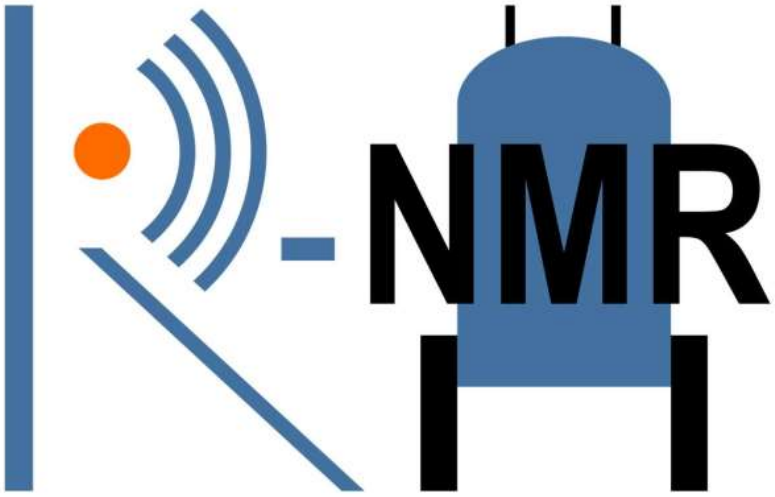

Deliverable 3.1

Standard operating procedures for remote NMR measurements and sample shipment

Authors: James Tolchard, Tanguy Le Marchand, Anne Lesage, Guido Pintacuda (CNRS), Christina Redfield (Oxford University)

This project has received funding from the European Union's Horizon Europe research and innovation program under Grant Agreement N. 101058595

TECHNICAL REFERENCES

Project acronym:	R-NMR
Project Title:	Remote NMR: Moving NMR infrastructures to remote access capabilities
Grant Agreement number:	10105859
Project coordinator:	Prof. Dr. Harald Schwalbe
Organization:	J.W. Goethe Universität, Frankfurt
E-mail:	Schwalbe@nmr.uni-frankfurt.de
Project website address:	http://www.r-nmr.eu/
Deliverable No.:	D3.1
Lead Beneficiary:	CNRS
Type and dissemination level:	Report - Public
Due Date:	M18 (31 December 2023)
Delivery Date:	15 January 2024

CONTENTS

1. Introduction

This report aims at establishing Standard Operating Procedures (SOP) for remote NMR measurements and sample shipment, based on the analysis of the responses to the NMR User Survey conducted in early 2023 (Milestone 2.2). It will be of use for the implementation of standardized experiments for remote access to liquid and solid-state NMR within WP4.

The large variety of operational modes reviewed in WP2 for the partnership has served as the basis for defining a general workflow compatible with a variety of sample types (solutions, biofluids, powders, hydrated crystals, sediments, air-sensitive preparations), a large panel of instrumentation (solution probes, sample changers, MAS probes, DNP microwave sources) and the detection schemes of nuclei with specific properties and various abundance.

The report is structured in two sections. In the first section, protocols for sample shipment are described. In the second section, standard workflows are proposed for remote NMR measurements from reception of the sample to data transfer to the user. In the third section, a pool of standard samples as well as a list of experiments are proposed to ensure the quality assessment of the instrumentation.

2. Project submission

Regardless of the sample type or chosen access mode, requests for remote access should adhere to a common initial procedure. This process will begin by initiating the project through an established route for platform access. This could involve direct communication with an NMR institute or utilizing various institutional or transnational infrastructure online submission systems already in place to support external users accessing NMR facilities, such as ARIA used by iNEXT-Discovery and INSTRUCT ERIC.

Once access to the platform is confirmed, a standardized set of project information needs to be transmitted between the host institute and the user. This information can be conveyed through direct communication, or the initial submission platform. In either case, the information should encompass a project description (including the title, a concise scientific project overview, including preliminary NMR spectra, if available, expected NMR outcomes, and funding source), details regarding hardware requirements and the desired level of remote access, a comprehensive account of the user's practical experience (for further information, refer to R-NMR Deliverable 3.2), a list of materials to be transferred to the host institute (such as samples, tubes, rotors, etc.), and contact information for both the responsible scientist and their primary investigator.

