Standard Operating Procedure for 1D NMR Data Collection on the Varian/Agilent NMR Spectrometers

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Safety Requirements

Access to the lab will be revoked if you do not follow these safety procedures.

1. While working in the NMR/EPR Laboratory, researchers are always required to wear Personal Protective Equipment (PPE). The appropriate PPE include safety glasses, long pants/skirt covering the legs completely, and closed-toe shoes. All required PPE needs to be supplied by the user.

2. During normal times, gloves should not be used with the workstation keyboards. During a special health crisis, use the special color-coded gloves that should only be used within the LIC.

3. Food and beverages cannot enter the lab. Never eat or drink inside the lab.

4. Samples should be prepared in the user’s chemistry lab. The outside of sample tubes should be cleaned with an appropriate solvent in the user’s lab and allowed to dry. Then the tubes can be transported to the NMR/EPR laboratory in an appropriate secondary container.

5. Should a sample breakage occur, it is the user’s responsibility to clean the spill and broken glass if it occurs outside the magnet. It is also the responsibility of the user to report all sample breakages in the NMR/EPR lab to the manager. If the breakage occurs inside the magnet, do not try to clean it, but place a note across the workstation keyboard for that instrument and notify the manager immediately thereafter. If you are unsure how to clean up the spill, contact the lab manager or the LIC Director (Dr. Westrick). If the spill occurs after hours with personal injury, please contact WSU police department (7-2222) and Dr. Westrick, immediately.

6. All researchers working in LIC must complete the EH&S initial course for Laboratory Safety Training and show proof of completion. Users whose safety training has expired will not be permitted access to the laboratory.
7. In case of an instrument malfunction, place a note on the instrument workstation keyboard indicating that the instrument is not working and contact the lab manager immediately.

8. Be aware that the NMR/EPR Laboratory has a red binder containing the Chemical and Laboratory Safety Information. It is located on the bench adjoining the sink in the southeast section of the lab.

**Magnetic Fields and Cryogens**

1. The NMR spectrometers utilize superconducting magnets. The superconducting nature of these magnets is achieved with extremely cold temperatures using liquid nitrogen and liquid helium. Should you witness a large amount of gas, produced when one of these cryogens boils off rapidly, venting from a magnet, report this to the manager and Judy Westrick immediately.

2. The lab is equipped with oxygen monitors. Should the monitors detect a low concentration of oxygen in the lab, a loud alarm will result. Should an alarm sound, evacuate the lab immediately, and inform the manager and Judy Westrick from a safe location.

3. The magnets of the NMR spectrometers, as expected, generate high magnetic fields. People with pacemakers or other medical implants should not enter the NMR lab.

4. The floor around the magnets have blue carpet around the magnets to indicate the furthest extent of the 5 gauss fringe field around the magnets. Inside these areas, electronic devices and cards with magnetic strips may be affected. It is best to leave these items outside this area.

**Access and Training**

**LIC Authorization Information**

1. Contact the lab manager for training.

2. All users must have an Infinity account, have joined their PI’s lab in Infinity and been assigned a valid index number assigned by their PI.

3. When a user is checked out on the lab instruments, they may reserve time for the instruments in the Infinity system.

**Check Out Procedure**

1. The first step in the check out procedure is to contact the lab manager for training.

2. The initial training session will introduce the user to the NMR instruments and gain familiarity with the procedures for acquiring data.

3. Subsequent supervised sessions may take place with either the manager or with previously trained users from the PI’s lab.

4. When the user is well acquainted with the routines for collecting data, the user should schedule a check out appointment with the manager. At this meeting, the user will demonstrate the ability to use the instrumentation without supervision.

5. If a user has previous experience using similar instrumentation from another institution, the manager may, at his discretion, check out the user at the initial training meeting.
6. Once checked out, the user will be asked to reset the password of their account so that they may access it at any time. The manager will then arrange for the user to be given access to the lab door scanner. From this point on, the user may reserve the instrument and use it independently.

Overview
1. Nuclear Magnetic Resonance spectroscopy uses a large magnetic field and radiofrequency waves to study the nuclear spin of atoms with magnetically active nuclei.

