Check-out Procedure for the GN500

1. If you have a "Financial Responsibility Agreement form (Authorization form)", handout UGL013, on file in the Molecular Spectroscopy Lab (usually referred to as the NMR Lab), then it is applicable to all spectrometers in the lab. If you have not yet turned in such a form, obtain one out of the blue file boxes in 146 RAL, fill in the requested information, have your research director sign it, and then give it to Tracie Chilton or one of the MSL staff.

2. If you have not yet had your picture taken for MSL records, ask Keith Carriker to do it.

3. If you have not yet read handout UGI017 ("Basic NMR Information, Level 2 Checkout, XL200, QE300"), obtain a copy from the blue file boxes in room 146. Study this handout until you can answer all the questions at the end of it ("IV. NMR Basics Test"). Then, have Vera Mainz give you a blank copy. You are expected to answer the questions from memory, while under Vera's supervision, in about one-half hour. Vera will keep your completed test, grade it, and inform you of the results in about one work day.

4. Fill out form UGL014 ("MSL User Reslog Access Code Request Form"), and give it to Vera Mainz. She will create a training (TR/...) code for the GN500 and mail it to your group expert. Ask this person to show you how to reserve time using Reslog and how to log in and log off of an instrument.

5. The practice samples (NMR tubes) for the GN500 are out on the lefthand side of the console.

6. Using handout UGN501 and the practice sample NMR tubes, your group expert (or any previously checked out user) is to train you on the GN500. One should never train or practice on the GN500 without someone present in the room to supervise and answer questions (this is the group expert's primary responsibility). Unsupervised people using the GN500 will have their training codes deleted. Training takes from six to twelve hours before a person can complete the checkout procedure within a two hour time frame.

7. When you think you're ready to schedule a checkout, present a complete set of spectra, which you've obtained from the GN500, to Vera Mainz. If they are acceptable, she will schedule a two hour checkout time on the GN500. During this two hour time block, you will be expected to generate an entire set of spectra according to the instructions given in handout UGN501. If they are acceptable, the GN500 will be added to your permanent Reslog code.

8. Once you have completed this checkout, you may use the GN500 spectrometer for routine 1 pulse experiments such as proton decoupled $^{13}$C and proton experiments. However, the user will NOT be checked out for VT use of the GN500 or multinuclear applications such as $^{31}$P or $^{15}$N. There are separate checkouts for VT and these advanced experiments. If you want to perform any 2D experiments or non-routine 1D experiments, you should read the appropriate handouts and ask MSL staff for help the first time you do the experiment.

The GN500 check-out procedure consists of a complete set of plotted data for the following:

**PROTON:**
A) A proton spectrum of 0.1% ethylbenzene in CDCl$_3$, measuring the signal-to-noise (sensitivity) of the quartet.
B) A proton spectrum of 0.1% ethylbenzene in CDCl$_3$ with integration.
C) Two homonuclear decoupled proton spectra of the 0.1% ethylbenzene in CDCl$_3$.
D) A proton spectrum of 1% CHCl$_3$ in CDCl$_3$, measuring the linewidth of the CHCl$_3$ and percent spinning sidebands.
CARBON:
A) A carbon-proton coupled spectrum of 40% dioxane in 60% C₆D₆ (ASTM), measuring the signal-to-noise (S/N) of the C₆D₆ triplet.
B) A carbon spectrum of 40% dioxane in 60% C₆D₆ (ASTM) with the decoupler on, measuring the linewidth of the dioxane and percent spinning sidebands.
C) Four ¹³C data sets of 57% menthol in CDCl₃, demonstrating the effects of line broadening and number of acquisitions upon the experiment time and the spectrum's signal-to-noise ratio.

PROTON

Part 1. 0.1% Ethylbenzene in CDCl₃

A.

