

TEACHER REFERENCE PAGES - YEAST FERMENTATION LAB

USE OF GAS CHROMATOGRAPH FOR TOPS LABS

Part 1: Get the Computer Set:

1. Put PAPER TRAY TOP (from Red GC supplies box) onto the printer.
2. Attach the KEY BOARD and MOUSE (from blue box) to the computer, matching icons on cords to icons on the back of the computer. Be sure to use the appropriate attachments: #1 MOUSE & KEYBOARD to #1 computer, etc.

Part 2: Get the Helium Gas lines connected:

1. Get the 2 regulators and 1 crescent wrench from the Blue GC supplies box.
2. Match regulator A to the "A tank", and regulator B to the "B tank" (labels for each GC are on the front door of the machine).
3. Screw in the brass fitting until finger tight.
4. Push to click the "swage lock" quick release link from regulator to GC...it makes sense after you do it...have the resource teacher show you.
5. Never touch (never change) the regulator knobs themselves.
6. Tighten the connectors to the regulators with the wrench (that you previously did to finger tight) and make sure with a soapy film that there is no leakage. A gas leak will show up as bubbles when soap solution is put on the connector area. If necessary get someone stronger to tighten with the wrench.
7. Turn on the tank valve (look at arrows on valve). Note pressure gauges (tank pressure and line pressure). Tank pressure (the one on the right) should be greater than 300 lbs and the line pressure (the one on the left) should be approximately 50 lbs.
8. Put the wrench away. You will need it when disconnecting all of this at the end of the experiment to send back in van. Don't leave it out for some happy student to play with.

Part 3: Get the Front of the GC Set:

1. Plug in the extension cords safely for both GC units and computers. Turn GC on using power switch (small black switch)

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located at right rear of the GC.

2. Front display on GC should say "passed self test" after it has run through several diagnostic checks. Check the column head pressure gauge on front of the GC; it should be above 200 lbs.

Part 4: Get the Computer System ready:

1. Make sure computer is also plugged in. Turn **monitor** on by pushing in the gray button on lower right side of the monitor. Turn computer CPU on by pushing in white button to the lower left of disk drive.

2. After self testing, a **C:\>** will appear on the computer screen. On keyboard, type in **HPCHEM** and press **enter** to load the software. This may take a moment.

3. When software is ready, the screen will read **HP3365:ANALYZER1-LABNAME.MTH**. Read blue menu bar at top of screen to make sure you have the program that goes with the Yeast Lab (**alcohol.mth**). The TOPS labs have different methods (**alcohol.mth**, **esters.mth** & **spearmint.mth**) with different settings for their analysis. If the correct method is not loaded, you must load it by selecting it under the method menu (the resource teacher can do this for you).

4. If necessary to load the correct method, use the mouse to click and drag to **LOAD** on the **METHOD** menu. When **LOAD** menu appears, choose the appropriate method by clicking with the mouse to highlight the method, then click on **LOAD**. When this process is complete, the name of the method will be displayed on the title bar at the top of the box.

Part 5: Turn on the detector

1. **NEVER TURN THE DETECTOR ON UNLESS THE HELIUM GAS LINE IS ON** and you have checked to see that there is pressure on the head column gauge. The pressure must read a value ≥ 100 kpa. Turning on the detector without sufficient gas flowing through the system can cause very expensive damage to this sensitive component.

2. Click and drag on the **INSTRUMENT** window to **DETECTOR**, then release.

3. You will see the Detector Window. In the box labeled **DETECTOR A**, click the "detector on" box located at top of screen, then

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click on the "OK" box located at the bottom of screen. You should begin to hear the detector of the GC, it makes a audible rapid clicking noise.

4. The numbers on the side of the "signal 1" window need to be "2" for Attn (attenuation) and "6" for Time. The "attn" can be adjusted up or down to change the vertical scale of your signal. This is useful if you are looking to magnify very small peaks in your printout. Have the resource teacher demonstrate this function for you. You normally will not need to adjust this setting.

5. Wait for the green "ready" bar to appear in the **RUN STATUS** window. Zero the signal by clicking the "zero" box if necessary.

