ANKOM RF
GAS PRODUCTION SYSTEM

RF2000 Operator's Manual

07/13/26

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ANKOM TECHNOLOGY

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Table of Contents

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1. Introduction

Congratulations on your purchase of the ANKOM^{RF2000} Gas Production System (RF2000). We are confident that this product will effectively serve your needs.

The RF2000 is designed to provide an easy-to-use method for monitoring and measuring gas production from anaerobic and aerobic microbial activity.

ANKOM Technology designs, manufactures, and markets analytical instruments and support products used by laboratories around the world in food, feed, agricultural, environmental, and bioenergy industries. Our products allow for the analysis of fat-soluble vitamins, cholesterol, dietary fiber, crude and detergent fiber, crude and total fat, digestibility studies and more.

ANKOM Technology is committed to total customer satisfaction, designing every product based on a thorough assessment of customer needs.

2. Warranty

ANKOM Technology warrants the RF2000 Gas Production System against any defects in workmanship or material for one year after the original date of purchase. This warranty does not include damage to the instrument resulting from neglect or misuse; moisture damage voids the warranty. During the warranty period, should any failure result from defects in workmanship or materials, ANKOM Technology will, at its discretion, repair or replace the instrument free of charge. Extended warranties are available upon request.

3. Contact Information

We are committed to supporting you and value your feedback. For any questions or suggestions regarding your instrument, please contact us at: www.ankom.com

- For Sales Support: sales@ankom.com or https://www.ankom.com/contact-us
- For Technical Support: www.ankom.com/contact/technical-services
- For Analytical Support: www.ankom.com/contact/analytical-services
- Telephone: (315) 986-8090

NOTE:	Please review the entire contents of this manual before you begin operating this product.

4. Instrument Description

4.1 - General Description

The ANKOM*RF2000* Gas Production System is designed to automatically measure the kinetics of microbial fermentation. This is accomplished by recording the gas pressure within system's Modules and storing the data in a database. This data can be exported to spreadsheet for analysis. Each Module is equipped with a pressure sensor, temperature sensor, and a pressure release valve, allowing each unit to control and monitor the important aspects of the reaction. The system can include up to 75 individual Modules that communicate information to a computer using radio frequency (RF) transmission. Using the computer interface, the operator can monitor the status of each Module in real time and configure test parameters, including data logging intervals and automatic pressure venting within each Module.

4.2 - Instrument Applications

Current and potential applications for the ANKOM*RF2000* Gas Production System include, but are not limited to, rumen nutrition, human digestion, yeast activity, biomass-to-fuel, biodegradability, biochemical oxygen demand (BOD), soil quality, and other analytical applications.

4.3 - System Components

The ANKOM*RF2000* Gas Production System includes the following components:

1 – RF2000 Base Coordinator (Part # RF2a)
1 – Charging Hub Station (Part # 7320)
1 – RF2000 GPM Software (Part # RF3a)
1 – Vent Valve Cleaning Kit (Part # RF22a)
2 – Luer Duckbill Check Valve (Part # 7139)
2 – Male Luer Cap (Part # 7147)
10 – O-Rings (Part # RF5a)
1 - Positive Control Capsules (Pkg includes 5 Blue Capsules & 5 Clear Capsules)

RF2000 Modules (RF1a) and bottles are sold separately.

Items Sold Separately	Part Number
Gas Production Module Purchase up to 75 Modules per Base Coordinator.	**(Part # RF1a)**
100ml Glass Bottle, Coated, Clear	**(Part # RF56)**
100ml Glass Bottle, Coated, Clear, w/Septa port	**(Part # RF36)**
250ml Glass Bottle, Coated, Clear	**(Part #RF57)**
250ml Glass Bottle, Coated, Clear, w/ Septa Port	**(Part # RF37)**
500ml Glass Bottle, Coated, Clear	**(Part # RF58)**
500ml Glass Bottle, Coated, Clear, w/Septa Port	**(Part # RF38)**
1000ml Glass Bottle, Coated, Clear	**(Part # RF59)**
1000ml Glass Bottle, Coated, Clear w/Septa Port	**(Part # RF39)**
1.8L Wide Mouth Bottle with adapter Assembly	**(Part # RF42)**
1.8L Wide Mouth Bottle and Tightening Ring	**(Part # 7181)**

4.4 - System Capabilities

The RF2000 has a modular design that allows the user to increase or decrease the number of samples tested. The Base Coordinator provides the pressure and temperature references to monitor and record atmospheric pressure and communicates with up to 75 Modules, each programmed with a unique ID number and address.

If you have a system with less than 75 Modules, more can be ordered to complete your system. If your system is complete with 75 Modules and you would like to add more Modules, an additional RF2000 is required.

NOTE:	Each RF2000 network requires a connection to a unique laptop or desktop computer. The computer does NOT have to be dedicated to the ANKOM system.

4.5 - RF Communication

Radio Frequency (RF) technology allows the Modules to be located away from the Base Coordinator without the need for cumbersome wire connections. The RF signal from the Modules can be detected at distances of up to 30 yards. For the best signal reception, it is recommended to have the Base Coordinator as close to the Modules as possible with minimal physical obstructions.

5. Safety Precautions

This system is designed to meet or exceed the applicable standards of CSA, NRTL and OSHA.

WARNING: For the 250ml – 1000ml glass bottles, never apply pressure exceeding 10 psi while purging your system (eliminating oxygen), or allow the pressure in the bottles to exceed 10 psi (68.9476 kPa) during an experiment**.**

For the 1.8 L wide-mouth bottles, never let pressure exceed 1 psi.

Always wear safety glasses and appropriate lab protection when handling the Modules.

Using this system or its components in a manner not specified by the manufacturer voids the warranty and may result in harm to the user.

6. Instrument Setup –Software and Equipment

Minimum Computer Software Requirements

Recommended: IT Department should make sure the computer running the GPM software is not connected to the internet or an internal network during a test run (i.e., disable WIFI and or network card). This is will prevent interruptions from occurring during the run.

To run the ANKOM GPM Software your computer must have the following programs.

- Windows 10 or later
- Microsoft Excel 2003 or later
- Microsoft.Net v8.0 or later
- Recommended: IT Department should make sure the computer running the GPM software is not connected to the internet or an internal network during a test run (i.e., disable WIFI and or network card)
- Processor – x86_64 (Intel 64 or AMD64)
- RAM – 4 GB

To begin using the RF2000 System, install the GPM software provided by ANKOM. To install your GPM software, follow the steps below.

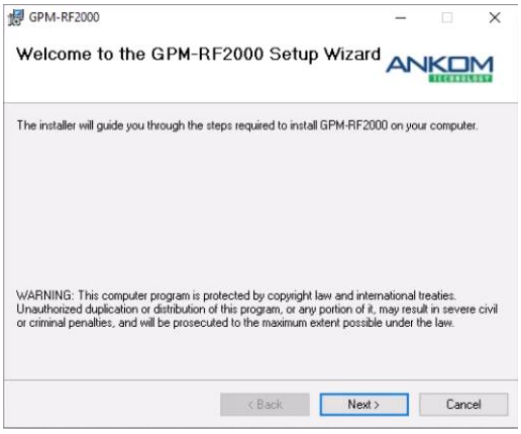
6.1 Follow the link below to go to the password protected ANKOM website page.

https://www.ankom.com/private/gpm-rf2000-software

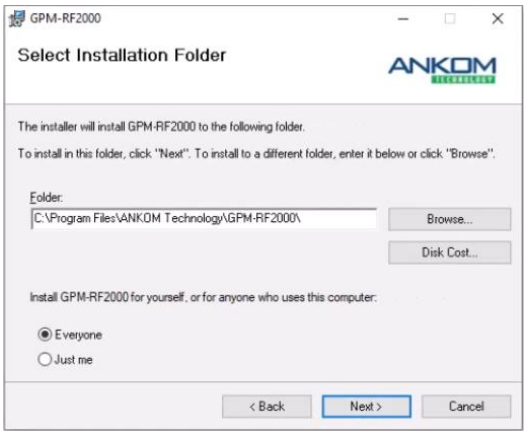
6.2 Enter the password provided by ANKOM. The password is sent in an automated email after purchasing the RF2000 Gas Production System online. It is also provided with a manual insert that arrives with the system. Please contact ANKOM if password is not found.

6.3 Begin downloading the GPM software. Click on the software file provided on the webpage. A message box for the window installer will appear and begin preparing to install the GPM Software.

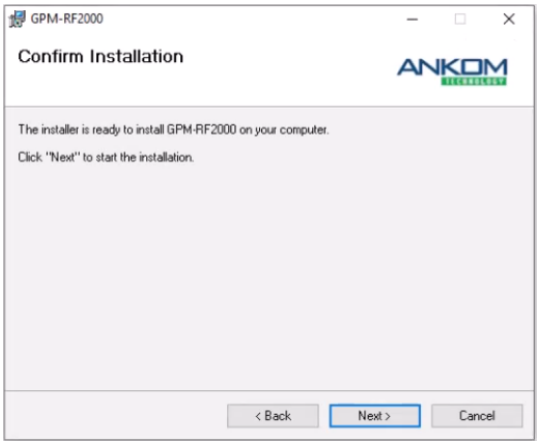
The following screen will be displayed:



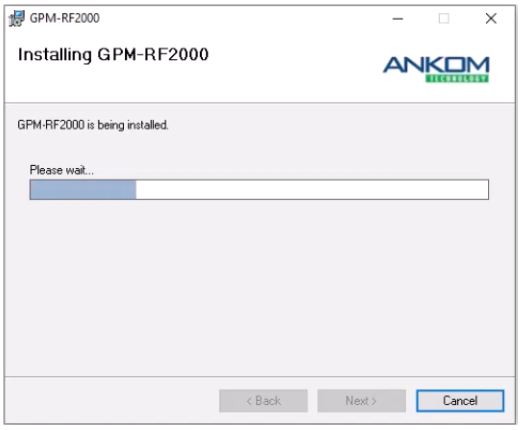
6.4 In the message box above, click on the **Next**. The following message box is displayed:



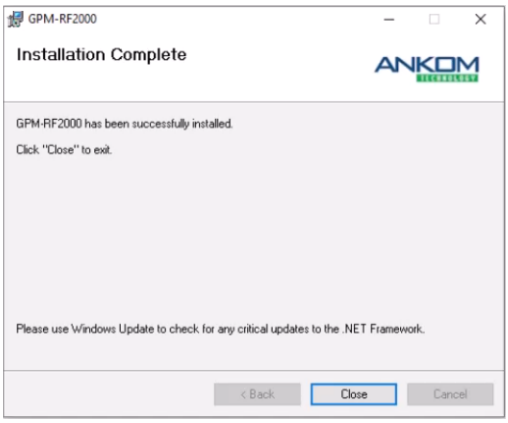
6.5 In the message box above, the **Installation Folder** box shows the name of the folder path in which your GPM software program will be stored. If you would like to change the folder, type in the desired folder, or click the **Browse** button to navigate to the desired folder to store the program. Once you confirm the location, click on the **Next** button. The following message box is displayed:



6.6 In the message box above, click on the **Next**. The following message box is displayed when the software is installing.



