Lab Management Plan for Cell Culture Facility (CB02) in the MPACC facility 2019-2020

Introduction:

The MPACC Cell Culture Facility is a Containment Level 1 laboratory. Due to the multi-user nature of the facility only work with hazard group 1 and/or GMO class 1 organisms is permitted. All work MUST be approved by the Departmental Biological Safety Committee PRIOR to starting and only trained personnel have access to this room.

This LMP provides guidance for the maintenance and usage of the lab; however, it is the responsibility of the individual group leaders to ensure that their group members understand fully the work they are undertaking. Before being allowed access to the MPACC Tissue Culture Facility, users must attend a Basic Cell Cuture Induction course (at the Cancer Research UK Cambridge Institute (Addenbrooke's Site). Once completed, a general induction to the lab is given by the assigned MPACC TC principal administrator and the new user will be assigned rota duties. All users must ensure that, prior to any usage of the facility, they complete a daily checklist of duties and also carry out their assigned rota and administrator duties. Tissue culture users MUST adhere to stated protocols, checklists and rotas. If they fail to do so, a penalty system is in operation: First offence – yellow card; Second offence (within 6 month period) – ban from the lab for 1 month.

For all general inquiries about the MPACC and specifically the Cell Culture Facility, your first point of call is the MPACC Research Technician, Mrs. Annette Steward (mpacc@ch.cam.ac.uk), please note that Annette is in the department Monday through Wednesday and therefore correspondence should not be directed to her personal e-mail address.

Mrs. Anita Andreou (ak629@cam.ac.uk) is our Technician/Cleaner for the MPACC facility. Please note that Anita works part-time (9am-1pm; daily).

The Tissue Culture lab will have Principal Adminstrators (currently, Sigitas Mikutis sm2029@cam.ac.uk and Lavinia Dunsmore lgd29@cam.ac.uk) who will set the TC weekly rota table and give new users an introduction to the lab.

The Departmental Safety Officer and Biological Safety Officer is Dr. Richard Turner (rmt35@cam.ac.uk).

Dr. Janet Kumita (jrk38@cam.ac.uk) is the MPACC facility manager. Please direct all correspondence to mpacc@cam.ac.uk, as it is received by all relevant personnel.

The Tissue Culture mailing list is: **chem-mpacc-tissueculture-users@lists.cam.ac.uk**

1.0 Usage of the Laboratory

1.1 General

- It is the responsibility of each individual group to ensure that approved Biological Risk Assessments cover all work (information is available on the departmental website - http://www.ch.cam.ac.uk/safety/biological-safety) and that all users are listed on the group "Biological Worker Database".

  MPACC Research Technician will maintain an up-to-date register of users of TC facility. Access via Mifare only.

- No one is allowed to be inside the Tissue Culture facility without having attended the Cancer Research UK induction course. To register for the course, please e-mail Dr. Janet Kumita (jrk38@cam.ac.uk).

- The Chemistry Department is only approved for work which pertains to GM Class 1 Organisms and Hazard Group 1 pathogens as defined by HSE guidelines (http://www.safety.admin.cam.ac.uk/files/hsd111b_rev3.pdf).

- **NO BACTERIA, YEAST or associated reagents** are to be brought into the facility, spun in the centrifuge or stored in the fridge or incubators.

- All Outdoor coats/bags should be taken to your own labs/offices prior to coming to the MPACC facility as they are NOT ALLOWED inside the lab.

- Lab coats and safety specs are to be worn **at all times** when doing experimental work in the Cell Culture Facility. Protective gloves should be worn for all experimental work.

- Lab coats must be light blue Howie-style labcoats (elasticated cuffs) and will be changed fortnightly by Anita Andreou (ak629@cam.ac.uk).

- All samples stored in the TC room MUST have your name, CRS ID and date written on it. Any unlabelled items will be discarded.

- All personal items (autoclaved tubes, tips) must be in covered plastic containers labelled with CRS ID and stored on the available shelving.

- Do not exchange items (e.g. pipette buoy) between the general laboratory area and the cell culture facility. Use ONLY the designated equipment for the facility within the room for

experiments. This is particularly important for maintaining the sterility of the Class II hood, as any spores that might be brought in through contaminated equipment may become trapped in the hood.

- Avoid bringing cardboard boxes into the TC lab whenever possible (exceptions eg boxes of nitrile gloves)

1.2 Housekeeping

- GENERAL CLEANING: Departmental cleaners should not clean the room (often just leads to redistribution of dust). A hand-held hoover is available and weekly rota will include a quick clean using this.

- Floors will be cleaned by Anita Andreou (ak629@cam.ac.uk) bi-weekly on Tuesdays and Fridays.

- Benches and shelves are to be cleaned with 70% ethanol once every 3 months. Annette Steward will organise this, all registered users are expected to remove all non-communal items to enable the effective cleaning of benches/shelves.

- NONE OF THE BINS (general waste, biological waste, recycling) should be overfilled. All bins should be emptied when 2/3 full. Emptying of bins are monitored on the daily checklist, any bins that are close to 2/3 full must be emptied as part of this checklist.