Part 6: How to Use the GC needles correctly:

1. Use a 25 microliter (μl) syringe and note where 1 μL is measured, at the second line on the syringe glass barrel scale.

2. Pull in more of the sample (about 5-10 μl worth) and push out the excess liquid onto a kimwipe. Do this three or four times for each new sample, to make sure the needle has only that set of chemicals in it. Then push down your sample to the 1 μl line. Wipe off the excess on the needle tip with a "kimwipe" if necessary.

3. Position the needle above the GC port to do the injection. Use both hands, but do not let your fingers touch the actual metal of the port. You will get burned. It's hot.

4. The injection needs to be done quickly and smoothly. The sequence is to push down on the syringe body until the needle is fully inserted into the injection port, quickly push the plunger and then quickly pull the entire syringe out of the port and push the start button on the GC front to begin the analysis. The reason you do this fast is to prevent damage to, or blockage of, the needle caused by the high temperatures inside the GC. Try to push the needle straight in and pull it straight out to avoid bending needles. You may have another student push the start button when you complete the injection.

5. There is a rubber septum that the needle goes through when inserted into the port. If you want to show students what is happening inside of the machine and why they feel a resistance when they push the syringe into the injection port, there are spare septa in the supplies box. Have students talk the process through to you and then do it. They can also have their lab

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partner push the computer start button if the student feels the injection of sample is enough to worry about.

6. After repeated injections, holes may form in the septum, allowing gas to escape. If this occurs (you can hear the gas hissing out of the septum, and the head column pressure drops), you will need to replace the septum. This is to be done only by teachers, after a demonstration by the resource teacher. Spare septa are in the supplies box.

7. The computer and GC take over now. Each "run" takes about 6 minutes to do. All they can do is wait for their computer printout to come into the printer paper tray. The computer screen is showing you what is happening during this time. Most students stay and watch the process. This is a chance to explain the time axis and the time # on the screen, why you did some "standards" of alcohol and where water is, etc. You can have them copy down the numbers from the machine standards and control, into their lab while they wait. Be sure they copy the standards run on the same machine as their sample. Just before the 6 minutes is up, get your next group ready and show them how to put sample into the needle and get ready to inject, so that you do not waste time.

Part 7: Get the Computer ready for next group:

1. After the printout begins to appear in the printer's paper tray for the current run, open the "tabulate" menu located at top left corner of screen. Select **CLOSE**. This will clear the screen and return you to the original screen. When you see the green "ready bar" you can proceed.

2. Do this after each run during the day. There is a screen saver on the monitor, if it appears, you can get the program back by touching any key.

3. If another copy of your report is needed, open the "data analysis" menu and select "print report". Click the "OK" button when the printing is complete. Do this before clearing the screen.

Part 8: Get the whole thing safe for over night storage and/or ready to return to OXY at the end of your lab:

1. Get the detector turned off first. On screen, open "instrument" menu, select "detector", click the "detector on" box, click the "OK" box. The detector will stop its clicking sound.

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2. Open the "run control" menu, select "exit". Click "yes" button to close this instrument session.
3. Close all icons which appear on bottom left of screen. Do this by clicking on them and then selecting "close" to exit. When the "exit windows" box appears, click "OK". You should now be in DOS.
4. Turn the computer off and turn off the monitor by pushing their buttons.
5. Turn off the GC power switch.
6. Open the GC door of the unit (small release button located at right and bottom of door) so that it can start to cool off. It needs to cool off for about 30 minutes **BEFORE** you can disconnect the Helium gas lines.
7. After 30 minutes, turn off the valves on gas tanks. Twist it to close, as best as you can do, be strong.
8. Pop (disconnect) the swage valve fitting and listen for "gas sound". You do not want to have the Helium gas running all night. If there is any apparent gas flow (hissing sound) tighten the valves on the gas tanks. Do through step eight of this process for overnight storage if you keep the equipment for more than one day.
9. If you are getting ready to send the equipment back to OXY, then you need to take the regulators off the tanks with the wrench. Put the paper tray tops back in the red box, along with the syringes, standards and extension cord. Take the mouse and keyboard off the computers and put back into the blue box along with the regulators. Re-pack all the other lab equipment back into their boxes.