NIHR | King's Clinical Research Facility

Use of the Centrifuges for the Processing of Clinical Trial Samples in the King's Clinical Research Facility

Document Detail	
Document type	Standard Operating Procedure
Document name	CRF-LAB-SOP-1: Use of the Centrifuges for the Processing of Clinical Trial Samples in the King's CRF
Document location	Q-Pulse \ CRF Documents
Version	6.0
Effective from	5th March 2020
Review date	5th March 2022
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Authorised by	Professor Peter Goadsby, CRF Director
Related documents	Eppendorf 5702R Operating Manual Rotina 380R Manual CRF-LAB-SOP-2: Procedure for Dealing with Biological Sample Spillage in the King's CRF CRF-LAB-SOP-3: Processing, Storage and Shipment of Samples in the King's CRF CRF-LAB-FRM-1: Centrifuge Maintenance Log CRF-LAB-FRM-7: Centrifuge Maintenance: Monthly Rota CRF-HS-COP-1: King's CRF Health and Safety Code of Practice CRF-HS-SOP-1: MRI Access Control and Safety Procedures in the King's CRF
Keywords	Centrifuge, biological sample, maintenance, calibration, CTF, EMF
Supporting references	See Section 6.0

Change History

Date	Change details, since approval	Approved by
19th December 2013	1. Amended text in SOP title from “Clinical Research Facilities” to “King’s Clinical Research Facility” 2. Amended name of Director to reflect new Director 3. Amended logos to update to current CRF letterhead template 4. Amended document number from CRF SOP006 to CRF-LAB-SOP-1 to comply with QPulse document numbering system 5. Amended numbers of documents referred to throughout the text to reflect revised QPulse/CRF numbers 6. Section 3.3: Added additional locations of Eppendorf centrifuges 7. Section 4.3: Added new section stating requirement for staff using centrifuges in MRI and 2nd Floor QC lab to undergo training and induction to these areas	E. Giemza
March 2015	1. Update to Sections 5.8.2 and 5.8.3 for daily maintenance and to amend weekly maintenance to fortnightly maintenance. Updated process for Section 5.8.3 2. Section 5.8.1.4: Updated SOP reference number to correspond with all Q-Pulse documents 3. Updated documents / references in Section 6.0 4. Minor administrative changes to the text	E.Giemza
January 2016	1. Updated ‘related documents’ to include new/amended CRF documents 2. Sections 3.3, 4.3, 5.3: updated location of the centrifuges within the EMF and CTF 3. Section 5.6: ‘daily’ maintenance has been amended to ‘weekly’ maintenance and ‘fortnightly’ maintenance has been amended to ‘monthly’ maintenance, as per CRF practice 4. Section 5.6.3.1: correction to the dilution ratio for the disinfectant 5. Section 5.7: updated process for the allocation and documentation of the weekly/monthly maintenance of the centrifuges 6. Administrative changes to the text throughout the SOP for clarity	E.Giemza
February 2018	1. Updated locations for the CRF centrifuges 2. No changes to the processes are required 3. Minor administrative changes to the text for clarity	E.Giemza
March 2020	1. Operating and maintenance procedures for LaboFuge 400R centrifuge was changed to reflect operating and maintenance procedures for Rotina 380R centrifuge as per purchase of new model Centrifuge.	E. Giemza

Review History		
Date	Review details	Approved by
19th December 2013	Review of v1.0 conducted by Lara Edwards, CRF QA Manager, superseded by v2.0 (effective date 03rd January 2014)	E Giemza
March 2015	Review of v2.0 conducted by Georgia Bullock, CRF QA Manager, superseded by v3.0	E.Giemza
January 2016	Review of v3.0 conducted by Georgia Bullock, CRF QA Manager, due to a change in CRF practice. Changes made as per 'Change History' and re-issued as v4.0.	E. Giemza
February 2018	Review of v4.0 conducted by Georgia Bullock, CRF QA Manager, as per the review date. Changes made as per 'Change History' and re-issued as v5.0.	E. Giemza
March 2020	Review of v5.0 conducted by Olabisi Awogbemila, Clinical Research Practitioner, as per the review date. Changes made as per 'Change History' and re-issued as v6.0	E. Giemza

1.0 Background

1.1 All biological samples (e.g. plasma, serum) collected for the purposes of clinical research within the King's Clinical Research Facility (CRF) will be centrifuged and separated in compliance with the trial protocol to ensure consistency and continuity in the sample processing. The processing of samples must also adhere to Good Clinical Laboratory Practice (GCLP), the MHRA 'Good Clinical Practice for Laboratories' guidelines and local Health and Safety policies. Centrifuges processing biological material can create significant health risks through liquid spillage and droplet dispersion. It is thus important to ensure that they are correctly sited, installed, operated and maintained.