1.3 Spills and Decontamination

- All spills are to be cleaned with a 1:20 (5%) solution of Chemgene HLD4L (Fisher UK) and then 70% ethanol. Any towels, tissues or materials used should be disposed of in red biological waste bin.

- Nothing is to remain in MSC or on the benches after completion of work. Decontamination to take place both before and after use with a 1:20 (5%) solution of Chemgene HLD4L (Fisher UK) and then 70% ethanol.

- All problems with equipment/suspected contamination should be reported to Annette Steward at MPACC@ch.cam.ac.uk. Laboratory Inductions and lab rotas are carried out and maintained by the TC principal administrator. Please note all new users must attend a training session at Cancer Research UK – registration is done by contacting Dr. Janet Kumita (jrk38@cam.ac.uk)

- **Any signs of contamination in any of the equipment and/or problems with cell lines should be reported IMMEDIATELY to Annette Steward** MPACC@ch.cam.ac.uk **AND all active TC**

users to ensure that the problem does not escalate – use the tissue culture mailing list:
chem-mpacc-tissueculture-users@lists.cam.ac.uk

- Waste must be disposed of appropriately, for liquid waste this is treatment with 1% Virkon solution (it must be pink) for 20 min and then washed down the sink with plenty of water or for solid waste it must be autoclaved (see Section 3.3 and Section 3.4 of this LMP).

- Information for the Class II Microbiological Safety Cabinet (MSC), CO_2 Incubators, Waterbaths and Liquid Nitrogen Storage Dewars is found in Section 4.0 of this LMP.

2.0 Cell Lines and Liquid Nitrogen Dewars

- All cell lines brought into the TC facility must be certified (ATCC/ECACC). Non-certified cell lines, e.g. products from other labs, **must** be quarantined and tested for mycoplasma before use in the TC facility. Details of the cell lines must be recorded in the dewar directories

- Mycoplasma testing can be done by sending samples to the Cambridge Stem Cell Institute where they perform a PCR assay. Information on who to contact is on the following site: http://www.stemcells.cam.ac.uk/about-us/facilities/tissue-culture-facility/sci-services.

- Antibiotics should be avoided unless absolutely necessary.

- When you are culturing cells, you must place your name in the appropriate column (indicating which incubator) on the Wipeboard in the TC room.

- Three liquid Nitrogen storage dewars are present MPACC lab CB04. Dewar "Yellow" is for storage of currently in-use cell lines and Dewar "Red" is for long term storage of cell lines. Dewar "Purple" is for storage of currently in-use cell lines and for long term storage of cell lines. A record of cell lines/groups will be maintained in a binder in MPACC lab CB04. In this binder, we can record Dewar number, Rack number (6 racks per dewar) and box position (top→bottom (1→5)). Each user is requested to e-mail Annette Steward (mpacc@ch.cam.ac.uk) when any new cell line is received either from a supplier/collaborator. The information requested should consist of name, Cambridge CRS ID, group, cell strain (and classification) and date of receipt. Once any cell line/strain is used/ disposed of likewise a note MUST be made in the binder and an e-mail sent confirming disposal of sample/strain.

- **Eye protection must be worn when using the liquid N2 dewars particularly when removing samples stored in screw-cap vials**

3.0 Waste Disposal

All biological waste must be treated and properly disposed of. After every use of the TC lab, the levels of the general waste, autoclave waste and Virkon-treated liquid waste bins must be evaluated. Any bin that is 2/3 full must be emptied in accordance with this checklist. For information about autoclaving waste contact Anita Andreou (ak629@cam.ac.uk) or Annette Steward (mpacc@cam.ac.uk) for protocols and procedures.

3.1 General Waste

- Anita Andreou (ak629@cam.ac.uk) empties the general waste bins in the MPACC facility. If you have abnormally high levels of waste, please speak to Anita so that it is not left unattended.

- General waste is any non-biological waste. This includes paper, packaging materials, plastic bags etc. All large items of packaging material are to be placed in the corridor outside of the Tissue Culture Facility for collection. Please flat-pack any cardboard if at all possible.

- NOTE: all tubes, gloves, plates and flasks MUST be disposed of in the biological waste autoclave bin regardless as to what it has come into contact with!

3.2 Sharps

- Sharps are defined as glass Pasteur pipettes, glass slides, glass cover slips and needles.

- All sharps (whether biohazardous or not) must be placed in dedicated sharps-only bins (yellow bin). When full, contact Annette Steward (mpacc@ch.cam.ac.uk) who will take these to the Chemical waste store. This is done in accordance with the Department's waste policy.

- For plastic serological pipettes, they go to the tall biological waste bin (white bin) which is double lined with autoclavable bags.

- DO NOT place any sharps in the normal rubbish bins or the biological waste red bin (for autoclaving).