6.7 Once installed, Click the **Next>** button. The following message box is displayed.



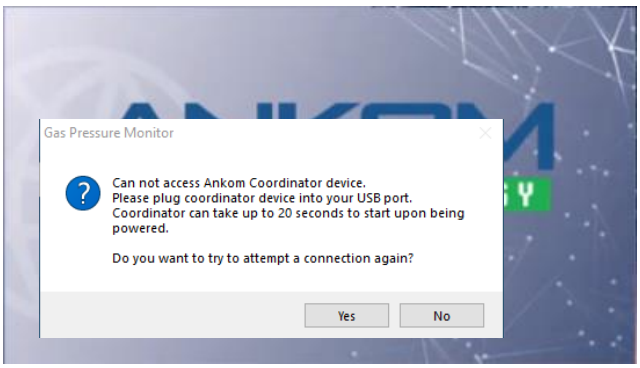
6.8 To complete the installation, click the **Close** button in the message box above.

6.9 After completing the software installation, connect the Base Coordinator to the PC and turn it on by pressing and holding the button on top for 1 second. The blue LED light will turn on.

IMPORTANT: **Do not use a USB cable that has not been provided or approved by ANKOM.**

6.10 The following GPM icon is placed on your desktop. To use the GPM software, double-click the GPM icon.

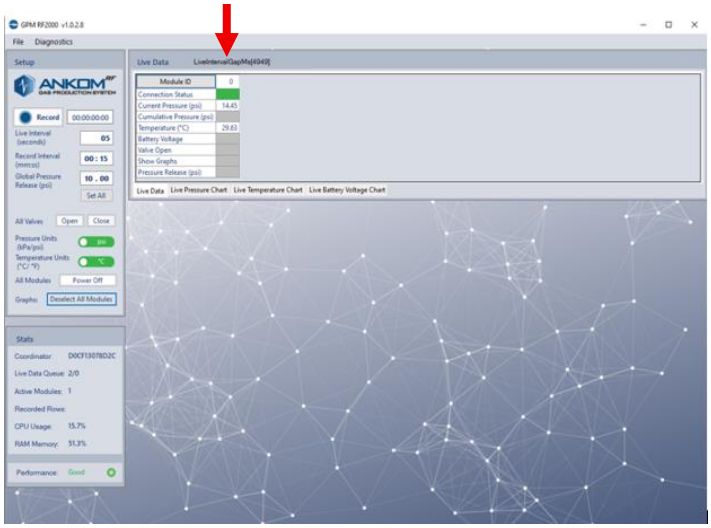
If no coordinator is detected on the USB port, a pop-up will appear as displayed below. Confirm that the coordinator is connected to the USB and turned on, then press "Yes". The system will attempt to look for the coordinator again.



6.11 Make sure your RF Modules are sufficiently charged. Refer to the section **Charging Modules**.

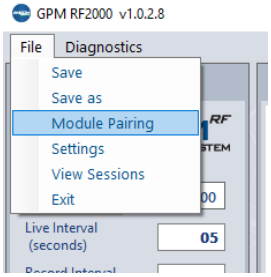
6.12 **Pair the Modules.** Once the GPM software is opened, it will display the Base Coordinator connection status in green. To begin pairing your RF Modules, press and hold the power button on top of the Module for one second.

NOTE:	Pairing the Modules is only necessary for initial set-up. Once paired, the Module will automatically connect wirelessly to the coordinator when turned on and will appear on the software.



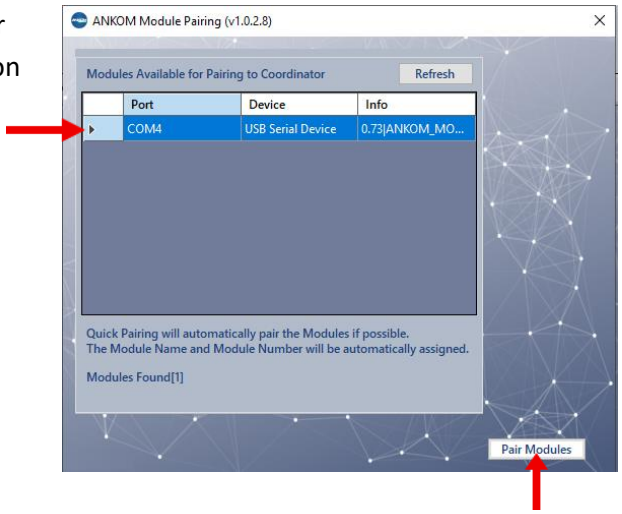
6.13 Plug in the USB cable to the module top and connect to any available USB port on the PC. A module must be connected to the PC to pair. The charging station hub can also be used to pair up to 16 connected Modules at one time. The charging station hub must be connected to the PC through USB and plugged into a power source.

6.14 On the software go to **File** and then **Module Pairing**.

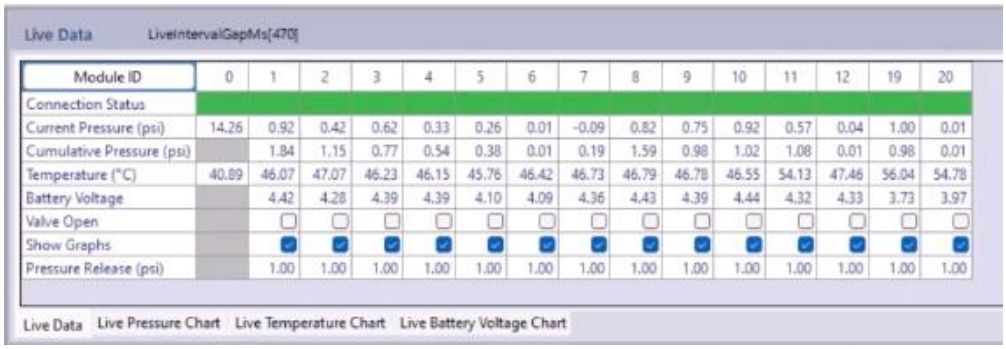


6.15 A dialog box will appear displaying modules available for pairing. If no modules are listed, press the **Refresh** button to try again.

6.16 Press **Pair Modules**.



6.17 A pop-up will confirm that the module paired successfully. Press **OK**. The module will now appear in the live data section of the software and is able to pair up to 75 modules to one coordinator. Once paired you are ready to begin your study by following Operating Instructions section. Modules can now be disconnected from the PC.



7. Instrument Software Functionality

7.1 Color Coding Definitions

The GPM software uses a color-coding scheme to help you clearly identify specific conditions within your data. As you use your RF2000 System, reference the following color-coding definitions.

Module ID

White – A cell is shaded WHITE when the Module is running as expected.

Orange - A cell is shaded ORANGE when the GPM software has failed to receive five (5) transmissions in a row from the Module and is considered no longer in communication with the Module.

Connection Status

Green - A cell is shaded GREEN when the Module is connected and communicating properly.

Yellow – A cell is shaded YELLOW when the GPM software is intermittently communicating with the Module. If prolonged and/or multiple modules appear yellow, it is likely an indicator of an issue.

Grey - The GPM software has missed more than five (5) transmissions in a row and is considered no longer in communications with the Module.

Current pressure

White – A cell is shaded WHITE when the Module is running as expected.

Light Blue - A cell is shaded LIGHT BLUE when the pressure within that Module climbs above the Pressure Release value specified for the respective Module. When this happens, at the next live interval the valve briefly opens and vents the gas for the specified valve open time (default 250ms). This is repeated until the pressure is below the Pressure Release setting.

Battery Voltage

White - When a cell is shaded WHITE there is no need to charge the Module. The cell will show WHITE until below 3.4v.

Yellow - A cell is shaded YELLOW when charging the Module is recommended. This will prevent the battery voltage from reaching the RED indication where valve control is disabled. The cell will show YELLOW between 3.4v and 3.2v.

Red - A cell is shaded RED when the battery voltage is too low to control the valve opening. The Module must be charged. The cell will show RED below 3.2v.

7.2 Module LED Information

LED Color	Explanation
Light off	When the LED light is off, this means the module is not turned on or is asleep because it is not transmitting data.
Faint solid green	The Module is off but is plugged in and charging. The Module is not yet fully charged; The light will turn off when fully charged.
Flashing blue then magenta	The Module is turned on and has sent data to the coordinator (blue) and the GPM software has responded confirming it received the data (magenta). This light will flash at the live interval setting.
Solid or flashing blue	The Module is communicating with the coordinator but not the software. To fix, ensure your GPM software is turned on.
White light flashes on then off Or White light stays on	To turn off the Module, the user must press and hold the on / off button on the top of the Module until the white LED light flashes on then off. Once the light is off, it indicates the Module is off. If the white light remains on, it means the Module is still on. The user must press and hold the on / off button until the light turns off. Once the light is off, it indicates the Module is off.
Solid green light	When the Module is on, a green light will illuminate when the valve is open releasing pressure. The light will turn off when the valve closes.
Solid yellow light	A yellow light will illuminate when the user selects "Identify Module" on the GPM software. This allows for quick identification of the desired Module.
Solid or flashing red	The Module cannot initialize the sensor. Call ANKOM Technology for assistance.
Flashing blue then yellow	This means the Module and Coordinator are not communicating typically due to one of three reasons: 1. The coordinator is not powered on. Turn on the coordinator. 2. The Module is not paired with the system. Following the pairing module instructions. 3. The Module is out of range of the Coordinator. Move the Module closer to the Coordinator.