1. Insert and lock the sample. The lock frequency of CDCl₃ is 7.26 ppm. Load the correct shim library for the ¹H/¹³C Dual probe by typing AH, L, L 10, <>, <>, Q, X. Type LD and go to sweep display by typing S. Center the sweep in the window using O, and check the phase (P) Check the lock gain (~ 650) and the lock transmitter power (~120 for CDCl₃) in the LD window display. Activate the lock by typing S (to go to the lock level meter display) then L. Make sure you are in the lock fast mode to shim. Read Handouts UGN506B and UGN506C on Locking and Shimming for details about these operations. To shim while in LD, type K, and select the number for two shims (e.g., for Z1 and Z2, type 1, 2). To activate the coarse shims, type C. To activate the fine shims, type F. The suggested order for maximizing the spinning shims is as follows:

- Z₁C and Z₂C interactively to a maximum,
- Z₄ to a maximum, then Z₂ to a maximum
- Z₃ to a maximum,
- Z₂ to a maximum,
- Z₁ and Z₂ interactively to a maximum

After you are finished shimming the spinning shims, shim the nonspin shims. In LD, turn off the spinner air by setting the airflow to zero, A 300 <>. Type K, then choose X and Y and shim interactively; choose XZ and YZ and shim interactively, ending by shimming X and Y interactively. Turn airflow on, A 1120 and reshir Z₁ and Z₂ interactively to a maximum (S 12).

2. Run Macro #2 (XM, 2, Y, enter your initials at the US prompt) to set up standard ¹H conditions, check the line broadening by typing LB and change the line broadening to 1.0 Hz.

3. To change the gain with GN, type GN, T, 200, <> . To check the gain visually, type ZG to take one scan. By using the up-down arrows on the right of the keyboard, set VS16 at the top of the screen. If the gain is adjusted correctly, the FID should be about one inch high on the far left of the screen when you take a scan. If you notice that the transformed spectrum has a rolling baseline or that the sides of the peaks are distorted, the gain is set too high. The automatic SG command will also work but for some experimental applications it chooses too large a gain value. Do not use it for the S/N measurement in this section.
4. Set P2 equal to the last recorded 90° pulse width for 0.1% Ethylbenzene in CDCl₃ found in the logbook. Type ZG to obtain a spectrum and transform it (BCEMFTPS). Check the reference. The ethyl triplet should be centered at 1.24 ppm. Reference the spectrum if necessary by zooming in on the triplet (ZO, ^E <>, PP, S, R(L), O 1.24P <>). Reset the transmitter offset and the sweep width to a 10 ppm to -0.5 ppm window (ZO F10P -0.5P, ^E, W, ^F). Wait about 5 x T₁, or 60 seconds, for complete relaxation and then accumulate the spectrum using GS instead of ZG. Save the spectrum as HSNXXX.001 where XXX are your initials and enter appropriate text to describe the experiment. When completed, recall the spectrum with GA and process it and then zoom (ZO) on just the ethyl quartet (ca. 2.6 ppm), type <>, YS, ^F, AM, Y. Record the resulting number as the AM signal-to-noise. This number should be > 150:1. Zoom in on the 4 ppm - 6 ppm range (ZO, F6P 4P, ^E, N (to set noise), <>, ^F), S N. Record the resulting number as the 4-6 ppm signal-to-noise value. Next, zoom in on the 5 ppm - 6 ppm region (ZO, F6P 5P, ^E, N, <>, ^F), S N and record the 5-6 ppm signal-to-noise value.

5. Set up the plotter by putting a pen in the Zeta plotter's pen carriage, position the perforation 1cm below (in front of) the pen, press "clear". Type NP. The pen carriage should go to the same relative position on the paper, but a page forward. If it did, type RZ, and you are ready to plot. If it did not, press "clear", reposition the pen carriage, and type NP, RZ again.

6. Type ^F and check the phasing before plotting. Then type YS, XY = 3, PL. Add a 10x registered plot of the 5 ppm - 6 ppm region by typing ZO, F6P 5P, ^E <>. Wait for PL to finish, then type RP and answer the questions (50 <>, 10 <>). Type LC to add an axis, and label the spectrum. Move the pen to the center of your spectrum with MP, and adjusting the pen position with the A and B knobs. Type PT (plot text) to enter the text mode. The PT subcommands are:

\^Z<> to erase the screen
\^D to Print
\^P to exit

To print your three signal-to-noise values on your spectrum, type PT, ^Z (if necessary), your measured values [e.g., P2(90)=30USEC, S/N(AM)=185:1, S/N(4-6P)=165:1, S/N(5-6P)=160:1], ^D, ^P, NP.

7. Position the pen in an open area (MP) and print out the shim values for this sample on your spectrum (PZ); tape them neatly to the spectrum.

B. Integration

1. Reset standard proton parameters (XM2, Y <>). Set NA=16, type SG to scale the gain, and then take the spectrum using GS and save to INTXXX.001.

