NIHR | King's Clinical Research Facility

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

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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
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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
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May 2024	1. Reference of CRF-LAB-FRM-1 and CRF-LAB-FRM-7 deleted due to forms being obsolete 2. Reference of CRF-LAB-FRM-17 and CRF-LAB-FRM-18 were added 3. Mandatory requirement of training added in section 4.2 4. CRF-HS-SOP-1 has been replaced with DOC60-Out of Hours MRI Access DH-MRI-10	E. Giemza

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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19th December 2013	Review of v1.0 conducted by Lara Edwards, CRF QA Manager, superseded by v2.0 (effective date 03rd January 2014)	E Giemza
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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 centrifuges are situated in the EMF (two Eppendorf 5702R centrifuges in the Treatment Procedures Room, one Eppendorf 5702R centrifuge in the MRI suite) and three centrifuges in the CTF (one Hettich Rotina 380R and one Eppendorf 5702R in the CTF lab, and one in the CTF sluice room).

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 including the operational processes described in sections 5.3.4.8 and 5.3.4.11 that they are conducting. A Centrifuge Memo Log (*CRF-LAB-FRM-18: Centrifuge Memo Log*) has been developed to ensure recording of all CRF core staff and CRF users' understanding of the procedure described in this SOP prior to their first use of centrifuges.

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 *DOC60: Out of Hours MRI Access DH-MRI-10*).

4.4 It is the responsibility all CRF staff delegated to clean the centrifuge every 2 weeks as per the cleaning instructions outline in Section 5.64 of this SOP, as per the manufacturers cleaning manual. The cleaning rota, drawn up by the Lead Research Nurse specifies the CRF staff member responsible for cleaning a specific device.

4.5 It is the responsibility of the delegated CRF staff member to sign and date when the cleaning is complete.

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 Please refer to the cleaning instructions below. (Section 5.64)

5.2.4 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**

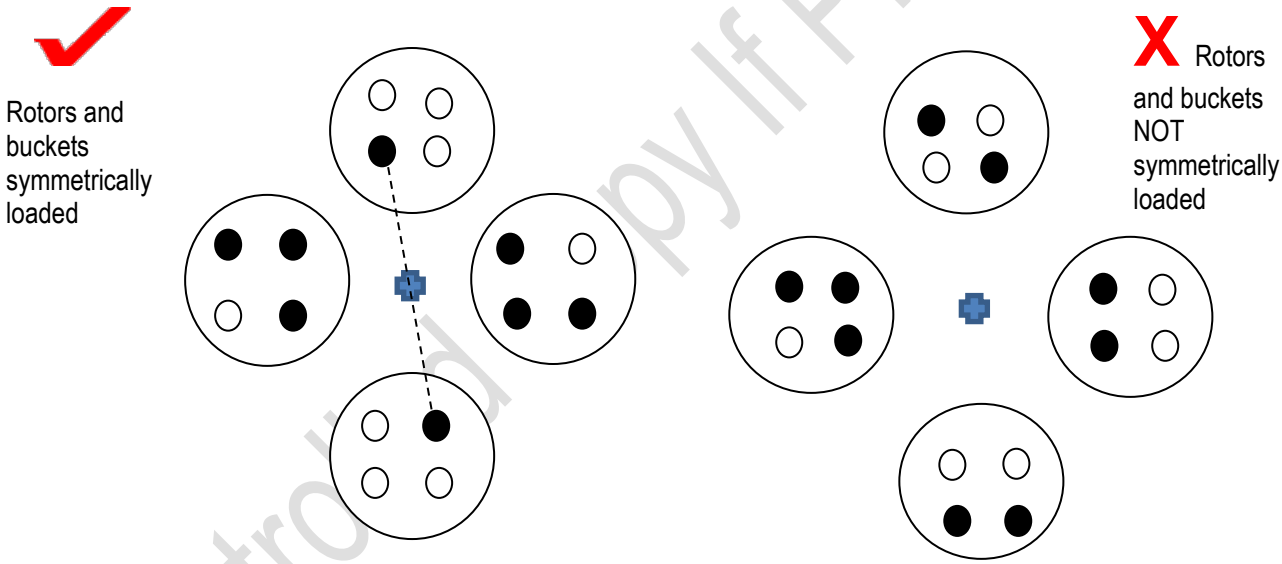
5.3.3.1 The following buttons are displayed on the front of the centrifuge:

- 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.

