Establishing human embryonic stem cells master cell banks under gmp conditions

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Introduction

The increased interest in using human pluripotent stem cells for advanced therapy medicinal products (ATMP) has revealed the urgent need for safe and GMP compliant established hESC Master Cell Banks. The culture conditions for human pluripotent stem cells have evolved since 1998, leaving the use of mouse feeders and animal components behind, and instead one employ defined, feeder free culture conditions. However, the absolute majority of the derived human ES cells are made with feeders and outside the GMP environment, with animal components. Further, the starting material, the blastocysts, are not sourced according to FDA's guidelines, lacking proper donor testing, and/or sourced in non-prion free regions of the world.

In order to derive human embryonic stem cells under GMP and sourcing the starting material according to also FDA's requirement we have built up the infrastructure required for a successful establishment of human ES cells for clinical applications. Initially we developed a new, xeno-free defined culture medium, that together with a defined coating substrate was successfully evaluated for derivation of hES cells. The xeno-free medium was then produced and released under GMP.

We then established appropriate sourcing procedures, e.g. sourcing according to FDA's requirement and with proper donor consents that allows the future use and needed analyses as well as significant pre-screening of the donor couples before they were approved as eligible donors. In parallel we built the infra structure and processes that rendered us to become a registered tissue establishment and with a manufacturing license for deriving and banking human ES stem cells from the Swedish MPA (according to the Swedish LVFS 20018:12 and LVFS 2004: 7, Eudralex Volume 4 GMP).

Tissue Establishement

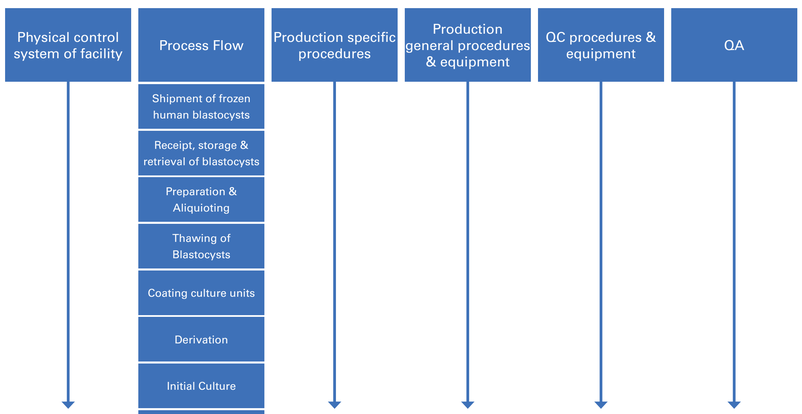
- For Tissue Establishment we applied, according LFVS 2008:12 on quality and safety norms when handling human tissue and cells.
- We are a registered Tissue Establishment since Sept 2017.
- (US) IRB and local (Swedish) ethical permit
- Import license
- Document storage > 30 years
- Validated transport company
- Control of donors
- Controlled dry shippers
- Storage of donor material
- Tissue Establishment license

GMP Facility

- Cell therapy is classified as an Advanced Therapy Medicinal Product (ATMP).
- Manufacturing License, according to LVFS 2004:7 and inspection on Eudralex Volume 4, EU GMP guidelines.
- Manufacturing License for the manufacture and storage of Master Cell banks for use in Clinical trial material

- Eudralex Volume 4, EU GMP guidelines (Annex 1 and 15)
- Manufacturing License (Annex 16)
- Quality Risk Management according to International Conference on Harmonization, ICH Q9 (Part 3 EU GMP)
- Continuous Environmental Monitoring
- Aseptic Process Qualification
- Process Flow Validation
- Material flow
- Environmental control system (Air, Equipment)
- Continuous Gowning Qualification
- Life Cycle Management
  - validations, (IQ, OQ, PQ)
  - equipment,
  - maintenance

Processes surrounding the hESC establishment workflow



Sourcing of Blastocysts

- Starting material:
  - Human fertilized eggs has to be sourced from FDA approved regions ("prion free"), such as USA, Iceland, Israel, Australia or New Zeeland)

- Ethical approval and donor consent
  - Donor consent including the right to commercialize products

- Recruitment of donor couples
  - Signed donor consent

- Selection of donor couples
  - Medical history interview
  - Donor testing for infectious diseases/viruses*
  - Physical examination
  - Fulfillment of all acceptance criteria

- Sourcing of material
  - Cryopreservation
  - Transfer to Takara Bio Europe AB

*Donor screening maximum 7 days before/after IVF donation of blastocysts/embryos by Clinical Laboratory Improvement Amendments (CLIA) certified laboratories according to FDA recommendations.

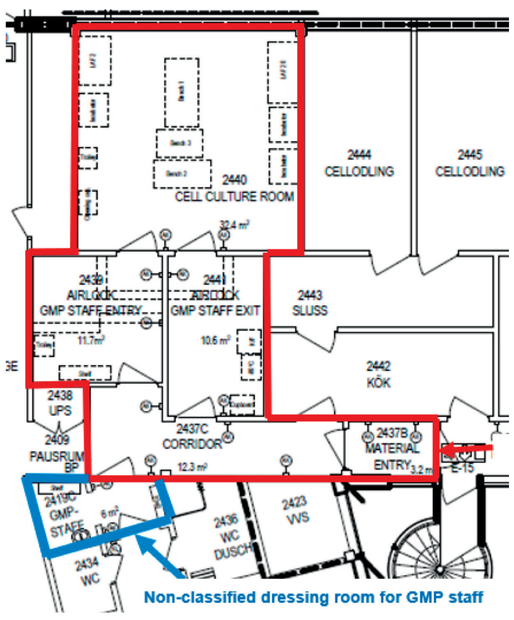
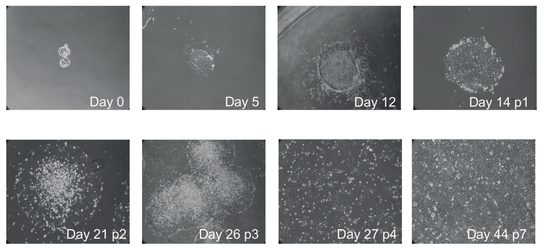


Figure 1. Detailed overview of GMP facility for ATMP. HEPA filtered air with regulated and controlled over-pressure (from Area B to D), togehter with control of strict gowning procedures, personell-and material flow and continuous Environmental Monitoring (static and dynamic) will assure the GMP set demands.

Previously Established hESC (non-GMP)



Quality Control and Release

Assessment of **production** data:

- Environmental control results from QC
- Lists and alarms from Production regarding Surveillance systems
- All Deviations from Manufacture and their Status
- Operational readiness document status
- Review of Batch records against the 'Batch tree' and GMP
- Review of QC assessment of analytical results against Product specification, CoA
- Release of SEED / MCB in relation to the reviewed documentation from Production and QC.
- Issue Certificates CoC, followed Manufacturing license, customer Quality agreement and GMP as expressed through TBEAB Quality system
- BSE/TSE according to EMA 410/01/revision 03 (cuirrent revision)
- Certify in a register telling where documents are stored and tht released for further manufacturing of Clinical trial material

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