

Standard Operating Procedure

SOP114

Title: Use and Maintenance of the Heraeus HERAcell 150i CO₂ incubator and HERAcell 150i Multigas incubator and the HERAcell 150i CO₂ copper interior incubator.

Location: CBE Laboratory Unit and CBE Tissue Engineering Laboratory T208B

1. PURPOSE

The purpose of this SOP is to describe the procedures for the use and maintenance of a Heraeus HERAcell 150i CO₂ incubator, Heraeus HERAcell 150i CO₂ Copper Interior Incubator and the Heraeus HERAcell 150i multigas incubator in order to ensure its correct use for the incubation of human cell lines, human primary cells or enzymatic assay material. (H29 only- incubation of bacteria)

2. SCOPE

This SOP applies to procedures for the operation and maintenance of the *Heraeus HERAcell 150i CO₂ incubator and Heraeus HERAcell 150i CO₂/ multigas incubator* sited in the CBE Laboratory Unit (laboratories H23, H25 and H29) located at Holywell Park. The HERAcell 150i CO₂ Copper interior incubator is located in the CBE Tissue Engineering laboratory T208B in the Wolfson school. The SOP describes controls, operating rules and limitations, the principles for maintenance and procedures for record keeping, reporting and emergency shutdown of the equipment. These incubators are suitable for work with any Hazard Group 1, Hazard Group 2 Biological Agents or Class 1 GMOs, providing there are no other airborne hazardous substances present. They are also suitable for HTA relevant material, when appropriately labelled. They are also used for bacterial work in H29.

The environmental operating conditions are set at 37C and 5% CO₂.

3. RESPONSIBILITIES

3.1. Laboratory Personnel:

1. Shall complete proper training before using the incubator.
2. Shall ensure that they are familiar with the incubator, its controls, and emergency procedures by reference to this SOP and the Manufacturer's Operating Instructions.
3. Shall ensure that the incubator is suitable for the work they intend to carry out before commencing.
4. Shall carry out the routine inspection and maintenance of the incubator, as required.
5. Shall record any adverse events and alarms that indicate non-conformance or malfunction on the Equipment Maintenance Record and notify the Laboratory Manager/Responsible Person.

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6. If there is a contamination within the incubator to alert the responsible person so that the incubator can be cleaned and decontaminated and that the appropriate Contamination form can be filled out and given to the Laboratory Manager.

3.2. The Responsible Person/Laboratory Manager:

1. Shall ensure laboratory personnel are given suitable information, instruction, training and supervision in the correct use and maintenance of the incubators. The requirements for competence to use the incubators should include instruction in the appropriate and inappropriate use, safe working procedures, calibration and decontamination procedures.
2. Shall coordinate routine inspection and maintenance duties to be performed by laboratory personnel (according to SOP004).
3. Shall investigate any reported problems, adverse event, alarms or non-conformities associated with incubator usage.
4. Shall organise the maintenance, repair or servicing of the incubators by trained and authorised contract / service personnel.
5. Shall ensure that prior to authorising the commencement of maintenance, repairs or servicing that the incubator is suitably disinfected and that a "Decontamination Certificate" is issued.

4. EQUIPMENT AND MATERIALS

- 1) Heraeus HERAcell 150i CO₂ incubator/ Heraeus HERAcell 150i Multigas incubator with decontamination routine
- 2) Copper Sulphate Solution
- 3) 1:20 and 1:50 Chemgene
- 4) 70% (v/v) IMS solution
- 5) Sterile water

5. PROCEDURE

5.1 Operation and Use

NOTE: The incubator should be switched on at all times except when it is being cleaned.

Before adding or removing a culture vessel or equipment (e.g. spinner flask platform) check to ensure the following:

Version 006

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- (1) The screen on the door is on and displaying CO₂ and temperature readings (if this is not the case contact the Responsible Person for advice).
- (2) The vessel or equipment is not dirty or contaminated with spilt reagents; in this event clean with 1:50 Chemgene before putting inside the incubator.
- (3) All culture vessels to be entered into the incubator are labelled (refer to SOP005). If an incubator is to be used for HTA relevant material, then the appropriate HTA label needs to be attached to the incubator being used. This also goes for any incubators being used for quarantined, or highly sensitive work.

NOTE: The environmental operating conditions are set at 37C and 5% CO₂. If these need to be changed for an experimental procedure, talk to the relevant lab leader beforehand (give fair notice). The incubator will also need a sign putting on the front which states- The temperature, the CO₂ concentration, your initials, the start date of the experiment and the end date of the experiment.

Hypoxic Use- The stand alone incubator in H25 can also be used as a Hypoxic incubator. In order to use this, all lab users need to be notified in advance. Lab management also need to be notified, so that they can order nitrogen gas if needed and also so that they know that the cylinders may need changing.