2.0 Purpose

2.1 The purpose of this Standard Operating Procedure (SOP) is to describe the procedures for the safe operation and routine maintenance of the Eppendorf 5702R and Rotina 380R model centrifuges which are located within the Experimental Medicine Facility (EMF) and Clinical Trials Facility (CTF).

3.0 Scope

3.1 The CRF encompasses the Clinical Trials Facility (CTF), the Experimental Medicine Facility (EMF) and the Cell Therapy Unit (CTU). CRF SOPs will apply to the CTF and EMF only and staff working in those areas should work to all relevant CRF

SOPs. The CTU will continue to control and use its own policies and SOPs to ensure compliance with Good Manufacturing Practice (GMP).

3.2 All core CRF staff and users of the CRF who are operating and/or involved in maintaining the centrifuges in the EMF and CTF areas of the CRF are bound to adhere to the procedures outlined in this SOP.

3.3 Three Eppendorf 5702R centrifuges are situated in the EMF and one in the CTF and the Rotina 380R centrifuge is situated in the CTF.

4.0 Responsibilities

4.1 It is the responsibility of the Principal Investigator (PI) or appropriate delegate to ensure that the sample processing/centrifugation procedures defined in the study/trial protocol are adhered to. In the absence of study-specific details, this SOP will be used.

4.2 All core CRF staff and users of the CRF who are required to use any of the CRF centrifuges must be trained in the use of the equipment and in the sample processing procedures that they are conducting.

4.3 All staff wishing to use the centrifuge in the MRI area of the CRF must also have completed the MRI safety training and completed a MRI Safety Questionnaire (see *CRF-HS-SOP-1: MRI Access Control and Safety Procedures in the King's CRF*).

5.0 Procedure

5.1 General Information

5.1.1 Prior to centrifugation, visually inspect tubes for signs of material damage. Damaged tubes must not be centrifuged.

5.1.2 Seal tube lids down tight prior to centrifuging. Lids of unclosed tubes can rip off during centrifugation and damage the centrifuge.

5.1.3 Ensure that the rotor and buckets are free of spillage and damage.

5.1.4 Ensure that blood collection tubes filled with water are used to balance samples when there are an odd number of samples to spin.

5.1.5 Always run a centrifuge with the full complements of buckets. Failure to do so can distort the rotor.

5.1.6 Balance the samples and distribute them evenly or diametrically around the rotor.

5.1.7 Hand-tighten the lids securely on all buckets. Do not over-tighten as the lids can cross thread and over-tighten while spinning.

5.1.8 Stay with the centrifuge until it gets up to its set speed. If the blood tubes are not properly balanced or there is a problem with a bucket or the rotor, the centrifuge will make an unusual noise and shake. If this happens, stop the centrifuge, open the lid and assess what caused the imbalance, correct the problem and start again.

5.1.9 Do not circumvent any of the safety features (such as lid closure override switches). They are there to protect the user.

5.1.10 Do not move or knock the centrifuges while in operation.

5.1.11 Do not lean or place items on the instrument while it is operating.

5.1.12 Condensation built up in a centrifuge bowl will damage the motor and buckets. Allow this to evaporate by leaving the lid open at night or when not in use, with the power switched off.

5.2 **Sample Spillage/Maintenance Issues**

5.2.1 If, when the centrifuge is opened, a sample has broken, close the lid immediately and leave the centrifuge closed for at least 30 minutes (1 hour is preferable). This allows aerosols to settle and lowers the risk of inhaling any harmful aerosols released from the broken samples.

5.2.2 After 30-60 minutes, open the lid and remove the bucket containing the broken tube and remove the lid from the bucket. Remove the broken tube, (with forceps if necessary) taking care not to cut yourself, then remove the bucket, immerse the bucket and the lid in disinfectant for at least 15 minutes

and then rinse and leave to dry. Wipe the centrifuge clean of any spillages by spraying with disinfectant, wiping and leave the lid open to dry.