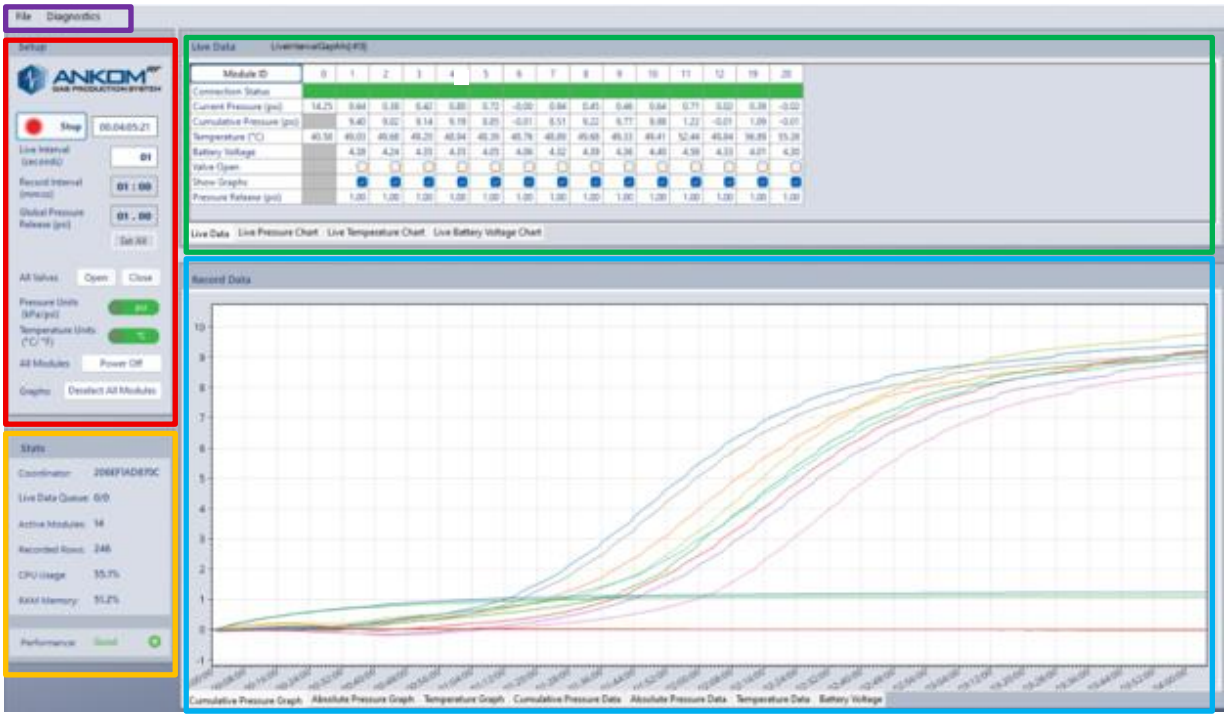
7.3 Temperature Control

The Module Assembly which includes the Module connected to a glass bottle, can be placed in a cabinet incubator to maintain appropriate temperatures. A water bath can also be used however; it is important to note that ANKOM recommends using a dry incubator to prevent moisture and vapor contaminating the modules.

Do not allow water into the Module: When placing a Module Assembly in water, DO NOT allow the water level to reach the vent tube or water damage will occur. When using a water bath, DO NOT cover the bath and the RF modules with a lid as the trapped humidity will be detrimental to the electronics. Instead, use bath balls (RF48) to cover the surface of the water and control the temperature and evaporation.

8. Software Screen layout

The screen below contains the controls and data associated with the operation of this system.



The main screen is divided into four (4) sections and two (2) dropdown menus:

- **Setup**
  The Setup panel located on the left side of the GPM screen displays the adjustable settings of the program. Settings in the setup panel are saved when you exit the program.

- **Stats**
  The Stats panel located on the left side of the GPM screen displays the current status of the program and PC.

- **Live Data**
  The Live Data is at the top of the GPM screen. It displays the current status of the Modules. The status will be updated based on the time interval specified in the **Live Interval** box on the Setup panel.

- **Record Data**
  The Recording View is in the center part of the GPM screen, and it displays the data recorded during the use of the system. Data graphs are automatically generated to provide the user with a visual indication of the recorded data during the gas study. The graphs are NOT automatically stored after the study is complete. See the "Auto Graphing" section in this manual for further details.

- **File and Diagnostics**
  These dropdown menus allow the user to save files, adjust settings and view more information.

The following is a more detailed description of the individual components displayed on the GPM screen.

8.1 - Setup

Record and Stop – When you click this button you either start recording the pressure and temperature data or you stop recording the pressure and temperature data therefore stopping the study.

Elapse Time – Next to the Record button, this stat displays the period of time that the system has been recording data.

Live Interval – This box contains the time (in seconds) between each instance of Module-to-computer communication. This interval can contain values from 1 to 60 seconds. This value does have an impact on battery life; the faster the live interval, the quicker the battery loses charge.

Record Interval – The record interval determines the frequency of the data points that are stored. The Record Interval should typically be set shorter for fast reactions and longer for slower reactions. Changing this value has no impact on battery life.

Best Practice:	The system will default to three (3) live intervals per Record Interval. When manually overriding the system defaults, best practice is to set the **Live Interval** so that there are at least two (2) Live Intervals for every **Recording Interval**. (e.g. Live Interval = 60 seconds, Record Interval = 2 minutes). Refer to the performance and connection status indicators if further adjustments to your interval settings should be made.

Global Pressure Release – This is a convenient way to set the pressure release for ALL of the Modules. Enter the value and then click the **Set All** button to apply to all the Modules. You can still change the value for each individual Module if you desire, by clicking in the Pressure Release row for a specific Module.

IMPORTANT:	The GPM software will not allow a release pressure greater than 10 psi. For 1.8L wide-mouth bottles, set the release pressure to a value no greater than 1 psi.

All Valves - Open – When this button is clicked, at the next live interval, the valves in ALL the Modules will open at one time. After clicking this button, you will see a check mark (☑) in each cell of the **Valve Open** row that has a live Module. The valve will remain open for the specified amount of time in the user-controlled setting or until the All Valves Close button is pressed.

All Valves - Close – When this button is clicked, at the next live interval, the valves in ALL the Modules will close at one time. After clicking this button, you will see a blank box (☐) in each cell of the **Valve Open** row.

Pressure Units – You can select PSI or kPa units to apply to the value entered into the **Global Release** box and to the values being recorded by the system.

Temperature Units – You can select Fahrenheit or Celsius.

NOTE:	Set your **Pressure and Temperature Units** before you click the **Record** button. You can NOT change them while the experiment is running and the system is collecting data.

8.2 - Stats

Coordinator – This displays the status of the Base Coordinator and its MAC address.

Live Data Queue – This is a performance indicator metric showing the programs ability to keep up with information coming in from the Modules. The closer the value is to zero the better. If the program starts to fall behind, this number will increase indicating that some adjustments to your setup (e.g. increasing the Live interval) should be made.

Active Modules – Number of Modules that are currently paired and communicating to GPM software.

Recorded Rows – This is the number of rows that have been recorded so far in your current session and represent data points that have been collected.

CPU Usage – This is the current PC's CPU usage in its entirety (not based solely on just our GPM program).

RAM Memory – This is the current PC's RAM usage in its entirety.

Performance - Four color indicators shows systems status – Hovering the mouse pointer over the word "Performance" will provide a brief description of the performance status and any action to take. Below is additional performance information:

Green – Good, GPM software can properly collect data with your Live Interval setting, quantity of Modules and PC performance.

Orange – Warning, consider increasing your Live Interval because the system is near the point of losing data points. At this time, the GPM software can properly collect data with your Live Interval setting and quantity of Modules and PC performance.

Red – Critical, the system is losing data. To resolve this issue, increase your Live Interval setting by several seconds. This condition can be caused by a slow PC or using too many Modules for the current Live Interval setting. The solution in either case is to increase your Live Interval setting. Please note that after increasing the Live Interval, it may take several minutes for the system to start performing correctly.

Purple – Error, a Module is communicating with a coordinator that was removed from the system database. Turn off the module or connect it to the PC via a USB cable and perform a "Module Pairing". This situation can occur when a user with multiple GPM systems exchange Modules between setups and a Module is deleted from one system but not paired with another system.

8.3 - Live Data

The following data or charts will be displayed according to the tabs clicked: **Live Data, Live Pressure Chart, Live Temperature Chart** or **Live Battery Voltage Chart**. By clicking on the tab, the user can monitor all the active Modules but not the inactive ones.

Live data tab:

Module ID/Name – Cells within this row display the Module identification number or name. By default, these cells display numbers but can be changed to display names by clicking on Module ID/Name. The Module names and ID numbers can be edited under **File** > **Settings** > **Edit Module**. For each individual column, this cell becomes **orange** when the Module has not communicated with the computer for at least 5 messages. If a Module that had previously lost communication regains communication, the Module ID/name cell will return to **white**.

Connection status – This provides feedback on the strength of the RF signal from each Module to the Base Coordinator. This can be affected when Modules are too far away from Base Coordinator, or there is too much radio frequency (RF) interference.

Connection Status – Green: The RF communications used by the system is working efficiently and all Modules have good communication with the Base Coordinator.

Connection Status – Continuous Yellow: The RF communications used by the system is struggling to maintain communications. This can be caused by RF interference in your area, the Modules positioned more than 30 yards away, or too many physical barriers between the Base Coordinator and Modules.
To improve connection, reposition the Modules closer to the Base Coordinator. **Yellow** indications can also occur briefly when the user decreases the Live Interval; this is normal and expected.

Connection Status – Grey: This Module is not communicating with the Base Coordinator. Possible Causes:

a. Dead Module battery – resolve by connecting the unit to the charging station hub to charge.
b. Module is too far away from the Base Coordinator. To resolve, move the Module closer to the Base Coordinator.
c. Module is placed in an area with too much RF interference or too many physical barriers between the Base Coordinator and Modules. To improve connection, reposition the Modules closer to the Base Coordinator.

Current Pressure – Cells in this row display the current pressure within the bottle relative to the readings by the Base Coordinator and represents the most recent pressure reading reported during the last **Live Interval**. A **light blue** cell indicates that the **Current Pressure** is above the **Pressure Release** threshold.

