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The Stem Cell Laboratory: Design, Equipment, and Oversight

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Abstract

This chapter describes some of the major issues to be considered when setting up a laboratory for the culture of human pluripotent stem cells (hPSCs). The process of establishing a hPSC laboratory can be divided into two equally important parts. One is completely administrative and includes developing protocols, seeking approval, and establishing reporting processes and documentation. The other part of establishing a hPSC laboratory involves the physical plant and includes design, equipment and personnel.

Proper planning of laboratory operations and proper design of the physical layout of the stem cell laboratory so that meets the scope of planned operations is a major undertaking, but the time spent upfront will pay long-term returns in operational efficiency and effectiveness. A well-planned, organized, and properly equipped laboratory supports research activities by increasing efficiency and reducing lost time and wasted resources.

Keywords

pluripotent stem cell laboratory; establishing a cell laboratory; cell culture; human PSC culture laboratory

1. Introduction

Establishing a well-functioning laboratory for the culture of human pluripotent stem cells (hPSCs) provides the foundation for successful culture and experimentation. This chapter will describe the major considerations for establishing a successful PSC research-grade laboratory (see Chapter 11 for considerations of a clinical-grade laboratory) (see Fig. 1). While the culture of hPSCs is carried out in a laboratory that is not much different than one used to culture other types of human cells (1, 2), due to the special status of these cells, there is a higher degree of oversight, review, and reporting. We have found that a nearly equal amount of time and effort is required to establish initial protocols and seek approval for culturing and obtaining human pluripotent cell lines as is required to design and equip the laboratory.

Whether retrofitting an existing laboratory or designing a new laboratory from shell space, the basic issues are the same (3–5). One must consider the equipment, number of people, and type of activities to be performed in order to achieve the best design (within budget constraints). Using a modular design, both in terms of laboratory benches and equipment, allows the laboratory to be expanded as needed by adding additional tissue culture modules and personnel to manage the work load (6). The key considerations when setting up the laboratory include (1) defining of the scope of the work which includes the numbers and types of cell lines to be cultured and (2) determining the number of people who will work in the laboratory and their specific tasks. One must consider, for example, how the lines will be maintained and characterized. If more than one PSC line will be cultured simultaneously,

what safeguards will be put into place to prevent cross-contamination? How will new lines be introduced to the laboratory? Is there an area in which to quarantine these new lines? Accommodations must be made for the receipt of incoming materials and their testing and storage, as well as proper disposal of waste material. Culturing stem cells is very much like culturing other types of cells, with some additional special techniques. Specifically, the PSC research laboratory, at a minimum, requires space and equipment for: tissue culture, microscopy, and standard biochemistry and molecular biology.

2. Equipment

This section lists the minimal equipment required for basic culture and characterization of hPSCs. A given program may require specialized culture equipment, such as incubators that allow culture in low oxygen tension, or characterization and analysis equipment. This section lists the equipment required to establish a standard hPSC culture laboratory.

2.1. Tissue Culture Laboratory

1. Class II Biosafety Cabinet (BSC) (see Note 1).
2. CO_2 incubator (see Note 2).
3. Pipettors.
4. Vacuum flask/aspiration device.
5. Water bath (37°C).
6. Low-speed centrifuge (clinical grade, for spinning cells).

2.2. Microscopy

1. Phase–contrast microscope.
2. Dissecting microscope.

2.3. Storage

1. Cabinets and shelves for the storage of tissue culture supplies (see Note 3).
2. Refrigerator (4°C).

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[1]The type of biosafety cabinet (BSC) that one installs depends on the type of work being conducted in the laboratory (7). The Class II BSC is typically utilized in the hPSC laboratory. Class II BSCs are partial barrier systems that rely on the directional movement of air to provide containment. They provide protection to both the worker and the material that is being manipulated in the cabinet when properly maintained. They provide the microbe-free work environment that is necessary for cell culture. Class II BSCs are available in four different types: Types A1 and A2 re-circulate the HEPA-filtered air back into the room. Types B1 and B2 are hard-ducted into a (preferably dedicated) exhaust system that carries the HEPA-filtered air to the outside. Class II A2 is the most common type of BSC installed in cell culture laboratories. Since the safe operation of the BSC relies on the HEPA filter, they are usually tested and certified on an annual basis or before any work commences following the relocation or movement of the BSC (see ref. 3 for an excellent review of the safe use and operation of BSCs). Generally, Class II BSCs can be used to work with *nonvolatile* and *nonhazardous* chemicals or gases; however, only type A2-exhausted or Types B1 and B2 can be used for preparing small amounts of volatile chemicals and minute quantities of hazardous chemicals, since they exhaust to the outside.

