Lockable door with controlled access.
❑ Temperature controlled.
❑ Good ventilation or added ventilation.
❑ 4 Standard (110V) power outlets (2 for the GeneXpert system, 1 for the centrifuge, and 1 for the dedicated refrigerator). May also consider a backup power supply.
❑ 2 Sturdy tables or benchtops with non-porous surfaces that are easily cleaned and at least 1.8m / 6ft long each.

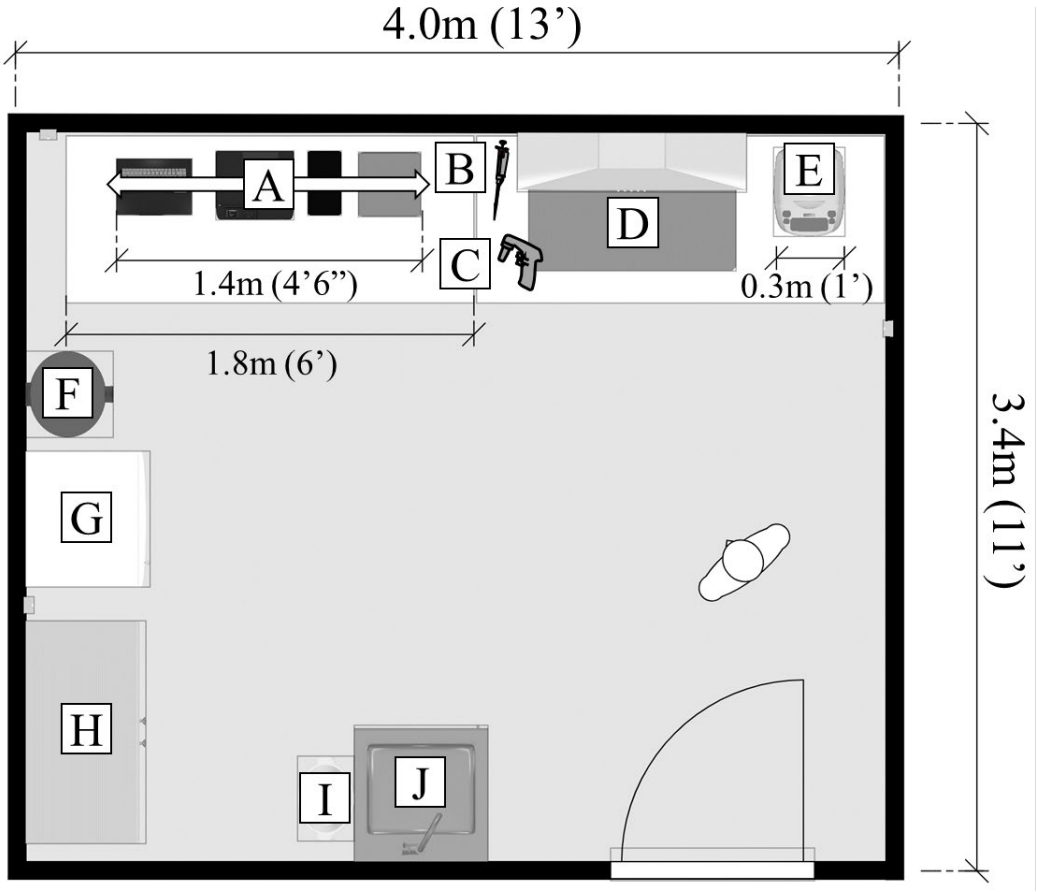

Figure 2. Example of a wastewater workspace layout

Minimal Testing Workspace Equipment Checklist

Equipment	Description
❑ A. GeneXpert System	Installed in a low-traffic area, next to the sample testing area, on a stable surface.
❑ B. Micropipette (P200)	To transfer liquid volumes from 20 µL to 200 µL.
❑ C. Serological Pipette Controller	To transfer liquid volumes from 1 mL to 50 mL.
❑ D. Designated Testing Area	Must be well ventilated to minimize contamination risk as wastewater can be aerosolized.
❑ E. Centrifuge	Installed close to the sample testing area on a stable surface.
❑ F. Storage for Biological Waste	Must be specifically for biological hazardous waste.
❑ G. Dedicated Refrigerator	To store wastewater samples.
❑ H. Storage for Testing Supplies	Must be accessible to Testing Staff.
❑ I. Eyewash Station	For rinsing eyes in case of biological splashes.
❑ J. Sink	For handwashing.

Installation and Maintenance of the GeneXpert System

Instructions for installation and maintenance of the GeneXpert system can be found in Appendix E.

5.3 Receiving Wastewater Samples

After receiving a wastewater sample, it must immediately be stored in a dedicated 4°C refrigerator. Sample containers must remain upright to prevent leakage.

5.4 Testing Wastewater Samples

Wastewater Testing Using the GeneXpert System

Wastewater testing should be performed as soon as possible while the sample is still fresh or ideally within 48 hours. The GeneXpert wastewater testing protocol takes approximately 2.5 hours with about 15 minutes of hands-on time. Multiple samples can be tested at a time. The detailed testing protocol can be found in the Appendix F.

Quality Control Considerations

Quality control measures help to provide reliable and consistent results. These measures may vary depending on local guidelines and type of testing workspace. Basic examples of quality control measures include proper staff training, routine cleaning, instrument maintenance and calibration, and record keeping. More basic recommendations regarding management and quality control can be found in Appendix D.

Waste Flow and Disposal

Biological waste must be disposed according to local guidelines. Consult with local institutions (e.g., facilities that handle wastewater, environmental/medical laboratories) about their hazardous waste disposal procedures.

5.5 Sending Results

Test results can be exported as a PDF report or a .csv file with additional information, a detailed protocol for exporting results can be found in Appendix F. Results must be sent to the designated individual(s) promptly after testing.

Part 6 – Confirm Results with External Laboratory

For in-community testing, results should initially be checked for 1-2 months by another laboratory to confirm that testing is working as expected – a process known as split sample testing. Split sample testing involves testing the same wastewater sample in-community and at a central facility (such as a Provincial or Federal Laboratory). The sample must be thoroughly mixed, evenly divided and cold shipped to the testing facility with enough volume for testing (approximately 500mL). The shipping process is detailed in Appendix C. Split sample testing provides confidence in future results and the results can be used to adjust/improve the program.

After this initial confirmation period, the program is considered established and may continue to test wastewater without split sample testing.

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Appendices

Appendix A – Supplies Required for Community-Led Wastewater Surveillance

- Sampling Supplies
- Shipping and Transport Supplies
- General Testing Supplies *If testing on-site*
- GeneXpert Testing Supplies *If testing on-site*

Appendix B – Sampling Protocol

- Safety Guidelines for Sampling Wastewater
- Wastewater Sampling Protocol

Appendix C – Shipping Protocol

- Equipment and Materials
- Packaging Protocol
- National Microbiology Laboratory Shipping Address

Appendix D – Basic Testing Standards

- Good Working Practice
- Micropipettes
- Serological Pipettes
- Centrifuges

Appendix E – GeneXpert Installation and Maintenance

- Where to Install the GeneXpert System
- GeneXpert System Components
- APC-UPS Installation Protocol
- GeneXpert Installation Protocol
- Creation of additional user accounts
- GeneXpert System Maintenance

Appendix F – Wastewater Testing Using the GeneXpert System Protocol

- GeneXpert Test Cartridge Precautions
- Wastewater Testing Using the GeneXpert System Protocol
- How to View and Report/Export GeneXpert Results

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Appendix A – Supplies Required for a Community-Led Wastewater Testing Program

Sampling Supplies

Equipment	Note
❑ Autosampler	Required for automatic sampling
❑ 20L Bucket, Rope, Extending Telescopic Rod with Pitcher	Required for grab sampling
❑ Refrigerator (Preferred) or Cold Box	
❑ Personal Protective Equipment	As per local regulations
❑ Permanent Marker	

Consumable Supplies	Note
❑ Plastic Sampling Bottle (500 mL)	1 used per sample
❑ Label for Bottle	1 used per sample

Shipping and Transport Supplies

Equipment	Note
❑ Refrigerator (Preferred) or Cold Box	
❑ Freezer	
❑ Personal Protective Equipment	As per local regulations

Consumable Supplies	Note
❑ Testing Requisition Form	1 per sample
❑ Bottle Seal or Parafilm Strip	1 per sample
❑ Re-sealable Plastic Bag	1 or 2 per sample
❑ Absorbent Pad	1 per shipment
❑ Flat Ice Packs	2 or 3 per shipment
❑ Insulated Shipping Cold Box	1 used per shipment
❑ Brown Kraft Packing Paper	
❑ Bubble Wrap	
❑ Packing Tape	

General Testing Supplies (*If testing in-community)*

Equipment	Note
❑ Personal Protective Equipment	As per local regulations
❑ Spray Bottles for Disinfectant	

Consumable Supplies	Note
❑ Gloves	
❑ Disposable Paper Towels or Kimwipes	
❑ Absorbent Bench Liner	
❑ Biohazard Waste Bags	

GeneXpert Testing Supplies (*If testing in-community)*

Laboratory Instruments	Note
❑ Centrifuge	Must be able to spin 50mL conical tubes
❑ GeneXpert System • APC – Uninterrupted Power Supply (UPS) • Includes a Laptop, Scanner, and Printer	

Equipment	Note
❑ Benchtop Biohazard Waste Bag Holder	
❑ Pipette Controller	
❑ Large Conical Tube Rack	
❑ Microcentrifuge Tube Rack	

Consumable Supplies	Note
❑ 50mL Conical Tubes – Falcon	1 per sample
❑ 25mL Sterile Serological Pipettes	2 per sample
❑ P200 Pipette Tips (96 tips/box)	2 per sample
❑ 2.0mL or 1.5 mL Microcentrifuge Tube	1 per sample
❑ Amicon Ultra-15 10-KDa Centrifugal Filter	1 per sample
❑ Nuclease-free Water	200µL per sample
❑ 10% v/v Tween-80	40µL per sample
❑ GeneXpert Express Single Use Pipette	1 per sample
❑ GeneXpert Test Cartridge (10 tests/box)	1 test per sample

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Appendix B – Wastewater Sampling Protocol

Contents

Safety Guidelines for Sampling Wastewater

The following section describes general good principles when handling wastewater, however, these are always overruled by local guidelines. Consult local guidelines to ensure sampling personnel are following location specific safety guidelines when sampling wastewater.

Strict adherence to ideal safety standards may not be possible while working with wastewater in remote locations, however, understanding the principles of good safety practices is essential to create a safe work environment for all sampling personnel.

Basic Safety Guidelines

Safety guidelines for handling wastewater adapted from Wastewater Sampling for Epidemiological Surveillance (Environment and Climate Change Canada)
Conduct a risk assessment/hazard analysis of the site prior to starting work to identify hazards which may lead to injury and implement mitigating measures.
At all times, follow all safety procedures, whether pictorial, written, or verbal.
Workers must receive training on disease prevention/mitigation including information on good hygiene practices, use and disposal of personal protective equipment (PPE), and proper handling of wastewater.
Clean and disinfect sample containers after sample collection to avoid any possible transmission of potential hazardous.
Sampling personnel should receive vaccinations that are in-line with recommendations for workers exposed to wastewater in consultation with local health authorities. Vaccines (e.g., Tetanus) should be up to date.
Samplers should promptly seek medical attention if displaying signs or symptoms of infection (e.g., vomiting, stomach cramps, and watery diarrhea).
Avoid splashing wastewater and, if possible, minimize exposure time in areas where splashing may occur. Always wash hands with soap and water immediately after handling wastewater.
Avoid touching the face, mouth, eyes, nose, open sores, or cuts while handling wastewater.
Do not smoke, eat, or drink while handling wastewater.
Cover open sores, cuts, and wounds with clean, dry, waterproof bandages and further protected with PPE.
Consult with a physician prior to engaging in a task if you suffer from any previous medical conditions that could be worsened by exposure to wastewater or other hazards present.

Personal Protective Equipment Use Guidelines

PPE must be available and used in alongside proper training to prevent injury and avoid self-infection. Wash hands with soap and water immediately after removing PPE. Keep contaminated reusable PPE in a leakproof bag for washing or disinfecting when required.

Minimal recommended PPE
❑ Splash-proof face shield that protects the eyes, nose, and mouth from potential splashes
❑ Protective face mask to prevent inhalation of aerosols
❑ Liquid-repellent coveralls, rubber safety boots, and waterproof gloves to protect against contamination of clothing and body

Wastewater Sampling Protocol

Sampling Equipment and Materials

Equipment
❑ Personal Protective Equipment (PPE)
❑ Refrigerator or Insulated Cold Box (to store wastewater samples post-collection)
❑ Autosampler*
❑ 20L Bucket, Rope, Extending Telescopic Tod with Pitcher*

*Dependant on sample collection method.

Materials
❑ Plastic Sampling Bottles
❑ Label for Bottle
❑ Permanent Marker

Testing Requisition Form

Please complete the provided requisition form prior to sampling if the samples are to be shipped to the National Microbiology Laboratory for testing.

Sampling Protocol

A. On the sampling day, label the sampling bottle using a permanent maker with the following information:
    a. Where the sample was collected.
    b. The date and time of collection.
    c. The type of sample collected (e.g., influent, effluent etc.).
B. Wear PPE as per the sampler's training and/or existing community protocols and policies.
C. Remove the sampling bottle lid. Ensure it is protected from contamination.

D. Fill the sampling bottles just below the shoulder according to sampling method chosen. **Do not fill the bottle completely to prevent issues upon freezing/storage.**

   a. **Composite sample from an autosampler**:

      i. If the autosampler **collects composites samples:** directly fill the sampling bottle just below the shoulder (approximately 500mL). Composite samplers need to purge following the collection of each discrete sample to prevent accumulation of solids in the sampler tubing.

      ii. If the autosampler **collects individual fractions** in separate tubes: combine sample fractions into a bucket, close lid, and mix the fractions well. Fill the sampling bottle just below the shoulder (approximately 500mL).

      iii. If the autosampler **collects a single large fraction:** gently swirl the sampling vessel and mix well. Fill the sampling bottle just below the shoulder (approximately 500mL).

   b. **Composite grab sample from the wastewater flow or a lift station**:

      i. Using a bucket, draw samples of approximately the same volume (1L) from the flowing wastewater every 10 minutes for an hour (approximately 6L total).

      ii. Seal the lid and mix the sample well to ensure that suspended solids are thoroughly mixed.

      iii. Once mixed, draw a subsample by filling the sampling bottle just below the shoulder (approximately 500mL).