3.3 Biological Waste for Autoclaving (Red Bin)

- Anita Andreou will check the red bins and remove and autoclave the waste accordingly. If you notice that the bins are full, we would

appreciate you packaging the bag and placing in the prep room (CB16) for autoclaving. Write TC lab and date on autoclave tape on bag

- Non-biological waste should be disposed in the general waste bin.

- Biological waste consists of biological organisms and any material that has come in contact with living cells. This includes the following:
    - Cell cultures
    - Cell culture plates and flasks
    - Paper towelling used to clean up spills
    - Pipettes used for transfer of media and cells (disposed as "sharps")
    - Cell culture media and media bottles
    - Tubes (15 mL, 50 mL, eppendorfs) – empty
    - Gloves

- All non-sharp biological waste must be disposed of in a double-bagged designated biological waste bin (red bin). Use only autoclave bags to line these bins (use two bags). These bags are available on request from Anita Andreou (catalogue number: 649201; Greiner Bio-One Ltd. marketplace item).

- All liquid material, such as media from plates, must be removed prior to disposal into the bins. **DO NOT** put liquid into the bags as they are not sealed well enough to prevent seepage.

- All double bagged biological waste for autoclaving must be transferred to the autoclave on a trolley or in a plastic bin.

- When the bin is 2/3 full it must be removed and autoclaved using the Waste 2 cycle (126^{o}C for 12 minutes) in the MPACC Autoclave (waste-only). Allow the bags to remain partially open during sterilisation to ensure steam penetration. The bags MUST be sealed. If any doubt as to how the bags need to be sealed prior to autoclaving please contact the administrator.

- **IMPORTANT – strictly only users trained by Anita Andreou are permitted to operate the large Priorclave autoclave (CB16)**. If you are not trained, leave the waste in the metal tray beside the autoclave for Anita

- Once autoclaved, waste bags are transferred to the black bin located in the MPACC preparation room (CB16). The bin is checked daily by the Anita Andreou. Any questions / concerns related to general Lab cleanliness in the MPACC / Tissue Culture Facility are to be directed to Annette Steward (mpacc@ch.cam.ac.uk).

3.4 Liquid Biological Waste

- All liquid should be transferred to a separate waste bottle/container such as an old/empty media bottle or the plastic containers provided adjacent to the sink.

- The liquid waste should be treated with 1% Virkon for 10-20 min at room temperature to ensure that all biological material is neutralised. Daily users are responsible for emptying the disinfected liquid biological waste down the sink with plenty of water.

- **NOTE:** Effective Virkon concentrations are 1% solutions and the solution must be pink – if colour has faded or is lost, the efficacy is also lost!

3.5 Chemical Waste

- To dispose of chemicals or chemical waste, please ensure that they are in an appropriate container and that the proper disposal of hazardous waste form is filled in (in DUPLICATE) before asking Annette Steward (mpacc@cam.ac.uk) to take it to the waste chemical storage. The form is available on the Departmental website - **http://www.ch.cam.ac.uk/safety/waste-recycling**

4.0 Maintenance of Equipment in the Tissue Culture Facility

4.1 Vacuum Line and Aspirated Liquid Waste in MSC

- The chamber which collects aspirated waste should contain two Virkon tablets (1 tablet per 500 mL). Prior to, and after usage of the vacuum lines, a 1% Virkon solution should be aspirated through the lines followed by 70% Ethanol. Daily Users are responsible for monitoring the level of accumulated treated liquid waste in the chamber – this must be emptied when it reaches 750 mL and two Virkon tablets added to the empty chamber.

4.2 Class II microbiological safety cabinet (MSC)

- The Class II microbiological safety cabinet (MSC) will be cleaned ***fortnightly*** by Annette Steward (this will include removing the steel insert, cleaning all surfaces with Chemgene and 70% Ethanol and clearing any debris that has been pulled under the work surface; fans speeds and needle position will also be checked). In addition, the Class II MSC will be serviced every 6 months and fumigated via vapourised hydrogen peroxide (VHP) once every year (organised by Annette Steward) and records kept.

- The microbiological safety cabinet is bookable on:
  http://bookings.ch.cam.ac.uk/mpacc-tissue-culture-hood

All useages MUST be registered on the on-line system (this includes evenings and weekends). When booking on-line please specify your name or CRSID plus the initals of your research group leader (eg CMD). Contact **(mpacc@cam.ac.uk)** to be added to this booking calendar

4.3 Maintenance of CO_2 Incubators

Principal Administrators:
Antibiotics Incubator – Juri Konc (jk690@cam.ac.uk)
Non-antibiotics Incubator – Katarina Pisani (kp476@cam.ac.uk)
Quarantine Incubator – Lavinia Dunsmore (lgd29@cam.ac.uk)

- Water for incubators must be autoclaved and treated with an antimicotic and copper sulphate for bacteria. It is the responsibility of the weekly rota to check the water levels and report any issues to mpacc@ch.cam.ac.uk.