Cumulative Pressure – Cells in this row display the sum of the incremental changes in pressure for the specific Module recorded throughout the entire study. This pressure is NOT positively or negatively affected by manual or automated valve releases.

Temperature – Cells in this row display the value reported by the temperature sensor in the specific Module.

Battery Voltage – Cells in this row display the current battery voltage for the specific Module. The cell is shaded **white** when the voltage is 3.4 volts or higher. The cell is shaded **yellow** when the voltage is between 3.2v and 3.4v. The cell is shaded **red** when the battery voltage is below 3.2v. When a cell is shaded **red** the battery voltage is too low to control the valve opening and needs to be recharged.

IMPORTANT:	Although a Module will function at 3.2 volts, it may not have enough power to consistently operate the valve. Therefore, before starting a study, ensure that your rechargeable batteries are fully charged to 4.0 volts or higher. If batteries are getting low, you can connect a USB cable from the Module to the Charging Hub Station. This will charge the batteries while the unit continues to run as long as the test requires.

Valve Open – Clicking a cell within this row puts a check mark in the box (☑) and opens the vent valve for that specific Module for a default of 1 minute, the user can adjust this time to a maximum of 10 minutes. This can be adjusted under **Settings** in the section **Valve Open Time** > **User Controlled (sec)**. Clicking the cell again removes the check mark (☐) and closes the specific vent valve. This function allows you to open and close valves for individual Modules. You can open and close all valves at once by using the **All Valves Open** and **Close** buttons in the Setup Panel. **NOTE: When the vent valve is open the system will NOT build any pressure.**

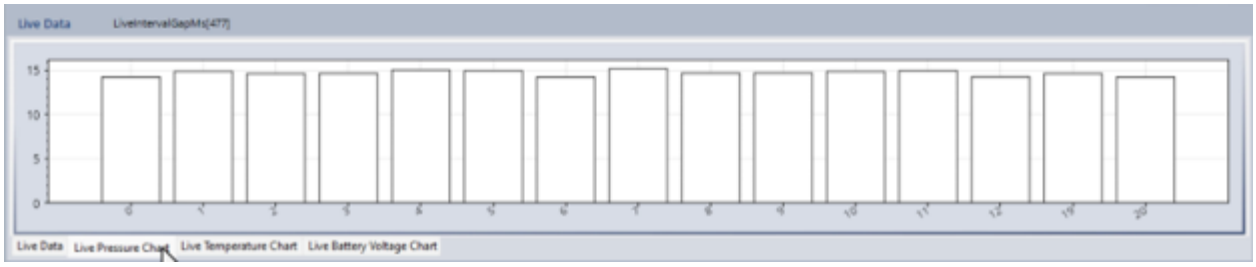
Show Graphs – Clicking a cell within this row puts a check mark in the box (☑) for that specific Module. Clicking the cell again removes the check mark (☐). The Auto Graphing capability will only display a graph for a Module that has a check mark (☑) in its cell within this row. You can disable or enable all Modules from displaying their graphs by clicking **Graphs: Deselect All Modules / Select All Modules** button in the Setup Panel.

Pressure Release – Cells in this row display the maximum pressure setting for the respective Module relative to the reference Base Coordinator. If pressure within a Module climbs above this value, the valve will open for a default of 250 (ms) to vent the gas at the next live interval. The user can adjust this time to a maximum of 1000 (ms). This can be adjusted under **Settings** in the section **Valve Open Time** > **Global Pressure Release (ms).** The **Current Pressure** cell will turn **light blue** when this occurs. The computer automatically accounts for pressure loss during this valve venting. **NOTE: To update the individual Module's Pressure Release value, enter the value into that specific Module's cell and then press <Tab> or <Enter> on your keyboard to enter the value into the system.**

IMPORTANT:	The GPM software will not allow a release pressure greater than 10 psi. For 1.8L wide-mouth bottles, it is recommended that you set the **Pressure Release** to a value no greater than 1 psi. The software sets a default pressure release to 2.5 psi.

Live Pressure Chart Tab:

This tab shows a bar graph of live pressure readings for every Module.

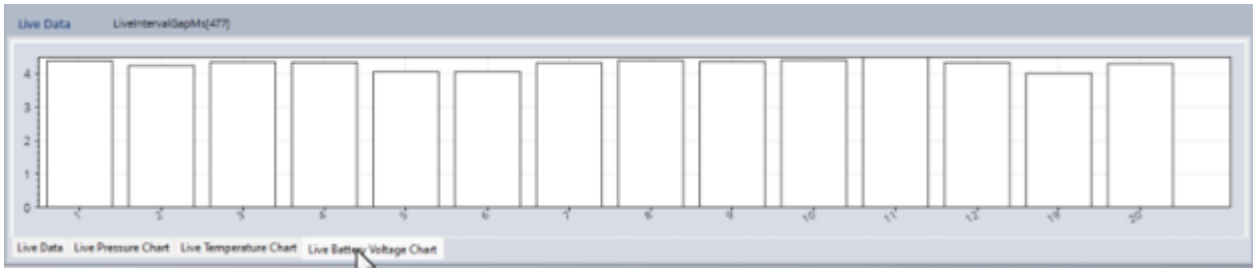


Live Temperature Chart Tab:

This tab shows a bar graph of live temperature readings for each Module.

Live Battery Voltage Chart Tab:

This tab shows a bar graph of live battery voltage for each Module.

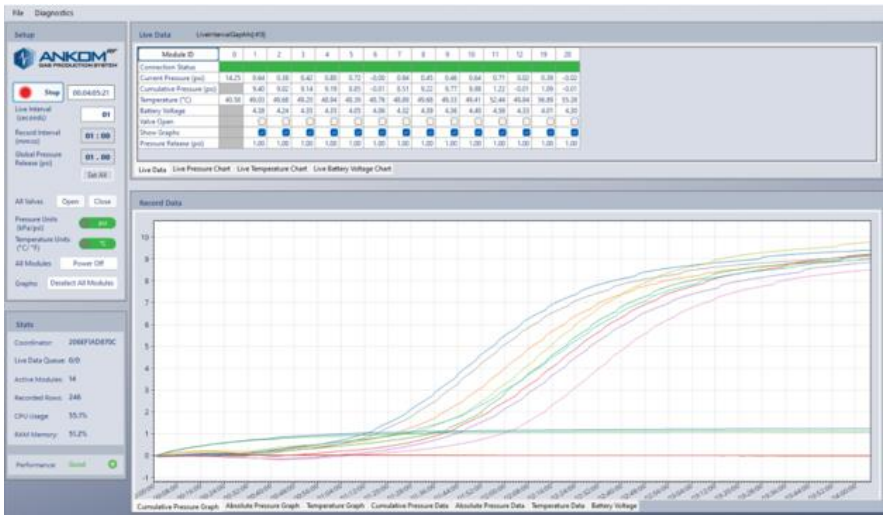


8.4 - Record Data

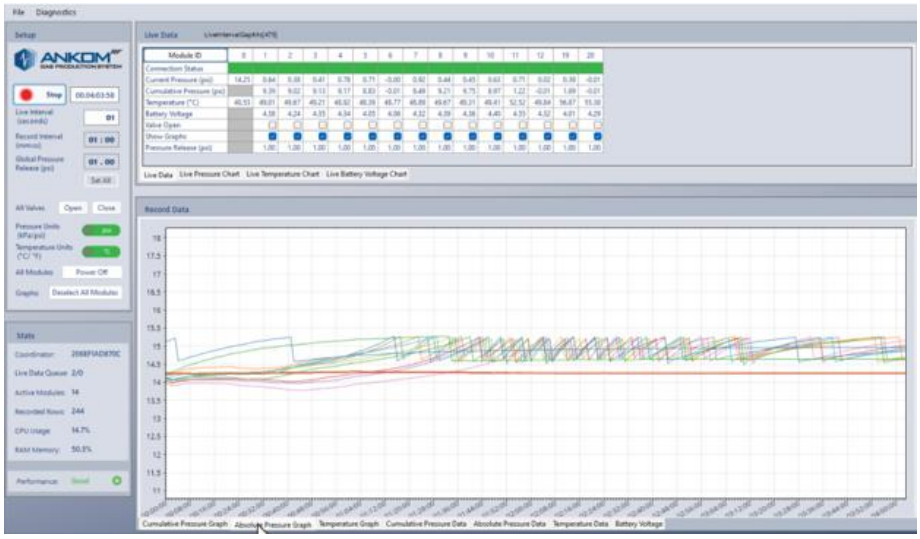
Auto Graphing

In addition to capturing the data points for pressure and temperature, the GPM software has an Auto Graphing capability that displays line graphs for the **cumulative pressure, absolute pressure** and **temperature** for each selected Module within a study. The pressure and temperature graphs can be viewed on separate tabs. Examples of these graphs are shown below.

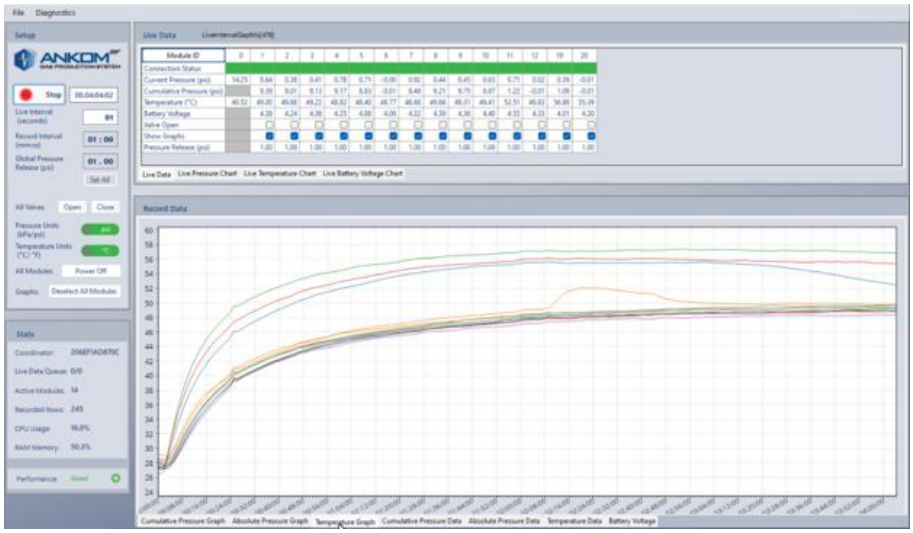
Cumulative Pressure Graph – When you click this tab you will see the cumulative pressure graph for the times specified by the **Recording Interval**. This is the default data view for the program.