E. Place the sampling bottle's lid back on and firmly close the lid.

F. **Samples must be kept cold** by placing them in a designated refrigerator or in a cold box during collection, storage, and transportation.

G. Clean the sampling equipment:

   a. Rinse all sampling equipment with water before disinfection (e.g., buckets, grab samplers, and funnels). Equipment may be disinfected using a 10% household bleach solution. Contaminated items should remain wet with the bleach solution for at least one minute before rinsing with water.

H. Remove PPE and wash hands using soap and water after sampling and equipment cleanup.

Appendix C – Sample Shipping Protocol

Contents

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Wastewater Shipping Protocol

This protocol provides guidance for packaging wastewater samples to be shipped. The shipment of wastewater in Canada is not regulated under the Transportation of Dangerous Goods (TDG) Act. In an abundance of caution, the following shipping practices are drawn from the regulations governing the transport of UN3373 Category B dangerous goods.

To minimize spills, sample lids must be sealed with a bottle seal. Additionally, "double bagging" the sample with a primary and a secondary resealable bag as well as using absorbents can help contain spills. Cold packs are the preferred method of controlling the temperature. The use of wet ice is discouraged but, if necessary, should be contained within a sealed double bag. Sample bottles are kept upright inside the box with packing paper or cold packs. Finally, ensure directional "up" arrows are visible on the outer packaging.

Shipping Equipment and Materials

Equipment
❑ Personal Protective Equipment
❑ Refrigerator or Insulated Cold Box (to store wastewater samples post-collection)
❑ Freezer (to freeze cold packs)
❑ Permanent Marker

Materials
❑ Testing Requisition Form
❑ Bottle Seal, Parafilm Strip, or Tape
❑ Re-sealable Plastic Bags
❑ Absorbent Pads
❑ Flat Ice Packs
❑ Insulated Shipping Cold Box
❑ Brown Kraft Packing Paper
❑ Bubble Wrap
❑ Packing Tape

Packaging Protocol

A. Check that the broken sterility sticker is not obstructing the seal. Then, seal the bottle by stretching and wrapping one parafilm strip or alternative bottle seal along the bottle lid and body. The film should be wrapped in the direction as if tightening the lid.

Figure 3. Stretching and applying the parafilm along the bottle lid

B. Place the sealed bottle(s) into the first resealable bag between the absorbent strips. Fold over the top of the bag and remove as much air as possible before fully sealing the bag closed.

Figure 2. One or two bottles in primary resealable bag with absorbent strips.

C. Place the sealed bag with bottle(s) into the second resealable bag. If shipping a single bottle, put a bubble wrap “buddy” roll inside the secondary bag to keep the sample upright.

Figure 3. Double bagged single bottle and a buddy roll (left) and two bottles bagged (right)

D. Place the double-bagged sample(s) standing upright with lid at the top, then fill the void space within the cold box with flat ice packs and packing paper to minimize movement.

Figure 4. Arrangement of ice packs in the cold box

E. Cover the cold box with the provided Styrofoam lid, place the completed testing requisition form on top, and then seal the outer cardboard with tape.

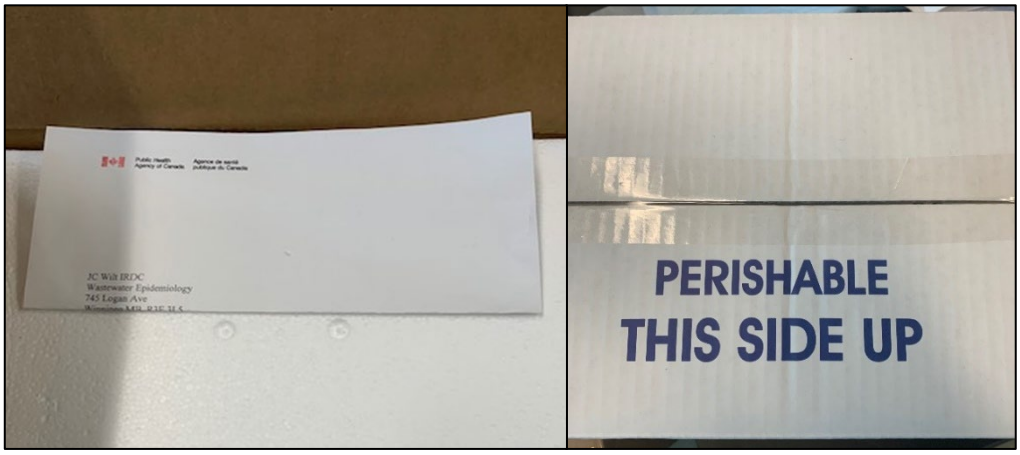

Figure 5. Placement of requisition form in cold box and sealing of outer box.

National Microbiology Laboratory Shipping Address

Arrange for express shipping to the address below:

ATTN: Dr. Chand S. Mangat
Public Health Agency of Canada
JCWIDRC rm J1103
1015 Arlington St.
Winnipeg, MB, R3E 3R2
Tel: 204-789-6508

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Appendix D – Basic Testing Guidelines

Contents

Good Work Practices

This section describes general guidelines to work safely and effectively while testing wastewater. This includes basic working safety guidelines, aseptic (clean) working techniques, general guidelines for handling and testing wastewater samples, as well as workspace management.

Strict adherence to ideal safety standards may not be possible while working with wastewater. Regardless, an understanding the principles of good working practices is essential to ensure a safe working environment for all testing personnel.

Assessing Hazards and Reducing Risk

The purpose of safety practices and controls in a workplace is to reduce risk. This is especially important when working with potentially infectious materials such as wastewater. Potential hazards should be assessed while following the Hierarchy of Controls to reduce the risks associated with the hazard:

1. **Elimination**: The hazard is removed from the scenario.
2. **Substitution**: The hazard is replaced with a lower risk option that still retains the required properties of the original hazard.
3. **Engineering controls:** Physical changes to materials, workstations, and/or other relevant aspects of the work environment to prevent/reduce hazard exposure.
4. **Administrative controls:** Policies and guidelines used to control risks/hazards.
5. **Practices and procedures:** Activities/processes that have been demonstrated to be effective in risk reduction.
6. **Personal protective equipment (PPE):** Equipment/garments worn by the individual to protect against hazard exposure.

Training Personnel

All testing personnel must receive training on disease prevention including information on basic hygiene practices, use and disposal of PPE, and proper handling of wastewater. This training must meet the local guidelines for personnel who handle wastewater samples.

General Safety and Good Microbiological Practices

Good microbiological practices are techniques and practices that aim to minimize the contamination of handled material into the environment. Following these procedures also provide a basic level of protection to the individual worker. These practices can be found on the next page.

Good Microbiological Practices adapted from The Canadian Biosafety Standard
Eating, drinking, smoking, applying cosmetics, handling contact lenses, storing food or utensils in the work area is strictly prohibited.
Hair that may become contaminated while working should be restrained (e.g., tied back or fastened with a clip) or covered.
Jewellery (e.g., rings or long necklaces) that may come in contact with biological material or that may puncture protective gloves should not be worn while working.
Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings.
Workstations (e.g., benchtops) should be kept free of clutter to avoid cross-contamination and to facilitate cleaning and disinfection.
All personnel, including visitors, should wear suitable footwear (e.g., shoes that cover the entire foot with no or low heels) and PPE (e.g., lab coats, aprons, gloves, protective eyewear) appropriate to the procedure.
Personal belongings (e.g., purses, bags) and street clothing (e.g., coats, boots) should be stored separately from PPE and from workstations where biological material is handled.
Aseptic (clean) techniques should be used when manipulating open samples to provide basic containment and quality control.
Work surfaces should be cleaned and decontaminated using a suitable disinfectant and an appropriate contact time after work is complete.
All items that come into contact with biological material, including liquid or solid wastes, should be decontaminated before disposal or reuse. Hands should be washed with soap and water or otherwise disinfected after handling specimens that contain microorganisms, immediately after removing gloves, and before leaving the work area.
Disposable gloves should be discarded after use and never reused; all contaminated clothing and PPE should be decontaminated before laundering when a known or suspected exposure has occurred.
PPE should be removed in a manner that minimizes the spread of contamination to the skin and hair.
Procedures for the safe use of sharp objects should always be followed (e.g., avoid use whenever possible, use safe alternatives or safety-engineered sharp devices, avoid bending, shearing, breaking, or recapping needles, and discard used sharps in a puncture-resistant sharps container).

Guidelines for Handling and Testing Wastewater Samples

In a wastewater testing workspace, the primary exposure risk is to wastewater that may be contaminated with disease-causing microorganisms, including SARS-CoV-2. As this risk cannot be eliminated or substituted, other measures must be put into place to reduce its severity. Recommended guidelines for safely handling wastewater samples include the following:

Safety guidelines for handling wastewater adapted from Wastewater Sampling for Epidemiological Surveillance (Environment and Climate Change Canada)
All work performed with the wastewater should be in (a) designated area(s) with adequate ventilation that can be cleaned or disinfected frequently. The area(s) must be disinfected before and after testing wastewater sample(s) with an appropriate disinfectant (as per local regulations).
Avoid/minimize aerosolizing wastewater.
Ensure ventilation systems are functioning properly in areas where wastewater may be aerosolized, and chemicals are used.
Avoid touching face, mouth, eyes, nose, or open sores and cuts while handling wastewater.
Always wash your hands with soap and water before eating, drinking, or using the toilet.
Do not smoke, eat, or drink while handling wastewater.
Sample containers should be cleaned and disinfected after sample collection and testing to avoid transmission of potential hazardous materials during subsequent handling/analyses.
Pipette tips should be rinsed with an appropriate disinfectant after transferring wastewater.
Personnel should receive vaccinations that are in line with recommendations for workers exposed to wastewater. Local health authorities should be consulted. Vaccines should be kept up to date (e.g., Tetanus).
Consult a physician before engaging in the task involving wastewater if there are any prevailing medical conditions.
Personnel should promptly seek medical attention if displaying any signs or symptoms of diarrhea (e.g., vomiting, stomach cramps, and watery diarrhea).

Minimal PPE recommended when testing wastewater:
❑ Protective body covering (e.g., lab coat or gown).
❑ Splash-proof face shield or safety glasses to protect eyes, nose, and mouth.
❑ Protective face mask to prevent the inhalation of aerosols.
❑ Gloves (double gloving is recommended when handling wastewater).

Workspace Management

Good workspace management is essential for quality and timely results. Below are examples of basic practices for effective workspace management:

Inventory Management
❑ Refrain from using expired chemicals, reagents, plastic consumables, PPE, and kits.
❑ Store chemicals and supplies in a secure location with proper storage conditions (correct temperature, humidity, etc.).
❑ Keep a sufficient reserve of consumable testing supplies (pipette tips, Falcon tubes, gloves etc.) for approximately 1 month of testing or more.
❑ Request/order additional supplies ahead of shortages.

Instrument Maintenance
❑ Ensure personnel are trained to use equipment properly.
❑ Disinfect equipment that is exposed possibly infectious materials on a regular basis (before and after testing samples etc.).
❑ Refer to manufacturers' manuals for specific ways to set up and maintain equipment and instruments.

General Cleanliness
❑ Schedule regular (month, bimonthly, etc.) cleaning duties.
❑ Maintain general organization of supplies and workspace.
❑ Wash gowns/coats and other PPE regularly.

Record Keeping
❑ Make backups of GeneXpert data on another device.
❑ Document daily working events such as receiving and testing samples.

Micropipettes

What is a Micropipette?

Micropipettes are precision instruments used to transfer small volumes of liquids (< 1 mL) (Figure 1.). These pieces of equipment may be easily damaged if used incorrectly.

There are 7 main components of a micropipette:

1. Push button/plunger
2. Tip ejector button
3. Thumbwheel
4. Volume display
5. Main body
6. Tip ejector
7. Tip holder

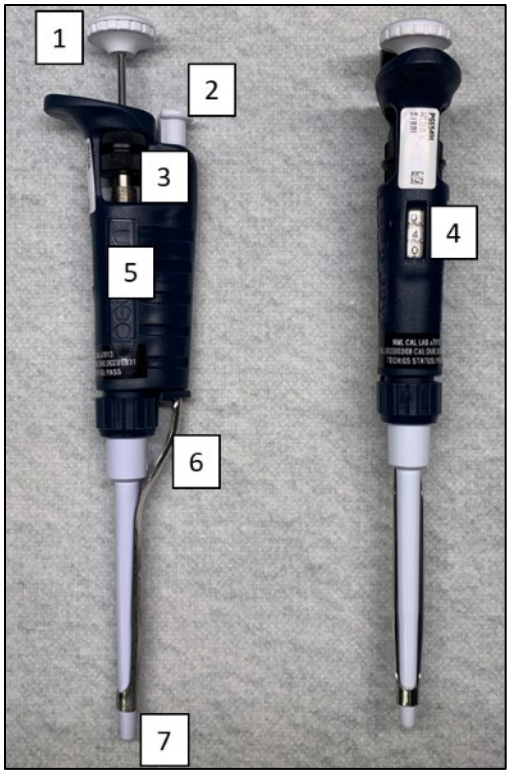

Figure 1. Components of a micropipette

Setting the Volume on a Micropipette

The volume display (Figure 1., #4) is made up of three vertically stacked numbers (Figure 2.). These numbers are read from top to bottom.

To change the pipette's volume, you will need to turn the thumbwheel clockwise (decreases the volume) or counter-clockwise (increases the volume) to the desired value (200 µL or 40 µL).

The maximum volume that may be transferred with a P200 pipette is 200 µL and the lowest is 20 µL; **NEVER turn the pipette past these values as this may damage the pipette.**

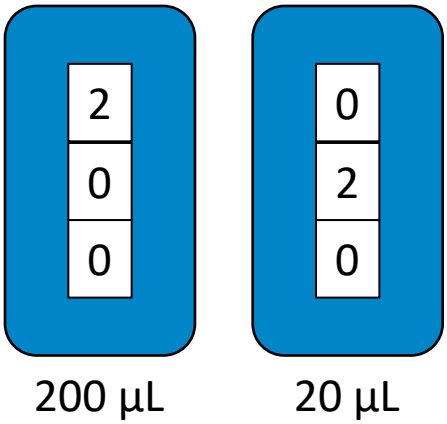

Figure 2. Display of the minimum and maximum volumes on a P200 micropipette

How to Use a Micropipette

1. Using your dominant hand to hold the pipette by its body (Figure 1., #5), fit the tip onto the pipette by pushing the tip holder (Figure 1., #7) into a pipette tip with a slight twisting motion.
2. Press the push button (Figure 1., #1) to the first position/stop.
3. With the pipette held vertically, immerse the tip into the liquid ready to be transferred.
4. Allow the push button to return slowly to the starting position. This will cause the liquid to enter the tip of the pipette.
5. While the plunger is still in the starting position, insert the filled tip inside the wall of the desired tube at a 10° to 40° angle. Once in the tube, slowly expel the liquid into the new tube by pressing down on the push button to the first position.
6. Move the tip above the dispensed liquid and press the push button to the second position to remove any residual liquid from the tip.
7. Remove the tip from the newly filled tube and then slowly guide the push button back to the starting position. Close the tube. Eject the used tip into a waste container by firmly pressing the tip ejector button (Figure 1., #2).

