University of Sussex
Life Sciences

MRC Genome Damage and Stability Centre

Human Tissue Act
SOP – Biopsies – practical procedures

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Version	Date	Reason for Change
2.0	29/5/15	New LN2 database, review of procedures in line with HTA at annual review.
2.2	13/04/2018	Annual review

1.0 Purpose
The purpose of this standard operating procedure is to outline the procedures to be followed in the laboratory for the preparation of received human biopsy material, and the storage of any surplus material. All work is covered by HTA license 12119.

2.0 Introduction
Biopsies are received by the Genome Centre and are documented, checked and given a unique code all according to SOPs 1,5 and 8. These can be located on the Life Sciences webpage:
http://www.sussex.ac.uk/lifesci/internal/servicesandsupport/ethics/humantissue
They are then taken into the lab where they are processed by trained staff only.

3.0 Safety
All samples are deemed Category Level two and should be manipulated in the designated quarantine hood in the quarantine laboratory (G2.26). Personnel should wear Blue Howie- style laboratory coats (side fastening, elasticated cuffs, high necked), which should be buttoned up fully, and nitrile gloves worn. Disposal of surplus human biopsy material is described in the HTA SOP 12 which can be found on the Life Sciences website:
http://www.sussex.ac.uk/lifesci/internal/servicesandsupport/ethics/humantissue

Compliance with the University of Sussex 'Science Schools Safety Handbook' is required at all times. This can be found on the University website: http://www.sussex.ac.uk/hso/healthandsafety/science-safety-procedures-and-guidance/sciencesafetyproceduresandguidance
Specific risk assessments and COSHH assessment forms associated with this procedure can be found in the HTA folder in the TC office (G2.07)and on the Intranet

4.0 Materials

- Two T25cm^2 filter-capped flasks
- Mammalian Growth Medium: MEM (500ml) supplemented with 1% pen/strep solution and 1% L-glutamine 200mM 15%FCS
- 1.5 ml Cryovial
- 144mm culture dish
- 100mm culture dish
- Glass pasteur pipettes
- Plastic serological pipettes
- Sterile disposable scalpel
- Rubber Bulb
- Universal tube
- DMSO

5.0 Equipment

- Class II tissue culture safety cabinet (designated "the quarantine hood") situated in room G2.26.
- Manual Pipette Aid
- Sharps Bin
- Water Bath set at 37°C
- CO_2 Incubator set at 37°C and 5% CO_2

6.0 Procedure

To be carried out in the Quarantine Laboratory (G2.26)
6.1 Prepare 15% mammalian growth medium by adding 90mls of FCS to 500ml of MEM. Supplement with 5ml of pen/strep solution and 5ml of L-Glutamine. Label with your name and date prepared. Store in fridge in G2.26 and discard after 6 weeks from this date.
6.2 Label both T25cm^2 flasks with date received, the biopsy code (BR number) and either A or B. Add 2.5ml of sterile mammalian growth medium to each flask and ensure that the bottom surface area has been coated with media.

Position flasks in a tilted position (for example by resting end- to- end on a large (144mm) culture dish).
6.3 Prepare 1ml of 10 % DMSO solution in sterile mammalian growth medium and place in a cryovial. Label cryovial with BR number and date.
6.4 Inspect biopsy vessel for leaks and possible contamination. If contaminated follow decontamination procedure: SOP/NER/2.0 .
6.5 Using the bulb attached to a glass pasteur pipette carefully suck up the biopsy from the travel vessel and place in the 10mm sterile culture dish.
6.6 Identify the skin surface from the subcutaneous fat and carefully cut the latter away with the sterile disposable scalpel. Push fat to one side but keep on dish.
6.7 Using a downward motion (not sawing motion) cut the biopsy in half.
6.8 Pick up one half and place in the 10% DMSO in the prepared cryovial. This is stored securely at -80ºC (G2.15) overnight in a designated cryovessel and then transferred to the liquid nitrogen.
6.9 Cut the remaining biopsy section into small pieces (>10) again using a downward motion to prevent shearing of the cells.
6.10 Add more transport medium (medium from vessel in which the biopsy arrived) as required if plate becomes dry and to facilitate sucking up the pieces.
6.11 Suck up half of the biopsy pieces (>5) and transfer to one of the T25 flasks by dotting onto the bottom surface of the flask above the medium level so they don't float away. Repeat with the other flask and the remaining biopsy pieces.
6.12 Carefully transfer the dish with the tilted flasks to the incubator.
6.13 Leave for approximately 3 days before lying flat.
6.14 Seal the culture dish containing the discarded fat with tape and place in autoclave waste bag to be autoclaved and disposed of in the clinical waste stream. Place scalpels in original packaging and put all into sharps bin.
6.15 Cells should be visible under the microscope after 1-2 weeks. If after 6 weeks there is no cell growth then thaw the frozen section and reseed.
6.16 When there is approx. 1cm^2 of cell growth around any biopsy piece the flask is ready to be trypsinized and expanded into a larger flask.

7 Training

All staff working with HTA samples should first be trained by a HTA persons designate or a PI. They should sign off the SOP in the office G2.07.

8 Documentation

Receipt of human tissue in biopsy form is controlled and regulated by the HTA under licence 12119 in the Genome Damage and Stability Centre. Please see the link on the Life Sciences website:
http://www.sussex.ac.uk/lifesci/internal/servicesandsupport/ethics/humantissue
Personal patient information is recorded on the biopsy database which is kept on a restricted access computer in the tissue culture office (G2.07). A "BR" number is allocated to the biopsy and this becomes the cell line's designation unless a particular test outcome warrants an additional clinical designation.

Hard copies of consent forms are filed numerically as per the BR number allocated along with any clinical details of the patient and kept in a locked filing cabinet in the same office (G2.07), which is kept locked out of hours. Thus samples are linked-anonymized both here and on the biopsy database only.

8.1 *Cell Sheet*

A paper cell sheet is created for each biopsy under the BR designation .A or .B depending on which biopsy section is being used. Cell sheets must not contain patient information, only the BR no. All patient information must be with the consent form locked in the filing cabinet. Cell sheets are continually updated documenting exactly what has been done to each flask to ensure a complete historical provenance for the cell line established.

9.0 Records

9.1 *Cell Lines In/Out Book*

The arrival date, address of sending laboratory and referring consultant and any comments for each biopsy are recorded under the BR designation in the "Cell lines in/out" book kept in the tissue culture office (G2.07).

9.2 *Liquid nitrogen database*

Location of the frozen sections in the liquid nitrogen storage facility is recorded in the liquid nitrogen database using the BR designation. The location is also recorded in the biopsy database. Copies of completed consent forms ,SLAs and NRES approval certification are also linked electronically on this database.

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Biopsy preparation