Chapter 8

Affordable Clean Air Facility for (Stem) Cell Therapy in an Academic Setting

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Additional information is available at the end of the chapter

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Abstract

Together with her colleagues, the author has worked extensively on the differentiation potential of mouse embryonic stem cells (ESCs) to blood islands containing embryoid bodies (EBs) in culture. Such EBs contain hematopoietic stem- precursor cells (HSCs) committed to lymphoid, myeloid lineages and cells possessing Natural Killer (NK) cell marker. Further differentiation of such EBs to mature B-lymphoid cells can be demonstrated in a second stage of tissue culture, by co-culturing separated EBs with a mitogen, Lipopolysaccharide (LPS). The demonstration of differentiation of such cells to mature T- and B-lymphoid cells was performed in vivo, i.e., by implanting EBs subcutaneously in nude mice. The differentiation of ESCs to EBs needed to be scaled up to obtain enough HSC-containing EBs for a second stage of differentiation, in vitro and in vivo. Selection of HSC-containing EBs must be performed manually under a microscope outside the tissue culture incubator. For research purposes, the cell culture medium contains antibiotics to inhibit the growth of bacteria. However for clinical application, the medium should be free of antibiotics. A conventional research laboratory environment is not sufficient for such an application. A GMP (Good Manufacturing Production) facility with clean air became necessary. Commercial clean air facilities used in the operation room and/or by industry were over budget. The attention of the author has shifted to developing an affordable GMP facility for clinical research in academia. These efforts were successful and the price for such a GMP facility is 10% of a comparable industrial model. After that experience, "a GMP facility for cell therapy on wheels" was further developed. This chapter will describe and share with academic colleagues our experience of constructing such affordable GMP facilities from scratch, starting with the purchase of high-efficiency particulate arrestance (HEPA) filters for the clean air facility.

Keywords: cGMP, clinical application, mobile HEPA filter, mobile clean air facility, mini-clean air box

1. Introduction

cGMP (current Good Manufacturing Production) for (stem) cell therapy is the making (growth, propagation, expansion, scaled up production) of (stem) cells for clinical purposes. The procedures and requirements include two parts: hardware and software.

Here, I shall discuss and share some experiences that we have conducted and performed by converting a regular research tissue culture room-facility, especially the hardware part, into