Additionally, the user should verify the following with the facility staff:

- Whether the sample can be accepted and analyzed at the facility, considering its safety level.
- The documentation required by the facility to certify the safety level of the sample, and whether this should be provided together with the sample or in advance.
- To whom the sample should be addressed.
- That no national/local holidays or strikes may affect the timing of delivery.

In response, users should receive contact details of a representative from the host site, insights into the expected scientific outcomes, proposed experimentation dates, shipping specifics (including address, courier details, and any lawful restrictions, etc.),

confirmation of the determined remote access level, and, if required, instructions for establishing remote connections and mechanisms for data transfer.

3. Procedures for sample shipment

Having reliable standard operating procedures for sample shipment is key for remote access. According to the surveys carried out in WP2, most NMR facilities currently lack defined standard operating procedures for sample shipment and do not provide users with written instructions on how to package samples for shipment. Building on information collected in WP2, we provide below guidelines and flowcharts for sample shipment.

Shipping samples for solution NMR

Sample stability will determine the best state in which to ship a sample for solution NMR.

1) If the sample can be rapidly frozen and then thawed without damaging the material, then shipping the frozen sample in a small plastic (or glass) vial is probably the best method for sample shipment. The sample can be packed in dry ice for shipping. It is desirable that the solution already contains the necessary deuterated solvent for locking so that the only sample manipulation required at the remote NMR facility is to thaw the sample and transfer it to an NMR tube supplied by the user.

2) If the material can be lyophilized and redissolved without damaging the material then this is another good method for sample shipment. The remote user should provide appropriate buffer (containing deuterated solvent) to redissolve the material by facility staff once it has arrived at the remote facility. If adjustment of sample pH is required then this will need to be discussed with facility staff. Depending on the sample stability, the lyophilized powder can either be shipped in dry ice or at room temperature.

3) If the sample cannot be frozen or lyophilized then it will be necessary to ship the sample as a solution, ideally in an unbreakable tube/vial, at either ambient temperature or cooled (but not frozen). Shipment at ambient temperature is straightforward but may result in sample degradation. Shipment at cooler temperatures will be more difficult depending on the door-to-door shipment time. It is desirable that the solution already contains the necessary deuterated solvent for locking so that the only sample manipulation required at the NMR facility is to transfer it to an NMR tube supplied by the remote user.

4) Shipment of solution NMR samples in NMR tubes may be the least desirable method for shipment due to the fragile nature of NMR tubes, possible leakage of the sample at the cap/tube interface and the possible movement of the liquid along the NMR tube which may result in bubbles forming. However, this method of shipment may be necessary for air-sensitive samples placed in NMR tubes under controlled conditions.

5) Samples for metabolomic studies may need to be shipped to the NMR facility in a 'sealed' state so that no manipulation is done once they arrive. For facilities with sample changers, the samples may be put into 'short' NMR tubes loaded into the 96-tube racks with a top on the rack to hold the tubes in position. If possible, the rack should be kept upright during shipment. If the samples can be manipulated at the remote NMR facility, then it may be better to ship the samples in a frozen state and then transfer to tubes/racks at the facility.

6) At the end of the experiments, the samples are returned to the user in the form of a solution, either directly in the NMR tube with the associated risks mentioned above, or in an unbreakable tube/vial, at either ambient temperature or cooled.

The remote user should provide the following information with the sample that is shipped: Sample Name, Quantity/Concentration, Solvent composition (buffer type and pH, ionic strength, amount of deuterated solvent), Toxicity, Conditions for sample storage prior to NMR experiments.

Shipping samples for solid-state NMR

A variety of samples can be considered, from powdered materials to sedimented proteins or cell extracts.

1) Samples can be packed into solid-state NMR rotors by the remote user prior to shipment. This is possible if the remote user has the appropriate rotor in-house and the necessary tools to pack the rotor. The remote user should discuss appropriate rotors and rotor packing procedure with the NMR facility before preparing the sample.

2) Samples can be shipped and packed into the appropriate rotor at the NMR facility. This may be the preferred procedure at a facility if visual inspection of the material is required before packing the rotor. Samples can be shipped in dry ice, in a cold package or at room temperature.