Absolute Pressure Graph – When you click this tab you will see the absolute pressure graph for the times specified by the **Recording Interval**. An example of this screen is shown below.



Temperature Graph – When you click this tab you will see the temperature graph for the times specified by the **Recording Interval**. An example of this screen is shown below.



Sections of each graph can be enlarged using a few different methods (Note: the screen will go back to normal size when the next data point is recorded):

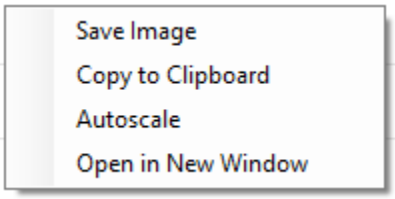
1. Placing the cursor over the section of interest and using the center scroll button to enlarge.

2. Placing the cursor over the section of interest and then holding the center mouse button and dragging so that a red square appears over what will be enlarged.

Saving Graphs

IMPORTANT:	All raw data can be exported to MS-Excel format which easily allows the user to generate graphs and analyze the data. The Auto Graphing capability is meant to provide the user with a visual indication of the data being recorded during the gas study. **However, the graphs themselves are NOT automatically stored after the study is complete.** To save the graphs from the Auto Graphing feature, you can either: (1) Save before ending the recording. (2) Save immediately after ending the recording. (3) Do not start a new recording or the graphs will not be saved, only the raw data.

To save the graphs, the user right clicks on any of the graphs and the following menu will be displayed:



From this menu, you have the following options:

- **Save Image**– Saves the graph in a selectable image format.
- **Copy to Clipboard** – Saves the graph temporarily in the PC memory (RAM). This allows the user to quickly paste the information in other locations. Copying something new will override the previous item.
- **Autoscale** - Automatically adjusts the graphs X or Y axes to fit displayed data. This helps make all the data visible in the plotted area.
- **Open in New Window** – Opens a smaller version of the graph in a new window.

Data Collection

When an experiment begins and the recording button is clicked, the GPM software will begin capturing data points from every paired Module.

Cell Color coding Definitions – The recorded data points will capture every Modules communication and pressure status during the study, and the colors below represent events that happened during the study.

Cumulative and Temperature Data

Orange - A cell is shaded ORANGE when the GPM software has failed to receive 5 messages in a row from the Module.

Absolute Data

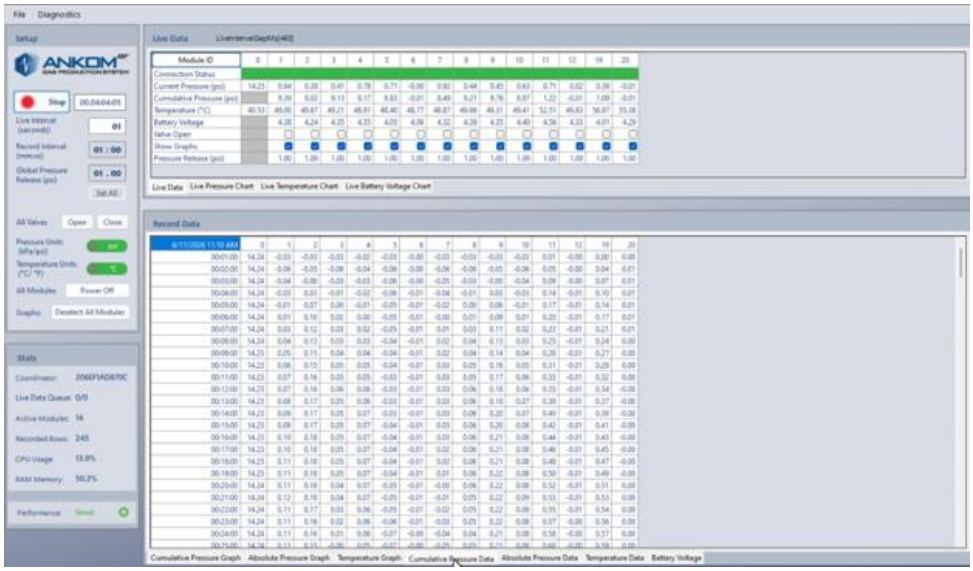
Orange - A cell is shaded ORANGE when the GPM software has failed to receive 5 messages in a row from the Module.

Light Blue - A cell is shaded LIGHT BLUE when the pressure within that Module climbs above the Pressure Release value specified for the respective Module. When this happens, at the next live interval the valve briefly opens and vents the gas until the pressure is below the Pressure Release setting.

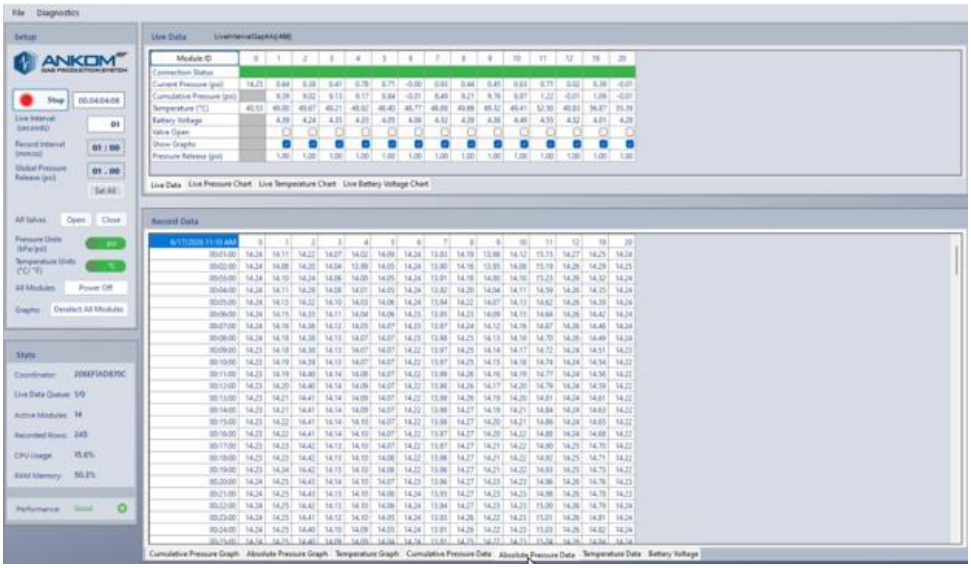
Battery Voltage

Yellow - A cell is shaded YELLOW when charging the unit via USB is recommended. This will prevent the battery voltage from reaching the RED indication where valve control is disabled. The cell will show YELLOW between 3.4v and 3.2v.

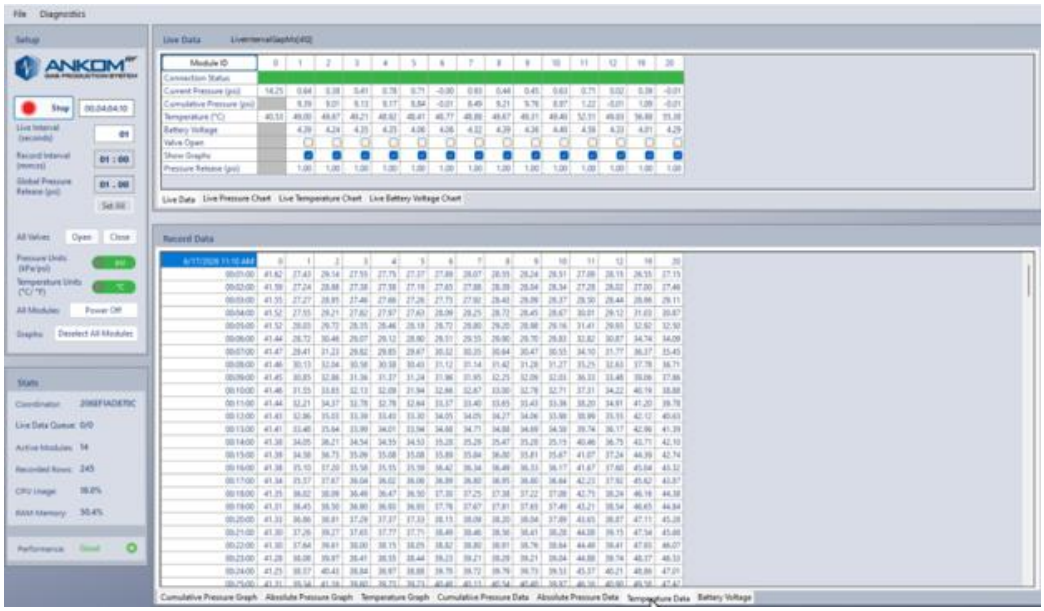
Cumulative Pressure Data – When you click this tab you will see the cumulative pressure data for the times specified by the **Recording Interval**. An example of this screen is shown below.