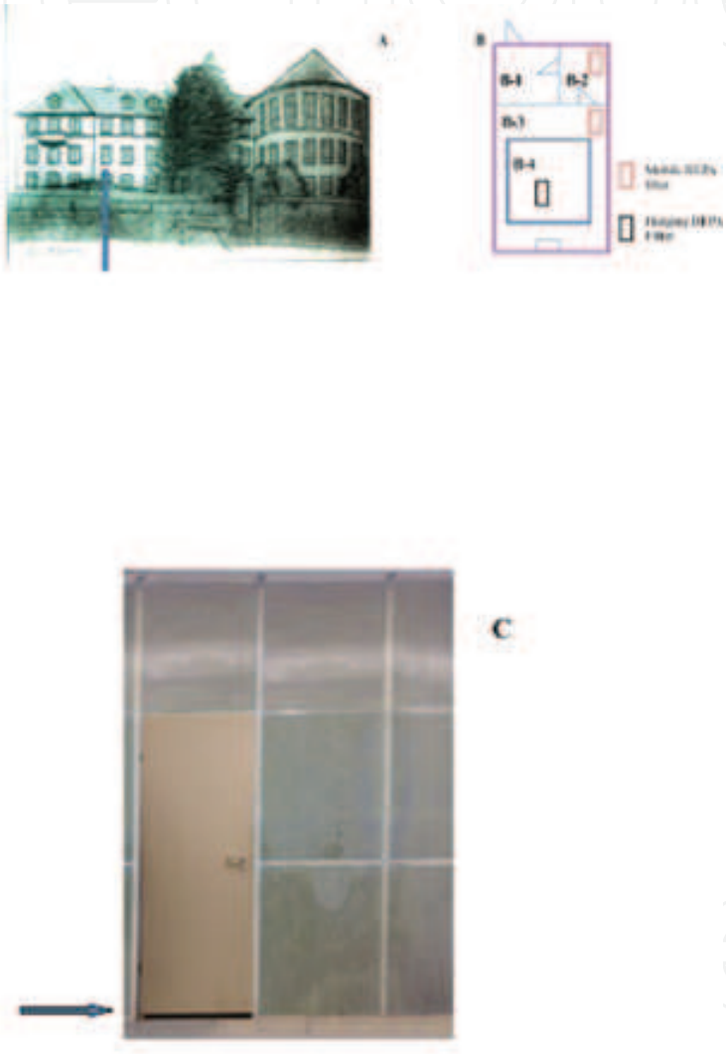

Figure 1: GMP facility. A: Biochemistry Institute Building in Uni. Clinic Giessen, cold needle graphic by U.C. Blue arrow points to the location of GMP facility. B: Planning of the GMP facility: Space 1-B-1: A front room; Space 1-B-2: Air lock; Space 1-B-3: main space of the GMP facility; Space 1-B-4: Soft wall where clean bench was located. Red long square: Mobile HEPA filter, Black long square: Hanging HEPA filter.**C. Washable wall and doors for the facility.** The existing wall of the space (1-B, purple color) was coated with transparent lacquer. The newly constructed wall and doors were these between entrance preparation room (1-B-1) and airlock (1-B-2); airlock and the main room (1-B-3) of the facility. The material was iron frame coated with white color paint and acryl glass (shown in 1-C). A row of brushes (black color, indicated by a blue arrow) at the bottom of the door (blue triangles in 1-B-1 and 1-B-2) for directing the outward air flowing.

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an open system clean air facility inside an old building (**Figure 1**), and then transferring the facility in a container (**Figure 5**) to become a "GMP facility on wheels".

2. Procedures and results

2.1. Software

The software includes: Documentation, Standard Operational Procedures (S.O.P.), QA (Quality Assurance), QC (Quality Control) tests.

The criteria and requirements can vary from country to country and be updated periodically. One must be in contact with the relevant regulatory authority regularly to achieve the standard required. There are guidelines constantly updated from regulatory authorities such as FDA [1]. The information provided by the UK stem cell bank could also be of interest [2]. The appointment of a QA person, possibly from industry working in the field, who can guide the production, give advice and suggestions, deal with update of the guidelines, and communicate with regulatory authorities, is highly recommended.

Standard Operation Procedure (S.O.P.)-1 (**Figure 7**) and S.O.P.-2 provide examples on how the clean air facility operation procedures were written, practiced, and controlled by the staff members. Our S.O.P. were updated whenever necessary and as often as possible to describe the real steps of practical exercise so that new/any staff members could hold a printout sheet in their hands, read, and follow the instructions, step by step, to meet the requirements and to operate properly.

"S.O.P. 1: Into the clean air room" (**Figure 7**) instructs the staff on what to do in the entrance preparation room, including how to change clothes, pin up hair, wear protective glasses; on how to take air shower when entering the airlock, on how to operate first in the clean air facility such as to measure particles counts, to use particle collectors, to place agar plates for collection of particles for microbiological sterile tests, before starting the cell culture experiments. Any leak and/or contamination of the air particles from outside will be detected by these procedures.

"S.O.P. 2: Out of the clean air room" (**Figure 8**) describes the reverse steps of "Into the clean air room", stating how to operate before leaving the facility, what to take out and where to place the samples collected, how to document the operation and to be double checked by a supervisor of the operation..

S.O.P. 3 (**Figure 9**) provides the regular and routine control of the clean air in the facility and describes, step by step, how and where (in clean bench, HEPA Filter 1, HEPA Filter 2, air lock) agar plates should be placed, when to be removed, where and how long they should be tested. The procedure describes how to clean the benches and what should be done before and after the cleaning, namely, agar plate tests. It describes when and where to measure particles using particle counter and particle collector. Any inappropriate operation in the entire procedure or contamination is documented and double-triple controlled by the operators and supervisor.