3) Air sensitive samples require additional care. The samples will need to be placed in a sealed container under an inert atmosphere (oxygen free or other) prior to shipment and the NMR facility will need to have a glove box available to transfer the sample to a rotor. A rotor placed in a sealed container can also be directly shipped to the NMR facility.

4) At the end of the experiments, the samples are returned to the user, either directly in the NMR rotor or in their solid form, at ambient temperature or cooled.

The remote user should provide the following information with the sample that is shipped: Sample Name, Quantity/Concentration, Sample composition, Toxicity, Conditions for sample storage prior to NMR experiments.

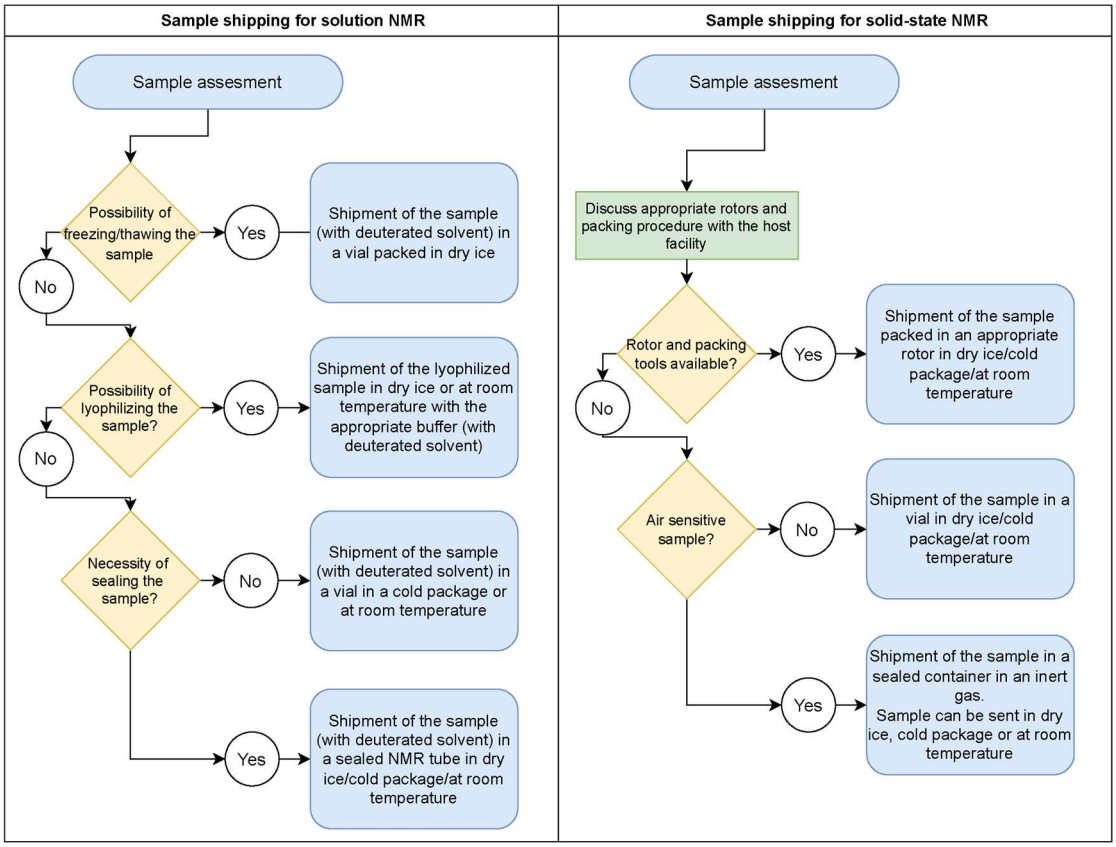

Figure 1: Work flowchart for sample shipment.

4. Work flowcharts for remote access

The practical implementation of solution and solid-state NMR experiments has been dissected, yielding standardized workflows encompassing the full extent of practical experimentation and remote access (addressing three different access levels). These are descriptive but suitably broad to accommodate a wide range of samples, NMR approaches and research themes. These workflows have been created as a series of flowcharts and have been shared with the community. Two distinct flowcharts were designed for solution NMR and solid-state NMR work, each divided into four sections: admission of the sample ahead of experiments, pre-acquisition settings, data collection, and post-acquisition operations.