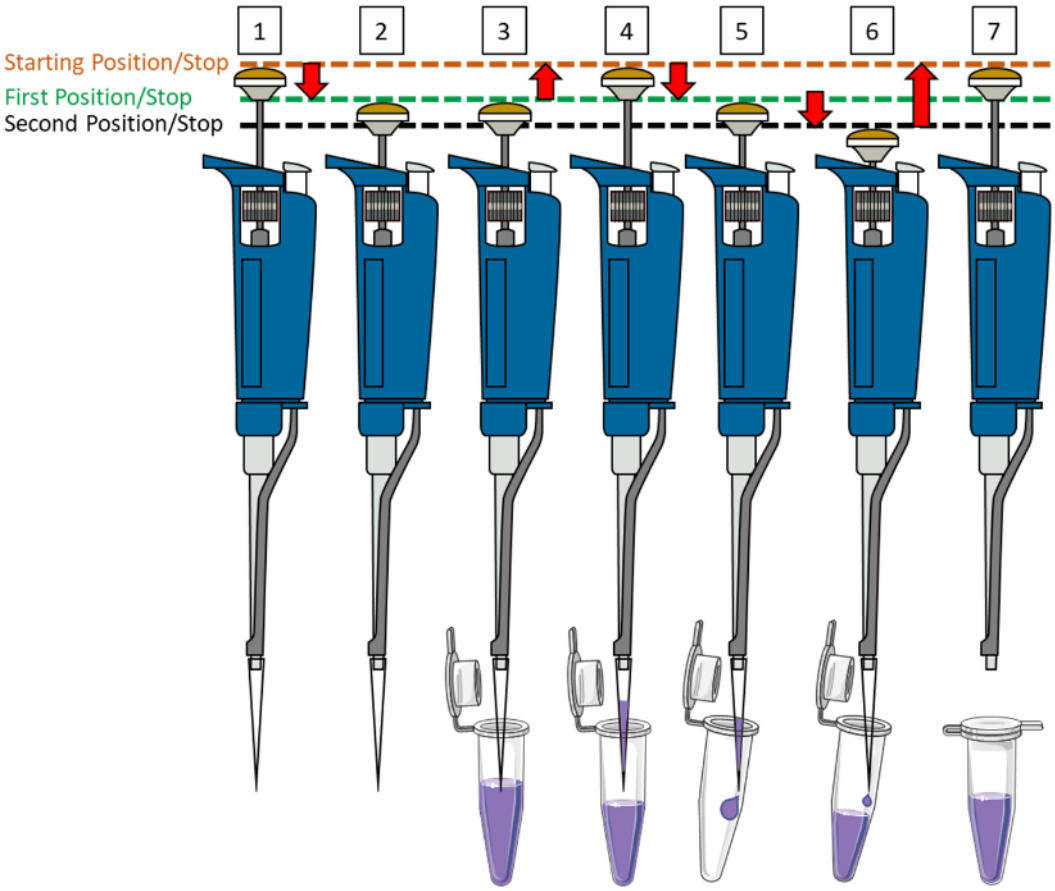

Figure 3. How to use a micropipette

Serological Pipettes

What is a Serological Pipette?

Serological pipettes are used to transfer large volumes of liquid (> 1 mL) (Figure 4.). A serological pipette has two sets of evenly spaced lines on its side; one set counts up to the maximum volume and the other counts down to the minimum volume.

The liquid's volume is read at eye level from the bottom of the meniscus (the curve at the surface of the liquid; Figure 5.). The total volume of liquid that is taken up in a serological pipette must be dispensed to ensure the correct volume. This includes any liquid that may remain in the tip.

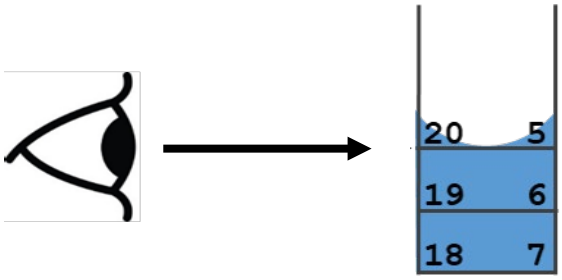

Figure 5. Reading a pipette from the bottom of the meniscus (curve at the surface of the liquid)

Best Practices When Using a Serological Pipette

Wrapped plastic serological pipettes are sterile (clean) and single use; once the pipette is removed from its wrapper, it should not be placed on a dirty surface.

To reduce the risk of contamination, handle the pipette by the top portion once it is out of its wrapper.

The serological pipette should remain upright when containing liquid to prevent leakage into the pipette controller.

The serological pipette may only be used multiple times if transferring the same liquid into the same vessel immediately after the first transfer.

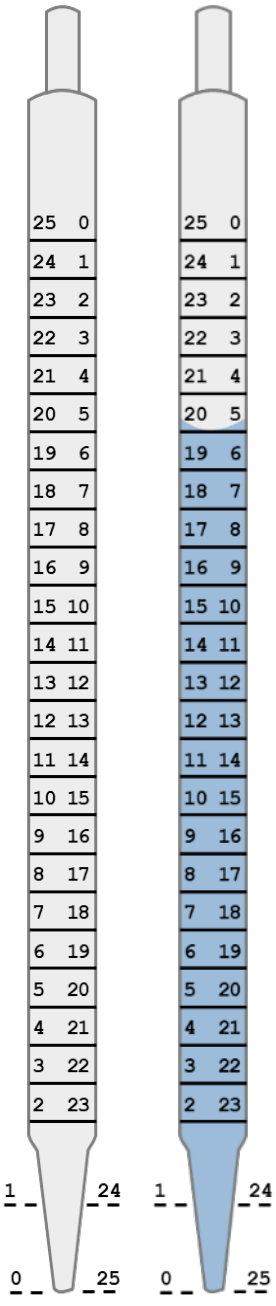

Figure 4. A 25 mL serological pipette, empty (left) and filled to 20 mL (right)

Electric Pipette Controllers

Pipette controllers allow for easy and accurate transferring of liquids with pipettes (Figure 6.). The controller must be charged by plugging into a wall power outlet. Pressure applied to buttons changes how fast the liquid is drawn up/dispensed from the pipette.

Components of a generic electronic pipette controller include:

1. Filling/liquid drawing button
2. Dispensing button
3. Nosepiece, where the top of the serological pipette is inserted.
4. Speed and mode controls (if applicable)
5. Charging socket (bottom of the unit)

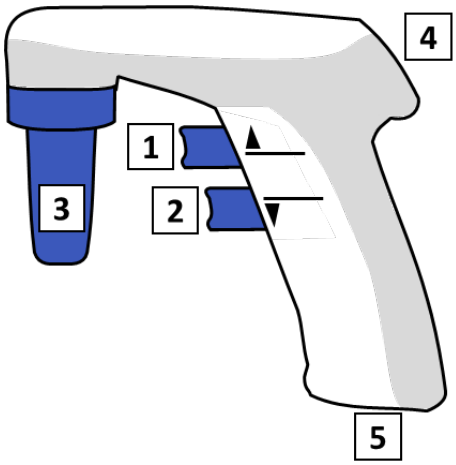

Figure 6. Electronic pipette controller

How to Use an Electronic Serological Pipette Controller

1. Ensure all containers are open and easy to access.
2. Unwrap the serological pipette from the wrapper and attach it to the controller by pushing and twisting the top end until it is fully inserted.
    - **Do not use excessive force when inserting the serological pipette as it may crack.**
3. Rotate the pipette gently so the measurement marks with the largest volume at the top faces you.
4. Immerse the serological pipette tip approximately two thirds into the container with the liquid to be transferred.
5. Gently push the top button on the controller to draw the liquid up into the pipette. Ensure the serological pipette tip stays immersed in the liquid to draw up the correct volume.
    - Adjust to the desired volume by switching between the two controller buttons.
6. Remove serological pipette from the container and cover the container with its lid.
7. Transfer the filled serological pipette into the target container and hold the tip on the wall of the container at a slight angle.
8. Dispense the liquid slowly by pressing the bottom button gently.
    - Avoid dispensing liquid with the tip immersed into liquid as this can make bubbles and aerosols.

OPTIONAL

Repeat steps 4 to 8 if transferring the same liquid into the same vessel right after.

9. Ensure all the liquid in the pipette is dispensed and the pipette is empty by fully pressing the bottom button.

10. Remove the serological pipette from the controller by twisting it down gently and dispose of it into an approved waste container.

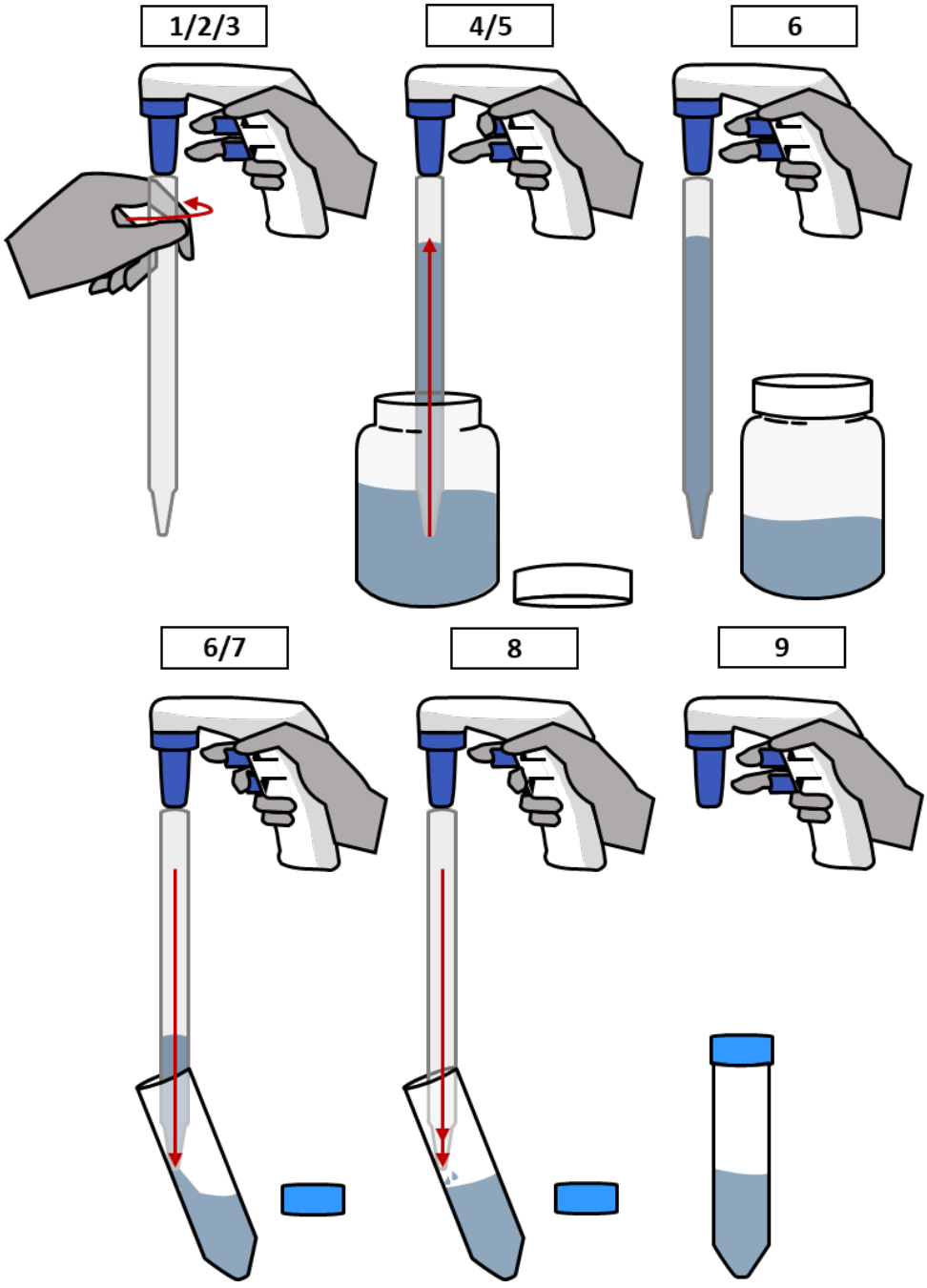

Figure 7. How to use an electronic pipette controller

Centrifuges

What is a Centrifuge?

Centrifuges are used to separate components in a fluid based on density. Centripetal force is generated through a rapidly spinning rotor which separates the denser components of a liquid. This leaves a visible “pellet” at the bottom of the sample tube.

A swing-bucket rotor has notches that fit metallic buckets. White plastic adaptors are inserted into each bucket allowing the correct size of sample tubes to fit securely. Rough handling or mixing the sample tube after centrifugation can dislodge the pellet and undo the desired separation. If this occurs, repeat the previous centrifugation step.

The centrifuge manual contains maintenance instructions and information specific to the model of the centrifuge.

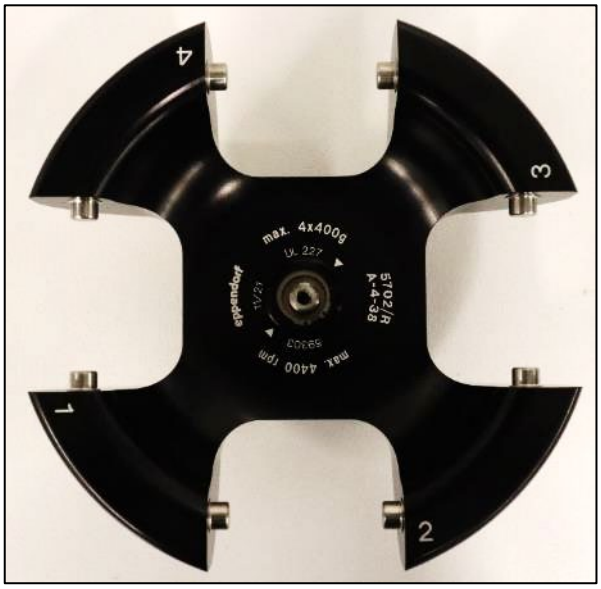

Figure 8. Centrifuge motor

Figure 9. Bucket with a 50mL falcon tube adaptor

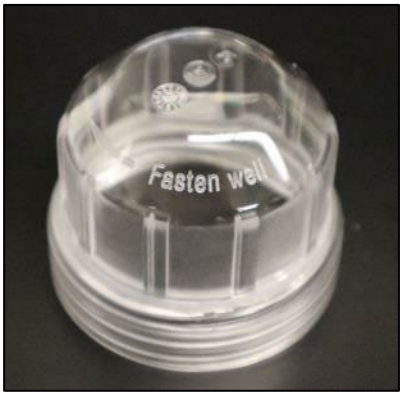

Figure 10. Aerosol cap for centrifuge bucket

Figure 11. Aerosol cap attached to centrifuge bucket

Symmetric and Balanced Loading of a Centrifuge

Centrifuges **must be balanced** to ensure the rotor spins properly (Figure 12.).