2.2. Hardware

The hardware includes: standardization, inspection, and routinely periodic control of all equipment used for tissue culture, including temperature and cleanness control of refrigerator, accuracy control of pipette-aids, temperature and CO_2 content of CO_2 incubator, Laminar Flow-clean bench, etc. One goes through such inspection routinely once a week, and documents the results on the so-called Monday S.O.P. (S.O.P. 3).

The equipment control is essential. The equipment do not have to be purchased new, though new equipments are under guarantee by the manufacturing companies and require less work, but the existing equipments must be inspected, (re)-standardized, and controlled routinely. Tissue culture working area should have no (bacterial–fungal) contamination (S.O.P. 3), i.e., clean air 100/ISO 6 (see below under criteria and in **Table 1**).

Class	Maximum particles/m^3						FED STD 209D
	≥0.1 μm	≥0.2 μm	≥0.3 μm	≥0.5 μm	≥1 μm	≥5 μm	equivalent
ISO 1	10	2.37	1.02	0.35	0.083	0.0029	
ISO 2	100	23.7	10.2	3.5	0.83	0.029	
ISO 3	1000	237	102	35	8.3	0.29	Class 1
ISO 4	10,000	2370	1020	352	83	2.9	Class 10
ISO 5	100,000	23,700	10,200	3520	832	29	Class 100
ISO 6	1.0×10^6	237,000	102,000	35,200	8320	293	Class 1000
ISO 7	1.0×10^7	2.37×10^6	1,020,000	352,000	83,200	2930	Class 10,000
ISO 8	1.0×10^8	2.37×10^7	1.02×10^7	3,520,000	832,000	29,300	Class 100,000
ISO 9	1.0×10^9	2.37×10^8	1.02×10^8	35,200,000	8,320,000	293,000	Room air

Table 1. ISO 14644-1 cleanroom standards vs US FED STD 209D standard.

2.3. Clean air facility: closed vs open systems

Two systems for cell culture work at the clean air bench: closed- system vs. open- system. Companies such as Phoenix in N.Y. sell such closed clean air benches. For our purpose of growing cells and selecting ES-cell derived embryoid bodies under a microscope, we could only use the open clean air bench located inside a clean air room.

Clean air rooms are classified according to the number and size of particles permitted per volume of air. There are basic codes of classification for cleanrooms:

1. US FED STD 209D and 209E cleanroom standards [3]:

It denotes the number of particles of size 0.5 μm or larger permitted per cubic foot (ft^3) of air. For example "Class 100/A" is 100 particles/ft^3, "class 1000/B" is 1000 particles/ft^3, "class 10,000/C" is the normal air environment. For a tissue culture bench, it is recommended to operate under condition A, i.e., 100 particles or less/ft^3. One m^3 is 35 ft^3. This criteria of clean room

classification was cancelled by the General Services Administration in 2001 [4, 5]. However, this system is still commonly used by many organizations, including us.

2. ISO 14644-1 standards [6]:

International Organization for Standardization (ISO) codes ISO 14644-1 cleanroom standards are the most commonly used. ISO classifies cleanrooms on a scale from 1 to 9, with 1 being the "cleanest" air. ISO classification 1 cleanrooms have a maximum of 10 particles smaller than 0.1 micrometers in a cubic meter of airspace. Class 9 is used to describe normal room air.

3. BS 5295 cleanroom standards and standards of other countries:

There are many other standards, such as Britain BS 5295, Australia AS 1386, France AFNOR X44101, and Germany VD I.2083. Table 6 in [7] describes the details.