- The decontamination cycle for the CO_2 incubators will be carried out twice a year. Annette Steward will coordinate with the principal administrators for the incubators. A notion is to co-incide this decontamination cycle (which takes in the region of 18 hours) with the servicing of the Class II Microbiological Safety Cabinets. All Tissue Culture Facility users are to be notified *via* e-mail with as much notice as practically possible.

4.4 Maintenance of Waterbaths

Principal Administrator – Florian Buhr (fdb22@cam.ac.uk)

- Water baths must be kept covered and checked by daily users (as part of the checklist). It is recommended that they are switched on only when required. Leaving water baths uncovered overnight may cause total evaporation of water and is a potential fire hazard.

- Water Baths will be cleaned bi-weekly. Water for the waterbaths must be autoclaved and treated with an antimicotic and copper sulphate for bacteria.

4.5 Maintenance of Liquid Nitrogen Storage Dewars

Principal Administrator – Benedetta Mannini (bm475@cam.ac.uk)

- Annette Steward (as376@cam.ac.uk) backed up by Kevin Judd (ktj20@cam.ac.uk) will top off the dewars weekly. If any issues are noticed with the dewars they should be IMMEDIATELY reported to mpacc@ch.cam.ac.uk and also e-mail the TC-user mailing list.

- Contact Annette (as376@cam.ac.uk) for storage boxes for these dewars (Marketplace item cat. no. 10739331 – they are 5 X 5 holding 1.2/2.0 ml vials).

4.6 Fluorescence Microscopes

Principal Administrator for the old Olympus – to be confirmed

Principal Administrator for new EVOS system – Lizzie Li (hl493@cam.ac.uk)

4.7 Cell Culture Lab Rota and Daily User Checklist

- A weekly rota will be set by the Principal Administrator and all users must take part to ensure that the lab runs efficiently. Duties include retrieving autoclaved dH_2O (1L bottles) from CB16, checking the water levels in the CO_2 incubators and the general cleanliness (and liaising with Annette Steward regarding any issues), ensuring bench tops are tidy (and hoovered), check the communal supplies, re-fill the racks and shelves with the supplies stored in the MPACC and send relevant ordering details to Annette Steward for ordering. **Items and consumables required for the TC lab should be written on the white-board and Annette will order these.**

- To ensure that the lab remains at a high standard of cleanliness and safety, a Daily User Checklist is set up (see Appendices). Every time you use the TC facility you must complete the list (posted on the back of the door). Failure to leave the room in an acceptable state will result in yellow carding and eventual loss of access to the facility. If the next user feels that this has not been left in an adequate state, they are to contact mpacc@ch.cam.ac.uk immediately so that penalty can be issued. NOTE: refusal to leave the room in good condition even if the previous user failed to do their tasks will also result in yellow carding. EVERYONE needs to take on responsibility. The daily users are responsible for:

    - Benchtops left clean and tidy
    - Consumables are re-stocked from the contingency stock (otherwise put on wipeboard for re-ordering)
    - general and autoclave waste removed/treated if 2/3 full
    - ensuring the MSC is left clean and tidy
    - vacuum trap is emptied if ~ 750 mL full
    - liquid waste is Virkon treated and disposed
    - virkon and 70% ethanol stocks are renewed

5.0 Ordering and Stocks for Cell Culture Facility

- The consumable ordering will be divided up based on the number of active users per research group. To determine the proportion of

charges per group, we will base this on hours of booked time for the MBSC's.

- **For consumable purchases through iProc and Chem stores, please record the catalogue number, name and quantity of an item on the TC laboratory wipe board**. Annette will raise the requisition through iProc for external purchases and items from Chem stores. All items will be charged to the MPACC grant code and your PI will be informed about contributing towards these communal costs.

- ALWAYS ensure that there are sufficient communal reagents/materials to last until the replacement order arrives. Time your purchase orders with this in mind. Checking stock levels is part of the maintenance rota.

- NOTE that all plasticware should be **STERILE** for use in the TC facility, DO NOT stock communal goods with non-sterile items.

Table 1: Reagents

Reagent /product code	Pack Size	Stock Quantity	Location/Storage	Brand	Source
Virkon	tablets	1 x 500mL	4ºC (Fridge)	Gibco (Invitrogen)	Stores
Virkon 95015661	5g tablets	10 x 5g		Anachem Ltd	iProc
Virkon 95015659	500g Shaker	500g x 6		Anachem Ltd	iProc
Chemgene HLD4L XTM301-C	750mL	6 x 750mL	Shelf storage (once started 2 months)	StarLab UK	iProc
Chemgene HLD4L XTM305-AS	1L – conc.	2 x 1L		StarLab UK	iProc
SigmaClean® SS525-4OZ	4fl. oz.	4fl. oz.		Sigma	iProc

6.0 Standard Operating Procedures for Equipment in TC room

The Cell Culture Facility (CB02) has a number of pieces of communal equipment. Provided in this section are standard protocols for the use of these items along with other useful protocols. These include:

- Operating the Class II Microbiological Safety Cabinet Hood
- Using the Binder CO_2 Incubators
- Use of Waterbaths
- Liquid Nitrogen Dewars

SOP for the Class II Microbiological Safety Cabinet Hood

General Information

- This facility houses a 1200 mm BioMat Thimble Class II Biological Safety Cabinet from Contained Air Solutions (serial number: MC9992-3) and a Thermo Fisher Safe 2020.