All buckets with adaptors must be put in the rotor even if they do not contain a sample tube.

The rotor buckets and the volume in the sample tubes must be placed symmetrically in the centrifuge in respect to the centre of the rotor (Figure 12.).

If there is an odd number of sample tubes, a "**blank**" must be placed opposite of the odd tube to balance the centrifuge.

- A blank is made using the same type of sample tube filled with a volume water that will weigh the same as the opposing sample tube; should be within one gram of the odd sample's weight.
- Blanks may be kept and reused but must be replaced periodically or when damaged.

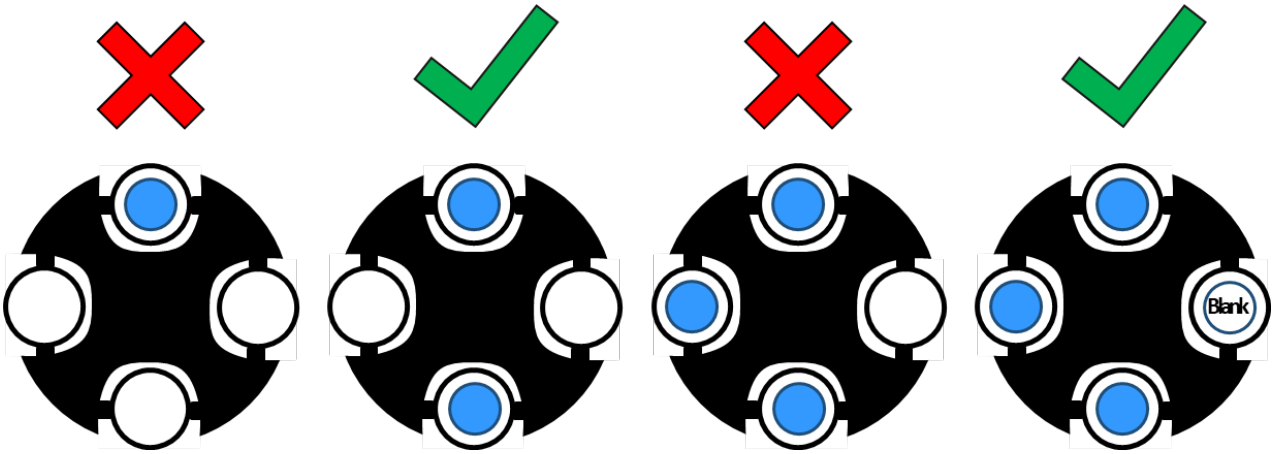

Figure 12. Correct placements of sample tubes (blue) in a four-place centrifuge rotor

How to Use an Eppendorf 5702 Centrifuge

1. Turn the centrifuge ON by using the power switch found on the back of the machine.
2. Press the OPEN button to release the lid.
3. Check that the rotor is secure and properly set.
    - If required, tighten the rotor using the rotor key (included with the instrument).
4. Insert the adaptors into all the buckets and then load each of the tightly sealed sample tubes.
5. Attach the aerosol-tight caps to all buckets (even if there is no sample tube in it).
6. Place all buckets in the rotor by aligning the two notches on the sides of the buckets into the corresponding notches on the rotor.
7. **Ensure balanced sample tubes are placed opposite of each other in the rotor.**
    - If required, tighten the rotor using the rotor key (included with the instrument).
    - If there is an odd number of sample tubes, place a blank on the opposing side of the sample tube without a pair.
8. Gently close the lid. It will seal automatically, and you will hear an audible “click” when it is set into place.
9. Set the desired time and speed by turning the respective knobs.
    - Assure that the centrifuge is in RCF and **NOT** RPM by pushing the blue “Speed” nob; the screen should display RCF.
10. Press the time knob to begin the centrifuge run.
11. During the run, press the time knob to end the run early.
    - Check for excess noise and shaking as the rotor begins to speed up. If this occurs, stop the centrifuge, and ensure the sample tubes are balanced and rotor is secure.
    - If the centrifuge runs too long while unbalanced or with its rotor dislodged, a sample tube may break, and a spill may occur. A spill in a centrifuge can create aerosols that may need time to settle before cleaning.
12. The centrifuge lid will unseal automatically and play an “alert” once the time has elapsed.
13. Remove the sample tubes carefully and keep them upright to prevent dislodging the pellet.
14. Insert the sample tubes into a conical tube rack.

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Appendix E – GeneXpert Installation and Maintenance

Contents

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GeneXpert System Installation

Where to Install the GeneXpert System

The GeneXpert system should ideally be installed in a low traffic area of the room, next to the sample preparation area on a stable, and non-porous surface. It should be installed away from vents and direct sunlight with 5-10cm of clearance around the instrument. The instrument must be near a standard (110V) power source with a standard wall outlet with two available plugs.

GeneXpert System Components
A. GeneXpert Instrument
B. APC – Uninterrupted Power Supply (UPS) B1. Main Battery Unit B2. Secondary Power Unit
C. Laptop
D. Scanner
E. Printer

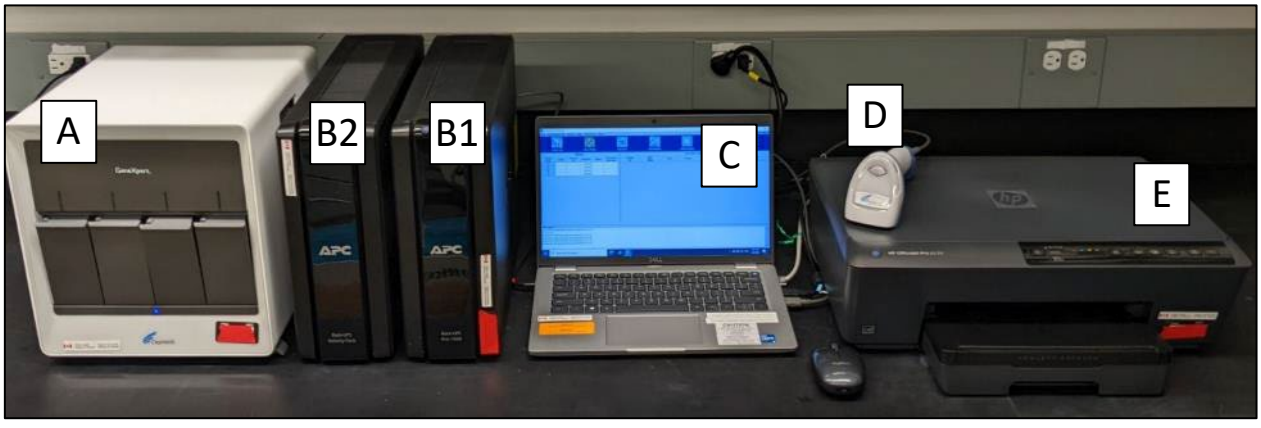

Figure 1. GeneXpert® System

APC – UPS Installation Protocol

Caution – the APC-UPS is very heavy (approximately 60 lbs.)

The APC – UPS provides back-up power if there is a temporary power-outage (Figure 2. and Figure 3.). For transportation purposes, the battery is shipped in an inactive state. Before first use, the battery pack of the *Main Unit* must be connected to enable battery power.

Figure 2. Back – UPS Pro 15000: Main battery unit

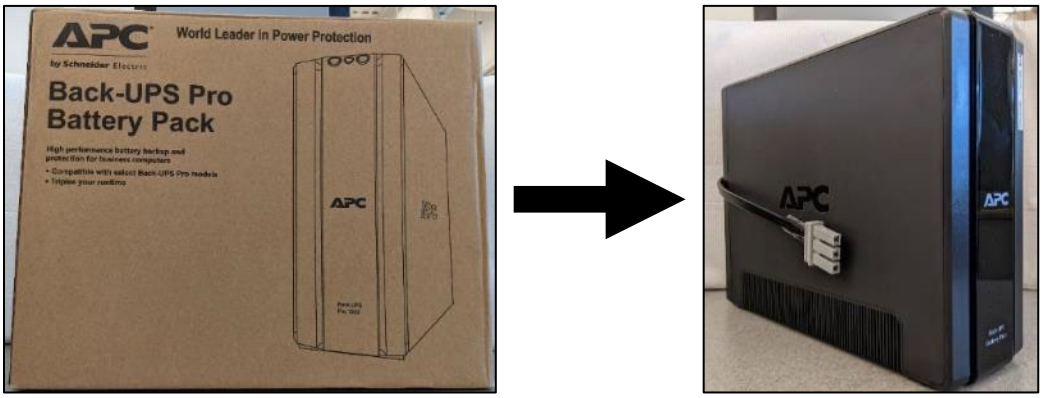

Figure 3. Back-UPS battery pack: secondary unit to connect to main unit

A. On the *Main Unit*, locate the yellow sticker and place the unit on its side with the yellow sticker facing up.

B. Locate the removable panel and slide the panel up to reveal the battery.

C. Lift and pull both tabs at the same time to remove the battery.

D. Flip the battery over so that the green side is facing upwards and insert the battery back into the unit as indicated by the “INSERT THIS WAY” arrows on the label.

E. Firmly push the battery pack all the way back into the slot.

F. Re-install the panel and return the main unit to an upright position.

G. Battery power of the main unit is now active.

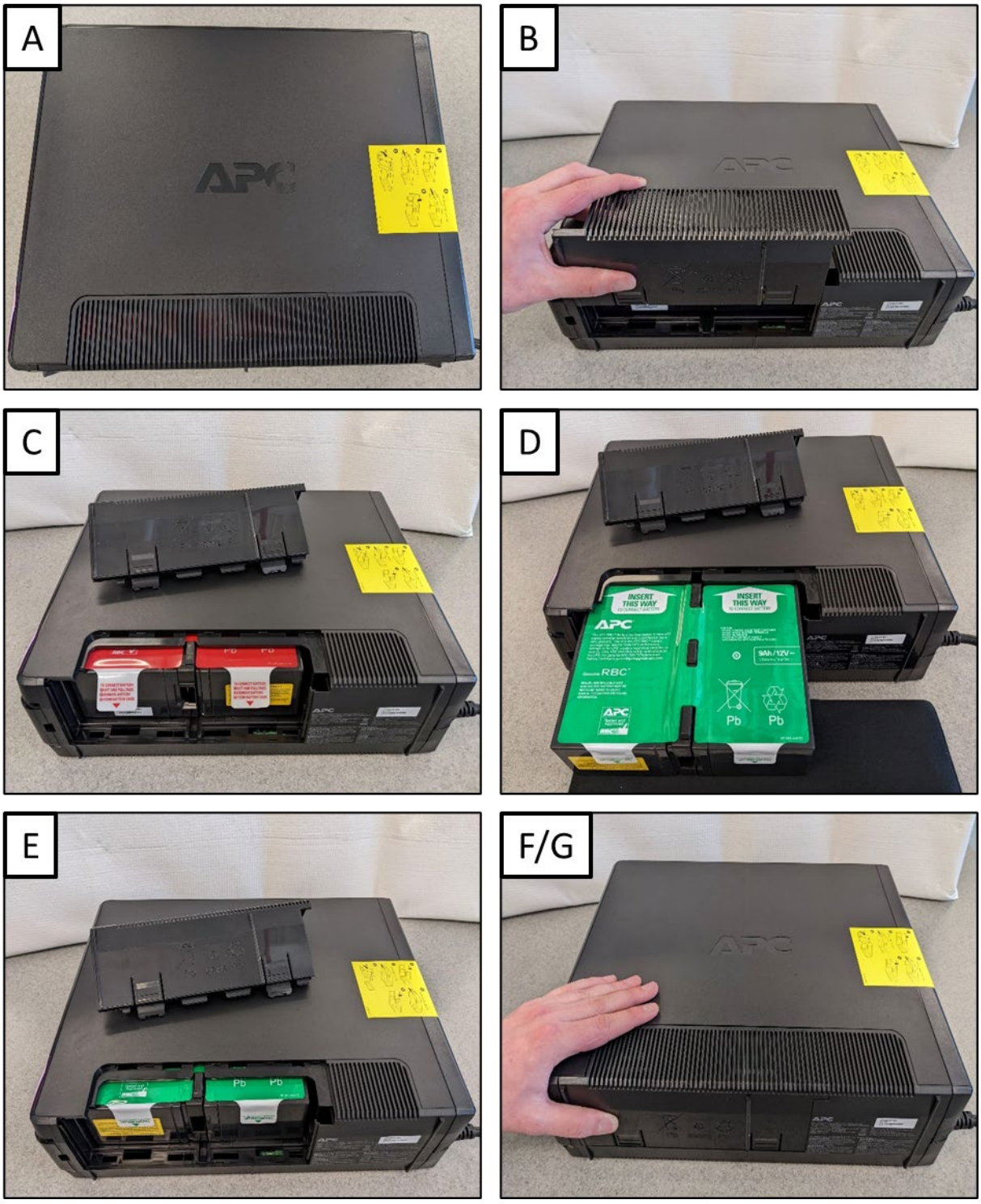

Figure 4a. Steps to connect APC – UPS

H. Locate the connector cable at the back of the *Secondary Unit*.

I. Plug the connector into the *Main Unit*.

J. Plug the *Main Unit* into a wall power outlet.

   a. *Main Unit* must have its own wall plug to remain fully charged.

K. Press the power button to turn on the APC – UPS.

   a. Battery symbol should indicate fully charged.