2. Reference to the triplet (PP, S, L(R), O 1.24P <>).
3. Plot the spectrum by setting XY = 0, YS. Zoom in on the 10 to -0.5 ppm region (ZO, F10P - 0.5P, ^E, <>). Type PL and LC. To plot the integral, type IS, and use D (decrease) or I (increase) to bring the integral on scale. Type ^R to clear any zeros, then use the A and B knobs to adjust phase and curvature. Set appropriate zeros using the C and Z subcommands. When ready to plot, type P, 10 <->, 20 <-><. Type NP to set the next page.

C. Homodecoupling (make sure the spectrum from INTXXX.001 is on the screen and properly referenced).

1. Make F2 = SF by typing SF, noting the value, then typing F2 and entering the value for SF.

2. Type EF, <control> R, and move the cursor to approximately 10ppm (any region of the spectrum that has no peaks), and type D <>. Zoom in on the quartet and triplet region (ZO, F2.75P 1P, ^E, <>). Type EF, move the cursor to the center of the triplet, type D <>, move to the center of the quartet, type D <>, then <>. Using these procedures, you have arrayed three decoupling frequencies.

3. Type CD + and <> through the list. The three frequencies listed should be those you just set in part 2 above. The fourth value on the list should be Ø.

4. Type DC

   DECOUPLER OUTPUT OFF OK? +
   DECOUPLER OUTPUT (0-82 DB) = 0 55 <->
   HETERO DECOUPLING ON OK? -
   MODULATION MODE = 4
   POST DELAY = (1...57 msec): = 9 <->

5. Type DN. Make sure both the DEC PULS status light and the HOMO DEC status light are on (on the status panel on the front right panel of the console).

   (If these lights do not come on now - don't give up yet. Go into monitor MO, >GEM, take one scan ZG, MO, >GEM16. Then check F2 = SF and CD + (look at your list by <returns>'s). Then repeat step #6 and then DN. If the lights do not come on now, please notify MSL personnel and do not attempt this decoupling experiment).

6. Set NA=1, and start the arrayed experiment by typing GS=3 and saving the spectra to DECXXX.001. When finished, GA DECXXX.002 and transform (BCEMFTPS). Check to see if the triplet is fully decoupled. If it is not, redo step 4, increasing the decoupler output by 2dB steps, i.e., the first value is 57. Reacquire and check as before. When power level is set correctly, set NA=4, and reacquire final set of spectra. After spectra have been acquired, turn off the decoupler (DF) and decoupler offset list (CD-).

7. Process spectrum DECXXX.001 first (BCEMFTPS), properly phase, then type AK, S. Process the complete set using the link LI = GABCEMFTPSAISB by typing A U=3, DECXXX.001, <->, DECXXX.501, <->.
8. **GA=DECXXX.501**, zoom in on the quartet and triplet region (ZO, F2.75P 1P, \(^E\), <>), and then type **YS**. Set XY=3, CM=20, 5, <>, and PO Ø, <>, 55, <>. To preview the plot, type **SV DECXXX.501 <> <> N <>** (<> to exit preview). To plot a stacked plot of these three spectra, type **SP DECXXX.501 <> <> N <>**. Put on an axis and label with **AX, LA, and NP**.

**NOTES:** When CD (Decoupler offset list) is turned on, the decoupler offset frequencies in Hz may be entered manually. The offset value is added to the current value of the decoupler frequency (F2). A zero (Ø) entry will terminate the list. It is usual to set F2 equal to the same value as SF. Also, check DN each time you start the experiment and make sure the decoupler light is on. If all else fails, reload software as described on console.

**Part 2. 1.0% CHCl\(_3\) in CDCl\(_3\)**

**A. Determine the 1/2 height resolution**

1. Insert and lock the sample (LD). Hand shim and execute Macro #2 (XM2 Y <>). Check the gain with (GNT#) or **SG** and set it to an appropriate value.

2. Take one scan, set LB = Ø since you are measuring resolution and lineshape, and process the FID (BC, FT, PS). Find the CHCl\(_3\) peak and set the transmitter offset slightly off to the right of this peak (TH, move the cursor, T — or you can set an appropriate zoom window (ZO, \(^E\), W, \(^F\), <>)). Set SW = +/- 500, NA = 1, and CB = 16KN.