Sample Preparation
1. Samples should be prepared in the user’s chemistry lab.
2. In general, samples used for solution state NMR should be prepared in a deuterated solvent, suitable to dissolve the sample of interest. There are many choices, and the stockroom has several different solvents in stock.
3. Solutions should be prepared with the solute fully dissolved in solution at room temperature. Multi-phase samples will tend to show poor spectra. Heat should not be used to create a solution that is supersaturated upon cooling back to room temperature.
4. Concentrations above 50 mM should generally be avoided.
5. High quality NMR tubes with a 5 mm O.D. and 7” or 8” in length should be used and are available in the stockroom.
6. A solution height in the NMR tube of 50 mm is ideal for the Varian/Agilent spectrometers. This equates to a volume of about 700 μL.
7. The NMR tube cap should be securely fixed on top of the NMR tube. Use a Kimwipe wetted with a suitable solvent like Methanol or IPA to clean the outside of the tube.
8. When the tube is clean and dry, it may be transported to the NMR lab in a suitable secondary container.

Instrument Operation

General
- All the Varian/Agilent spectrometers are using the same version of software; thus, the operation of all the instruments will be very similar.
- There is a correlation between field strength and resolution. Thus, a higher field yields better resolution than a lower field instrument.
- There is also a correlation between field strength and sensitivity. However, this is also greatly affected by probe design. Discuss this with the manager to discover which instruments might be better suited to the experiments you wish to perform.
(For example, the sensitivity for 1D 1H experiments most often performed in the lab is ranked from best to worst as: A600>A400>V500>M4.)
LUMIGEN INSTRUMENT CENTER NMR/EPR LABORATORY:
WAYNE STATE UNIVERSITY

Data Collection

1. The general components of the NMR spectrometer include: the magnet, the probe (affixed inside the magnet), the console, and the workstation.

![Figure 1](image1.jpg)

**Figure 1:** The magnet with associated probe and electronic utility boxes. Samples are inserted in the magnet from the top.

![Figure 2](image2.jpg)

**Figure 2:** The NMR console, where the majority of the electronic components are housed for generating and collecting radio waves for the experiments.
Figure 3. The NMR workstation for running the experiments. This uses Linux (CentOS), and the users will have their own accounts and passwords.

2. Sit at the workstation and log into your account using your Access ID as your username and your previously set password. The desktop environment should look something like Figure 4.

Figure 4. Typical CentOS desktop after login.

3. Double click on the VnmrJ icon in the upper left. The VnmrJ GUI should look more or less like Figure 5.
Figure 5. The VnmrJ GUI screen upon opening.

4. Carefully start to push the NMR tube through a spinner. The spinner contains o-rings which hold the sample by friction. This fit may be a little looser or a little tighter depending on the tube. It is best to hold the spinner in one hand and the tube in the other until the tube is all the way through the spinner.

5. Place the spinner/tube assembly in the depth gauge. Then push the tube through the spinner until it reaches the bottom of the depth gauge. The window of the depth gauge indicates what is the ideal amount of solution to have in the tube, approximately 50 mm. Figure 6 shows the tube, spinner, and depth gauge.

Figure 6. On the left image, the spinner is shown on the left, the depth gauge in the center, and the NMR tube on the right. In the right image, the tube is in the spinner and lowered to the proper depth.

6. Pull the tube/spinner assembly out of the depth gauge and click on the Eject button in the VnmrJ GUI. A stream of nitrogen gas will be heard flowing through the upper barrel of the magnet. Climb the steps of the step stool to place the tube/spinner assembly in the
stream of gas.

**DO NOT HOLD ONTO OR LEAN AGAINST THE MAGNET!**

7. Return to the workstation and click on the **Insert** button in the VnmrJ GUI. The gas flow will adjust to lower the sample into the upper barrel, rapidly at first, then more slowly, until the tube/spinner assembly is fully inserted. At that point, the sample volume in the bottom of the NMR tube will be located within the RF coils of the probe.

8. In the Protocols section in the upper left of the VnmrJ GUI, expand the Common section of the Experiment Selector Tree. Double click on the PROTON selection, as seen in Figure 7, to load the default 1H experimental parameters, or double click on the CARBON selection to load the default 13C parameters.

![Figure 7. The Common experiments of the Experiment Selector Tree with the PROTON parameters highlighted.](image)

9. In the parameter input section of the VnmrJ GUI, located in the lower center portion of the screen, ensure that the Start tab is selected, and the Sample Info selection is highlighted. If they are not, first click on Start, then on Sample Info. Then, select the identity of the deuterated solvent used to dissolve your sample. The common solvents, as shown in Figure 8, of DMSO, CDCl3 and D2O have push buttons for selection. Other solvents may be selected by clicking on the Solvent pull down menu.