[2]CO_2 incubators come with a variety of options. Incubators can come as two-gas (CO_2:air) or three-gas models (CO_2:O_2:N_2), the two-gas being the most common and least expensive. Chamber temperature is maintained by a water jacket or an air jacket. It is important that the shelving and the hardware securing it be easy to remove and clean. Although copper shelving and interior walls can inhibit the growth of organisms, it is expensive and, unless all hardware components are of copper construction, thorough cleaning is still routinely needed. Routine cleaning with a disinfectant and ethanol rinse will help to reduce sources of contamination. The chamber, however, should be allowed to equilibrate overnight after a thorough cleaning prior to returning the cultures as the volatile components of the cleaning agents may kill the cells in culture. Use a portable RTD thermometer for accurate temperature determination and a Fyrite unit for CO_2 calibration.

[3]Storage cabinets for tissue culture supplies are usually overlooked when designing the laboratory; however, sufficient storage allows for smooth and safe laboratory operations. Sufficient space should be made available that accommodates large bulky boxes so that supplies can be properly stored off of the floor and off of the equipment, allowing for easy cleaning and accessibility.

3. Freezer (−20°C, nondefrosting).
4. Low-temperature freezer (−70 to −85°C).
5. Cryogenic freezer (storage below −140°C, usually liquid nitrogen).

2.4. Molecular Biology Laboratory/Quality Control Laboratory

1. RT-PCR.
2. Flow cytometer (might be in a Core facility).
3. Fluorescence microscope (might be located in a Microscopy Core).
4. Confocal microscope (might be located in a Microscopy Core).

2.5. Quarantine Laboratory

1. Class II Biosafety cabinet..
2. CO_2 incubator
3. Phase–contrast microscope.
4. Water bath (37°C).
5. Low-speed centrifuge (clinical grade, for spinning cells).
6. Pipettors.
7. Aspiration/vacuum flask.
8. Sink.

2.6. Additional Access to Common Equipment or Core Facilities

1. Microscopy.
2. Flow cytometry.
3. Microarray gene expression.
4. Genomics.
5. Proteomics.
6. Virus production.
7. Vivarium.

3. Methods

3.1. Laboratory Design and Layout

When building out new laboratory space, the design team usually includes an architect, a contractor, a builder, an electrician, a mechanic, a plumber, and a laboratory director or manager. The design team for retrofitting existing laboratory space is usually smaller and less formal, but is still comprised a number of people with specialized skills including the builder, electrician, mechanic, plumber, and the laboratory manager or director. Since the costs for building-out average laboratory space is 3–5 times that of office space, the budget is a key consideration and should be established as part of the preplanning.

The dynamic nature of biomedical research and the cost of laboratory construction have resulted in the prevalent use of modular design that allows reconfiguration of the laboratory as needed while keeping construction costs to a minimum.

3.1.1. Key Considerations for Planning and Design

1. Budget
    a. How much money to spend and over what time?
    b. Return on investment (what is expected to be gained through this investment).
2. Space
    a. What type? Is it new construction, build-out of shell space, rehab of existing laboratory or office?
    b. How much space is available?
    c. Phased construction, how do the phases relate to each other? Should the plan include drawings for eventual build-out of all of the space?
3. Type and scope of work to be performed
    a. Biosafety level of laboratory.
    b. Major equipment.
    c. Number of people.
4. Major functional areas
    a. Tissue culture laboratory.
    b. Quarantine laboratory (optional, but desired).
    c. Molecular biology/quality control laboratory.
    d. Microscopy laboratory.
5. Personnel
    a. How many people?
    b. In which functional area will they work?
    c. How many offices?
    d. How many desk spaces, shared or dedicated?
    e. Break room.
6. Freezer rooms
    a. How many and what types of freezers.
    b. Back-up generator.
    c. Alarm system.
    d. A separate cryogenic freezer room with limited access.
7. Storage areas in and adjacent to the laboratory
    a. Cabinets.
    b. Shelves with 1-in. lip to prevent objects from falling.
    c. Closets.
8. Information technology

a. IT closet.

3.1.2. Key Construction Considerations—Described and listed below are very high-level considerations that are meant to stimulate thinking by the laboratory manager and director as they begin working with their design or construction team to establish a new laboratory for the culture of human PSCs.

