Formatting and Replication of Samples

National Phenome Centre

NPC.SOP.CC005 Version 2.1

Effective Date: April 2019

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1. Purpose

The purpose of this standard operating procedure (SOP) is to provide step-by-step instructions for the formatting of plasma, serum and urine samples. This includes the pooling of the study reference (SR) sample for use in ultra-performance liquid chromatography (UPLC) mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR) assays.

Human blood and urine are potentially infectious (Class 2) biofluids commonly handled within the facility. The aim of this SOP is to reduce the risk from handling biological materials, and to ensure accurate reproducible results through efficient and consistent sample handling.

2. Scope

This SOP describes the process of transferring samples from their original containers into 96-well plate format (“formatting”), and the distribution of that material into multiple plates, one for each assay type (“replication”).

3. Materials

Consumables

- Plate maps
- Roll of unique generated barcode stickers
- "Plate formatting recording sheet" *(see appendix I)*
- 20-300 µL tips
- 50-1250 µL tips
- 50-1250 µL elongated tips
- Self adhesive storage film
- 2 mL Deepwell plates, V-shape bottom
- 0.5 mL Deepwell plates
- 0.3 mL Microplate plates
- 50 mL freezable tert-butanol free containers
- Square shaped sealing mats
- 1.5 mL Eppedorf tubes
- Round shaped sealing mats
- 70% Industrial Methylated Spirit (IMS)
- Waste bottle containing Distel

Equipment

- Cooling platforms (stored at -80ºC)
- Balance
- Centrifuge equipped with a 96 well plate rotor
- 800 mL glass beaker
- 50-1200 µL 8 channel electronic pipette
- 15-300 µL 8 channel electronic pipette
- 1 mL pipette
- Seal mat roller

Personal protective equipment

- White laboratory coats
- Nitrile gloves
- Eye protection
- Class II biosafety cabinet

Prerequisite: study and aliquot information should already be uploaded to the centre LIMS and samples should have been sorted into their randomly generated LIMS order

Please note that this procedure involves the handling of a Class II rated biological hazard. Please ensure you have undergone all appropriate training & read all relevant risk assessments prior to any sample handling.

4. Procedure Day 1

4.1. Defrost

4.1.1. Thaw each batch of sorted samples intended for formatting at 2-8ºC for 17-20 hours (overnight).

5. Procedure Day 2

5.1. Preparation

5.1.1. Print a recording sheet (appendix I) and appropriate plate map from LIMS.

5.1.2. Attach a unique barcode plate sticker with a unique identifier number to a 2 mL deepwell plate. In addition, label the plate with the project name, biofluid type and plate number e.g. PROJECT_BIOFLUID_PARENTPLATE_PLATEXX.

5.1.3. Place the cooling platforms in the class II biosafety cabinet and cover with paper towel to absorb any condensation.

5.1.4. Keep the plate on the cooling platform to ensure the dispensed samples remain cool during formatting.

5.2. Parent plate production

5.2.1. Remove the samples from the fridge and place on the cooling platforms.

5.2.2. Remove a sample tube and check identification against the plate map.

5.2.3. Dispense up to 2 mL of the sample to the corresponding well (according to the plate map) of the 2 mL deepwell plate. (Samples sent in several aliquots should be combined in the 2 mL deepwell plate to a total volume of up to 2 mL).

5.2.4. Sample tubes with sample volume remaining should be retained for continued storage. *If agreed with study sponsor empty tubes may be disposed of appropriately.*

5.2.5. Once all samples have been transferred, seal the plate with self-adhesive storage film. *NB. Columns 11 and 12 remain empty until the addition of long term reference (LTR) and study reference (SR) at the sample preparation stage.*

5.3. Centrifugation

5.3.1. Weigh the plate; this is done within the class II biosafety cabinet.

5.3.2. Bring the centrifuge bucket to the class II biosafety cabinet and load the plate.

5.3.3. Once the bucket is sealed, wipe down with 70% industrial methylated spirit (IMS).

5.3.4. Fill a second plate with water. Ensure that the weight is within 1% of the plate containing the samples. This will be used to balance the centrifuge.

5.3.5. Place balance plate in bucket.

5.3.6. Centrifuge the plates at 3486 g for 10 minutes at 4ºC. (Acceleration: 9; Brake: 9).

5.4. Replication preparation

Whilst the parent plate is in the centrifuge:

5.4.1. Place a clean 800 mL glass beaker onto the cooling platform in the class II biosafety cabinet (for pooling the NMR-SR).