Table 1 lists the comparison of US and ISO systems (1 and 2, above). Small numbers refer to ISO 14644-1 standards, which specify the decimal logarithm of the number of particles 0.1 μm or larger permitted per m^3 of air. The table provides an immediate comparison and understanding of the criteria, such that an ISO class 5 clean room has at most 10^5 particles/m^3. In our facility, we succeeded in achieving (marked in red color) class 1000/ISO 6 for clean air room, class 100/ISO 5 for clean bench open working area.

2.4. A mini-clean air box

For the purpose of growing cells, flasks equipped with mini-filters are available. However, for picking ES-derived EBs, Petri dishes are preferable for the practical reason that an access to the open dish under the microscope is absolutely required. Therefore, we have developed and made "**mini-tissue culture boxes"**. They are self-made, from material such as ready-made plastic boxes or acryl glass derived from Mishell-Dutton cell incubation system, and equipped with an air in-let and out-let system for CO_2 gassing before they are placed into the CO_2 incubator (**Figure 6**).

2.5. Open clean air facility

The clean air facility was located on the second floor of an old building where the Biochemistry Institute of University clinic of Giessen is situated (**Figure 1-A**), having one window (blue arrow). The three windows to the right of this facility are those of my research laboratory.

2.6. Washable wall and doors for the facility

Due to financial considerations, the option to have a commercial hardwall facility inside the existing building wall was not chosen. The existing wall of the space (**Figure 1-B**, purple color) was coated with a transparent vinyl lacquer/paint to reduce the release of particles from plaster.

The newly constructed wall and doors were those between the entrance preparation room (1-B-1) and air shower-airlock (1-B-2); air shower-air lock and the main clean air room (1-B-3) of the facility. The material of the newly constructed wall was white-painted iron frame and acryl

glass (shown in **Figure 1-C**). The surface was smooth, washable, and inert, so that it will not attract and accumulate dust. A row of brushes (black color in **Figure 1-C**, indicated by a blue arrow) was fixed to the bottom of the door (blue triangles in 1-B-1 and 1-B-2) to direct the air flowing outward. Above the window was an air-conditioning apparatus (blue box).

The facility (**Figure 1-B**) was constructed and divided into the following spaces: an entrance preparation room for changing clothes and a storage shelf (Space 1-B-1), an air shower-airlock with a mobile high efficiency particulate air (HEPA) filter for taking air shower (Space 1-B-2), the main workspace (1-B-3) with a softwall cleanroom (Space B-4) with hanging HEPA filter from the ceiling, and a mobile HEPA filter moving inside and outside the softwall clean room. Inside the softwall clean room, a two-person-laminar-air-flow cabin/clean bench, and two chairs were placed for doing cell culture. The CO_2 incubator could also be placed inside the softwall cleanroom. The red long box indicated the place where the mobile HEPA filter was located. The black long box indicated the place where the hanging HEPA filter was located.

2.7. HEPA filter on wheels

The construction of the mobile HEPA filter is explained in this figure (**Figure 2**). It requires access to a metal workshop so that one can do the metal work by oneself, and/or contracting the design to a skilful mechanic who knows how to work with metal. A frame-in metal carrier with four wheels was designed with the precise size of the outer measurement of the HEPA filter. In this case, the HEPA filter from Zander (EU 3 and H 14, Zander, [8]) was purchased (**Figure 2-A** left image) and the designed construct was made by a mechanic (**Figure 2-A** right lower corner image). The HEPA filter could be lifted with the help of two persons and loaded to fit into/onto this carrier (**Figure 2-B**). The mounted HEPA filter could move around the space freely and securely, no extra screw was needed to fasten these two pieces together for this construct.