5.2.3 If the centrifuges have any problems that cannot easily be rectified, or when maintenance is required, then the unit must be immediately taken out of service, disconnected from the power source and clearly marked **DO NOT USE** until serviced. This notice should include the name of the person, the date, the reason and the signature of the CRF Manager or Nurse.

5.3 **Eppendorf 5702R. Locations: EMF, Ground Floor (MRI area); EMF, 1st Floor (Trial Procedures Room: 2 centrifuges); CTF (Sample Processing Area)**

5.3.1 **Switching on the Appliance**

5.3.1.1 To switch on the centrifuge, ensure that the device is plugged in and the power switch (black switch at rear of machine) is on. The stand-by switch located on the front right-hand side of the machine should now also be switched on.

5.3.2 **Switching off the Appliance**

5.3.2.1 Ensure that the centrifuge is switched off at the power switch and the mains. The display will take a few seconds after the machine has been switched off to go blank.

5.3.3 **Display Panel Buttons**

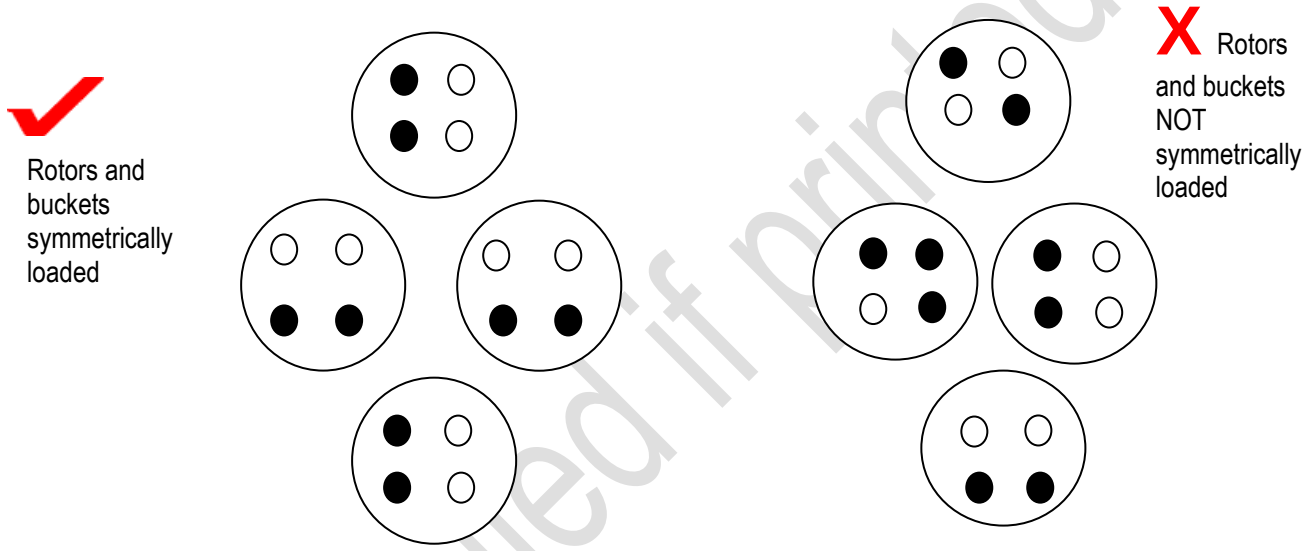
5.3.3.1 The following buttons are displayed on the front of the centrifuge:

- TIME - turn dial on left-hand side: alters the running time.
- SPEED - turn dial on right-hand side: alters the speed in increments of 100 1/min or rcf.
- START- (stand-by button on right-hand side): starts the run. The ■ symbol flashes while the rotor is running.
- STOP - (stand-by button on right-hand side): stops the centrifuge. The ■ symbol appears briefly as soon as the rotor comes to a standstill.
- STAND-BY- (stand-by button on right-hand side): centrifuge switched to stand-by mode.
- OPEN - (button on front panel labelled “Open”): releases the lid hatch.

- TEMP control - (buttons labelled ▲and ▼on front panel): increases or decreases the nominal temperature value.

5.3.4 Operation of the Eppendorf 5702R

5.3.4.1 When loading the buckets, ensure that the tubes and adapters are inserted symmetrically; the tubes opposite one another need to contain approximately the same filling quantity.
See the diagram below:



5.3.4.2 If the weight differences are excessive, the automatic imbalance detector will shut down operation and error message **"Inb"** will appear on the display panel.