5.4.2. Attach a unique barcode plate sticker with a unique identifier number to each required plate. In addition, label the plate with the project name, biofluid type, assay type, and plate number e.g. PROJECT_BIOFLUID_ASSAY_PLATEXX. See Table 1 for blood sample plate requirements and Table 3 for urine plate requirements.

5.4.3. Place the plates on the cooling platform in the class II biosafety cabinet in the appropriate order.

5.5. Replication

5.5.1. At the end of centrifugation bring the bucket to the class II biosafety cabinet, carefully *(without disturbing the pelleted material)* remove the plate from the bucket, then carefully remove and discard the self-adhesive storage film.

5.5.2. Remove any surface material from the supernatant of individual samples using a clean pipette tip. *(Discard the tip to the sharps bin).*

5.5.3. Programme the 50-1200 µL 8 channel pipettes as Table 2 for blood analysis and Table 4 for urine analysis.

5.5.4. Aspirate the samples from column 1 of the parent plate *(without disturbing the pelleted material).*

5.5.5. Dispense the discard step material into the NMR-SR beaker and dispense the remaining material across preparation plates according to volumes indicated in Tables 2 and 4. (*Should there be air bubbles in the tip, tap the top of the tips to remove air bubbles before dispensing).*

5.5.6. At the end of each dispensing series, eject the tips to the sharps bin.

5.5.7. Report any missing samples (marked as “M”), samples with low volume (marked as “L”), or no volume remaining (marked as “X”) on the recording sheet (appendix I).

5.5.8. With a clean set of tips for each column aspirated, repeat the above steps for columns 2 to 10.

5.5.9. For urine, repeat steps 5.5.4. to 5.5.8. with the 15-300 µL 8 channel pipette respectively.

5.5.10. Using a roller, seal each plate with an appropriate sealing mat:

- square sealing mat for parent plate
- round seal mats for all other plates

5.5.11. Decant the NMR-SR into a contamination free container, e.g. a tested 50 ml centrifuge tube, labelled with the study name, biofluid type, “NMR-SR” and date. Store the tube at -80 °C. Whenever sample replication is carried out across multiple days and multiple users, new pooled sample should be added to the frozen quantity.

BLOOD ANALYSIS

Table 1: Plate requirements for blood analysis.

Number of plates	Plate Type	Intended Analysis
1	0.3 mL Microplate plate	MS-SR
1	2.0 mL Deepwell plate	NMR
1	0.5 mL Deepwell plate	MS-Lipids
1	0.5 mL Deepwell plate	MS-HILIC
1	0.5 mL Deepwell plate	MS-RP
1	0.5 mL Deepwell plate	MS-Back-up

Table 2: Pipette order with volumes and analysis plate.
Use 50-1200 µL pipette only

Dispense Order	Sample Volume (µL)	Preparation Plate	Recipient format
1	Pipette discard step	NMR-SR	800 mL beaker
2	50*	MS-SR	0.3 mL Plate
3	350	NMR	2.0 mL Plate
4	50	MS-Lipid	0.5 mL Plate
5	50	MS-HILIC	0.5 mL Plate
6	100	MS-RP	0.5 mL Plate
7	50*	MS-Backup	0.5 mL Plate

** this can be increased to 100 µL if sufficient material is available*

URINE ANALYSIS

Table 3: Plate requirements for urine analysis.

Number of plates	Plate Type	Intended Analysis
1	2.0 mL Deepwell plate	NMR
1	0.5 mL Deepwell plate	MS-HILIC
1	0.5 mL Deepwell plate	MS-RP
1	0.3 mL Deepwell plate	MS-SR
1	0.5 mL Deepwell plate	MS-Back-up

Table 4: Pipette order with volumes and analysis plate.

Dispense Order	Pipette type (µL)	Sample Volume (µL)	Preparation Plate	Recipient format
1	50-1200	Pipette discard step	NMR-SR	800 mL beaker
2	50-1200	600	NMR	2.0 mL Plate
3	15-300	Pipette discard step	NMR-SR	800 mL beaker
4	15-300	75	MS-RP	0.5 mL Plate
5	15-300	25	MS-HILIC	0.5 mL Plate
6	15-300	50	MS-SR	0.3 mL Plate
7	15-300	150	MS-Backup	0.5 mL Plate

5.6. Post-proceedure steps

5.6.1. All samples and plates should be placed in the -80°C freezer ready for next use. *NB. LIMS can be updated by scanning the unique barcodes on the plates and recording the freezer location.*

5.6.2. Cooling platform, pipettes, and surfaces are to be cleaned with 70% IMS and left to dry in the class II biosafety cabinet.