- The cabinet is activated by a touch pad, however prior to any operation the base plate MUST be removed and correctly stored. The base plate is not to be stored on the floor.

- The hood contains a 13Amp plug socket for connection for electrical equipment.

- The cabinet is fitted with 2 UV lights which can be activated *via* the touch pad on the front of the system. Please note the UV lights are NOT a sterilisation device, they are only active to a distance of 400mm from source; they more usefully identify spills that require treatment. Should one of the UV lights fail please informAnnette Steward. In addition to this the cabinet is also fitted with 2 standard filament lights.

- To comply with British Safety Standards the cabinet is serviced twice yearly. Both of the service visits monitor air flow and check fans / HEPA filters (of which the system is fitted with two) and at one of the visits the cabinet is tested with a Potassium Iodide disc. Copies of the service reports are displayed on the cabinets

General Operational Protocols

1. All usage of the Class II cabinets in the Tissue Culture Facility MUST be booked using:
   http://bookings.ch.cam.ac.uk/mpacc-tissue-culture-hood
   To obtain an account you MUST be trained by the TC administrator. Please also indicate initials of your research group when booking on-line

2. The first thing to do is to remove the base cover plate; please store correctly in the shelf just under the working surface.

3. It is recommended to spray the cabinet with Chemgene (please see section on reagents) and then 70% ethanol prior to switching on the fans.

4. Once you start the fans the system will initially audio alarm (this can be muted should you wish using one of the buttons on the panel).

   For the **Biomat Class II safety cabinet**, this should cease once the needle position is in the “safe to work” zone. This should happen within 60 seconds of switching on the cabinet. Once the needle is the safe

position it is fine to work within the cabinet. Should the needle position drift at ANY time whilst you are working at the cabinet into either of the "unsafe" zones the unit will audio alarm. However the fans in the system will adjust speed to annul any problem.

Operating instructions for the **ThermoFisher 2020 Class II safety cabinet** are found on p21 – p25 of this document

If any alarm is evident whilst working at the hood please inform Annette Steward mpacc@ch.cam.ac.uk *via* e-mail since even if the fans within the system act to annul any such problem it may be symptomatic of another problem.

5. The cabinet functions to create a 1.2M sterile "air curtain", however every time you move an arm for example that curtain is broken. The advice is to restrict movements to as great a practical degree as possible whilst working in the cabinet.

6. After you have finished your work, remove all items from cabinet (spraying them with 70% ethanol on removal) and the clear work surface is to sprayed again with Chemgene and subsequently 70% ethanol. Please wipe the surface on application with 70% ethanol using Kimwipes which are available within the Facility. Please do not use Virkon in any form on the Stainless Steel work surface of the cabinet.

7. Finally please replace the base cover plate on the system on the Biomat cabinet

   If you are unsure of any features / usage of the cabinets please ask likewise please report all concerns to the system administrator, Annette Steward.

Note: All items are to be removed from the cabinet post work and the surface left completely clear.

SOP for the Binder and ThermoScientific CO_2 Incubators

General Information

- This facility houses two 150 L Binder CO_2 Incubators and a ThermoScientific CO_2 Incubator with an Infrared CO_2 Detector from Crown Scientific. One is for use with antibiotics, the other is antibiotic-free.

- The incubator is an air-jacketed incubator with an interior humidity well

- The incubator requires food-grade CO_2 which is regulated by a BOC gas regulator. The levels were preset on installation by a technician.

- An alarm sounds in MPACC office CB10 when the CO2 cylinder needs to be changed. Annette Steward mpacc@ch.cam.ac.uk will arrange with Kevin Judd (ktj20@cam.ac.uk) to have the cylinder replaced

- The control panel for the incubator consists of an LCD screen with buttons. The screen generally shows the level of CO_2 (this should be preset to 5%) and the temperature (37^{o}C). It can be adjusted to show parameters of interest, a temperature and temperature/CO_2 level vs. time graph etc.

- The infra-red detector (IRD) may require replacement every two years; Annette Steward will liaise with the manufacturer. We currently have one detector in use and a spare in storage. The second detector must be sterilised with 70% ethanol prior to use.

- A number of parameters for the incubator may be changed using the control panel (the code is 0001), however, note that some parameters can only be changed by authorised technicians (check with Crown Scientific, see contacts page).

- The incubator must have at least 50% humidity at all times. The levels of autoclaved de-ionised water in the insert trays are checked as part of the Weekly User Rota and if this is low, Annette Steward must be informed.

- **NO BACTERIA, YEAST** or related materials are to be stored in this incubator.

General Maintenance

- Swab any spills of media or culture inside the incubator immediately with Chemgene and subsequently 70% ethanol. If the spillage is isolated to a single shelf, then that shelf is to be removed and cleaned as outlined.