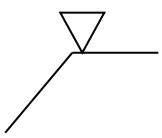
5.3.4.7 Both the round bucket and cap are autoclavable at 121°C, 20 minutes.

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.

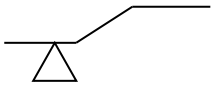
5.3.4.9 To start a **continuous run cycle**, turn the TIME dial to either above 99 minutes or below 0.5 minutes. The display panel will display “oo” to indicate that continuous running is active. Press STOP to end continuous running.

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 with Klercide 70/30 sterile denatured Ethanol spray and sterile wipes as per cleaning instruction below. (Section 5.64)

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 After cleaning your equipment take a small amount of

centrifuge lubricant (e.g. Centrifuge fat from Eppendorf, order number 5810 350.050) onto your finger. Lubricate bucket grooves, pivots of swing-bucket rotors, and rubber seals. Check if seals of aerosol tight lids/caps need
to be replaced.. Lubricate the threads of the fixed angle rotors after cleaning.

Figure 5:
Lubrication of bucket grooves and pivots of swing-bucket rotor equipment, rubber seals, as well as threads of fixed-angle rotors.

5.6.1.5 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.

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

5.6.2 Fortnightly/ Every 2 weeks Maintenance

5.6.2.1 Ensure centrifuge is not being used - switch off, then unplug the centrifuge and put on gloves and an apron.

5.6.2.2 Wipe the internal and external surfaces of the centrifuge with Klercide 70/30 sterile denatured Ethanol spray and sterile wipes

as per cleaning instruction below, (Section 5.64) and leave to dry.

5.6.2.3 Check for signs of damage/wear and tear.

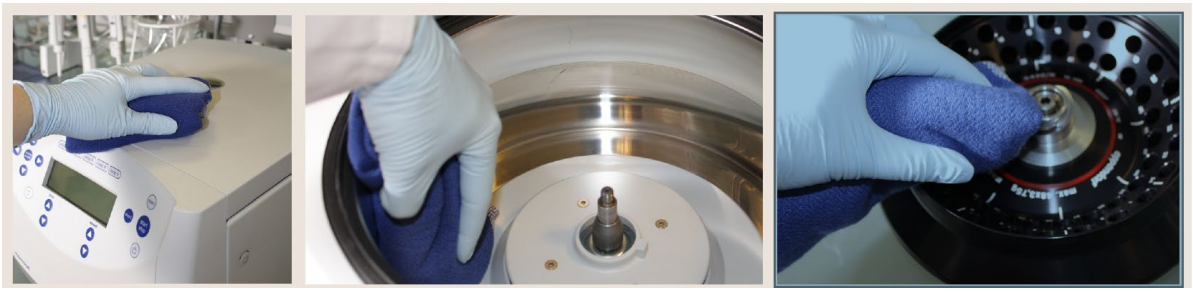
5.6.2.4 The centrifuges must be clean as per cleaning instructions below as specified on the cleaning rota.

5.6.3 **Cleaning Instructions**

5.6.3.1 **Centrifuges, rotors, adapters, buckets and rubber seal**

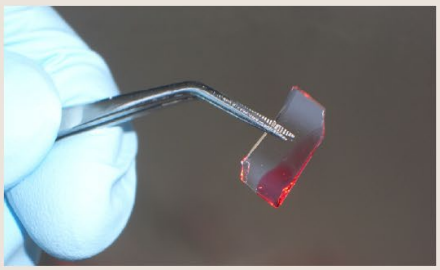
Carefully remove buckets and rotor if possible from the centrifuge. If applicable, for refrigerated centrifuges: Leave centrifuge lid open and defrost the ice on the rotor chamber surface. If your centrifuge is equipped with a water collection tray, empty and clean it with Klercide 70/30 sterile denatured Ethanol spray and sterile wipes,

5.6.3.2 Check the rotors and rotor bores visually for residue and corrosion. Rotors, buckets, lids or adapters, which have been subject to chemical or mechanical damage or which have exceeded their maximum operating life, should not be used any longer. Damaged tubes or plates should not be centrifuged, then wipe the rotor chamber with Klercide 70/30 sterile denatured Ethanol spray and sterile wipes. The outside of the centrifuge and the rotor chamber should be cleaned as well.