5.6.3. Once dry, place cooling platforms in -80°C freezer.

6. Study Reference (SR) preparation for Mass Spectrometry (MS-SR) – post NMR

6.1. Sample exclusion and pooling

6.1.1. Generate a list of samples to be excluded based on NMR results.

6.1.2. Thaw the MS-SR plates at 2-8°C for 2 hours.

6.1.3. Once thawed, centrifuge each MS-SR plate at 3486 g for 1 minute at 4ºC.

6.1.4. In a class II biosafety cabinet remove and discard to waste any excluded samples from each plate.

6.1.5. Transfer all remaining samples from the plates into a contamination free container, e.g. a tested 50 mL centrifuge tube, to pool.

6.1.6. Once all samples have been pooled in the beaker, gently swirl to ensure homogenisation.

6.2. Blood samples

6.2.1. Split the pooled SR into three equal aliquots. The three aliquots will be used to form the SR for the Lipid, HILIC and reversed phase (RP) assays.

6.2.2. Lipid assay: refer to NPC.SOP.MS003, section 5.2 for protocol.

6.2.3. HILIC assay: refer to NPC.SOP.MS004, section 5.3 for protocol.

6.2.4. RP assay: label container and store at -80ºC.

6.3. Urine samples

6.3.1. Split the pooled SR into a 3:1 ratio v/v. The larger volume (75%) will be used for the RP assay and the smaller volume (25%) will be used for the HILIC assay.

6.3.2. RP assay: refer to NPC.SOP.MS005, section 5.3 for protocol.

6.3.3. HILIC assay: refer to NPC.SOP.MS006, section 5.3 for protocol.

7. Related Documents

Document Number	Title
NPC.SOP.CC004	Sample Sorting and LIMS Logging
NPC.SOP.MS003	RPC UPLC-QTOF Analysis of Lipids in Human Plasma and Serum
NPC.SOP.MS004	HILIC UPLC-QTOF Analysis of Small Molecules in Human Plasma and Serum
NPC.SOP.MS005	RPC UPLC-QTOF Analysis of Small Molecules in Human Urine
NPC.SOP.MS006	HILIC UPLC-QTOF Analysis of Small Molecules in Human Urine
NPC.SOP.NMR003	Preparation of Human Urine Samples for NMR Analysis
NPC.SOP.NMR004	Preparation of Human Plasma and Serum Samples for NMR Analysis

8. Version History

Current Version

Version number	Author	Changes and justification	Section(s) updated
V2.1	VHS	Revision and minor corrections	all

Previous Version

Version number	Author	Changes and justification	Section(s) updated
V2.0	VHS/BC/ MD/SL	Changed for new diluted MS protocols, NMR plate correction	all
V1.1	VHS/BC	Revision and minor corrections	all
V1.02	VHS	Addition of Lipid protocol NPC.SOP.MS003 to text	6 and 7
V1.01	BJ	Correction of volume for urine NMR plate	1, 5, 7 and 10
V1	DB	New SOP	N/A

9. Responsibilities

Centre management is responsible for ensuring that laboratory technical personnel are appropriately qualified to perform the procedures outlined in this SOP. The appointed laboratory personnel are in turn responsible for conducting the procedure as outlined in accordance with health and safety standards.

Health and safety statement: before commencing any activities described in this document personnel must be adequately trained e.g. staff having completed local institution Chemical Safety Training and staff having read and understood the relevant risk assessments. Chemical, biological and general waste should be disposed of according to local policies.

10. Approval

Prepared by Dr Verena Horneffer-van der Sluis	Date
Reviewed by Dr Beatriz Jimenez	Date
Reviewed by Dr Maria Gomez-Romero	Date
Authorised by Dr Matthew Lewis	Date