- Ensure that there are no liquid droplets or puddles anywhere on the shelves, sides or door of incubator. Also please check for puddles of water around the insert water tray.

- Shut the door of the incubator quickly once you have removed/replaced cultures etc. Do not leave the door open for long periods of time unless cleaning

HUMIDITY

1. Humidity is maintained by evaporation of water in the incubator.

2. The bottom of the incubator contains a tray for water. In the centre is a well which gathers condensed water from the "cold spot" in the incubator. Water must be added to the tray but not to the well, which fills up over time. The well also has an indentation which shows the maximum level to which the tray can be filled.

3. Use only sterilised (autoclaved) tap water to refill this tray, distilled water causes oxidation.

4. Add 1 tablet of Aquasan to 1L autoclaved water to make a 10X stock – this is kept under the sink. Dilute the stock solution 10X before adding to the tray. This will help retard the growth of contaminating organisms like fungi and bacteria.

5. **IMPORTANT**: Always check the level of the water and ensure there is sufficient to last a weekend. Make it a routine to check at the end of each week.

6. **To clean the tray**:
    - Remove from incubator
    - Kill off any contaminants with Chemgene.
    - Tip water down sink, rinse tray well with tap water
    - Clean with 70% ethanol
    - Rinse, then give tray a final swab with 70% ethanol
    - Ensure tray is completely dry before adding 100 mL Sodium Hypochlorite and autoclaved tap water to just below the half-way point
    - Quickly replace in incubator

7. Always ensure that there is no condensate on the door or side panels. Wipe away any moisture well 70% ethanol.

8. Clean the tray if you spill media or cell culture in the tray.

CHANGING CO_2 CYLINDERS

SAFETY NOTE: Cylinders should only be changed by trained personnel. This includes Kevin Judd (ktj20@cam.ac.uk), Naomi Hobbs (nh457@cam.ac.uk) Gary Herrington (gmh31@cam.ac.uk).

1. Use vapour withdrawal full height CO_2 cylinders (product code 40-VK) which are purchased from BOC – Kevin Judd (ktj20@cam.ac.uk) will order these for us. Please copy in Naomi Hobbs (nh457@cam.ac.uk) on all emails to Kevin

2. Ensure that the CO_2 levels are at 5% in each incubator. The flow rate is automatically set and requires no adjustment.

3. Should anyone suspect a leak in the CO_2 supply please inform Annette Steward mpacc@ch.cam.ac.uk immediately.

CLEANING THE INCUBATOR

The incubator should be given a thorough clean out in the event of a suspected contamination of cultures. Additionally the incubators, which have built-in decontamination cycles, are cleaned routinely.

1. **Preventative maintenance:** To check whether the incubator is the cause of contaminations, duplicate a passage and store one culture in the incubator you suspect could be the cause of the contamination and the other in a second incubator. If you find contamination of repeated passages only from cultures grown in the suspect incubator, this is the cause of your contamination.

2. To clean incubator:
   - Discard or transfer cultures to a different incubator (discard for end of year break unless users are coming in)
   - Switch off incubator and remove IRD very carefully. Gently swab with 70% ethanol and keep aside.
   - Turning the incubator off will stop the CO_2
   - Remove all panels, water tray and attachments
   - Wipe down all surfaces of incubator thoroughly with 70% ethanol, particularly glass door, and seal
   - Rinse and clean tray (as above)
   - Clean all shelving and attachments with 70% ethanol.
   - Dry all components well, you may need to leave all the components for a while to be sure
   - Replace all components – shelves, tray – in incubator as set up before. Please ensure that no water is placed in the humidity tray in the instrument.
   - Double check that IRD has been removed – it will be destroyed by a high heat cycle. Run incubator through a decontamination cycle.

The entire cycle takes about 5 hours to heat up and cool down (best to leave for 24 hours).

- When complete and incubator has returned to room temperature, replace IRD, check CO_2, flow, switch the incubator on to the correct parameters.
- Replace water in tray
- Check a few times in the day to ensure that CO_2 and temperature levels are maintained.
- If the incubator is not required over the Christmas break, it is not necessary to replace the water until it is to be used again.

SOP for the Use of Waterbaths

General Notes

- Water Bath must be set to 37^{o}C. It may only be changed to heat inactivate FCS (56^{o}C)

- Water bath must be only filled with autoclaved tap water, distilled water can cause oxidation

- Ensure that there is sufficient water at all times, but do not overfill as media bottles will tip over (contamination risk)

- Add one tablet of Aquasan per week to approximately 10L of autoclaved water in the system.

- To operate, turn red switch on, it takes about 30 min to warm up

- Once the bath has reached 37^{o}C, place media bottles in water, ensure they are stable

- Lid coverage when using media bottles is optional (again, could keep lid off to reduce chance of contamination by condensate), but otherwise the lid must be left on the bath at all times

- Temperature is already adjusted to 37^{o}C, but monitor temperature with thermometer to ensure accuracy. Please ensure any theromometer is secured properly.