5.6.3.3 In the event of contamination caused by high-risk substances (bio hazardous or aggressive chemical reagents and radioactive reagents) wear a laboratory coat, gloves and goggles. If there is broken glass: Retrieve the bigger broken glass with forceps,

remove small and powder shards with a damp lint-free cloth. Absorb blood with gauze or paper towel and subsequently, seal the material in a biohazard bag for safe disposal. Wipe the contaminated parts with Klercide 70/30 sterile denatured Ethanol spray and sterile wipes, remove still contaminated rotor, rotor lid, buckets, and bucket caps out of centrifuge to decontaminate areas which are difficult to access.



5.6.3.4 Use Klercide 70/30 sterile denatured Ethanol spray and sterile wipes to clean your rotors and accessories. Wipe all contaminated parts.

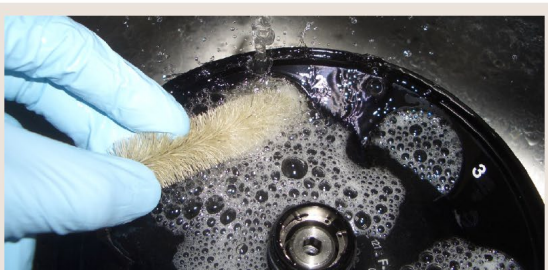
5.6.3.5 In case of spilling some aggressive liquid on your centrifuge equipment, please clean it immediately. If there is stubborn stain, clean with a plastic scrub pad or stiff test-tube brush that has end bristles and a non-metallic tip. Rinse equipment with distilled water. Avoid immersing the rotor in water as liquid could flow into rotor cavities, and dry thoroughly with
a soft cleaning cloth.

5.6.3.6 As salt crystals located on metal surface will corrode the surface, we strongly recommend cleaning rotors and buckets
immediately. If there is a need to clean the rotor's tube cavities or boreholes, use a stiff test-tube brush that has end bristles and a non-metallic tip.

5.6.3.7 For swing-bucket rotors, ensure that the grooves in which the buckets are fitted are free of contamination. Take care to ensure that the buckets can still swing freely.

5.6.3.8 Wipe down rubber seals and rinse with distilled water using a paper towel or cleaning cloth.

5.6.3.9 If parts are removed for cleaning, reassemble when dry and leave centrifuge lid open to let any moisture or condensate evaporate.



Caution

Do not use acetone, caustic detergents, or detergents that contain chlorite ions. Corrosion is most frequently caused by using chlorite ion solutions, such as sodium hypochlorite (household bleach). Do not use steel wool, wire brushes, abrasives, or sandpaper, since they may damage the rotor coating (anodized coating) and thus increase the risk for corrosion. We do not recommend usage of dishwashers for rotors or lids due to the aggressive cleaning agents used in these devices. These agents may result in corrosion.

5.7 **Allocation and Documentation of Maintenance**

5.7.1 The fortnightly maintenance of the centrifuges will be allocated to core CRF staff as part of the other checks carried out in the CRF. The checks shall be recorded using *CRF-LAB-FRM-17: Centrifuge Cleaning Log.*

5.8 **Servicing and Calibration**

5.8.1 Servicing and calibration of the Eppendorf 5702R and Hettich 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 Hettich Rotina 380R Operating Instructions

6.3 Eppendorf_Centrifugation_White-Paper_014_Centrifuges_Routine-Maintenance-Centrifuges

6.4 epservices-maintenance-of-centrifuges-poster_17

6.5 CRF-LAB-FRM-17: Centrifuge Cleaning Log

6.6 CRF-LAB-FRM-18: Centrifuge Memo Log

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

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

6.9 King's College Hospital Health and Safety information and guidelines on KingsWeb

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

6.11 DOC60: Out of Hours MRI Access DH-MRI-10

7.0 List of Appendices

N/A

8.0 Approval and sign off

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Position: CRF Experimental Nurse Team Leader

Signature: Date:

Approved by:

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