5.3.4.3 The specified maximum weight of 400g imprinted on the rotor is the gross weight rating of a bucket (including adapter, tubes and contents).

5.3.4.4 The maximum load (adapter, tubes and contents) of a round bucket is 190g.

5.3.4.5 Round buckets and the associated adapters are for the centrifugation of Falcon® tubes, blood withdrawal systems and other round bottomed tubes, NOT glass centrifugation tubes (rectangular buckets must be used for these).

5.3.4.6 The aerosol-tight caps should be used for all round buckets. Ensure that the silicone sealing ring attached to the lid is not removed or damaged and sits uniformly in the groove.

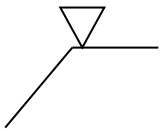
5.3.4.7 Both the round bucket and cap are autoclavable at 121°C, 20 minutes.

5.3.4.8 To run a **short centrifugation cycle**, load the buckets as described above and press the SHORT button with the lid closed to start a short run at maximum speed. The centrifuge stops when the SHORT button is released again.

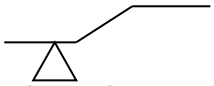
5.3.4.9 To start a **continuous run cycle**, turn the TIME dial to either above 99 minutes or below 0.5 minutes. The display panel will display “oo” to indicate that continuous running is active. Press STOP to end continuous running.

5.3.4.10 To set **rcf** of a cycle (“sometimes known as “g”) – turn the SPEED dial on the front of the machine to increase or decrease the speed in increments of 100 1/min or rcf. To toggle the display between 1/min (rpm) and (rcf) and vice versa, press the SPEED dial.

5.3.4.11 To set the **running time** of a cycle, turn the TIME dial on the front of the machine. The time can be counted immediately from the start or when the pre-set speed is attained. Pressing the START/STOP button for >2 seconds with the centrifuge lid open switches to the “at set rpm” mode, symbolised by:



5.3.4.12 To exit the “at set rpm” mode and begin counting centrifugation time immediately after starting the centrifuge, press the START/STOP button again for > 2 seconds with the centrifuge lid open until the following symbol is displayed:



5.3.4.13 To set the temperature of a cycle, press the temperature arrow keys on the front panel of the machine to increase or decrease the temperature.

5.3.4.14 Adjusting the **acceleration or braking ramps**: If there is a need to reduce the acceleration and braking ramps (if working with Ficoll® density gradients for example) press the SHORT key for >5 seconds while the centrifuge lid is open. The symbol **soft** appears on the display panel. The slower acceleration and braking ramps are now activated.

5.3.4.15 To revert to faster acceleration and braking, press the SHORT key for >5 seconds again while the centrifuge lid is open. **"br on"** will be displayed briefly in the display panel and signifies the reactivation of the faster acceleration and braking ramps.

5.3.4.16 **Parameter Lock**: to prevent pre-programmed parameters being adjusted, press and hold down the OPEN and SHORT buttons simultaneously for at least 5 seconds with the lid open. After 5 seconds, the dial adjuster is disabled and the parameters are locked. **"Lo on"** appears in the display panel together with a symbol of a locked padlock. To start cycle, load the buckets, close the lid and press START.

5.3.4.17 To enable the dial adjuster again simply press and hold down simultaneously the OPEN and SHORT buttons again with the lid open for at least 5 seconds. After 5 seconds **"Lo off"** and an open padlock symbol will be displayed.

5.3.4.18 It is possible to store two permanent programs in the machine. After setting the required programme data (time, speed, temperature and acceleration/braking ramp) press down either the **"PROG 1 or PROG 2"** buttons on the front of the machine

for 2 seconds until the button is no longer flashing and lights up continuously. The program is now stored.

5.3.4.19 To call up a pre-programmed program, load the buckets, close the lid and press either PROG 1 or 2 once, the button for the activated program will light up blue. Press START to start the cycle. Exit the programme again after the cycle by pressing the program button.

5.3.4.20 Press ‘Start/Stop’ to begin a run. The displays will then show the increasing rotor speed, the run time remaining, and the chamber temperature.

5.3.4.21 If you need to abandon a run before the end time, press ‘STOP” and switch main switch off.

5.3.4.22 To open the lid, press OPEN.

5.3.4.23 At the end of use, leave the lid slightly open (to allow any frost inside the chamber to disperse overnight), and switch power ‘Off’ at the wall socket.