- Check that element is switched off at the end of each day

- Alternatively, if you wish to leave the element on, ensure that there is enough water and that lid has been fitted properly

- Failure to carry out the measure in the last point runs the risk of the water evaporating and a possible fire.

CLEANING THE WATER BATH

1. If there is any fungal or bacterial growth evident please add Chemgene to the appropriate concentration and leave it to soak for approximately 30 minutes. If this is not sufficient dispose of old water and repeat for a further 1 hour period.

2. Dispose of old water down sink.

3. Remove the lid, interior grille and any racks and thermometers. Clean interior well with soft sponge or tissues. Do the same for the grille, racks, lid and thermometer.

4. Rinse with tap water, then swab with 70% ethanol.

Alternatively please run all stainless steel accessories through either the intense cycle in one of the facility's Laboratory grade dishwashers or through an autoclave cycle. After which, spray with 70% Ethanol.

5. Ensure that all components have dried thoroughly, then add the correct amount of water treatment solution, as advised in the previous section.

6. Add autoclaved tap water to desired level

7. Switch on.

Operating Instructions Safety Cabinet Safe 2020 / Maxisafe 2020

▶ **Switching the device into work mode:**

- Keep the ON key depressed until the ready signal sounds

▶ **Moving the front window up:**

- Press the △ key for approx. 1 second

  When the front window reaches the working position, the movement stops automatically. When the movement starts above the working position, the front window stops at the maximal opening position.

▶ **Stopping the upward movement:**

- Press, then release the △ key

  This function can be selected with any key except

  the key.

▶ **Lowering the front window:**

- Keep the ▽ key depressed

▶ **Stopping the downward movement:**

- Release the ▽ key

▶ **Switching the device to OFF mode:**

- Keep the ON key depressed until the ready signal sounds

6.3.2 Moving the front window to the working position

1. Raising or lowering the front window:
   - Press, then release the △ key for approx. 1 second
   - The red status indicator  on the display is illuminated
   - The audible alarm signal is on.
2. When the front window reaches the working position, the movement is automatically stopped.
   - The green status indicator  on the display is illuminated
   - The audible alarm signal is off if the airflow is steady.
3. If the movement starts above the working position, the front window must first be lowered below the working position and then be raised again. To lower the front window:
   - Keep the ▽ key depressed
4. To stop the downward movement:
   - Release the ▽ key

6.3.3 Silencing the audible alarm signal

When the front window is moved out of the working position or when the pressure sensors detect a safety-relevant change of the airflow velocities, the corresponding visual and audible alarm signals are issued. The alarm remains active until the front window has been moved to the correct working position or until the airflow velocity corresponds with the preset values.

CAUTION – Unsafe working!

When the alarm signals are activated, safe working is no longer ensured.

NOTE – Switching the alarm signals off

If the alarm signals fail to be switched off automatically, move the front window to the uppermost or lowest position and contact the Technical Service.

6.3.4 Switching the illumination on and off

In each operating mode, the sample chamber illumination can be switched on or off.

- To switch the illumination on or off:

  Press, then release the  key

6.3.5 Activating and deactivating the internal power supply

All outlets in the sample chamber can be activated (power supply on) or deactivated (power supply off) simultaneously.

1. To activate the power supply:

   Press, then release the  key

   The yellow status indicator □ ⏻ is illuminated.

2. To deactivate the power supply:

   Press, then release the  key

   The yellow status indicator □ ⏻ goes off.

6.4 Pilot switch

Fig. 17: The basic functions required for the operation of the device can be controlled with the pilot switch:

- switch the device on,
- Raising and lowering the front window,
- switching the device to OFF mode.

6.4.1 Moving the front window

The movement of the front window is controlled by depressing the pilot switch control element with the corresponding arrow symbol.

1. To raise the window, press the control element [1]. When the front window reaches the working position, the movement stops automatically. If the movement starts above the working position, the front window stops at the maximum opening position.
2. To stop the upward movement, release the control element.
3. To lower the window, keep the control element [2] depressed.
4. To stop the downward movement, release the control element.

NOTE – Switching functions (on/off)

If the device is started with the pilot switch, the chamber illumination is switched on.
If the device is switched off with the pilot switch, the chamber illumination is also switched off.

5. If the front window is not in the working position,
   - the red status indicator **FRONT WINDOW IS NOT IN WORKING POSITION** is illuminated.
   - The audible signal is silenced when the front window is completely closed.

6. When the front window reaches the working position after being moved upwards, the movement stops automatically:
   - The green status indicator **FRONT WINDOW IS IN WORKING POSITION** is illuminated.
   - The audible alarm signal is switched off.

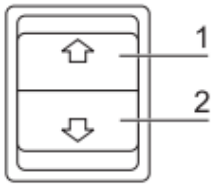

Fig. 15
Basic functions of the pilot switch

6.4.2 Switching the device to OFF mode

1. Move the front window to the upper and lower end positions.
2. Release the control element.
3. Keep the previously actuated control element depressed until the ready signal sounds.