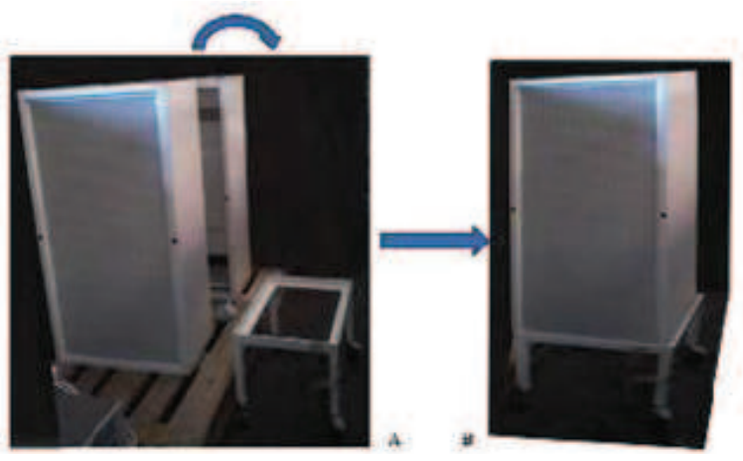

Figure 2. HEPA Filter on the wheels. The left image (A) contains two HEPA filters from Zander (No. EU 3 and H 14). To the right lower corner of these filters is the image of a self-made metal carrier with four wheels. The right image (B) shows a HEPA filter loaded on this wheeled metal carrier to become mobile.

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2.8. Soft-wall clean room self-construction

2.8.1. HEPA filter to hang from the ceiling

The same type of HEPA filter from Zander (EU 3 and H 14) was used for this construction (**Figure 3-A**). The HEPA filter was lifted onto self-constructed metal in-frames and suspended with additional metal support from the ceiling of the room (**Figure 3-B**).

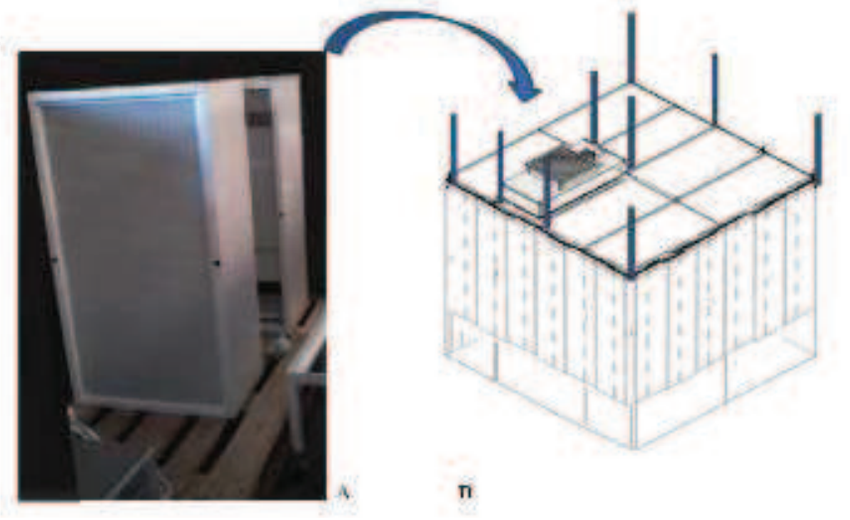

Figure 3. HEPA filter hanging from the ceiling. The HEPA filter from Zander (A) was lifted onto metal in-frames (B). For construction of soft wall, the additional material such as metal frame, ceiling metal hangers, plastic strips were locally available.

2.8.2. Self-constructed metal frame and heavy plastic hanging strips

If there is sufficient budget, one could purchase and install the commercially available softwall clean room, such as the one from the company, Cleanairproducts [9]. However, we have chosen a lower budget option: to purchase the HEPA filter and to have a skilful worker perform the rest of the construction.

For the construction of the softwall clean room, the additional material such as metal frame, ceiling metal hangers for suspension, heavy plastic strips, were purchased and constructed. The design of metal frame for the softwall clean room depends on the size of the room and the type of metal pieces available locally. **Figure 3-B** shows one option. Around the metal frames were heavy plastic sheets with free flip strips a few centimeters away from the floor to allow the air to escape.

2.8.3. Outfit for working in the clean air facility

Among all the items to enter the clean room, human beings carry the most dust. Thus, it is essential to remove as many dust particles as possible from the workers in the air lock before entering the clean room.