5.3.4.24 Ensure that all the centrifugation parameters selected for a cycle are compliant with those detailed in the sample processing section of the trial/study protocol and/or laboratory manual.

5.4 **Rotina 380R. Location: CTF (Sample Processing Area)**

5.4.1 Switch power ‘on’ at the wall socket. The centrifuge data of the last used program will be displayed.

5.4.2 Load the buckets, balance the tubes and secure the bucket lids tightly, as described in section 5.1.

5.4.3 To set **RPM:** Press the **TIME** key. The parameters **RPM** is displayed. Use the adjusting knob to set the value you want. Press the **RPM** or **START** keys to apply the setting to the display.

5.4.4 To set **RCF** (sometimes called 'g'): Press the **RCF** key as often as required until the parameters **RAD** and **RCF** are displayed and the value of the parameter, **RAD** is displayed in parentheses, **[ ] e.g RAD = [146] rcf = 3695.** The LED is lit in the key. Use the adjusting knob to set the centrifuging radius you want. By changing the centrifuging radius, the value adjusts automatically to the **RCF**. Press the **RCF** key again. The value of the parameter, **RCF** is displayed in [ ] parentheses, e.g RAD = **146 RCF = [3695].** Use the adjusting knob to set the **RC**F you want. Press the **PROG** key to save the set **RCF** value.

5.4.5 Adjusting the **acceleration or braking ramps**: Press the **brake** key ⎍ until the parameter ⎿ or **⌒t** is displayed. ⎿ = braking stage = B – breaking stage; **⌒ t** = run-down time. Press the **TIME** key to switch between the braking stage and run-down time. Set the desired stage or time with the rotary knob. If necessary, press the **brake** key ⎍ to set the next parameter. To apply the setting to the display, either press the **START** key or press the **brake** key ⎍ as often as is required until the centrifugation data are displayed. To set brake switch off speed, press the **brake** ⎍ key as often as necessary until the parameter **N Brake** is shown .Use the adjusting knob to set the value you want. Press the **brake** ⎍ or **START** key to apply the setting to the display.

5.4.6 To set **temperature:** Press the **T/^{0}C** key. Temperature is adjustable from -20 ^{0}C to +40 ^{0}C, in 1^{0}C increments. Use the adjusting knob to set your desired temperature.

5.4.7 To set the **running time:** Press the **TIME** key. The parameters **t/hms** is displayed. The minutes **(m)** are shown in parentheses [ ], and can be changed. Use the adjusting knob to set the value you want. Press the **TIME** key. The seconds **(s)** are shown in parentheses [ ] and can be changed. Use the adjusting knob to set the value you want. Press the **TIME** key. The hours

(h) are shown in parentheses [ ] and can be changed. Use the adjusting knob to set the value you want. To apply the setting to the display, either press the **START** key or press the **TIME** key as often as is required until the centrifugation data are displayed.

5.4.8 Opening and Closing the **Lid:** The Lid can only be opened when the centrifuge is switched on and the rotor is at rest. To close the Lid, place the lid and lightly press down the front edge of the Lid. The locking action is effected by the motor. The left LED in the button **OPEN/STOP** lights up. To open the Lid, press the button **OPEN/STOP**. The Lid unlocks via the motor and the left LED in the push button **OPEN/STOP** extinguishes.

5.4.9 Press **START** to begin the centrifugation run. The displays will then show the increasing rotor speed, the run time remaining, and the chamber temperature. The LED in the key is lit during the centrifugation run as long as the rotor is revolving.

5.4.10 If a run needs to be abandoned before the end time, press **OPEN /STOP.** The rotor decelerates with the pre-set rundown parameters. The right hand LED in the button lights up until the rotor is stationary. Once the rotor is stationary, the left hand LED flashes in the button. Unlock the Lid, the left hand LED in the button goes out.

5.4.11 To open the lid, press **OPEN/STOP**. Note that pressing the button twice will trigger the emergency stop.

5.4.12 At the end of use, leave the lid slightly open (to allow any frost inside the chamber to disperse overnight), and switch power ‘Off’ at the wall socket.

5.4.13 Ensure that all the centrifugation parameters selected for a cycle are compliant with those detailed in the sample processing section of the trial/study protocol and/or laboratory manual.