L. The APC – UPS is ready for use.

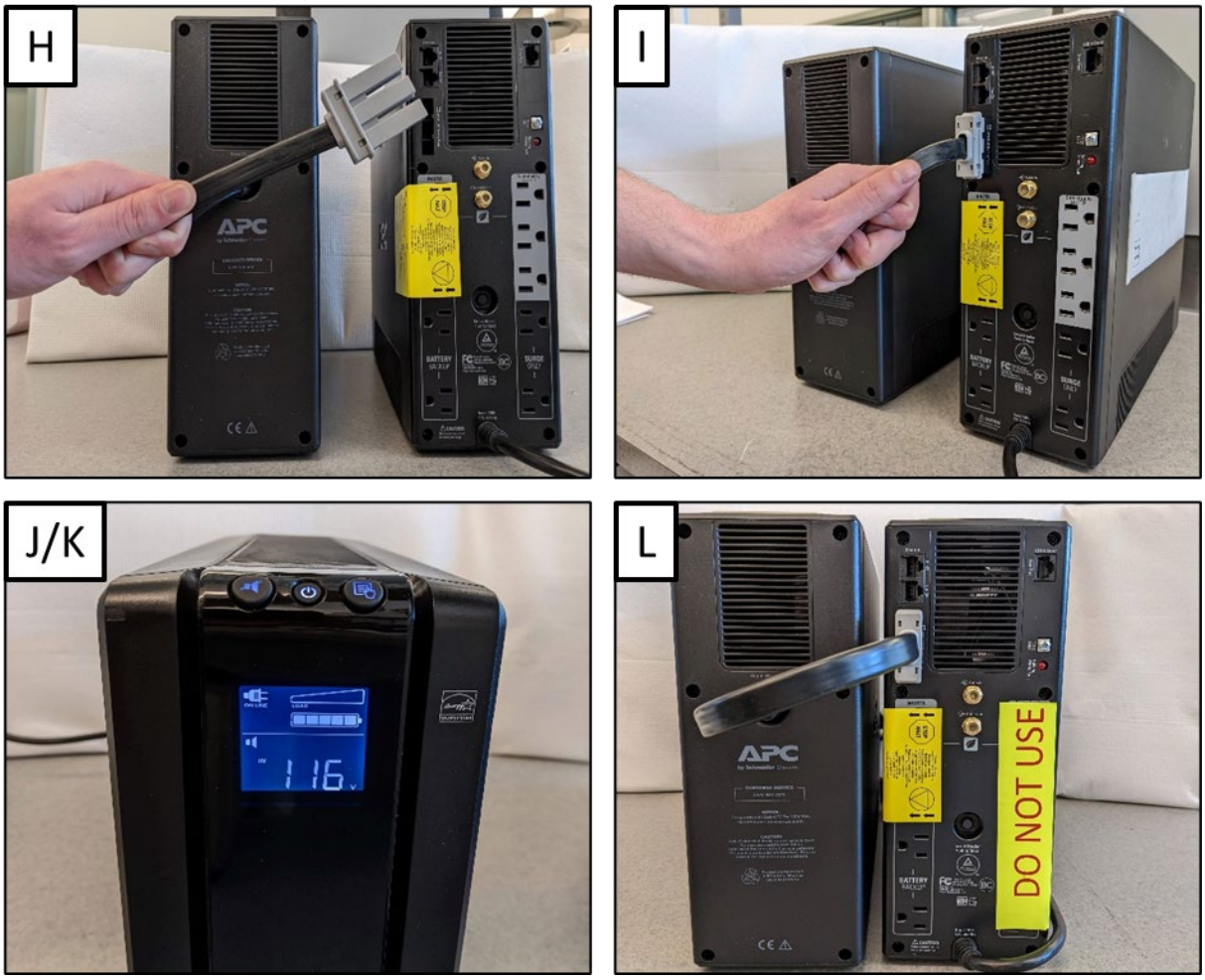

Figure 4b. Steps to connect APC – UPS

GeneXpert Installation Protocol

A. Connect the GeneXpert laptop and printer to the APC – UPS.

B. Turn the GeneXpert first. This makes sure that a connection is made between these two devices.

C. Power on the laptop and login.

D. Login credentials for both the laptop and GeneXpert Dx software are visible on a sticker present under the laptop's keyboard (Figure 5.).

   a. Windows Laptop:

      i. *User Name*: Cepheid-Admin

      ii. *Password*: covid19

   b. GeneXpert Dx Software:

      i. *User Name*: admin1

      ii. *Password*: covid19

Figure 5. Location of login credentials on the

E. The GeneXpert Dx software will automatically launch.

   a. The GeneXpert Dx software has been installed, formatted, and verified by the NML prior to the deployment of the system.

   b. If the software does not automatically launch, select the icon from the desktop (Figure 6.).

Figure 6. GeneXpert Dx icon on laptop desktop

F. After launching, the GeneXpert Dx software may display the prompt “Do you want to perform Database Management tasks?” (Figure 7.).

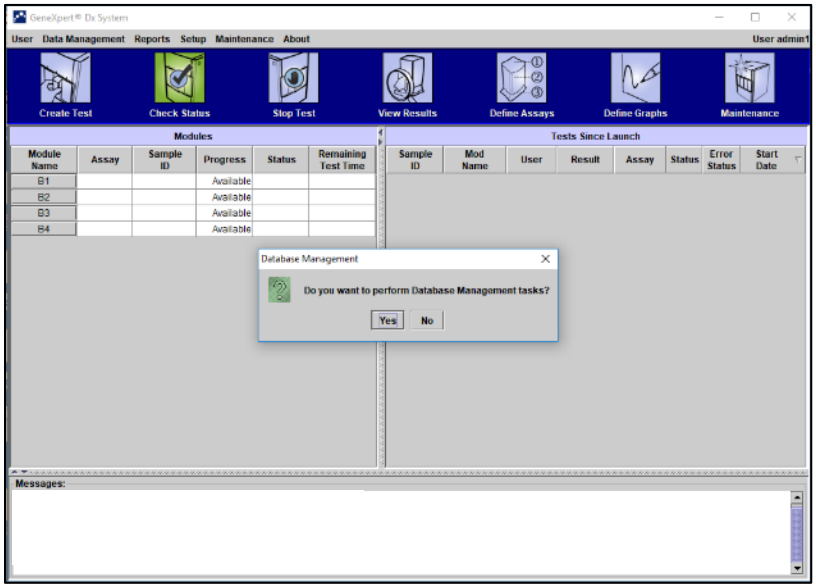

Figure 7. Database management task prompt

a. Select *No* if no backup is required.

b. Select *Yes* if a backup is required.

   i. Monthly backups are recommended.

G. Ensure the correct time and date is displayed on the laptop, if not, correct the date and time in the Windows setting app. This ensures that the GeneXpert Test Reports will have the correct date and time when printing results.

H. For tracking purposes, ensure that each user has their own login information.

I. The system is now ready to use.

Creation of Additional User Accounts

A. Log in with the default account information (*Username*: admin1, *Password*: covid19).

B. From the main screen, select the *Setup* tab.

C. Select *User Administration* (Figure 8.).

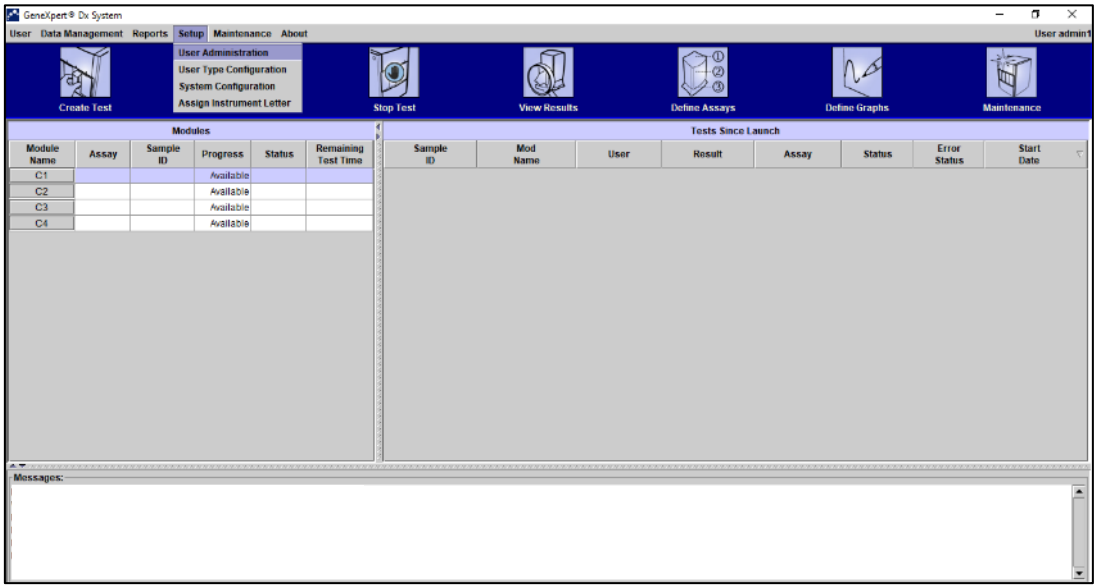

Figure 8. Setup and User Administration location

D. In the User Administration prompt, select *Add* (Figure 9.).

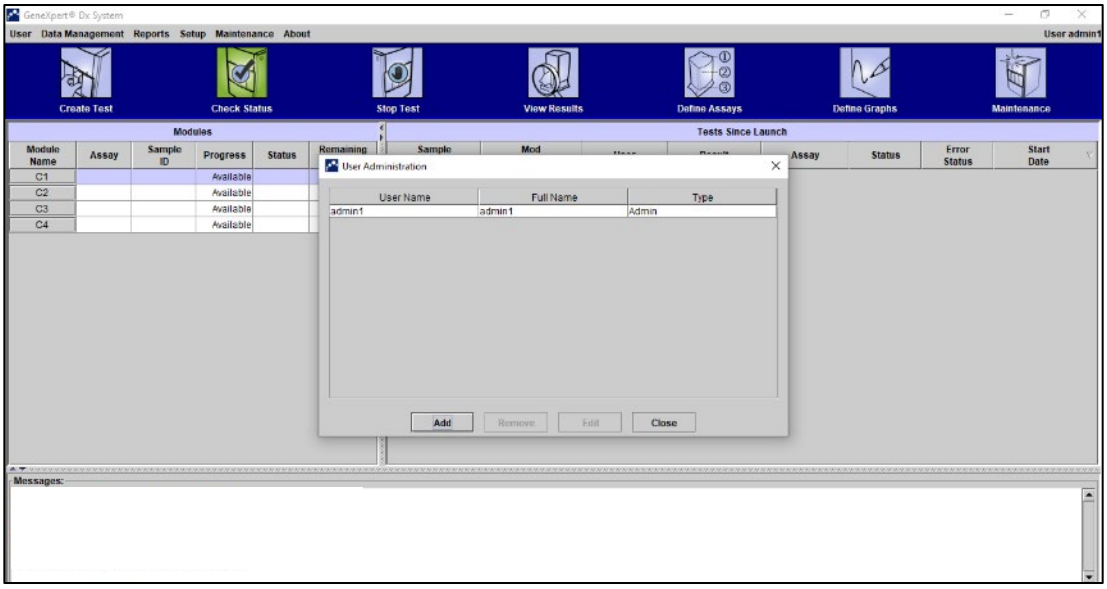

Figure 9. User Administration prompt

E. Fill in all the required fields. For User Type, select either *Detail* or *Admin* depending on the user setup required (Figure 10.).

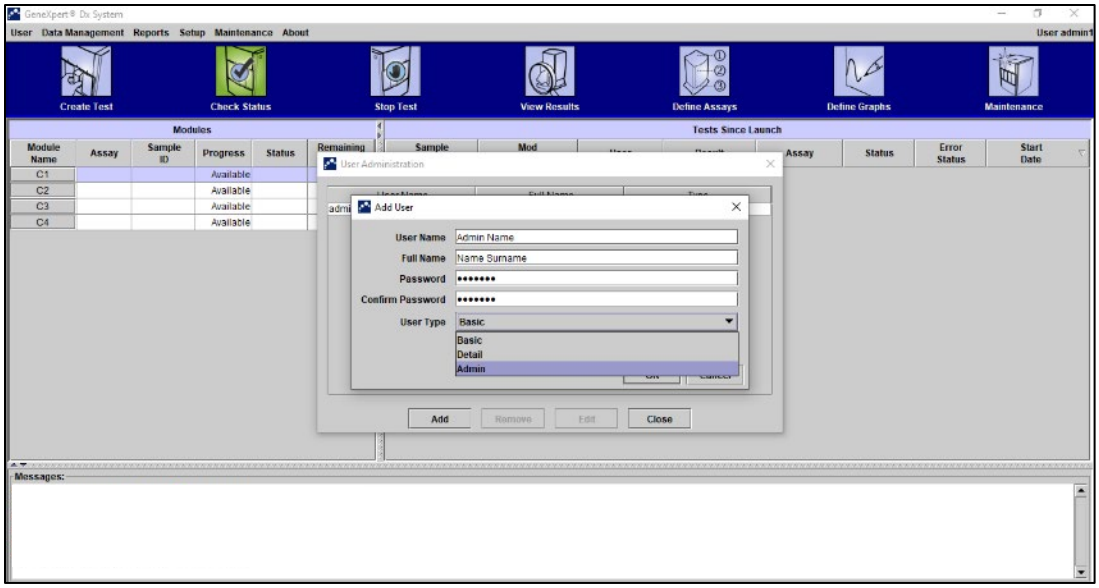

Figure 10. Add User prompt User Type options

F. Click *OK*. The new user is set up and ready to use the software.

GeneXpert System Maintenance

The GeneXpert system must be regularly maintained for reliable results and ensure its longevity and. There are three maintenance categories:

Daily Maintenance

❑ Remove test cartridges from the instrument after testing.
❑ Ensure modules are clean and free of debris.
❑ Close modules when not in use.
❑ Clean the surfaces of the instrument with an approved disinfectant.

Weekly Maintenance

❑ Fully shutdown (also known as “power cycle”) the GeneXpert instrument and laptop.

Quarter Yearly Maintenance

❑ Clean the cartridge bay(s).
❑ Clean the plunger rod(s).
❑ Clean the fan filter.

How Clean the Cartridge Bay(s)

A. Prepare a 10% solution of household bleach and moisten a paper towel.
B. Wipe the inside of the cartridge bay (Figure 11.).
C. Wipe the inside of the door.
D. Wait two minutes before repeating the cleaning a second time.
E. Once the second cleaning is complete, wipe the same surfaces with a clean paper towel that has been moistened with clean water.
F. The cartridge bay surfaces are now cleaned.