1. Mechanical/plumbing/engineering:
   a. Back-up generators: dedicated or shared, alarms and controlled access.
   b. CO_2 and LN_2 delivery: piped to the laboratory from "tank farm" or cylinders delivered to the laboratory and cryobank.
   c. Vacuum systems.
2. HVAC

   The numbers and types of BSCs will play a major role in the build-out of a tissue culture laboratory (7) and can add greatly to the cost of the build-out. Air handlers must be sized to accommodate the numbers and types of BSCs, as well as any other heat-producing equipment such as incubators and freezers. Efficient and comfortable conditioning of the air in the laboratory is one of the more challenging issues of the design and operation of the laboratory. It can be very uncomfortable working in laboratories, where the heat cannot be controlled due to the installation of HVAC system that is not of sufficient capacity to handle the heat produced by the BSCs and incubators. Air flow and its direction (negative, positive, or neutral) is a key consideration when designing the laboratory and is critical to the safe operation of the cell culture laboratory.
3. Electrical capacity and routing

   Accurately predicting the number of BSCs, freezers, and other major equipment and determining how much electricity they draw and heat they produce is critical to calculating the correct electrical capacity to maintain and safe working environment. The power requirements for all equipments as well as the location of power outlets, light switches, the determination of emergency power requirements, proximity of outlets to water faucets (GFC), and the coordination of the placement of outlets with the modular furniture designer is part of the detailed design. There need to be sufficient and dedicated circuits to handle all current equipments as well as future expansion. Incubators and freezers must be on back-up generator circuits and alarm systems.
4. Interior finishes:
   a. Vinyl flooring.
   b. Nonporous ceilings.
   c. Washable, impermeable paint and coatings.
   d. Impermeable bench-tops and furniture.

3.2. Tissue Culture Area

The tissue culture is comprised a minimal set of equipment, referred to here as the "tissue culture module" (see Table 1). It consists of one biosafety cabinet, one CO_2 incubator, one phase– contrast microscope, and a low-speed centrifuge, vacuum source, a water bath, two–2 L flasks, Pipet-Aid, micropipettors, and either a cabinet or cart next to the BSC for easy access to tissue culture supplies. If one keeps with the modular design concept, one can

increase output by increasing the number of tissue culture modules in the laboratory design. In the hPSC laboratory, one tissue culture module can accommodate 1–2 technicians. Access to cryogenic storage, centrifuges, microscopes, refrigerators, and freezers is a must for a functional cell culture laboratory; however, these frequently can be shared between multiple modules or laboratories depending on the goals of the overall laboratory program. If setting up a PSC laboratory contained within an existing cell culture laboratory, at least one tissue culture module is required. This will greatly improve productivity and reduce the chances of cross-contamination.

3.3. Molecular Biology Laboratory/Quality Control Laboratory

The molecular biology/quality control laboratory is comprised of the equipment and SOPs required to perform a predetermined list of characterization assays that allows one to systematically assess the quality of the cells in culture in the laboratory. Cultures are tested for the expression of specific markers, cytogenetic structure (karyotype or SKY), and the ability to differentiate, as described in many chapters throughout this book (and specifically in Chapter 2 describing the preparation of cell banks).

3.4. Quarantine Laboratory

The introduction of new cell lines into the laboratory is the major source by which cultures may become contaminated. Therefore, it is important that control systems are in place to minimize the potential for contamination. The quarantine control system can be a separate laboratory or cells can be quarantined through the use of a specific operating procedure where the incoming line is cultured at the end of the day by dedicated staff and grown in a dedicated incubator until it has been shown to be free of contaminates.

3.5. Storage

The proper import and storage of materials and reagents, including cell stocks, is key to the long-term success of the laboratory. When setting up a new laboratory, one has the opportunity to establish systems for logging incoming supplies and documenting their testing and use. Many of the reagents used in the PSC laboratory are derived from animal sources and therefore are subject to lot-to-lot variability that may necessitate in-house testing to determine which lot is suitable for use for specific applications in the laboratory. In addition, tracking materials for expiration dates and keeping the laboratory properly stocked is a critical function that can be facilitated through the use of databases and bar-coded inventory systems. The banking and retrieval of large numbers of small vials of frozen materials such as cell stocks and reagents can be a challenging and is greatly facilitated by developing an efficient easy-to-use tracking system.

3.6. Quality Control

The laboratory should be designed, equipped, staffed, and operated in a manner that allows for the production of reliable and reproducible experimental results. While the research laboratory must operate with enough flexibility to allow discovery to take place, establishing standard operating procedures and a quality control system can provide the foundation on which new discoveries can take place (see Fig. 2).