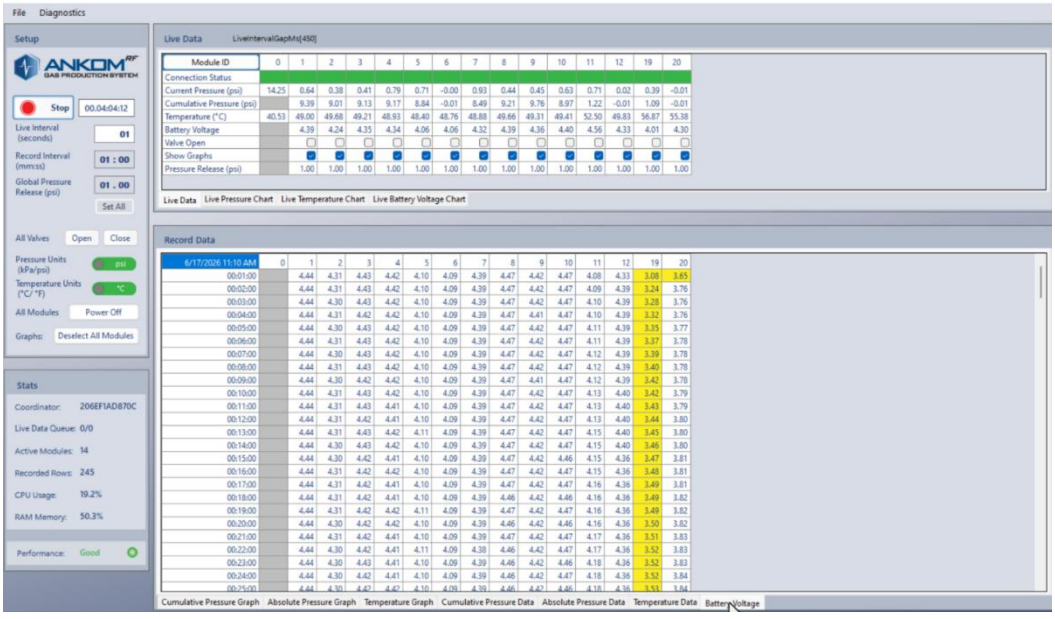
Absolute Pressure Data – When you click this tab you will see the absolute pressure data for the times specified by the **Recording Interval**. An example of this screen is shown below.



Temperature Data - When you click this tab you will see the temperature data for the times specified by the **Recording Interval**. An example of this screen is shown below.



Battery Voltage– When you click this tab you will see the battery voltage for each Module for the times specified by the **Recording Interval**. An example of this screen is shown below.



8.5 - File

Save or Save As – When you click **File** > **Save** this allows you to create an Excel file name and a file location within your computer system for the data to be stored. You can save the file any time during the run.

NOTE:	The data points in the saved Excel spreadsheet are time-stamped with incremental elapsed time based on the studies **Record Interval** setting.

Module Pairing – This opens a dialog box that displays the Modules available for pairing. If the Module does not appear after plugging it into the PC, click **Refresh**. Select the Module that you would like to pair. Click **Pair Modules**. **NOTE: Make sure that the Module is turned on when pairing.**

Settings – This opens a dialog box that displays a list of modules

- **Edit Modules –** This opens a dialog box that allows the user to alter the Module Name and the Module ID number. Any changes can be saved and then viewed in the Live Data section. The Modules can have matching names but cannot have matching ID numbers.
- **Delete Modules –** This will remove a Module that is paired to the coordinator. The Module can then be paired with another coordinator.
- **Delete All Modules -** This will remove all Modules that are paired to the coordinator. The Modules can then be paired with another coordinator.
- **Graphs / Display Options –** This section allows the user to adjust the display options of the recorded graphs and data tables.
    - **Show legend:** This turns On and Off the legend displayed on the graph.
    - **Max Viewable Data Points:** This is the number of data points that can be displayed on the recorded tables at one time. Once the data points exceed this number, the oldest data point will no longer be displayed.
- **Database Management –** This section allows the user to adjust the Live and Recorded session table layout.
    - **Max number of Live Sessions:** This is the maximum number of Live sessions the system will store before deleting the oldest session. Default is 99 sessions (range 5-99 sessions).
    - **Max Stored Rows in Live Sessions:** This is the maximum number of rows that will be recorded in a Live session table. Default is 5000 rows (range 5000 – 50,000 rows).
    - **Max Number of Record Sessions:** This is the maximum number of Record sessions the system will store before deleting the oldest session. Default is 99 sessions (range 5-99 sessions).
- **Valve Open Time –** This section controls the length of time the valve is open for manual and automatic operation.
    - **Global Pressure Release (ms):** This is the amount time the valve will automatically open when the Module's Pressure Release is reached. Default is 250 (ms) (range 250-1000 ms).
    - **User Controlled (sec):** This is the amount time the valve will open when the user manually opens the valve in the **Live Data section** or the **Setup panel** > **All Valves Open / Close Buttons**. Default is 60 (sec) (range is 1-600 sec).

- **Cumulative Pressure –** This is a time delay the software applies to the data collection to compensate for the adiabatic reaction when opening the Modules Valve.
    - **(PV=nRT) Delay (sec):** Default is 5 sec. (range 0-300 sec).

- **Advanced Settings –** This is the location of critical system settings. This should only be adjusted with complete understanding of software implications.
    - **Location of Databases and logs:** This is the file location of your GPM databases and live/recorded log sessions.
    - **Override Interval Minimums:** This turns off the automated adjustment to the Live and Recorded Intervals. This can be turned off when the user wants to adjust settings outside of the recommended ranges.
- **Save Changes –** If any adjustments have been made in the settings, make sure to click **Save Changes** to apply.
- **Cancel –** Click to Button to exit the settings dialog box without saving anything.

View Sessions – This is a table of all the recorded sessions stored in the GPM. A session can be exported to Excel in the selected pressure and temperature units. The table will store a maximum of 99 sessions before deleting the oldest session. The number of sessions that can be stored can be adjusted in **Settings > Max Number of Record Sessions.**

Exit – This closes and exits the software program completely.

8.6 - Diagnostics

Logs – This will open to the location where all the log files that have been automatically saved. This is set when the program is installed and can be viewed in **Settings > Location of Databases and logs.**

Live Interval Tables – This table is data collected from the Live Data Section. This table is logging senser data when the software is running but have not started recording. This feature is used for troubleshooting and diagnostic purposes and can be exported by the user. The maximum number of sessions and the maximum number of rows in the table can be adjusted in **Settings**.

Identify Module – If a user needs to locate a Module, they can select a specific Module ID. This will cause the LED indicator light on that Module to turn **yellow** for easy identification. To turn off the indicator light, exit out of **Identify Module** window.

9. Module Functionality

9.1 Charging Modules

The Modules will be partially charged when they are initially received. ANKOM recommends fully charging the modules using the provided Charging Hub Station before beginning a test. If the Module is turned on, (holding the power on/off for approximately one second), the status of the progress charging can be monitored using the GPM software. The module does not need to be turned on when it is charging. If the module is off, the LED indicator light will illuminate green to signal that it is charging. When the battery is fully charged the light will turn off. When the Module is on, there will be no green LED indicator light. To fully charge a module battery it takes approximately 8 hours.

A fully charged battery can last a long time. For example, if the software is set to receive Module information every 60 seconds and the solenoid is opened for 0.250 seconds every hour, a fully charged battery can last approximately three weeks before needing to be charged. The battery will need to be charged more frequently if the user sets the live interval and valve release at a higher frequency. The GPM software will inform users when the battery is low.

9.2 Unpairing a Module

Modules can be added or removed from different coordinators, customizing how many are being used in one session. If the user has multiple coordinators, a module can be unpaired from one and paired to the other in between the start of a session. This allows for flexibility in study sizes and the ability to pair up to 75 Modules with one coordinator.

1. To unpair a Module, go to **File** and then **Settings**.

2. A dialog box will appear displaying a list of all the paired Modules. Select the Module you want to unpair and press **Delete Module**.

3. A prompt will appear to confirm continuation. Select the **Yes**.

4. The module will disappear from the list of paired Modules. Select **Save Changes.** The Module can be paired before the start of a session to a new coordinator and can be assigned to any unused number.

9.3 Renaming Modules:

When a module is paired with a base coordinator it is automatically assigned an ID number and a name.

1. To change a module's name or ID number, go to **File** and then **Settings**.

2. A dialog box will appear displaying a list of all the paired modules. Select the module you want to rename or renumber. Press **Edit Module**.

3. A dialog box will appear. Any name can be entered into the **Module Name** box and the **Module ID** can be adjusted using the arrows or entered in. Modules cannot have the same Module ID number.

4. Press **Save**. The listed module will now show the updated name and ID. Select **Save Changes**. In the live data section, click on the cell to see the module name or module ID, and the entered value will be displayed.

9.4 Temperature Sensing

The standard sensor is mounted on the circuit board inside the Module electronics assembly. As a result, it will not directly measure the temperature inside of the bottle or the temperature of the material contained within the bottle. However, over time the temperatures of the outside environment, the inside of the Module electronics assembly, and the inside of the bottle will equilibrate. At that time, the standard sensor assembly will provide a reasonable representation of the temperature in the environment outside and inside of the bottle. The sensors are accurate to within ± 2° Cover a range of 5°C – 60°C.

IMPORTANT:	**The temperature sensor reading will increase approximately 5 degrees from ambient temperature. This will occur during the first 5 minutes of a Module being powered on. The temperature sensor reading will then return to ambient temperature.**

10. Operating Instructions

To conduct a study with the ANKOM*RF2000* Gas Production System, follow the steps below:

10.1 Clean and sterilize the Modules and Bottles before beginning operation.

It should be standard practice after each use to clean the underside of the blue module housing with warm soapy water. When this is done it is critical that water not get into the Module or the electronics will be damaged. Do not autoclave or submerse the module (even with the lid on). With the Modules cleaned, it is recommended that they be sterilized with alcohol before use. To expedite the process, the Modules may be dried at temperatures up to, but not exceeding, 60°C.

The glass bottles that come with your ANKOM RF Gas Production System are plastic-coated for safety. As such they are not rated for autoclave sterilization. As with the rest of the system, do not heat the bottles beyond 60°C. Clean the bottles with warm soapy water. Rinse and sterilize with alcohol. To expedite the process, the Modules may be dried at temperatures up to, but not exceeding, 60°C.

10.2 Charge each Module.

The parameters entered in the software set by the user will determine how long a battery will last. The frequency of the Modules releasing pressure, which is the opening and closing of the valve, will deplete the battery at different rates.

For example, if the software is set to receive Module information every 60 seconds and the solenoid is opened for 0.250 seconds every hour, a fully charged battery can last approximately three weeks before needing to be charged. For long-term studies (i.e. months) the RF2000 system can also be run with the Modules plugged into the charging hub station, so the battery charge is not a concern.

10.3 Connect and turn on the Base Coordinator to the PC.

10.4 Start your GPM software.

On your computer monitor, click the GPM icon to start the program.

10.5 Test that each Module is communicating with the computer.

Turn on each Module by holding the button on top down for 1 second until the indicator light turns on. If the Modules are paired and charged, the Module should automatically connect to the coordinator, displaying each Module in the Live Data section. If the Modules are not yet paired, refer to the "Pairing Modules" section in the manual.