As shown in **Figure 4**, the complete impermeable synthetic suit, either of tightly woven polyester or a nonwoven material, such as Tyvek, is preferable: Examples are polyester suit (Countdown clean systems, Derby, England), Jumpsuit (Tyvek Pro-Tech Classic, DuPontTM, US) was the recommended gown for working in the clean air room [10, 11]. In addition, hair cap, eye glasses (for non-glasses wearing workers), mouth mask, hand gloves, and washable plastic shoes were worn by the worker in the entrance preparation room before taking an air shower in the airlock. Most items were used once and disposed. Exceptions to this general rule were the protecting glasses and plastic shoes. The gown might be recycled a few times, if an X-ray machine is accessible to allow irradiation for sterilization.

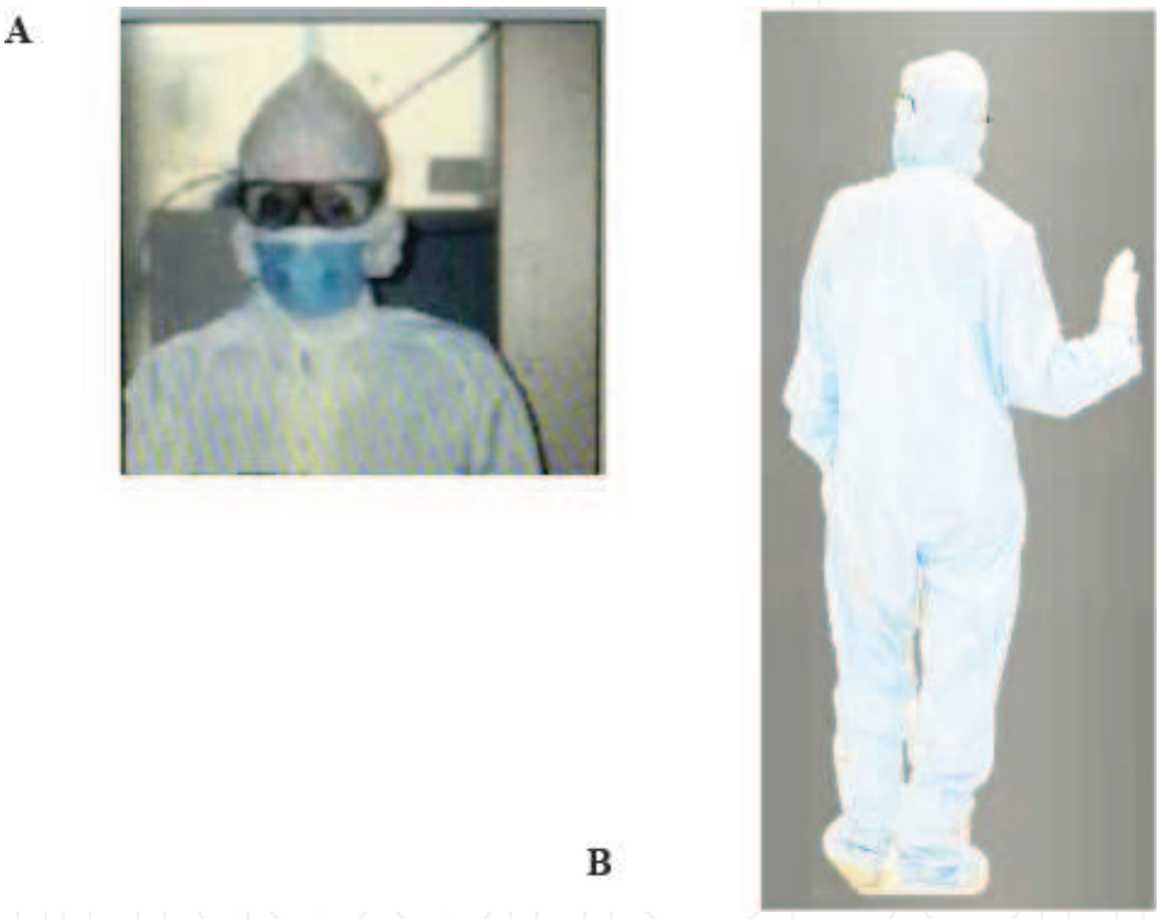

Figure 4. Outfits for working in the clean air facility. A: A staff member wearing glasses, mouth mask, hair hood to cover the hair and skin in the entrance preparationroom before entering the airlock. **Tyvek® Coveralls** gown was the clothes of choice [11]. B: A backview of the clean air gown with shoes (derived from [10]).

2.9. Mobile clean air facility/A clean air facility on wheels

Two containers, Variante 6 from ALHO [12] (**Figure 5-A**), were rented to place in a parking lot of Europaviertal, an industrial area outside the city of Giessen. The enlarged image (**Figure 5-B**) shows the location of HEPA filters (in red) and other items in the facility. The container was built from smooth surface metal material so that no extra coating process to repel dust

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was performed. The space was divided into the following working areas: The container to the left was the preparation space for storage and for changing clothes (Space B-1); an airlock with a mobile HEPA filter for taking air shower (Space B-2); the container to the right was the main clean air working space (B-3). The ceiling of the container was not high enough to accommodate any hanging HEPA filter. Instead, two mobile HEPA filters were placed inside this container. The two-person-clean bench (in black) and two chairs were placed there for cell culture. A CO_2 incubator was placed next to the clean bench for growing cells.