1. *Reliable techniques*: When establishing a PSC laboratory, identifying reliable techniques for the culture and characterization of cell lines is a critical function of the laboratory manager. Identifying techniques that are well-established and using cell lines that are well-characterized are the keys to establishing a solid foundation. New technologies and the rapid recent growth in the stem cell field require the laboratory manager to keep current with the literature, especially around the technologies that allow for the directed reprogramming of somatic cells to generate

induced pluripotent stem cells (iPSCs). There is a growing belief that one can "simply" generate some iPSC lines and that will be all that is required to establish a PSC laboratory. This is far from true. We strongly encourage hands-on learning in an established laboratory, core, or training center in the art and science of human embryonic stem cell culture prior to embarking on the establishment of a hPSC laboratory. Also, we strongly recommend the maintenance of a well-characterized hESC line(s) in laboratory as the "gold standard" for all subsequent PSC work.

2. *Validated reagents*: It is critical that the reagents used to culture and characterize hPSCs are validated and shown to be reliable. As described in Subheading 3.5, the testing of reagent lots, especially animal-derived products such as fetal bovine serum (FBS), Knockout serum replacer (KSR), and mouse embryonic fibroblasts (MEFs), is critical to successful and efficient operations in the PSC laboratory.

3. *Quality PSCs*: It is critical that the PSCs used in the laboratory are from well-characterized stocks and have the morphologic and genetic characteristics of high-quality PSC lines as described in several chapters of this book. We emphasize the use of well-characterized hESC lines as the "gold standard" in laboratories establishing iPSC cultures. Having these bona fide hPSCs in the laboratory will allow both the testing of reagents and techniques and the verification of iPSC properties.

4. *Checklist for confirming quality*: Establishing a system that allows for the periodic testing of cultures against known standards will facilitate long-term success and provides confidence and reliability in the experimentation. As described in other chapters in this book, hPSCs need to be free of contamination, have a normal karyotype, express defined markers, and be capable of differentiating into cells derived from all three germ layers.

3.7. Oversight Approvals

When establishing a new laboratory for the culture of hPSCs, gaining the proper approval for the culture of the cells can be a lengthy and involved process. Where one is located, the country, state, city and institution, all play a role in the type of approval(s) required prior to initiating culture. In most cases, applications will have to be filed with review boards and specialized committees, material transfer agreements will have to be obtained and executed, and personnel will need to be trained, all before the first hPSC can be cultured in the new laboratory. For this reason, we suggest that one begin the approval process before or during the laboratory design process.

3.7.1. Typical Institutional Review and Oversight for US Academic Institutions

1. Stem Cell Research Oversight Committee (SCRO).
2. Institutional Review Board (IRB).
3. Biosafety Committee Review (IBC).
4. Institutional Animal Committee (IACUC).

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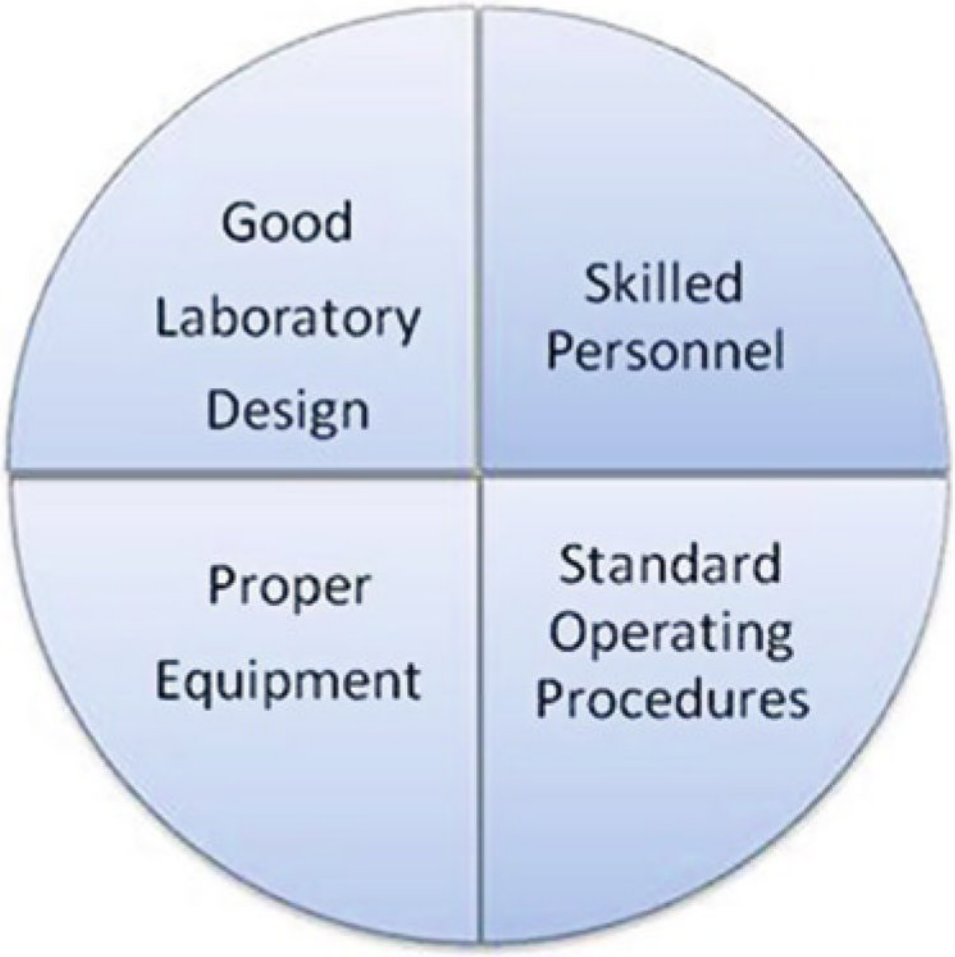