10.6 For ruminate studies, prepare the Buffer to be used.

See Appendix A for examples of Buffer Preparation used in Rumen studies.

10.7 For ruminate studies, prepare the Inoculum to be used.

See Appendix A for examples of Inoculum Preparation used in Rumen studies.

10.8 Prepare the Sample (substrate) to be used in your study.

Depending upon how fermentable the sample is, the quantity of sample will vary. In addition to the quantity of fermentable sample, the length of time the incubation is conducted must be taken into consideration. The quantities of sample and buffer may be sufficient for a 24-hour incubation, but they may not be sufficient for a 48 hour incubation. By measuring the pH at the end of the incubation period you can determine if the buffer maintained the proper pH throughout the incubation. This will allow you to alter the sample-to-buffer ratio to fit the desired incubation period.

See Appendix A for further information about Samples used in Rumen studies.

10.9 Add Buffer and Inoculum to the Blank to be used in your study.

When running a study using the ANKOM*RF2000* Gas Production System, corrections must be made for the following two factors:

- Gas produced by the inoculum.
- Gas lost by slight permeability of CO_2 through the elastomeric components of the system (in a pure CO_2 environment under 2 psi pressure, studies show that the permeability rate is 0.02 psi/hr).

Running a blank in your study will correct for both factors.

NOTE:	The gas permeability rate of 0.02 psi/hr is only a reference. This is NOT to be applied broadly. Use the results of your Blank as the correction factor.

Place buffer and inoculum in the Glass Bottle used as a Blank.

NOTE:	Do NOT use any sample (substrate) in the Glass Bottle used as the Blank.

10.10 Eliminate the oxygen from the Glass Bottle used as a Blank (i.e., purge the bottle).

If you are running a study that includes anaerobic organisms, then eliminate the oxygen from the Glass Bottle used as a Blank by following the procedure below.

NOTE:	You will need a source of CO_2 regulated to 8-10 psi for this procedure.

10.10.1 After adding inoculum to the Glass Bottle, reattach to its Module.

10.10.2 On your GPM screen, under **Live Data**, check the **Valve Open** box.

10.10.3 Under **Setup**, set the **Live Interval** to 1 sec to speed up the valve open and close operation. Set for 5 sec if there are more than 10 Modules connected.

10.10.4 Go to **File** and then **Settings**, under **Valve Open Time** set the **User Controlled** time to 30 seconds. Click **Save Changes**.

10.10.5 Select **Valve Open** check box in the **Live Data** panel to open valve to begin purging.

10.10.6 Holding a CO_2 supply against the Luer Port on top of the Module, add 8-10 psi of CO_2 to the Module's Glass Bottle (see below). Apply CO_2 for 30 seconds until the valve closes, allowing time for the CO_2 gases to purge O_2 from the system.

10.10.7 Ensure the pressure in the Glass Bottle is back to 0 ± 0.2 psi by opening the valve to release any residual pressure.

10.10.8 Remove / turn off the CO_2 supply and place the cap on the Luer Port.

10.10.9 Repeat this procedure for each Module.

10.10.10 Reset the **Live Interval** and **Valve open time** settings on the GPM screen based on the needs of the experiment.

WARNING: For 250 ml – 1000 ml narrow-mouth bottles, never allow the pressure in the bottles to exceed 10 psi. Always regulate incoming purge pressure down to 10 psi or less before purging bottles. For 1.8 L wide-mouth bottles, never allow the pressure in the bottles to exceed 1 psi. Always wear safety glasses and appropriate lab protection when handling the Modules and Glass Bottles.

10.11 Add Sample, Buffer, and Inoculum to all non-Blank Glass Bottles to be used in your study.

Place your sample, buffer, and inoculum into the non-Blank Glass Bottles.

10.12 Eliminate the oxygen from the non-Blank Glass Bottles.

If you are running a study that includes anaerobic organisms, then eliminate the oxygen from the non-Blank Glass Bottles by following the procedure detailed in steps above.

10.13 Configure your GPM software for your study.

10.13.1 In the **Setup Panel**, set the **Live Interval** duration. This is the number of seconds that the system will wait between each Module-to-computer communication. Longer live interval times increase battery life.

10.13.2 In the **Setup Panel**, set the **Recording Interval**. This is the number of minutes the system will wait between each data point that gets recorded to the GPM spreadsheet for each Module.

IMPORTANT: For best results it is recommended that the **Live Interval** be set so that there are at least 3 Live Intervals for every **Recording Interval**. For example, if you want to record data every 3 minutes, you should set the **Recording Interval** to 3 minutes and the **Live Interval** to 1 minute (60 seconds) or less. The GPM will set these interval minimums automatically unless disabled in **Settings > Advanced Settings > Override Interval Minimums.**

10.13.3 In the **Setup Panel**, set the **Global Pressure Releas**e value for all Modules. This is the pressure that when reached will initiate the opening of the Module's value.

10.13.4 In the **Setup Panel**, set the **Pressure Units**.

10.13.5 In the **Setup Panel**, set the **Temperature Units**.

10.14 Place the Module Assemblies in an incubator or water bath set to the appropriate temperature.

Do not allow water into the Module: When placing a Module Assembly in water, do NOT allow the water level to reach the vent tube or damage will occur. When using a water bath, do NOT cover the bath and the RF modules with a lid as the trapped humidity will be detrimental to the electronics. Instead, use bath balls (RF48) to cover the surface of the water and control the temperature and evaporation.

10.15 Start recording data.

IMPORTANT: Before you start recording data, charge batteries that show 4.0 volts or lower. It is highly recommended to use a fully charged battery.

On your GPM screen, click **Record** to start recording your data. At optimal system settings, a fully charged battery can run for approximately 3 weeks. For studies running longer than this, ANKOM recommends keeping Modules plugged into the charging station hub or plugging the Modules into a charger when the battery voltage drops below 3.4 volts.

10.16 Stop recording data at the end of the study.

16.1 On your GPM screen, click **Stop** to stop recording data and click **Yes** to end experiment.

16.2 To save the recorded session as an excel file, go to **File**, click **Save As**. Save the file in the location of choice on the PC. If the user forgets to save the data, they can go back later and export the session by **File** > **View Sessions**.

NOTE: If you want to create an Excel file during a run, just press File > **Save…** button on the GPM screen. The program will continue to run as normal.

11. Troubleshooting

The ANKOM Technology web site has the most current troubleshooting and replacement parts information. Therefore, if you have any questions about the operation of your ANKOM*RF2000* Gas Production System, or if you need replacement parts, please visit our web site at **www.ankom.com.**

To confirm the performance of the RF2000 Gas Production System, refer to the Validation Test Service Procedure (RFS004).

12. Periodic Maintenance

For information about periodic maintenance, please visit our website at **https://www.ankom.com/technical-support/rf-gas-production-system.**

Appendix A – Buffer, Inoculum, & Sample prep for Rumen studies

Buffer: Goering - Van Soest (see ref. 3)

The information contained herein comes from the following paper:

Goering, H.K. and Van Soest, P.J., 1970, Forage fiber analysis (apparatus, reagents, procedures and some applications), Agricultural Handbook No. 379 ARS-USDA, Washington, DC.

To prepare for the study of apparent digestibility within ruminant animals using the Goering – Van Soest buffer, follow the procedure below.

(1) Maintain all glassware at 39°C.

(2) Prepare the following solutions using Distilled Water throughout.

Resaruzin 0.1% (w/v) solution
Dissolve 0.1 g resaruzin into 100 ml H_20

In vitro buffer solution	
NH_4HCO_3	4 g
$NaHCO_3$	35 g
Bring volume to 1 L using Distilled Water	

In vitro macromineral solution	
Na_2HPO_4 anhydrous	5.7 g
KH_2PO_4 anhydrous	6.2 g
$MgSO_4 \cdot 7\ H_2O$	0.6 g
Bring volume to 1 L using Distilled Water	

In vitro micromineral solution	
$CaCl_2 \cdot 2\ H_2O$	13.2 g
$MnCl_2 \cdot 4H_20$	10.0 g
$CoCl_2 \cdot 6\ H_2O$	1.0 g
$FeCl_3 \cdot 6\ H_2O$	8.0 g
Bring volume to 100 ml using Distilled Water	

Reducing solution	
Cysteine·HCl	625.0 mg
1N NaOH	4.0 ml
$Na_2S \cdot 9H_2O$	625.0 mg
Bring volume to 100 ml using Distilled Water	

(3) Mix 2 g trypticase with 400 ml of water and 0.1 ml micromineral solution. Agitate to dissolve.

(4) Add 200 ml of buffer solution, 200 ml of macromineral solution, and 1 ml of resaruzin solution to the solution in step 3. Mix together to create your final buffer solution.

(5) Prepare enough buffer solution to support the planned number of Gas Production Modules (80 ml buffer per 250 ml bottle – adjust the amount according to this ratio if using different size bottles).).

(6) Add sample to each Glass Bottle (1 g per 250 ml bottle – adjust the amount according to this ratio if using different size bottles).

(7) Add buffer to each Glass Bottle used in the run (80 ml per 250 ml bottle – adjust the amount according to this ratio if using different size bottles).

(8) Allow the temperature of the Glass Bottle, buffer, and sample to equilibrate for 20 to 30 minutes at 39°C.

(9) Prepare the rumen inoculum while the buffer and sample are equilibrating.

(10) To remove O_2 from the buffer solution, add 2 ml of reducing solution. The buffer solution color should change from a red to colorless.

(11) Add rumen inoculum to each bottle (20 ml per 250 ml bottle – adjust the amount according to this ratio if using different size bottles).

Buffer / Mineral Solution: Cone (see ref. 4)

Solution	grams / liter
$NaHCO_3$	8.75
NH_4HCO_3	1.00
Na_2HPO_4	1.43
KH_2PO_4	1.55
$MgSO_4 \cdot 7H_2O$	0.15
Na_2S	0.52
$CaCl_2 \cdot 2\ H_2O$	0.017
$MnCl_2 \cdot 4\ H_2O$	0.015
$CoCl_2 \cdot 6\ H_2O$	0.002
$FeCl_3 \cdot 6\ H_2O$	0.012
Resazurin (optional)	0.125

Buffer: Kansas State (see ref. 5)

Mix 20 ml of Solution B with 1000 ml of Solution A and adjust pH to 6.8 by adding Solution B before each use.