3. Using Handouts UGN506B and UGN506C, set up FID autoshimming for Z1, Z2, Z3 (AH, U100 <>, 2 <>, 2 <>, F4 <>, S, R123 <>, QQX; LI=AH <>, A U=1 <>). Be sure to select an appropriate block size CB. SU should be >1000. When autoshimming is complete, enter LD and set the lock to slow sweep (research samples should always be acquired in the lock slow mode).

4. Accumulate a FID with GS saved as HLSXXX.001 where the text describes the experiment. Recall the FID with GA and process it (BC, FT, PE). Phase the CHCl\(_3\) peak, toggle the axis to hertz (\(^A\)), reference this peak to 0 Hz (PP, K, put the cursor on the peak, O = Ø), and then zoom in on the peak (3 Hz on either side — ZO, F3 <> -3 <>, \(^E\), <>). Type <>, **LF**, C to calculate the width at 1/2 height of this peak. This value should be < 0.60Hz. If it isn't, re-shim the sample. Record the final value and plot the line fit (Q, P). To exit LF, type ^O. Use the **PT** command to label this spectrum as "Proton Lineshape with Autoshimming". Position the pen in an open area (MP) and print out the shim values using **PZ**. Type **NP** to advance the page.

**B. Calculate the percent 1st order spinning sidebands**

1. Display the full spectrum with \(^F\). ZO to +/- 120 Hz on each side of the CHCl\(_3\) peak. Increase the vertical scale with the up-down arrows on the right side of the keyboard until you can see the spinning sidebands. The first order spinning sidebands (1st SSB) should be positioned at approximately +/- the spinning rate in hertz. Use **PP**, K to position the cursor on the larger of the 1st SSB and record its height and position in hertz. Record the height of the CHCl\(_3\) peak and calculate the percent height of the 1st SSB. If you can see 2nd SSB's in your spectrum, calculate the percent for them as well.
C. Calculate the lineshape.

1. Using the zoom window set in B, type CL and record the following:

<table>
<thead>
<tr>
<th>Resolution: the width at 50%</th>
<th>Lineshape: the width at 0.55%</th>
<th>the width at 0.11%</th>
</tr>
</thead>
</table>

Use PT to print these values on your spectrum.

CARBON

Part 1. ASTM in C₆D₆.

A. Determine S/N.

1. Insert the ASTM sample. The lock frequency for benzene is 7.15ppm. Shim and lock in LD (lock transmitter ~ 80). Start with the shims for the proton lineshape from the previous section and hand shim Z1, Z2, Z3 interactively to the lock level maximum.

2. Execute Macro #3 (XM3 Y <> ) to set up the standard carbon-13 parameters. Do not turn on the decoupler. Set CB=16KN, GNT1500, and LB=3.5 Hz. Take a single scan with ZG, work up the FID (BCEMFTPS). Set the reference to the middle C₆D₆ signal to 128 ppm (PP, S, R(L), O128P <>). Reset the transmitter and sweep width (ZO F150P50P ^E W ^F).

3. Set up and execute one autoshim on Z1, Z2, Z3 on the lock level meter (AH U 100 <>, 2 <>, 2 <>, L, 1 <>, S, R 123 <>, Q, X, LI=AH <>, AU=1 <>).

4. After the autoshim has stopped (about 3 minutes), clear the display by typing LD <> (lower the gain if necessary).

5. Set P2 equal to the last recorded 90° pulse width for ASTM. Accumulate the FID with GS and save the FID as CSNXXX.001 with the appropriate text. GA the file and process (BCEMFTPS). Determine S/N by typing YS, AM, Y. Record this AM S/N value, which should be >150:1. Zoom in on the 120-80ppm region (ZO F120P 80P, ^E, N, <>, ^F); type SN and record this S/N value, which should be >140:1.

6. Type ^F, check the phase, YS, XY=3, PL, LC. Manually center the plotter pen (MP) and use PT to label the spectrum with the AM S/N and 120-80ppm S/N values. Position the pen in an open area (MP) and print out the shim values (PZ). Type NP to advance the page.

B. Determine 1/2 height resolution

1. Turn the decoupler on by typing DN. Set P2 = 5 µsec, LB = Ø Hz, D5 = 3 sec, NA=1, GNT500. Check the gain and collect an FID using ZG. Process the FID (BCFTPS) and observe that the dioxane peak has collapsed to a singlet. Reference
the dioxane peak to Ø Hz (PP, S, R(L), OØ <> ) and then set the transmitter slightly to the left of the dioxane peak (TH, move the cursor, T), SW = +/- 500 Hz, CB=32K.