Sample admission. This comprises the steps of sample reception (assessing its integrity at the facility) and storage (assessing sample sensitivity to water, light, oxygen, temperature; labelling), together with the suitable arrangements at the spectrometer (booking of the NMR measurement session in agreement with the user). Information regarding the safety of the sample will be considered for storage and future handling. Any evidence of sample degradation will be immediately discussed with the remote user prior to data collection.

Pre-acquisition settings. These span the steps ranging from sample conditioning (NMR tubes for solution NMR, rotors for magic-angle spinning solid-state NMR), setup of the NMR spectrometer, including installation and configuration of the required probe head, to quality assessment of the instrumentation, and setup of a remote communication channel with the user to operate the spectrometer.

Data collection. This includes setting the working temperature, tuning, matching and shimming the probe head, field locking, RF calibration for the different channels, setup and acquisition of the required 1D (or nD) NMR experiments.

Post-acquisition operations. These comprise storing and/or shipping back the sample, transferring the data to the user and closing the remote access channel. Data processing is done by the users, with the help of the facility staff members when needed, depending on local access rules.

The flowcharts for remote solution and solid-state NMR are reported in Figures 1 and 2 below and are available as standalone high-resolution files on the R-NMR website (https://r-nmr.eu/category/outcome/). The steps where remote users may have full control without local assistance are highlighted by red boxes.