Fig. 1.
The keys to operating a successful laboratory. This diagram indicates the interrelated nature of the four key elements in a stem cell laboratory. While we cannot underestimate the importance of skilled personnel and established standard operating procedures (SOPs), this chapter will focus on laboratory design and proper equipment (SOPs can be formulated using other chapters found in this book).

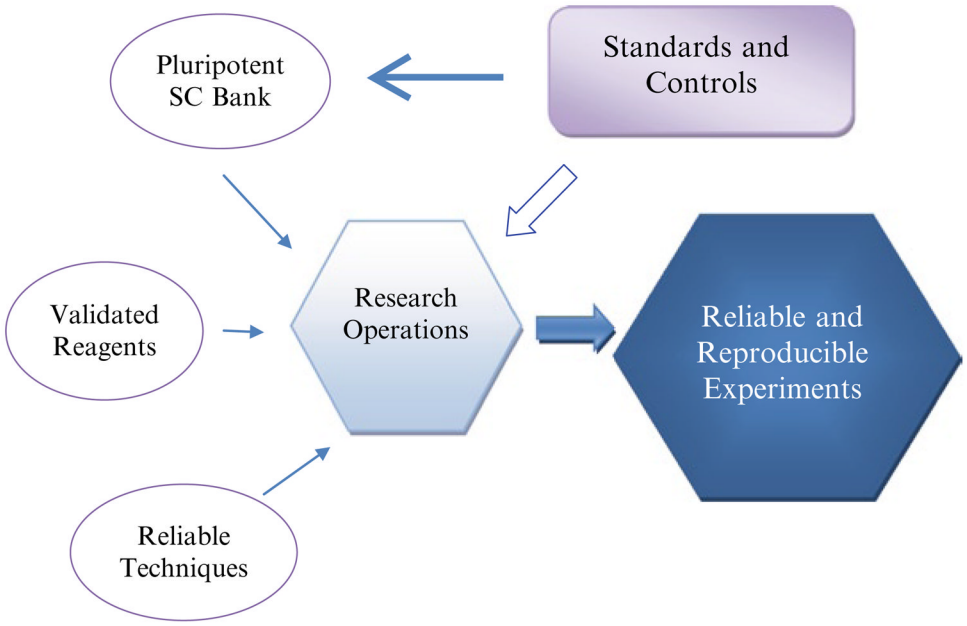

Fig. 2.
Quality control in the stem cell laboratory. This figure shows the key quality control systems that allow one to achieve reliable and reproducible experimental results (adapted from (4)).

Table 1

Tissue culture module

Equipment	Considerations
Biosafety cabinet	What class and type? Most commonly: Class II Type A2
Incubator	Water or air jacketed One gas (CO_2 and air) Two gases (CO_2, N_2, and air) Three gasses (CO_2, N_2, and O_2)
Microscope	Phase–contrast Photo port
Water bath	37°C constant
Low-speed centrifuge	Refrigeration not required
Vacuum source	Portable Supplied by the building
2-L Erlenmeyer flasks, in series, with in-line filter	Collect aspirate and protect vacuum system
Pipet-Aid	Automatic pipettor, cordless, rechargeable
Micropipettors	2, 20, 200, and 1,000 μL