2. Set NA = 1 and acquire a FID with GS. Save the FID as CLSXXX.001 and include the appropriate text. Toggle for the axis with ^A. Set XY = Ø, GA the FID and process it (BC, FT, PS). ZO the region +/- 3 Hz of the dioxane peak, type < >, LF, C. The resolution at 1/2 height should be < 0.30 Hz. If the peak shape is good and no more shimming is required, type Q, P. If more shimming is needed, type ^O to exit LF, shim on the lock level or FID, and re-acquire the spectrum (for FID shimming, set CB=8KN, then AH U100 <> 2 <> 2 <> F4; S, R 123 <>, Q, X, LI=AH, A U=1). Label this lineshape spectrum as "Carbon Resolution, NA = 1" with PT. Type NP to advance the page.

3. Set NA = 4 and acquire another FID with GS. Save the FID as CLSXXX.002 and include the appropriate text. Check the resolution and plot this spectrum; label it as "Carbon Resolution, NA = 4". Type NP to advance the page.

C. Determine Limeshape.

1. Increase zoom window to +/- 50 Hz around the peak. Type CL and record as below. Record these values on your spectrum using PT.

<table>
<thead>
<tr>
<th>Resolution: the width at 50%</th>
<th>________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineshape: the width at 0.55%</td>
<td>________________</td>
</tr>
<tr>
<td>the width at 0.11%</td>
<td>________________</td>
</tr>
</tbody>
</table>

Part 2. 57% Menthol in CDCl3

1. Insert the 57% menthol sample. Shim and lock using LD. The lock frequency for CDCl3 is 7.26. Start with the shims for the ASTM linewidth measurement above, or reload shim library 10 (AH, L, L, 10, <>, Q, X).

2. Execute macro #3 (XM 3 Y <> ) to set up standard carbon parameters. Type DN to turn on the proton decoupler. Set NA=1. Set LI=ZGBCEMFTPS <> . Type AU, 1, <> to execute the link. Phase properly (PE or AP) Y <> . Type YS to set the scaling and AM to determine the S/N. This value should be > 95:1. Record below. Repeat with NA=4, 16, 64, and record these values.

<table>
<thead>
<tr>
<th>Number of Scans</th>
<th>AM S/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>_____</td>
</tr>
<tr>
<td>4</td>
<td>_____</td>
</tr>
<tr>
<td>16</td>
<td>_____</td>
</tr>
<tr>
<td>64</td>
<td>_____</td>
</tr>
</tbody>
</table>
This questionnaire is to be completed by the potential user before checkout.

**Part One. General GN500 Information:**

The potential user and trainer should discuss these questions during the user's first training session. The trainer should demonstrate any part of this questionnaire to the user to help explain common problems and solutions to those problems.

1. Where do you check to see which probe is installed?

2. What is the only probe available for "walk-on" time?

   When is "walk-on" time on that probe?

3. How do you check to see when a probe is going to be available next on the GN500?

4. What should you do when you logon to the GN500?

5. Why is it important to run a "macro" to set up your experiment?

6. Sample insertion, spinning, ejection:
   A. How should you position your sample in the spinner turban?
   
   B. What is the correct depth for a sample in the 1H/13C Dual probe and where is this number posted?

   C. Where are the air flow gauges for the GN500?

   D. What are the correct flow rates for the 1H/13C Dual probe for:
      body air________
      VT air__________.

   E. What is the correct flow rate for the wall regulator?

   F. Type AA. What is the correct flow rate reading for the DAC?

   G. How should you adjust the spinning rate for a sample?
H. What is the correct spinning rate range for a 5mm tube?
I. Why shouldn't the tube be spun faster?

J. List several things you should check if your sample wouldn't spin:

K. What should you do if your sample wouldn't eject?

7. Software:

A. List several instances when it is necessary to REBOOT and/or reload software:

B. Write out explicitly how to REBOOT the computer:

   Remember that following a hard REBOOT, you should just go ahead and reload software as well.

C. Write out explicitly how to reload software:
D. Why is it necessary to go into GEM and take one scan in the software reloading sequence?

E. Why are normal experiments run in GEM16?

F. When would a user choose to run an experiment in GEM?

8. Common problems:
   A. What should you do if you type LD, S and you see no swept lock display?

   B. What should you check if the plotter doesn't start plotting after you type PL?

   C. What should you check if the keyboard is inactive?