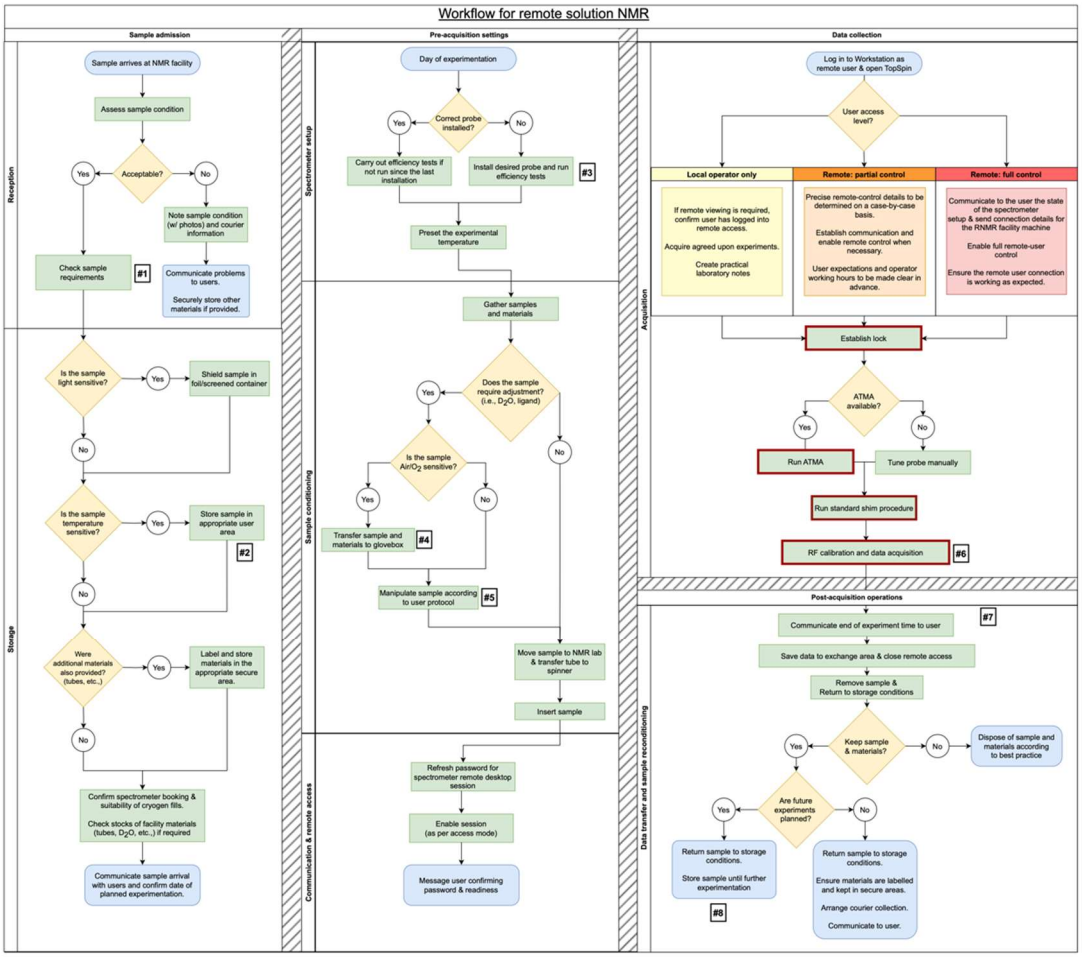

Figure 2: Work flowchart for solution NMR. #1) Sample requirements should be provided ahead of time by users. Unusual requirements should be discussed in the project brief. Any issue regarding sample safety should be addressed. #2) Secure areas, specific to remote users, should be identified or created for distinct temperature and atmospheric conditions (-80°C, -20°C, 4°C, room temperature, glovebox). These should be well organised and materials should be clearly labelled. #3) Standardised probe checks TBD in separate document. #4) Facilities with a glovebox should be made clear to the user when depositing a project. Any unusual equipment or tubes must be provided with the samples. #5) Detailed protocols for sample manipulation should be provided by users at project deposition. Any atypical reagents must be provided with the samples. #6) A standardized system to label the data (i.e., standard experiments vs. optimisation / popt exps / test planes) and the experiment metadata (sample, stage of manipulation, optimised pulses+delays etc.,) should be defined. This will be developed in T2 of WP4. #7) Data exchange mechanism to be determined, recommendations will be developed in WP4. #8) Facilities should be wary of becoming sample storage facilities. The recommendation could be that samples should only be stored when future experiments are explicitly planned. Red boxes highlight the steps where remote users may have full control without local assistance.

