**Health and Safety hazards and control measures**

* All staff and students must be trained and deemed competent in the safe use of the cryostat before commencing. Equipment manual must be read before initial use.
* **Biological hazard** – fresh human tissue. All users must have completed a Biological Agents Questionnaire and returned to Occupational Health before commencing work. This will determine whether the user requires further vaccination. Nitrile gloves must be worn at all times while using the cryostat. Any existing cuts or skin lesions must be properly protected. Information about infected tissue must be available for users in case of cross contamination (i.e. health screening information).
* **Sharps** - All staff and students must be trained in the correct use and disposal of sharps. The blade must be placed in last during set up and removed first when finishing. Machine lock to be engaged and blade covered when not rotating handle. Blades must not to be left in unused machines. Lone working is not permitted.
* **Repetitive Strain Injury** – Adjust chair to a comfortable position. Avoid working for long periods of time on the cryostat or for consecutive days. No more than 3hrs per day. A break must be taken every 1hr.
* **Low temperature** – Cold burns may occur from using the cryostat, cryogenic substances and from dry ice. Potential exacerbation of previous skin conditions. Wear nitrile gloves. Avoid leaving tools inside the cryostat when not in use. Take regular breaks.
* **Mechanical** - trapping from moving parts. Users must place rotators into machine lock position when not rotating and when machine is not in use.
* **Electrical –** electrocution or short circuit. Periodical visual inspection by the users. Spilled liquids must be cleaned immediately.
* **UVC radiation -** Ensure window sash is completely closed before turning on the UVC disinfection. Report any cracks or holes in the window sash immediately.
* **Mercury (Hg) -** The UVC lamp contains mercury which poses a health hazard when released. Any damaged UVC lamp should be replaced immediately.

**Cryosectioning human tissue protocol:**

**Note:** Cryostat is left on cool at all times. Before prepping the cryostat ensure machine lock for rotating hand wheel is on (positioned at 12 O’clock). Keep tissue samples on dry ice during set up. Ensure gloves are always worn.

1. Clean inside cryostat and utensils with 70% ethanol by spraying ethanol onto a piece of tissue and wiping surfaces down. Do NOT spray ethanol directly into the cryostat due to explosive hazard.
2. Using a pastor pipette add Milli-Q water to the specimen stage, place tissue on embedding medium and allow to solidify.
3. Place specimen stage with affixed tissue in the tissue block, adjust and lock the block in place.
4. Prior to inserting the blade, ensure there is space between the tissue and cutting block. If there is a large space, manually adjust the cutting block, moving it closer to the tissue block. You can also use the control buttons (marked with arrows) on the left-hand side of the machine to extend the tissue block forwards.
5. Insert the blade into the knife block. Cover blade with the knife guard at all times when not sectioning.
6. Prior to sectioning, ensure there is space between the tissue and blade. This is to ensure you do not take of large tissue chunks on the first rotation, and that the blade will not hit the tissue block. If there is a large space, use the control buttons (marked with arrows) on the left-hand side of the machine to extend the tissue block forwards.
7. To begin sectioning, remove the blade guard, unlock the hand wheel and begin to rotate hand wheel. If it becomes apparent that the tissue block needs adjusting/angling, lock the hand wheel, cover the blade, retract the tissue block and unlock it to adjust. Once adjusted, lock the tissue block, extend tissue block forwards using the control buttons, remove the blade guard, unlock the hand wheel and begin to rotate.
8. While sectioning the tissue, use smooth, constant pace movements to rotate the hand wheel.

Tissue shavings can be collected for homogenisation: Once you have accumulated tissue shavings on the cutting block, lock the hand wheel and cover the blade. Use a paintbrush to sweep shavings into a pre-cooled Eppendorf. Place the Eppendorf back on to dry ice and continue sectioning until enough shavings had been collected.

Tissue can be sectioned on to glass slides: Begin sectioning by using the trim function to ensure tissue is flat and even then, change to fine trimming. Use the anti-curl glass cover when sectioning. Remove the glass cover and use a paintbrush to flatten edges if necessary and press glass slide on top of tissue. Tissue will stick to the slide. Keep the slide in the cryostat until transfer to the -800c freezer.

1. After completing sectioning for one sample, cover the blade and lock the hand wheel machine lock. If a new sample is to be sectioned a new blade should be used and surfaces should be cleaned with 70% ethanol to avoid contamination. Retract the tissue block, uncover, remove and discard blade into a designated yellow sharps (WIVA) bin. Brush debris of the tissue and remove tissue from the tissue block. Remove tissue from the specimen stage – the stage will need to be gently warmed to allow the embedding medium to slightly melt allowing removal. Place tissue on dry ice. Clean utensils and inside of the cryostat with 70% ethanol. Dispose of tissue waste accordingly (Human tissue waste should be placed in a wiva bin labelled with “human tissue waste” and once full, disposed of in yellow eurocart on Ground floor).
2. Once finished, completely close the sliding window and turn on UVC light to disinfect the chamber.
3. Return tissue samples to -800c freezer.

**Accidental exposure/ first aid:**

In the event of a sharps’ injury, encourage bleeding while running cold tap water over injury. You must contact Occupational health immediately.

**First aid treatment for cold burn:**

If possible, place burn in lukewarm water. Clean plastic kitchen film or sterile dry dressings should be used to protect damaged tissues from infection or further injury. If necessary, seek medical attention.

**Lone working is not permitted.**

**Emergency support through security: 4444 (+442075891000)**

**In all instances** of accident OR near miss, notify the safety department and complete a SALUS report.

<https://www.imperial.ac.uk/safety/safety-by-topic/accidents--incidents/>

Occupational health contacts: <https://www.imperial.ac.uk/occupational-health/>

email: occhealth@imperial.ac.uk phone: +44 20 7594 9401

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Date** | **Name** | **Signature** | **Trained by** | **Supervisor/Lab Manager** |
| 17/06/2022 | Emily Adair | E.Adair | Nanet Willumsen |  |
| 17/06/2022 | Nanet Willumsen | N.willumsen | Karen Davey |  |
| 17/06/2022 | Callum Muirhead | R.Muirhead | Karen Davey |  |
| 17/06/2022 | Xiaowen Zhang | X.Zhang | Nanet Willumsen |  |
| 17/06/2022 | Thomas Chua | T.Chua | Nanet Willumsen |  |
| 17/06/2022 | Alessia Caramello | A. Caramello | Nanet Willumsen |  |
| 17/06/2022 | Vicky Chau | V.Chau | Nanet Willumsen |  |
| 12/07/2022 | Xingjian Wang | X.Wang | Nanet Willumsen |  |
| 25/07/2022 | Riad Yagoubi | R. Yagoubi | Nanet Willumsen |  |
| 19/10/2022 | Marianna Papageorgopoulou | M.I Papageorgopoulou | Nanet Willumsen |  |
| 07/11/2022 | Matilda Burridge | M. Burridge | Nanet Willumsen |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

|  |
| --- |
| *Review history*  |
|  | Review 1 | Review 2 | Review 3 | Review 4 | Review 5 |
| Date conducted |  |  |  |  |  |
| Conducted by |  |  |  |  |  |