5.4.14 Note that if no key is pressed for 8 seconds long after the selection or during the input of parameters, the previous values will be shown in the display. The input of parameters then has to be executed again. When you enter

several parameters, the **START** key does not have to be pressed until you have made the settings for the last parameter.

5.4.15 If parameters are changed, the program number is displayed in parentheses [ ]. This means that the centrifugation data in the display no longer corresponds to the centrifugation data from the program place that has been saved. You can discontinue entering parameters at any time by pressing the key **OPEN/STOP.** In this case, the adjustments are not saved.

5.5 **Troubleshooting**

5.5.1 For operational problems and in the case of error messages, please refer to the "troubleshooting" section of the relevant operating manual in the first instance.

5.5.2 If the error or operational problem cannot be rectified, please inform the CRF Manager or appropriate delegate.

5.5.3 If the problem cannot still not be rectified it will be necessary to call the relevant service engineers for that centrifuge. Contact details of these are held by the CRF Quality Assurance Manager.

5.6 **Routine Maintenance of the Eppendorf 5702R and Rotina 380R Centrifuges**

5.6.1 **General Maintenance**

5.6.1.1 Before cleaning, unplug the power plug with the lid open.

5.6.1.2 Surfaces must be dried immediately after cleaning.

5.6.1.3 Users are responsible for cleaning and decontaminating the centrifuge in the event of centrifuge contamination caused by infectious or high-risk material.

5.6.1.4 In the event of spillage of infectious or high-risk material, decontaminate the affected surface area. Do not use the centrifuge until all areas are completely dry. Refer to *CRF-LAB-SOP-2: Procedure for Dealing with Biological Sample Spillage in the King's CRF* and follow the procedure for dealing with a sample spillage.

5.6.1.5 In the event of condensation water formation, dry the centrifuge chamber by wiping it out with an absorbent cloth.

5.6.2 **Weekly Maintenance**

5.6.2.1 Wipe the internal and external surfaces of the centrifuge with disinfectant wipes and leave to dry overnight.

5.6.2.2 Check for signs of damage/wear and tear.

5.6.3 **Monthly Maintenance**

5.6.3.1 Dilute 500mls of concentrated **MicroSol $_{TM}^{3+}$** in 4.5 litres of water, or as per the manufacturer's instructions as required.

5.6.3.2 Immerse the buckets, lids, racks and other re-usable sample processing equipment into a bucket of the disinfectant for 10 minutes.

5.6.3.3 Ensure the grooves of the swing buckets are free of contamination. The buckets can be lubricated with the grease if necessary.

5.6.3.4 Check the rotor and buckets for signs of corrosion.

5.6.3.5 Rinse the centrifuge and its parts with a hand towel soaked in plain water (loose parts can be immersed in plain water).

5.6.3.6 Leave them on a dry surface to dry overnight.

5.7 **Allocation and Documentation of Maintenance**

5.7.1 The weekly maintenance of the centrifuges will be allocated to core CRF staff as part of the other weekly checks carried out in the CRF. The monthly checks can be allocated using *CRF-LAB-FRM-7: Centrifuge Maintenance: Monthly Rota.*

5.7.2 The weekly and monthly maintenance of all centrifuges must be documented on *CRF-LAB-FRM-1: Centrifuge Maintenance Log*.

5.8 **Servicing and Calibration**

5.8.1 Servicing and calibration of the Eppendorf 5702R and Rotina 380R centrifuges is carried out by Henderson BioMedical on an annual basis.

5.8.2 Records of the annual calibration / servicing of the centrifuges are kept by the CRF and can be provided as required.

6.0 Related documents & References

6.1 Eppendorf 5702R Operating Manual

6.2 Rotina 380R Manual

6.3 CRF-LAB-FRM-1: Centrifuge Maintenance Log

6.4 CRF-LAB-FRM-7: Centrifuge Maintenance: Monthly Rota

6.5 CRF-LAB-SOP-2: Procedure for Dealing with Biological Sample Spillage in the King's CRF

6.6 CRF-LAB-SOP-3: Processing, Storage and Shipment of Samples in the King's CRF

6.7 King's College Hospital Health and Safety information and guidelines http://kweb/kwiki/Health_and_Safety_Management

6.8 CRF-HS-COP-1: King's CRF Health and Safety Code of Practice

6.9 CRF-HS-SOP-1: MRI Access Control and Safety Procedures in the King's CRF

7.0 List of Appendices

N/A

8.0 Approval and sign off

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