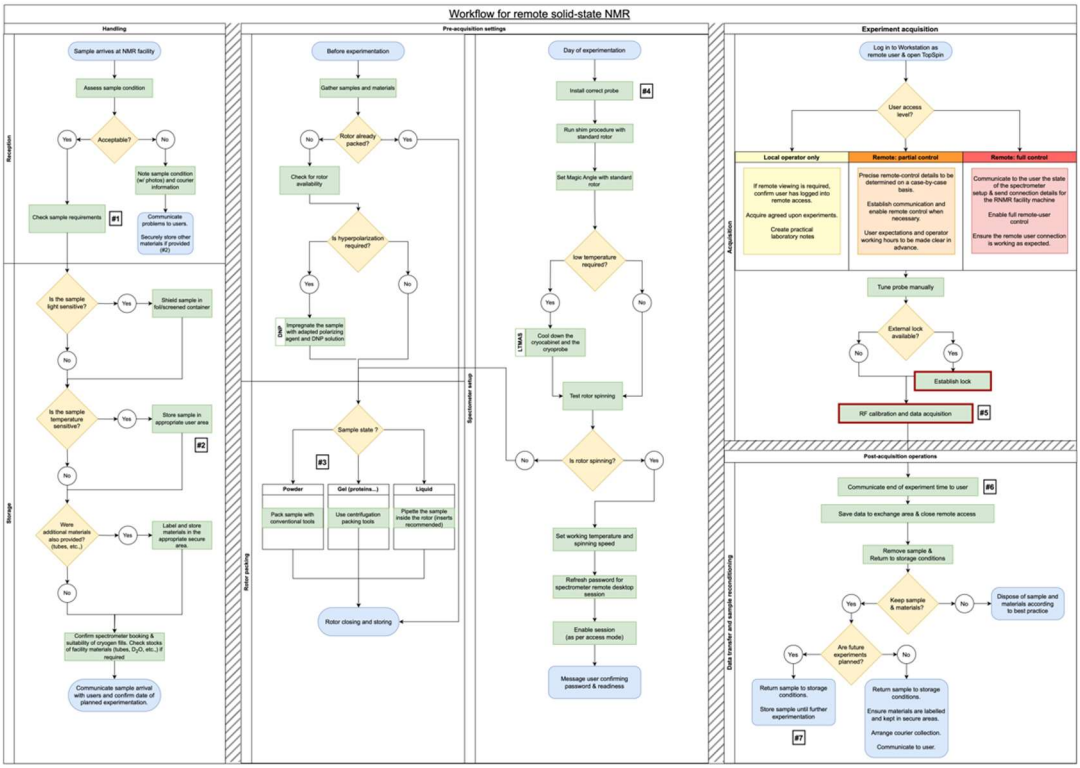

Figure 3: Work flowchart for solid-state NMR. #1) Sample requirements should be provided ahead of time by users. Unusual requirements should be discussed in the project brief. #2) Secure areas, specific to remote users, should be identified or created for distinct temperature and atmospheric conditions (-80°C, -20°C, 4°C, room temperature, glovebox). These should be well organised and materials should be clearly labelled. #3) Detailed protocols for sample manipulation should be provided by users at project deposition. Any atypical reagents must be provided with the samples. Facilities with a glovebox should be made clear to the user when depositing a project. Any unusual equipment or tubes must be provided with the samples. #4) Standardised probe checks TBD in separate document. #5) A standardized system to label the data (i.e., standard experiments vs. optimisation / popt exps / test planes) and the experiment metadata (sample, stage of manipulation, optimised pulses+delays etc.,) is necessary. This will be developed in T2 of WP4. #6) Data exchange mechanism to be determined, recommendations will be developed in WP4. #7) Facilities should be wary of becoming sample storage facilities. The recommendation could be that samples should only be stored when future experiments are explicitly planned. Red boxes highlight the steps where remote users may have full control without local assistance.

5. Quality assessment of the instrumentation - Standard samples and experiments

The quality of the instrumentation is evaluated by establishing (i) experimental sensitivity, (ii) required spectral resolution and (iii) radiofrequency pulse efficiency on different channels. We have therefore prepared a general list of standard reference samples, which are used both for hardware assessment and NMR experiment evaluation. These reference samples are largely inspired by the available reference samples provided by the primary spectrometer manufacturer (Bruker) as highlighted by the WP2 survey. In the table below, two shared pools of standard samples are chosen to evaluate the optimal performance of solution and solid-state NMR instrumentation. We focus on ^{1}H, ^{13}C and ^{15}N channels, which are the most commonly studied nuclei, while specifications for other nuclei would be provided in a case-by-case basis.

Test	Sample	Experiment
Solution NMR		
^{1}H lineshape	0.3% Chloroform ($CHCl_3$) in Acetone-d_6	^{1}H single pulse
^{1}H Sensitivity	0.1% Ethylbenzene (EB) in Chloroform-d	^{1}H single pulse
^{15}N & ^{13}C Pulse Calibration	100 mM ^{15}N-enriched Urea 100 mM ^{13}C-enriched Methanol in DMSO-d_6	^{15}N & ^{13}C single pulse
^{13}C Sensitivity & Resolution	40% Dioxane in Benzene-d_6	^{13}C single pulse
Gradient Recovery	0.1 mg $GDCl_3$/ml D_2O with 1% H_2O + 0.1% CH_3OH ^{13}C	^{1}H gradient echo
Temperature	Ethylene glycol (300-380K) & methanol (180-300K)	^{1}H shift
Spectrometer stability over time	^{13}C-^{15}N-labelled Ubiquitin	2D HSQC
Solid-state MAS NMR		
^{13}C lineshape	Powdered adamantane	^{13}C single pulse with decoupling
^{15}N & ^{13}C Pulse Calibration	Powdered ^{15}N & ^{13}C-enriched L-alanine	^{15}N & ^{13}C single pulse with decoupling
^{1}H pulse calibration	Powdered adamantane	^{1}H single pulse

^{13}C Sensitivity	Powdered $^{13}C\alpha$-enriched L-alanine	Cross polarisation
^{1}H Sensitivity (MAS >40 kHz)	Powdered adamantane	^{1}H single pulse
Magic angle	KBr	^{79}Br single pulse
Temperature	Water (protein samples; 250-320 K) / $Sm_2Sn_2O_7$ (>85K) / $PbNO_3$ (100-423 K) / KBr (20-300K) / CsI (<10K)	single pulse (^{1}H/ ^{119}Sn/ ^{207}Pb), R_1 (^{79}Br/ ^{127}I)