![Figure 8. The parameter input section of the VnmrJ GUI showing DMSO as the solvent of choice for this experiment.](image)

10. Now, click on the Lock selection under the Start tab. The parameter input selection
should look like Figure 9. There are 4 push buttons in the lower left portion of this pane (one push button is grayed out for the MR4 instrument). These buttons are meant to be used in order from top to bottom, allowing the spectrometer to complete each action in turn before continuing. This is to be done for every sample.

![Image of Lock pane](image)

Figure 9. The Lock pane contains 4 buttons: Read default shims, Auto Tune, Select lock signal…, and Gradient shim. These are meant to be used in order from top to bottom.

11. Click on the Read default shims button. This loads the default shims the manager has recently updated that works well as a starting point for nearly all samples. This action happens very quickly and you can move on to the next step.

12. Click on the Auto Tune button. A pop-up box will appear looking like Figure 10. The probe should be tuned in the low band (P31, C13, or N15) channel first, followed by the high band (H1 or F19). For a regular 1H experiment, click on the C13 button, and wait for the low band auto-tuning to complete.

![Image of Auto Tune pop-up box](image)

Figure 10. The pop-up box for probe tuning.

13. After the C13 tuning is complete, click on the H1 button, and wait for the high band auto-tuning to complete. As shown in Figure 11, the auto-tuning procedure is impedance matching the probe circuitry to minimize the reflection of the transmit/receive coil for the frequency and sample of interest. When the tuning is complete, click Close on the Tune
Probe pop-up box.

Figure 11. The ProTune pop-up box graphically shows the impedance matching to get the minimal reflection aligned at the frequency of interest.

14. Click on the Select lock signal… button. A pop-up box as seen in Figure 12 will appear. Click on the Measure 2H spectrum to initiate a deuterium scan.

Figure 12. The Interactive Find z0 pop-up box used to find the lock signal of the sample.

15. Wait for the deuterium scan to complete. When it does, the resulting 2H spectrum will appear in the graphics window as shown in Figure 13. There may be 1 or 2 red line cursors on the screen of the graphics window. The spectrum may contain one or more signals, based on the solvent in use. The single red line, or the leftmost of 2, should be placed near the top of the most intense signal in the spectrum.
16. With the red line near the peak of the most intense signal, click on Move cursor to nearest line, and then click on the same button again. Then click on Adjust z0 NOW! The Z0 value for the spectrometer will now be adjusted.

17. Click on the Close button of the Interactive Find z0 pop-up box. Then, back in the Lock pane, click on Gradient shim. Wait for the gradient shimming routine to complete. This may take multiple iterations.

18. When the gradient shimming routine completes, click on the Acquire tab in the parameter input section of the VnmrJ GUI. Under the Acquire tab, choose the Acquisition selection. The Acquisition pane should look like that in Figure 14.

19. Ensure that the Auto box is checked next to the Receiver gain input box. This will ensure that the gain is set to match amount of signal being generated from your sample and avoid overflow errors.

20. Now, click the green Go button, and wait for the experiment to complete.

21. When the experiment completes, the minimally processed spectrum will appear in the graphics window, as shown in Figure 15. Inspect it briefly to determine if the experiment appears to have worked correctly.
Figure 15. The processed spectrum of Quinine.

22. Save the data. Click the disk icon in the upper left of the GUI. A Save pop-up box will appear as in Figure 16. Navigate to whichever directory you prefer to save the data. Type a sample identifier in the File Name input box. Ensure that the Files of Type is set to .fid, or set it to that if it is not. Then click Save.

Figure 16. The Save pop-up box.

23. When the file is saved, return to the Start tab in the parameter input section of the GUI. Click on Eject to eject the sample from the upper barrel of the magnet. Retrieve your sample from the gas stream.

AGAIN, DO NOT HOLD ONTO OR LEAN AGAINST THE MAGNET!

24. Remove your sample tube from the spinner and place the spinner on the console. Click on Insert to stop the flow of gas.

25. If all experiments are complete, click on the File pull down menu in the upper left of the GUI and select Exit VnmrJ.

26. Then, in the upper left of the desktop, click on the System pull down menu and select Log Out <accessID>… Confirm that you want to log out, and the session should conclude.