The nitrogen will also need to be switched on in H25, See the user manual to change the incubator settings. A sign will need to be attached to the front of the incubator to remind other lab users, that it is being used as a hypoxic incubator.

Once the experiment has been finished, the incubator needs to be returned to the normal settings.

5.2 Routine Maintenance and Examination

NOTE: The incubator is self-maintaining once set up. No setup modifications should be made unless required as part of the calibration schedule.

Note: The HERAcell 150i copper interior incubator does not require the addition of copper sulphate solution to the water.

5.2.1 Weekly inspections

- (1) Check the water level at the bottom of the incubator. If water needs refilling (approximately 3 L) use autoclaved, distilled water and ensure 0.1 % (w/v) copper sulphate is added. (1g of copper sulphate per litre of water)

Standard Operating Procedure

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Title: Use and Maintenance of the Heraeus HERAcell 150i CO₂ incubator and HERAcell 150i Multigas incubator and the HERAcell 150i CO₂ copper interior incubator.

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- (2) Check the gauges on the CO₂ cylinders. If any of the gauges read empty, inform the Laboratory Manager or Responsible Person and follow their instructions.
- (3) Inspect the incubator for any visible microbial or fungal growth (or smell). If any growth is observed or smell detected, **follow the procedure described in section 5.2.2** and also ensure that any equipment inside is decontaminated with 1:50 Chemgene. Ensure that all culture vessels are thoroughly checked for potential contamination.

5.2.2 Monthly cleaning and decontamination procedure

The incubator should be fully cleaned and decontaminated depending on its usage, and whether experimental work will allow. Monthly intervals are recommended if there is high frequency work and this can be pushed to 2 monthly intervals if there is low frequency work.

Cleaning and decontamination of incubators can also be done prior to sensitive work being undertaken and must also be done after the incubator has been used for work that has been quarantined, and if there has been an infection in an incubator.

Note: Check the inner glass door of the incubator for any imperfections which could cause the door to shatter during the decontamination cycle. If any are found please notify the Lab managers.

- (i) Remove all culture vessels and relocate to another suitable incubator, remove any equipment and clean by wiping down with 1:20 Chemgene followed by 70% IMS.
 - (ii) Turn off the incubator
 - (iii) Use the small pump provided to remove the water from the incubator into a suitable vessel, allow to evaporate, and any remaining liquid can be absorbed, and got rid of through the yellow waste stream.
 - (iv) Remove everything from the interior of the incubator including the shelves and clean these and the interior of the incubator with 1:20 Chemgene followed by 70% IMS. Replace all shelving once clean. Also, clean both doors, inside and out.
 - (v) Turn on the incubator
- 1) Place 350 ml distilled water in the bottom of the incubator, turn on the incubator and run a decontamination cycle.

Standard Operating Procedure

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NOTE: The entire program run of the decontamination routine takes approximately 25 hours. During this routine, a hot and humid atmosphere at 90 °C with highly decontaminating effect is created inside the incubator.

- a) Press the **CONTRA-CON** key on the touch screen controller at the front of the incubator and press the **START** key.
 - b) Open both doors of the incubator until the buzzer sounds then close both doors and place tape across to limit ease of opening. Record on the tape: Date; Time; initials and Decontamination.
 - c) The next day, press the **END** key after the decontamination cycle is completed.
- (vi) The next day, remove the remaining water from the incubator and refill with water and copper sulphate as described in section 5.1.
- (vii) Activate the auto-start routine.
- a) Press the **auto-start** key on the touch screen, followed by the **START** key.
 - b) Open both incubator doors to air the workspace, wait until the audible alarm sounds, then close both doors. **NOTE:** Running the auto-start routine usually takes 5 to 7 hours. Ensure that the incubator is closed during the procedure by placing tape across the door. Record on the tape: Date; Time; Initials.
 - c) Press the **END** key after the auto-start routine is completed. The system returns to the main menu.
- (viii) If the incubator has failed to reach either of the set points, the Responsible Person should be notified immediately.
- (ix) Record maintenance procedures and outcomes on the Equipment Maintenance sheet, which is found on the outer door of each lab. This includes recording when the incubator is cleaned.

5.3 CO₂ Calibration

Once a decontamination cycle has been carried out on an incubator and the auto start has been run, the CO₂ should be calibrated. See the SOP for CO₂ calibration.

5.4 Spillage inside the Incubator

If any spillages occur inside the incubator, immediately clean with 1:50 Chemgene followed by 70% IMS and refer to SOP038 for further details. If in any doubt, consult the laboratory manager or responsible person.

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Before operating the incubator again clean and decontaminate according to the procedure described in section 5.2.2

5.5 Calibration of Incubator Temperature

NOTE: The incubator should be cleaned and decontaminated as per section 5.2.2 before any calibration occurs.