Solution A	grams / liter
KH_2PO_4	10.0
$MgSO_4 \cdot 7\ H_2O$	0.5
NaCl	0.5
$CaCl_2 \cdot 2\ H_2O$	0.1
Urea (optional)	0.5

Solution B	grams / 100 ml
Na_2CO_3	15.0
$Na_2S \cdot 9\ H_2O$	1.0

Inoculum Preparation Procedure

(1) Preheat two 2 L thermos bottles by filling with 39°C water.

(2) Empty heated water just prior to collection of rumen inoculum.

(3) Using the appropriate collection procedure, remove 600 to 1,000 ml of rumen inoculum and place in thermos. Include approximately two "fistfuls" of the fibrous mat from the rumen with your collection in one thermos.

(4) Empty the rumen inoculum and fibrous mat from the thermoses into a pre-warmed blender.

(5) Purge the blender container with CO_2 gas and blend at a high speed for 30 seconds. The blending action serves to dislodge microbes that are attached to the mat and assure a representative microbial population for the fermentation.

(6) Filter the blended digesta through 4 layers of cheesecloth into a pre-heated (39°C) flask. NOTE: Allow for extra cheesecloth around the edges to facilitate squeezing contents of filtered mat.

(7) The flask should be continually purged with CO_2 before and after the transfer of the inoculum.

(8) Be careful to maintain temperature to minimize cold shock of the microorganisms.

(9) Add the 20 ml of inoculum to the equilibrated buffer solution and sample in each Module's Glass Bottle.

(10) Purge the Glass Bottle with CO_2 gas for 30 seconds. A purge system can be purchased from ANKOM Technology which allows the purging to be accomplished in a closed Module. If you don't have an ANKOM purge system, remove the Glass Bottle and allow CO_2 to flow into it.

(11) Repeat process for all Glass Bottles to be used.

NOTE: Do not allow CO_2 gas to bubble through the buffered inoculum. Instead, use the CO_2 to form a gaseous blanket over the contents of the Glass Bottle.

Sample Preparation

The quantity of sample (substrate) to add to the Module will vary from 1g for a forage sample with minimal fermentable substrate to 0.25 g for a highly fermentable substrate such as a high starch diet. In addition to the quantity of fermentable substrate, the length of time the incubation is conducted must be taken into consideration. The quantities of substrate and buffer may be sufficient for 24 hour incubation but may not be sufficient for a 48 hour incubation. By measuring the pH at the end of the incubation period you can determine if the buffer maintained the proper pH throughout the incubation. This will allow you to alter the substrate-to-buffer ratio to fit the desired incubation period.

References

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Appendix B – Conversion of Pressure to Gas Production

Calculation of Gas Produced in ml at 39°C with pressure measured in psi

The ANKOM^{RF2000} Gas Production System is configurable to allow for gas pressure measurements in either psi or Kpa units. For the purposes of this discussion, we will refer to gas pressure measurements in psi.

The gas pressure measured during your study can be (1) converted to moles of gas produced using the 'ideal' gas law, and then (2) for graphing purposes, converted to milliliters (ml) of gas produced using Avogadro's law.

(1) Ideal gas law

PV = nRT

Where: n = gas produced in moles (mol)
P = pressure in kilopascal (kPa)
V = head-space volume in the Glass Bottle in liters (L)
T = temperature in Kelvin (K)
R = gas constant (8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$)

(2) Avogadro's law

At standard temperature and pressure (STP) (101.325 kPa, 273.15°K) ,1 mole will occupy 22.4 L. Therefore, gas measured in moles can be converted to gas measured in ml as follows:

Volume of gas produced (ml) = n mol x 22.4 L/mol x 1000 ml/L

ANKOM^{RF} Gas Production System – Glass Bottle Volume Capacity

The actual volume capacity of each glass bottle is greater than its rated volume capacity. Therefore, use the following Actual Volume Capacity for the specific bottle used in your study.

Labeled Volume (ml)	100	250	500	1000	1800	
Actual Volumes (ml)	137	310	614	1124	1950	Standard Bottle
	140	313	619	1130	N/A	Septa Bottle

Example

The Cumulative Pressure measured by the ANKOM^{RF} Gas Production System is 10 psi at 39°C.
The Glass Bottle in the study is rated at 250 ml (actual volume capacity is 310 ml).
The sample/solution/buffer uses 150 ml of the Glass Bottle.
The head-space volume in the Glass Bottle is 310 ml - 150 ml = 160 ml = 0.16 L.

P = 10 psi x 6.894757293 kPa = 68.94757293 kPa
V = 0.16 L
R = 8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$
T = 273°K + 39°C = 312°K

n = PV/RT
= (68.94757293 kPa x 0.16 L) / (8.314472 $L \cdot kPa \cdot K^{-1} \cdot mol^{-1}$ x 312°K)
= 0.004252553 mol

Volume of gas produced (ml) = 0.004252553 mol x 22.4 L/mol x 1000 ml/L
= 95.25717874 ml

Appendix C – PC Settings

Since your GPM software runs on a PC, power, operating system (e.g., WINDOWS) settings, other programs on the PC, and network connections can affect the GPM software.

Power

If your PC is being powered by a battery or an inconsistent AC power source, the power could turn off or spike while the GPM software is running. This could cause the GPM software to stop collecting data. You should always run the GPM software on a PC that is connected to an AC power source with surge protection.

Operating System Settings

Your PC screen can go blank as a result of the settings in your PC operating system for:

- Sleep
- Hibernate
- Screen Saver
- Automatic Updates

If your PC screen goes blank while the GPM software is running, the data collection can be impacted. To avoid issues associated with operating system settings, we recommend the following:

- Set the sleep setting to "Never."
- Set the hibernate setting to "Never."
- Set the screen saver setting to "None."
- Do operating system updates manually instead of automatically, as some operating system updates will reset the sleep and hibernate settings back to defaults after it performs the update.

IMPORTANT:	The names for the operating system settings could be different depending on the operating system. Also, be aware that the sleep and hibernate settings can still be automatically changed after a manual operating system update. Therefore, you should always check and correct these settings as needed after an operating system update.

Other programs on your PC

Other programs on your PC can impact your GPM software, especially programs that automatically run anti-Virus and anti-Malware. You should only update programs and run scans manually to avoid having them automatically run while the GPM software is running.

Network connections

If the PC running your GPM software is connected to a computer network, administrators of the network can make changes that impact the entire network. We recommend that the PC running the GPM software be disconnected from any computer networks (at least while the GPM software is running).

IMPORTANT:	If you lose data during a run with the ANKOM^{RF} Gas Production System, there is a possibility that user can recover some of the data from the Detail Log file. If running Windows 10 be aware that you must **DISABLE** "Mandatory Updates."

Appendix D – RF2000 Battery Charger and User Guide

ANKOM Technology warrants the ANKOM 16 Station Battery Charger against any defects due to faulty workmanship or material for six months after the original date of purchase. If the instrument is damaged because of defects in the workmanship or materials during the warranty period, ANKOM will repair or replace the instrument free of charge. This warranty does not include damage to the instrument resulting from neglect or misuse.

Using your Battery Charger

Before using your ANKOM 16 Station Charger, please read the information below.

- Only use this charger for the ANKOM RF2000 modules
- Connect the power supply to the back of the battery charger and then plug it into a standard electrical outlet (110v or 220v dependent on model ordered). Only use the power supply and cable supplied with the unit.
- Push the on/off buttons for the individual charger ports on the top of the charger to turn each port on. Blue LED indicator lights will show each ports status.
- Connect the RF2000 Module(s) to the charger using the USB-C to USB-A cords supplied with each Module.

NOTE:	RF2000 Module(s) do NOT have to be fully discharged before connecting to the charger

- When the RF2000 Module(s) are connected a Green LED located on top of the Module will turn on to indicate the module is charging. The Green LED will turn off when fully charged. If the Green LED does not come on after 5 minutes, the battery may be faulty or have a problem with the port/charging station, or the battery may be fully charged already.
- Disconnect RF2000 Module(s) when charging is complete. RF2000 Module(s) can be connected and disconnected from the charger with the charger power ON.

NOTE:	RF2000 Module(s) will NOT be damaged if you leave them connected to your charger after they are full.

- Keep the power OFF when the charger is not in use.

WARNING: Charge the RF2000 Module(s) with the ANKOM 16 Station Charger only. Charge RF2000 Modules in an operating environment of 0° C – 40° C. Only use batteries provided by ANKOM in RF2000 modules.

- Avoid excessive physical shock or vibration to Modules
- Do not expose Modules to fire
- Do not immerse Modules in water
- Keep out of reach of children
- Do not disassemble or deform Module

RF2000 Module Battery Information

Cycle Life	≥ 500 times	One cycle refers to one charge period and then one discharge period (Testing Temperature: 20±5° C)
 **Operation Temperature Range**	Charge: 0° C – 40° C Discharge: 0° C – 45° C	Keep RF2000 Module within prescribed ranges
Storage Temperature Range (If RF2000 Module is stored for longer than 6 months it is recommended to perform additional charge on module)	6 months: 5°C -25°C	Store RF2000 Module in a cool, dry and well-ventilated area
Battery Weight	Approx. 52g	-
Battery Dimensions	19.5 x 69.5mm	-
Battery Nominal Voltage	3.70V	-

If various usage conditions such as charging, discharging, and environment temperature are not maintained within the specified range, the expected lifespan of the battery may be shortened, and the RF2000 module(s) may be damaged due to electrolyte leakage. If the battery is unable to hold a charge for an extended period even after being correctly charged, it may indicate the need for a battery replacement.

Disposal- Regulations vary for different countries. Dispose of in accordance with local regulations.

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Automation saves time and money!

ANKOM Technology is an international company with products that include:

	**FLEX Analyte Extractor** • Simplifies fat-soluble vitamin and cholesterol analysis • Crude and total fat analysis capability coming soon • Eliminates chemical handling to improve safety • Eliminates bi-phase extractions • Provides ability to create custom methods
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	**DELTA Automated Fiber Analyzer with Pump System** • Crude Fiber (AOCS Ba 6a-05), ADF, NDF determinations • Automatically adds solutions and rinses • Batch process - up to 24 samples at one time
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