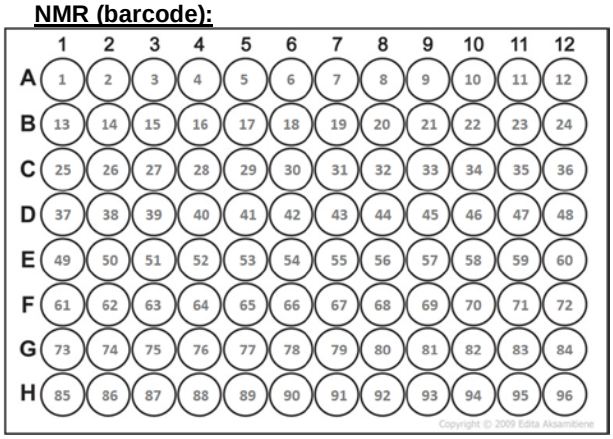
Appendix I - Plate formatting recording sheet – Biofluid_____

Project: **Date:**

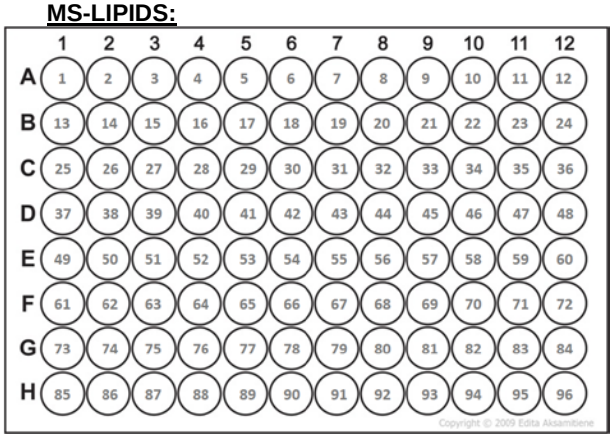
Plate number: **Aliquoted by:**

Storage Location: **Parent Plate barcode:**

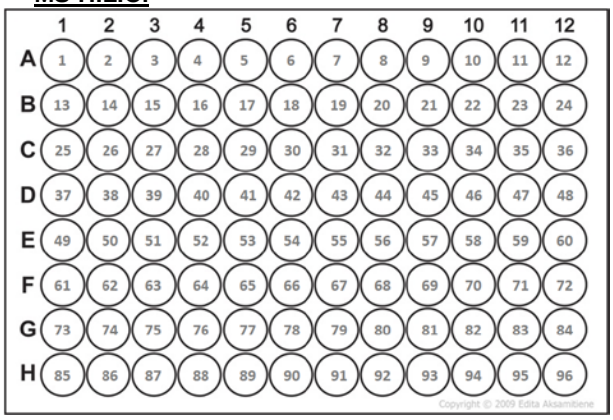
NMR (barcode):



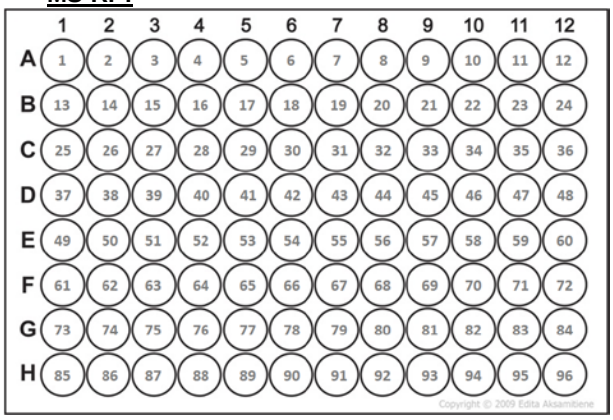
MS-LIPIDS:



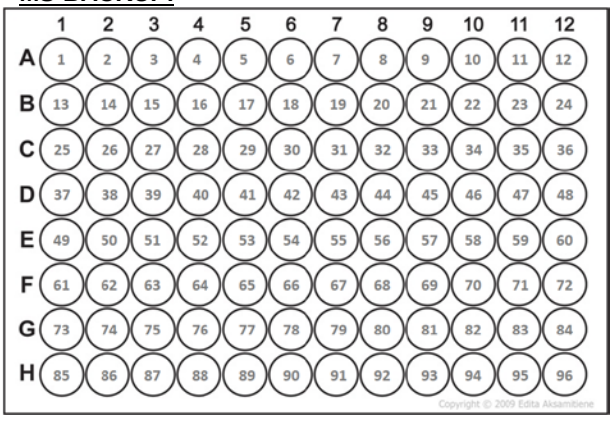
MS-HILIC:



MS-RP:



MS-BACKUP:



MS-SR (barcode):

Please cross through the MS-LIPID plate when analysing urine, this plate is not required.

KEY:

M = Sample missing (not received)

L = Low volume

X = No sample remaining

End of Document