The most appropriate calibration interval for the incubator should be agreed with the laboratory manager. This should take into account the implications of calibration failure - **6 monthly intervals are recommended**, and the calibration frequency must be recorded on the log sheet e.g. "retest due date" (Section 6).

The temperature set point for the incubator should be 37°C. The design specification is ± 0.5 °C of the set point in steady state conditions.

5.4.1 Test Procedure

- (1) Ensure the incubator has been switched on for at least 2hr before the start of the test and that it is empty of equipment and culture vessels.
- (2) Fill the reservoir in the base of the incubator with de-ionised water.
- (3) Place 3 temperature-data loggers in the incubator, one centrally on each of the shelves. The time between readings should be set to 1 minute.
- (4) Close the incubator door and leave 10-12 hours once steady state conditions have been reached – **DO NOT** open the door during this period.
- (5) After 10-12 hours retrieve the data loggers, download the data onto a computer and examine the data profiles for each logger. Observe only steady state data, data points from the end of the test when the door was opened to retrieve the loggers must be ignored and it is recommended that approximately 5% of the final data points are removed (~36 data sets).
- (6) Record the maximum and minimum temperatures for each of the data loggers in the calibration log table (section 6), together with the serial numbers/ID of the data loggers. Retain a dated copy of the raw data in the equipment file.

5.4.2 Analysis of Results

PASS = all the data points for each data logger are within ± 1.0 °C of the set point in steady state conditions. Attach a status label to the incubator showing date of calibration, who performed by and a retest date.

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FAIL = the minimum and maximum recorded temperatures fall outside the $\pm 1.0^{\circ}\text{C}$ of the set point in steady state conditions. Report this to the laboratory manager and place a “do not use” notice on the incubator. The manufacturer will need to be contacted so the incubator can be recalibrated by them.

5.6 Equipment Malfunction

- a. If the incubator alarm sounds, due to the door being recently opened, it should be silenced.
- b. If the alarm continues/repeats or any part of the incubator fails or malfunctions, seek advice from the laboratory manager. With permission of the Responsible Person, the user should consult the operator’s instruction manual to access fault finding and troubleshooting procedures.
- c. If any problem cannot be rectified according to the troubleshooting procedures a “do not use” notice must be put on the incubator and the laboratory manager informed. The manufacturer must be contacted for advice and external maintenance or servicing might need to be arranged.
- d. All problems and corrective actions must be recorded in the maintenance log (section 6).

NOTE: External maintenance or servicing of the incubator can only be performed after it has been suitably disinfected (refer to SOP003) and a “decontamination certificate” has been issued by the unit safety co-ordinator (see section 6).

5.7 SPECIAL NOTES: HEALTH & SAFETY

- (1) Observe the relevant biological risk and COSHH assessments associated with the work being carried out and ensure that the correct PPE is worn for the work.
- (2) Do not use radioactive, toxic or corrosive substances (e.g. chlorine-based substances such as sodium hypochlorite) inside the incubator
- (3) Do not use substances releasing explosive vapours, except 70% IMS (used for surface disinfection)
- (4) Wear protective disposable gloves, available in all change rooms, before putting your hands into the incubator.

6. DOCUMENTATION

The following forms can be found on the CBE internal website under forms from SOP’s.

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QS-form-005 : Incubator Calibration Test Record Sheet
QS-form-009 : Equipment Decontamination Certificate
Weekly housekeeping checklist
Equipment maintenance Record

The user manuals can be found on the CBE website, or can be found online.

Any maintenance or problems should be put on Equipment Maintenance Record and Housekeeping logs.

These records shall be filed in the Equipment File and stored in the CBE Office or otherwise archived for future review or retrieval.

SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	19 October 2012 Issued by A. Fotticchia	Corrections to the draft version were issued.	001
001	19 th January 2016 C.Kavanagh	i)Removal of reference to 70% IMS solution due to it being withdrawn as a disinfectant in the laboratory. ii)Addition of references to 1:20 & 1:50 ChemGene solutions where appropriate due to implementation (replacing 70% IMS).	002
002	1 st March 2016 Reviewed by C. Kavanagh	i)Addition of reference to 70% IMS due to re-introduction of 70% IMS into the laboratories for a 'rinsing stage' following use of Chemgene. (Chemgene can leave a residue)	003
003		i-Addition of the Heraeus HERAcell 150i CO ₂ incubator and the Heraeus HERAcell 150i	004

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		multigas incubator ii-Addition of the use of 1:20 and 1:50 Chemgene in cleaning and decontamination iii-Change of the frequency of cleaning and decon from every 2-3 months to every month depending on usage.	
004	12/03/2019 JB	Addition of :Check the inner glass door of the incubator for any imperfections which could cause the door to shatter during the decontamination cycle. If any are found please notify the Lab managers.	005
005	23/06/20	Addition of use of the hypoxic incubator as a hypoxic incubator. Amended how copper sulphate solution is disposed off.	006

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