NOTE – Switch-off function

If the device is switched to the OFF mode with the pilot switch, the chamber illumination is switched off. The device-internal power supply remains in the last functional state that it had been switched to.

6.4.2 Switching the device to OFF mode

1. Move the front window to the upper and lower end positions.
2. Release the control element.
3. Keep the previously actuated control element depressed until the ready signal sounds.

NOTE – Switch-off function

If the device is switched to the OFF mode with the pilot switch, the chamber illumination is switched off. The device-internal power supply remains in the last functional state that it had been switched to.

Appendices

6.1 Daily User Checklist

This check list should be completed BEFORE you complete your work to confirm that the room is in a safe and tidy state and fit for purpose. It there are any areas of concern please notify Annette Steward (and tell her the previous user on the list). It is your responsibility to ensure you leave the lab in an appropriate state with the consumables replenished (from the contingency stocks), benches clean and tidy, MSC clean and tidy, general waste and autoclave waste emptied if 2/3 full, vacuum aspirator trap emptied if ~750 mL, liquid waste discarded down the sink, virkon and 70% ethanol renewed.

Date	Time	CRS ID/ Group	Benches clean and tidy (tick)	MSC clean and tidy (tick)	General waste bin empty (tick)	Autoclave waste bin empty (tick)	Pipette waste bin empty (tick)	Vacuum trap empty (tick)	Liquid waste empty (tick)	Virkon EtOH renewed (tick)	signature

6.2 Appendix 2: Laboratory Containment Level Requirements

Laboratory Containment Level 1 requirements:

1. The laboratory must be easy to clean. Bench surfaces must be impervious to water and resistant to acids, alkalis, solvents and disinfectants.

2. Effective disinfectants must be available for immediate use in the event of spillage.

3. If the laboratory is mechanically ventilated, it is preferable to maintain an inward airflow while work is in progress by extracting room air to atmosphere.

4. All procedures must be performed so as to minimise the production of aerosols.

5. The laboratory door must be closed when work is in progress.

6. Laboratory coats or gowns must be worn in the laboratory and removed when leaving.

7. Personal protective equipment, including protective clothing, must be:
   (a) stored in a well-defined place;
   (b) checked and cleaned at suitable intervals;
   (c) when discovered to be defective, repaired or replaced before further use.

8. Personal protective equipment which may be contaminated by biological agents must be:
   (a) removed on leaving the working area;
   (b) kept apart from uncontaminated clothing;
   (c) decontaminated and cleaned or, if necessary, destroyed.

9. Eating, chewing, drinking, taking medication, smoking, storing food and applying cosmetics is forbidden.

10. Mouth pipetting is forbidden.

11. The laboratory must contain a basin or sink that can be used for hand washing.

12. Exposed parts of the body must be decontaminated immediately when contamination is suspected and before leaving the laboratory.

13. Bench tops must be cleaned after use.

14. Used glassware and other materials awaiting disinfection must be stored in a safe manner. Pipettes, for example, if placed in disinfectant, must be totally immersed.

15. Contaminated materials whether for recycling or disposal, must be stored and transported in robust and leak proof containers without spillage.

16. All waste material, if not to be incinerated, must be disposed of safely by other appropriate means.

17. Accidents and incidents must be immediately reported to and recorded by the person responsible for the work or other delegated person.

Laboratory Containment Level 2 Requirements:

Additional requirements above containment level 1 are listed below

1. Access to the laboratory must be restricted to authorised persons.

2. There must be specified disinfection procedures.

3. If the laboratory is mechanically ventilated air pressure must be maintained negative to atmosphere while work is in progress.

4. There must be safe storage of biological agents.

5. Laboratory procedures giving rise to infectious aerosols must be conducted in a microbiological safety cabinet.

6. There must be access to an incinerator for the disposal of infected clinical material.

7. There must be adequate space (24 m^3) in the laboratory for each worker.

8. Laboratory coats or gowns, which must be side or back fastening, must be worn and removed when leaving the laboratory suite.

9. Separate storage for PPE (for example, pegs) apart from that provided for personal clothing must be provided in the laboratory suite.

10. Bench surfaces must be regularly decontaminated according to the pattern of the work.

11. The laboratory must contain a wash basin located near the laboratory exit. Taps must be of a type that can be operated without being touched by hand.

12. An autoclave for the sterilisation of waste materials must be readily accessible in the same building as the laboratory, preferably in the laboratory suite.

13. Materials for autoclaving must be transported to the autoclave in robust, spill-proof containers.

All users of the MPACC Tissue Culture facility must sign before any lab work can be undertaken:

I confirm I have read, understood and will comply with the management plan for the MPACC Tissue Culture facility.

Name (email address)	**All GMO work is covered by a current Departmental GMO form** **Yes / No**	**All experimental work is covered by current chemical hazard & risk assessments** **Yes / No**	**Signature / Date**