Figure 11. Cleaning a cartridge bay

How to Clean the Plunger Rod(s)

A. Prepare a 10% solution of household bleach.

B. In the GeneXpert software, select the *Maintenance* menu tab from the top of the screen.

C. Select *Plunger Rod Maintenance* (Figure 12.).

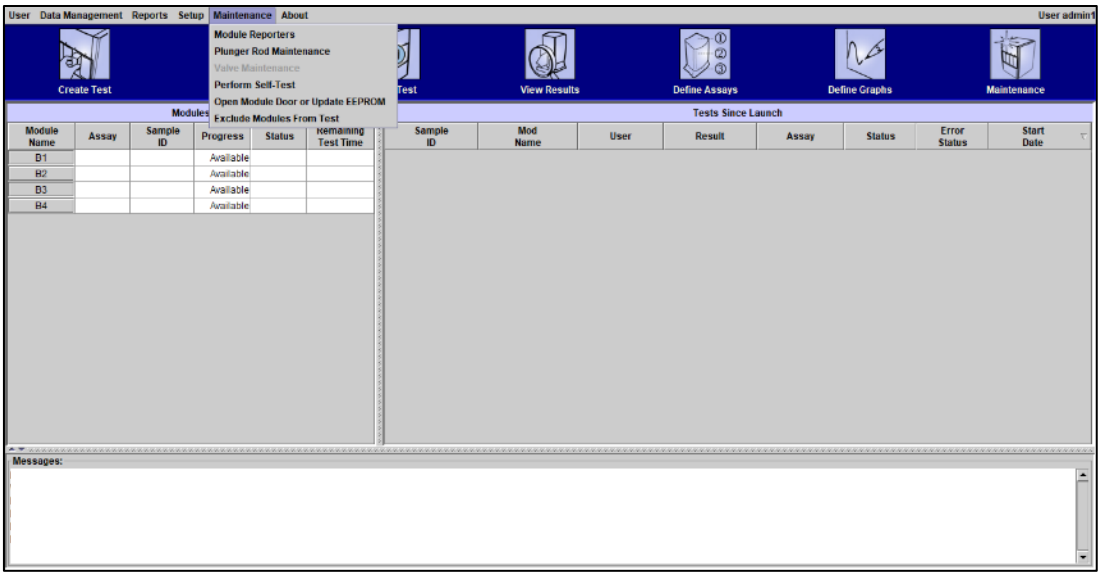

Figure 12. Plunger Maintenance location

D. In the *Plunger Rod Maintenance* prompt, select *Clean All* (Figure 13.).

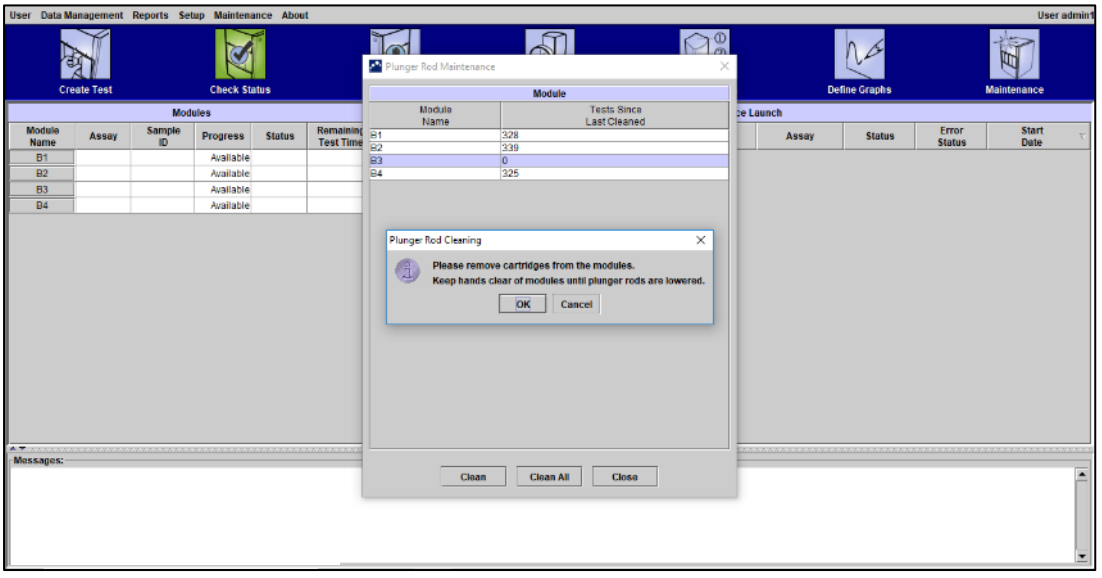

Figure 13. Plunger Rod Cleaning prompt

E. Follow the instructions in the prompt. This will cause the plungers from each module to lower.

F. Moisten a paper towel with the prepared 10% bleach solution and gently wipe the plunger rod(s) (Figure 14.).

G. Wait two minutes before repeating the bleach wash a second time.

H. Once the second cleaning is completed, gently wipe the plunger rod(s) with a clean paper towel that had been moistened with clean water.

Figure 14. Cleaning a plunger rod

I. Once wiped down, select *Move All* Up from the *Plunger Maintenance* window to return the plungers to their original position.

J. The instrument's plunger rod(s) are now cleaned.

How to Clean the Fan Filter

A. Assure that the GeneXpert instrument has been shut off.

B. Turn the instrument around as to have access to the fans at the back of the unit.

C. Unclip each of the four clips to release the fan's filter (Figure 15.).

   a. **Do not force the filter to be released**.

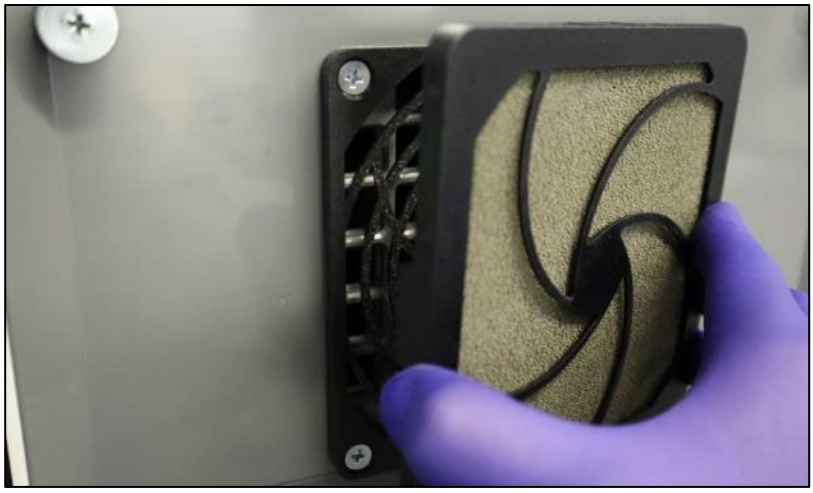

Figure 15. Removing the fan filter unit from the GeneXpert instrument

D. Remove the filter and wash with regular household soap and water (Figure 16.).

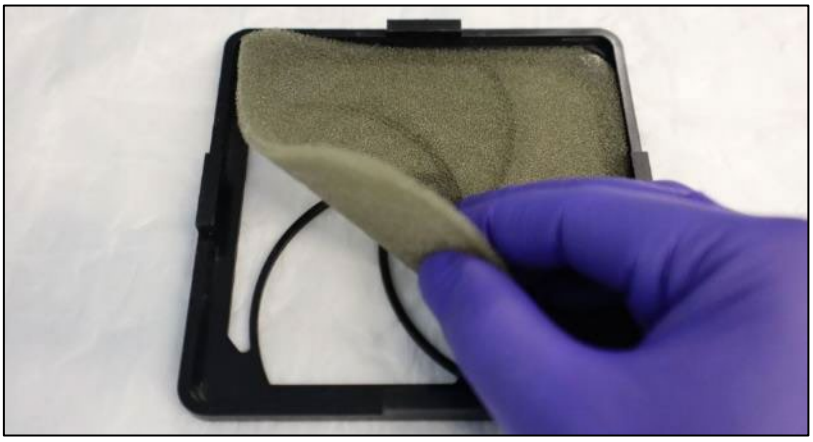

Figure 16. Removing the filter to be cleaned

E. If the GeneXpert instrument will be used immediately, fully dry the filter by pressing it between two paper towels.

F. If the instrument will not be used immediately, let the filter dry over night.

G. Once fully dry, return the filter on to the GeneXpert instrument.

H. The fan filter is now cleaned.

Appendix F – Wastewater Testing Using the GeneXpert System Protocol

Contents

GeneXpert Test Cartridge Precautions

- Store GeneXpert test cartridges in an upright position and at the temperature indicated on side of the test kit.
- If the ambient temperature of the workspace is above 28°C, store cartridges in a fridge (2 to 8°C).
- Cartridges and samples stored at 4°C must be brought to room temperature for 30 minutes prior to testing.
- Do not use a cartridge that has been damaged, leaked, dropped, or shaken (as seen by crystallization on lid; Figure 1.).
- Do not touch the *Reaction Tube*.
- Always handle the cartridge by the *Body* (see Figure 2.).
- Open a cartridge only when ready to add a specimen. **A loaded cartridge must be tested on the GeneXpert within 30 minutes.**
- Cartridges are single use. Do not attempt to open or re-use a previously used cartridge.

Figure 1. GeneXpert test cartridge, black arrow pointing to crystallization

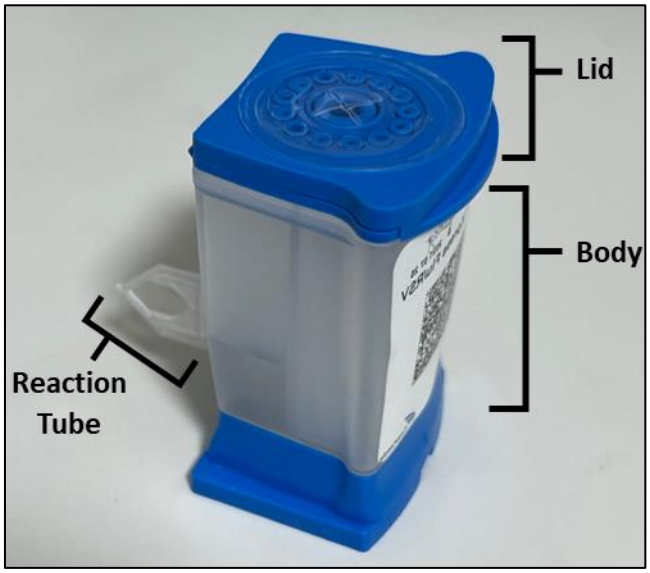

Figure 2. GeneXpert test cartridge components

Wastewater Testing Using the GeneXpert System Protocol

Equipment and Layout Within the Contained Work Area
• Personal Protective Equipment (Not shown in image)
A. Absorbent Bench Liner
B. Pipette Controller
C. P200 Pipette
D. P200 Tips
E. Alcohol-resistant Marker or Prepared Label for Test Cartridge
F. Microcentrifuge Tube Rack
G. Empty 50mL Conical Tube
H. Large Tube Rack
I. Biohazard Bag in a Rack

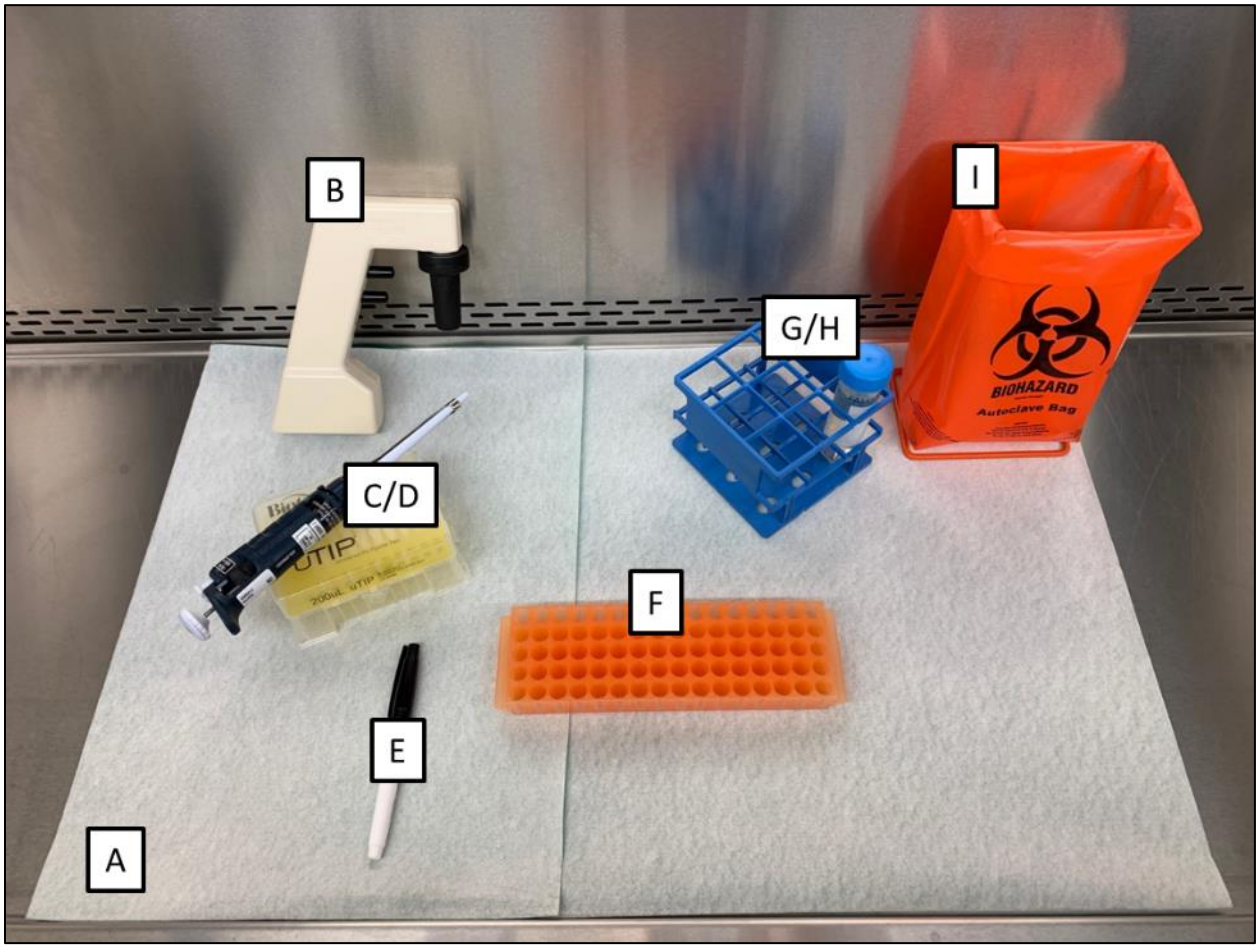

Figure 3. Contained work area layout

Materials Required per Wastewater Sample
A. 2 – 25 mL Serological Pipettes
B. 1 – GeneXpert Express Single Use Pipette
C. 1 – Xpert® Xpress Test Cartridge
D. 200 µL of Nuclease Free Water
E. 40 µL of 10% Tween-80 solution
F. 1 – 50 mL Falcon tube (or equivalent)
G. 1 – Amicon Ultra-15 10 KDa Centrifugal Filter Unit (UFC9010, Millipore) Unit or equivalent
H. 1 – Microcentrifuge Tube (1.5 or 2.0 mL volume)
I. The sample of wastewater (approximately 250-500 mL)

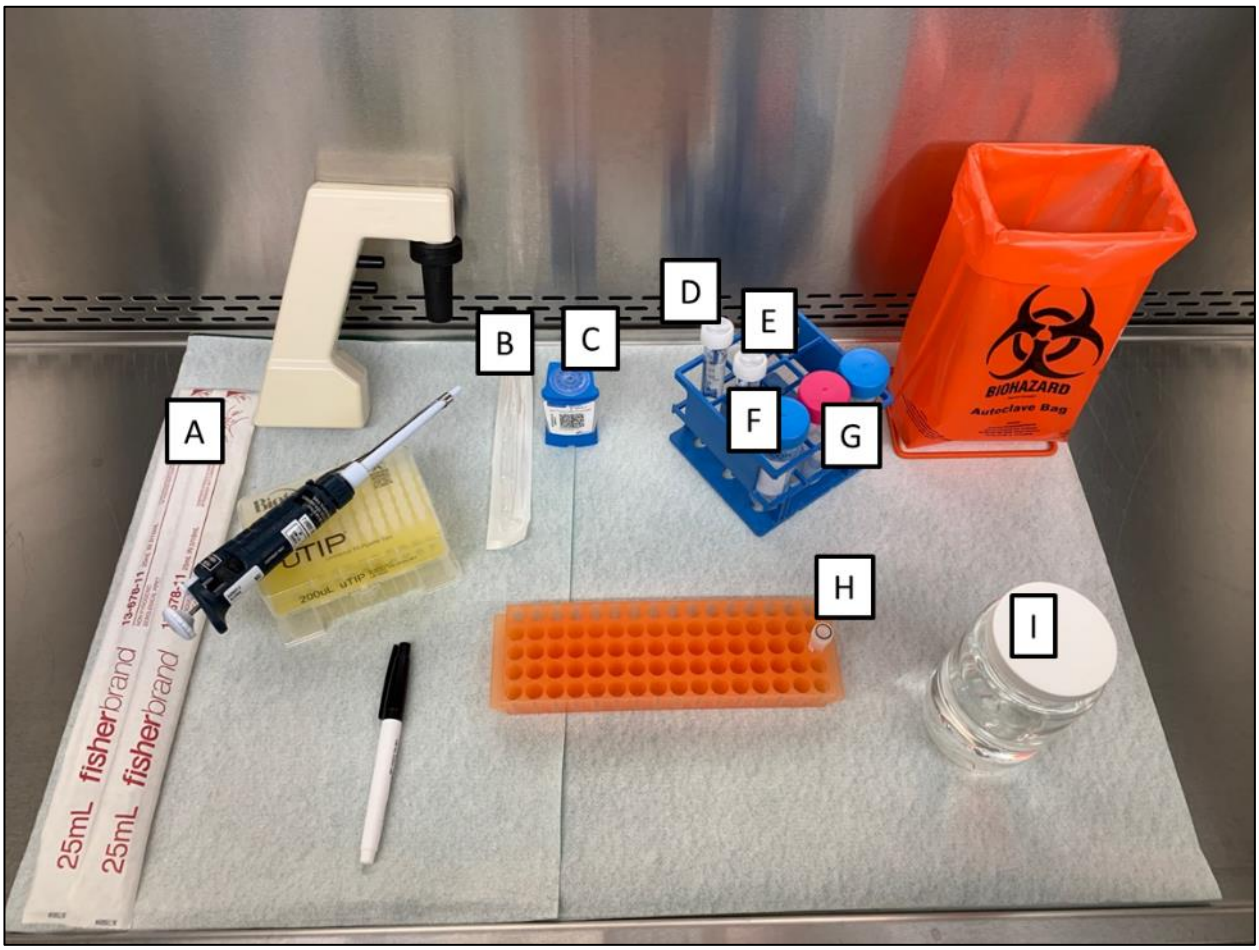

Figure 4. Items needed for testing one wastewater sample.

Wastewater Sample Processing Protocol

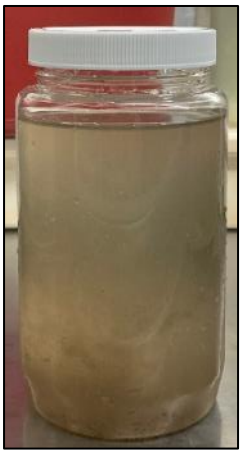

Figure 5. 500 mL of wastewater in a sample bottle

The work area must be thoroughly cleaned before testing a new sample

A. Conduct all tasks involving wastewater in a contained work area while wearing proper Personal Protective Equipment (PPE).

B. Label the side of cartridge with the *Sample ID* using a marker or prepared label/sticker.

    a. **Do not touch** the reaction tube, write on the lid, or obscure the digital matrix code on the front of the cartridge.

C. Thoroughly mix the wastewater sample by inversion for 10 seconds.

D. With a 25 mL serological pipette and a pipette controller, add 40 mL of wastewater (two transfers of 20 mL) into a 50 mL conical tube.

E. Add 40 µL of 10% Tween-80 to the 50 mL conical tube containing the wastewater sample.

F. Vigorously shake the mixture by inversion for 20 seconds.

G. Centrifuge at 2,900 RCF for 32 minutes to pellet any debris (Figure 6.).

    a. Samples must be centrifuged in pairs to ensure proper balancing of the sample.

        i. A “Blank” may be made by filling a 50 mL conical tube with an equal amount of water.

    b. If the pellet becomes loose or dislodges, centrifuge the sample again for 5 minutes at max speed.

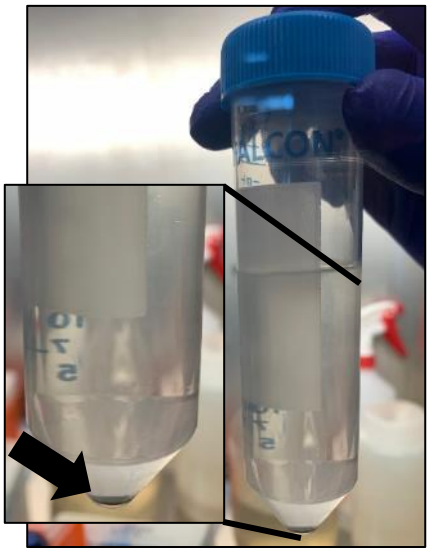

Figure 6. Wastewater after initial centrifugation, black arrow pointing to pellet

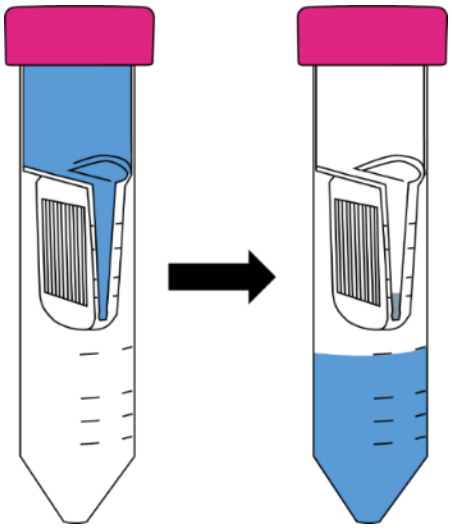

Figure 7. Amicon filter unit concentration of wastewater

H. With a 25 mL serological pipette and a pipette controller, carefully transfer 15 mL of the liquid into an Amicon filter unit while making sure that the pellet does not come loose.

I. Centrifuge the Amicon filter unit containing the wastewater at 2,900 RCF for 51 minutes. This results in approximately 200-300 µL of wastewater concentrate in the top of the unit (Figure 7.).

    a. If more than 600 µL of liquid remains in the unit, double-check the centrifugation settings. If this is a frequent issue, please contact PHAC for assistance.

J. With a P200 pipette and tip, add 200 µL of nuclease-free water into the microcentrifuge tube.

K. With a new tip, transfer 200 µL of prepared concentrated wastewater to a microcentrifuge tube containing nuclease-free water (Figure 8.). Mix by pipetting the mixture up and down ten times.

L. Mix the concentrated wastewater and the nuclease free water by inverting the closed tube for 10 seconds.

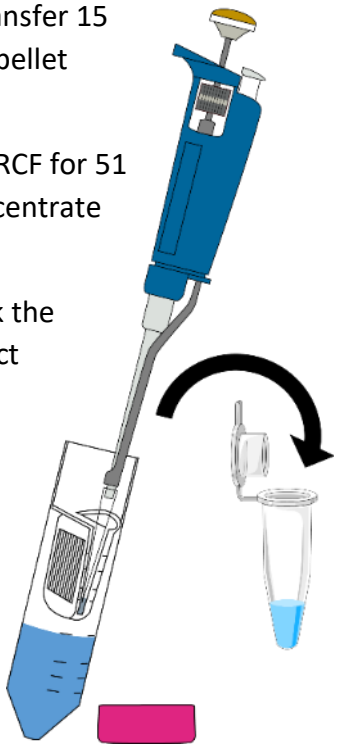

Figure 8. Transferring wastewater concentrate from the Amicon unit

M. On a flat surface, prepare the GeneXpert cartridge by opening, closing, and then opening the top lid of cartridge to ensure that the cartridge can be sealed.

    a. **The cartridge must be tested within 30 minutes of being open.**

N. With the single use pipette (Figure 9.), load 300 µL of the wastewater from the microcentrifuge tube into the GeneXpert cartridge, firmly snap close the lid of the cartridge.

O. The sample is now ready to be tested on the GeneXpert.

Using a Single Use Pipette

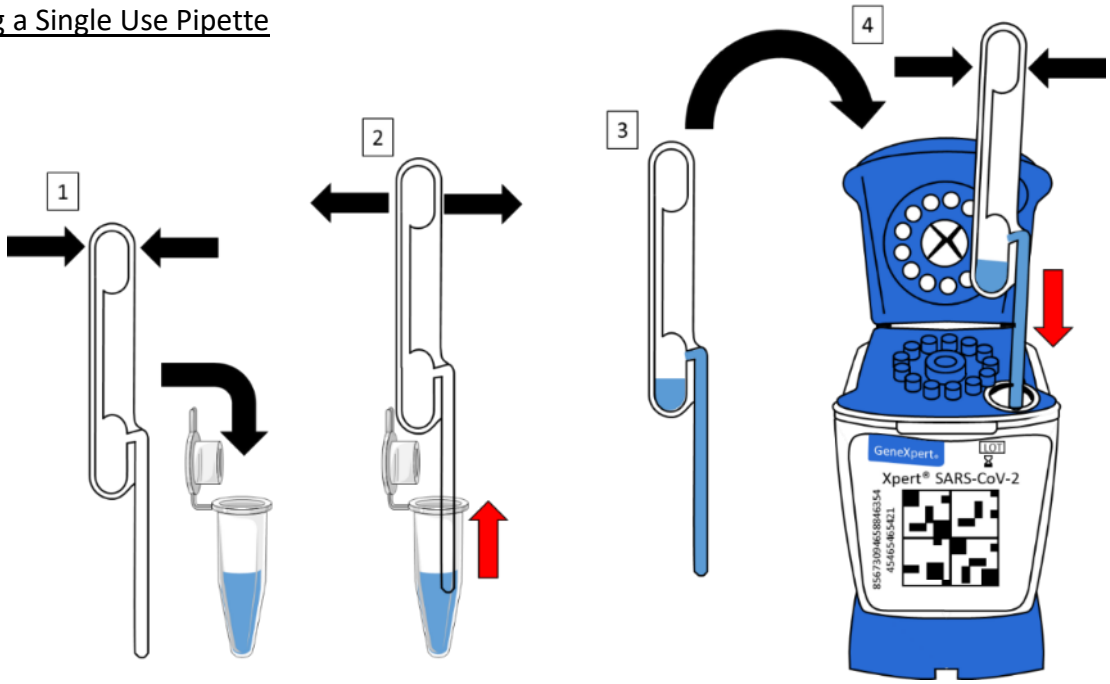

Figure 9. Use of a single use pipette

1. Compress the upper bulb of the single-use pipette and insert it into the wastewater mixture.
2. Release the compressed bulb to draw liquid into the single-use pipette so that some liquid enters the lower/overflow bulb.
3. Remove the single use pipette and insert it into the GeneXpert cartridge's large sample chamber opening.
4. Squeeze the upper bulb to completely empty the chamber's contents into the large sample chamber opening.

GeneXpert Testing Protocol

A. Turn on the GeneXpert using the switch located at the rear of the instrument.

B. Turn on the laptop and launch the GeneXpert Dx software using the desktop icon.

Figure 10. GeneXpert desktop

C. Enter your login and password.

   a. Default admin login information: *User Name*: admin1, *Password*: covid19

Figure 11. GeneXpert Login screen

D. Confirm that all modules that will be used are listed as *Available* under the *Progress* Column.

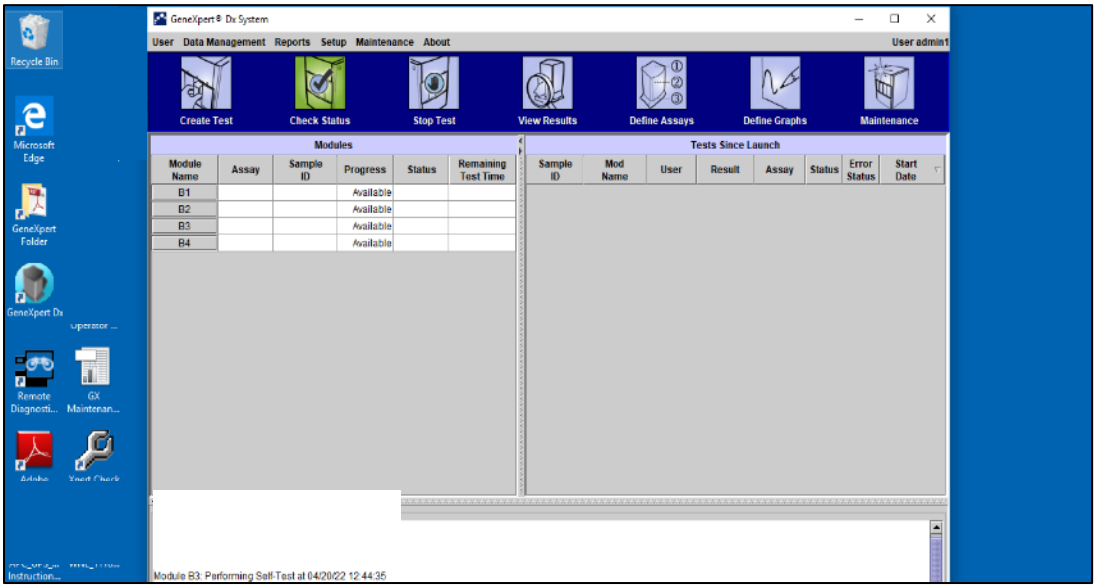

Figure 12. GeneXpert main screen

E. Click on the *Create Test* icon at top left of the screen.

F. A pop-up indicating to scan the cartridge barcode will appear; scan the barcode located on the front of the cartridge with the barcode scanner to continue.

G. *Select Assay* automatically populate with the correct assay name for the cartridge.

H. In the *Sample ID* box, enter the sample information **(*yyyy-mm-dd_location*)**

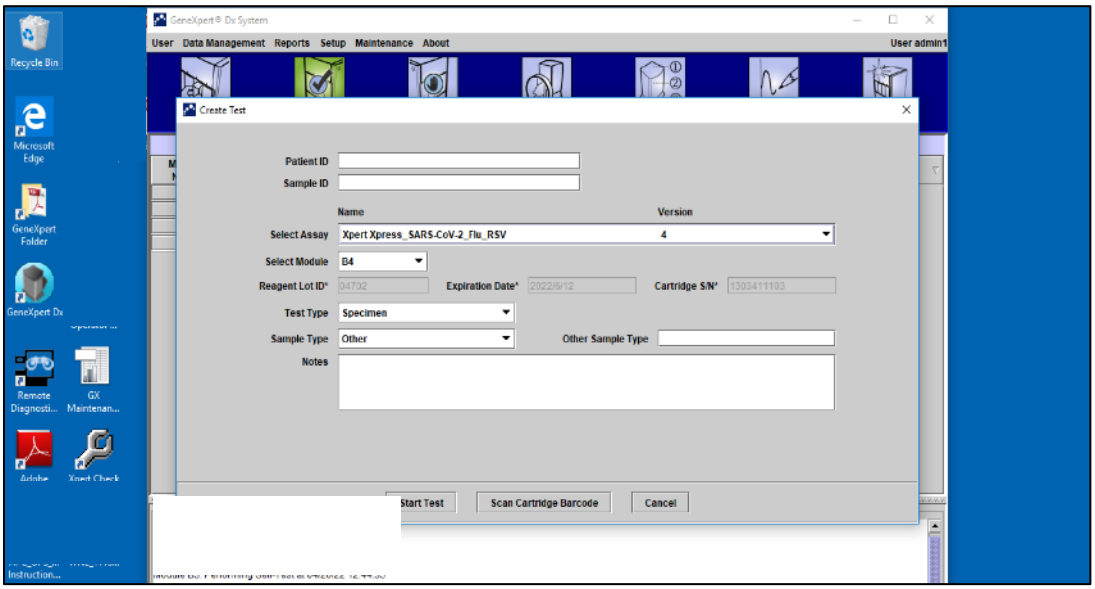

Figure 132. Create Test window

I. Look at the *Select Module* field to determine the module to use. The selected module's door will unlatch and a blinking light above the unit to indicate that it is active.

   a. If necessary, use the drop-down list to change the module.

J. Open the selected module door, ensure that it is empty, and then insert the new cartridge.

K. Push the door closed until it clicks, and the blinking green light remains solid.

   a. The timer for the run will start to count down.

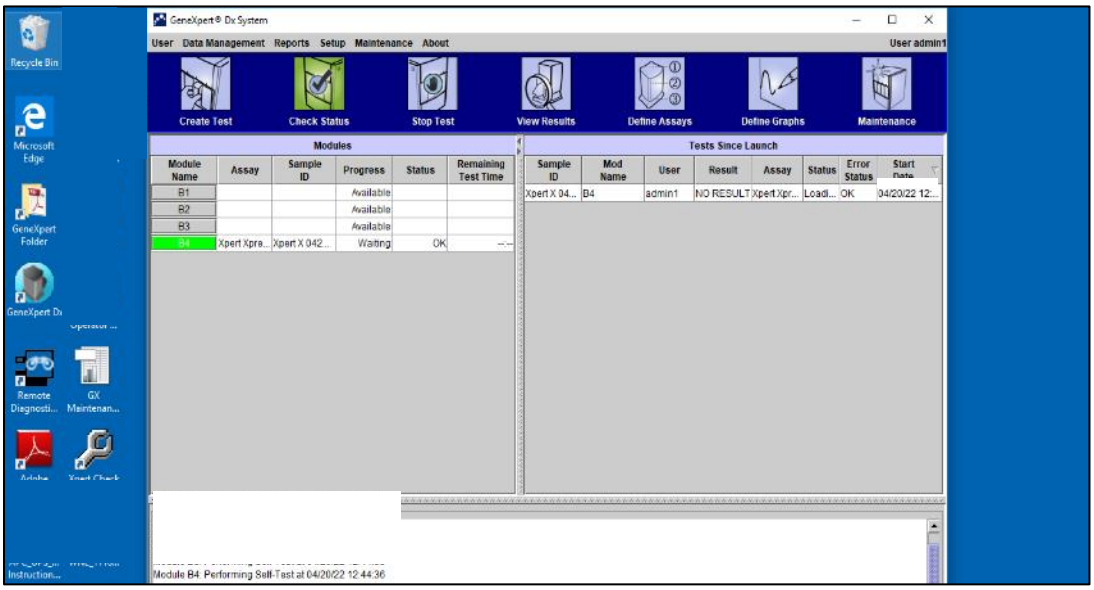

Figure 14. GeneXpert main screen while running a test

L. When the run is completed, the module door will unlock, and the light will turn off. Remove and dispose of the cartridge in accordance with local biosafety protocols.

Repeat Testing Criteria

Below is a list of criteria for retesting a wastewater sample on a GeneXpert. This list is not exhaustive and acts as guidance and further criteria may be added.

A. The test result is *Error*.

B. The test result is negative and the SPC value of the test is above 33.

How to View and Report/Export GeneXpert Results

Viewing Test Results

A. Results may be observed in real time during a test run by selecting the *View Results* icon from the top menu bar or by double clicking on the test result.

B. To view a different test, click *View Test* at the bottom of the screen, and select the test of interest in the pop-up window.

C. The selected test result will be displayed under the *Test Result* tab.

D. Select the *Analyte Result* tab to view CT values for the gene targets and for the internal quality control.

E. Error messages will be displayed under the *Errors* tab if applicable.

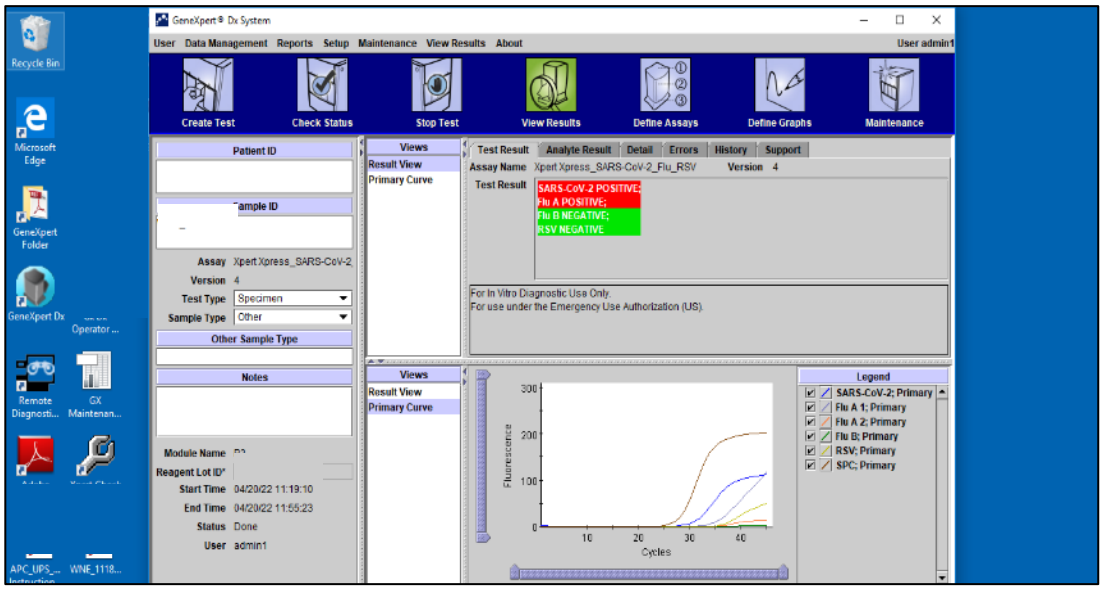

Figure 15. GeneXpert post run result screen

Reporting Test Results as a PDF

Reporting of test results is critical to the usage of the GeneXpert for Wastewater testing. Depending on the requirements of the program, results can be exported/reported as a .pdf or as a .csv file. **If the data is to be shared with the NML for trend analysis, please send both file formats.**

A. Click the *Report* button at the bottom of the screen.

B. Leave the *Analyte Result* at the default settings unless indicated otherwise.

   a. *Error Details* should be only checked box.

C. Check the box(es) adjacent to the *Patient ID* (the sample's name) of interest.

   a. A PDF test report may be generated for one or more selected samples.

D. Click *Preview PDF* to open a preview report before saving or printing.

E. Click *Generate Report File* and save PDF in the desired location.

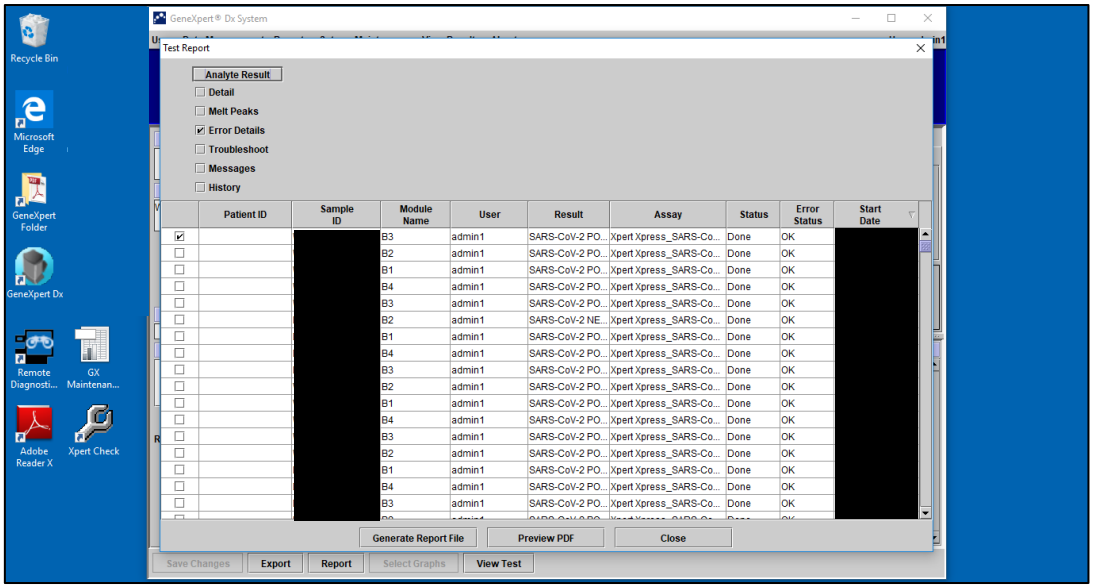

Figure 16. GeneXpert test report screen

Reporting Test Results as a .csv

A. Click the *Export* button at the bottom of the screen.

B. Leave the top boxes at the default settings unless indicated otherwise.

    a. *Test Result* should be only checked box.

C. Check the box(es) adjacent to the Patient ID (the sample's name) of interest.

    a. Multiple test results may be exported into one .csv file.

D. Click *Export* and save the .csv file in the desired location.

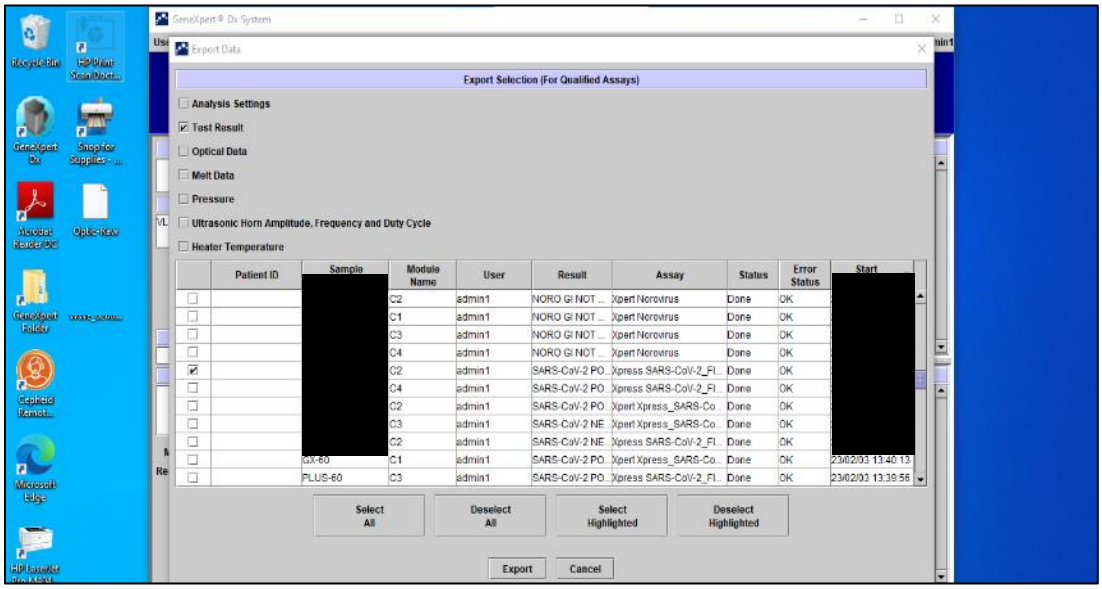

Figure 17. GeneXpert test export screen