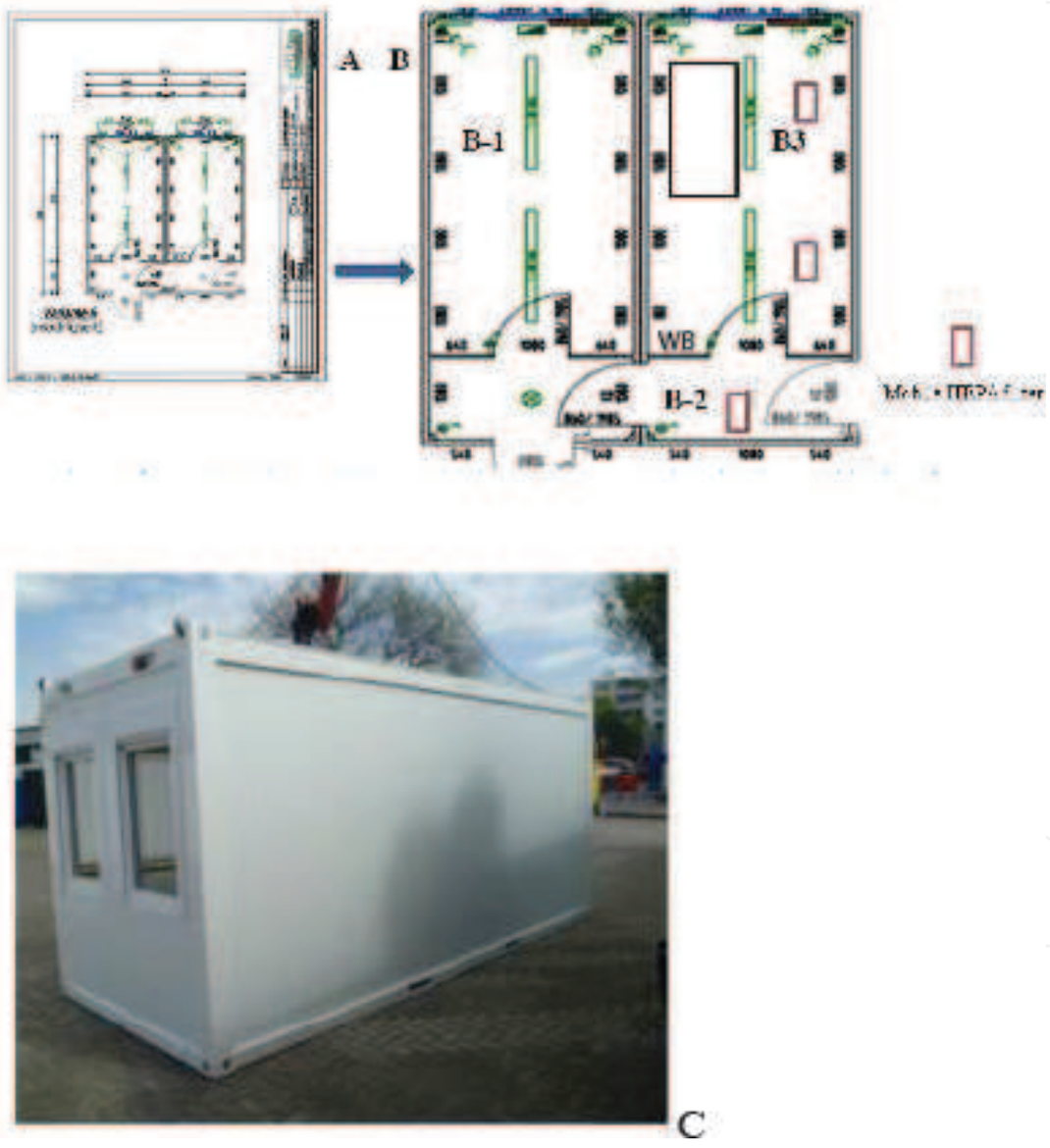

Figure 5. A hard wall clean air facility on wheels. Panel A shows the detailed plan of two containers with windows and doors from ALHO, Type Variante 6 [12]. The enlarged image (panel B) shows how the space was divided. The space was divided into: Space B-1: storage and clothes changing; Space B-2: air lock with a mobile HEPA filter (in red) for air shower; Space B-3: Main clean air facility equipped with two mobile HEPA filters (in red). A two-person-clean air bench (in black), two chairs, and a CO_2 incubator. Panel C shows one container from outside.

Figure 6. A clean air minibox.

3. Conclusion

It took the entire group almost two years to convert a regular cell culture laboratory in an old building located in a clinic campus into a clean air facility. It is well worth the effort to have undertaken such a project. A professional from Heraeus came to visit our clean air facility and did the particle count and particle collection himself. When he was about to leave the airlock, he shook his head and said that it is incredible that our clean air facility is so clean that even the industrial clean air facility could hardly achieve such a clean level. We consider our experiences valuable and worthwhile to share with members of the research community who wish to work in the direction of clinically-oriented projects such as ES cells and iPS. The total cost is estimated to be 10% of the commercial products, an affordable facility for many. In addition, we have moved the clean air facility into two containers and continued the work

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there. The advantage of "clean air facility on wheels" is that it can be shared by several laboratories in a nearby region for performing similar projects such as establishment of clinical grade stem cell lines. Our research work originated in 1987 while I was a scientific member of the Basel Institute for Immunology, and continued through decades in Germany from 1996, exploring different research directions and clinical applications including this clean air facility. For an overview of our research aims and outcome, please see review articles, Discussion Forum, and a World Patent on "Methods for growing stem cells" [13–16].

Acknowledgements

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Appendix 1: Examples of S.O.P.s on how to write, to operate, and to control the practice of a clean air facility. This appendix will have the following three figures:

Figure 7. S.O.P. -1. Into the clean air room

G.M.P./S.O.P.1 J.B.	J.B. / AG Chcn	written on: 11.01.01
		valid until: 11.01.03
Titel: In the clean air room		
	Filled by:_____	on (date):_____
	Controlled by:_____	on (date):_____

About one hour before the operation, one must turn on the HEPA filters to let them running (register in the log book).

In the entrance preparation room:

1. Lab coat, jacket, sweater, etc. remain in the entrance room (The clothes hangers are on the right side).

2. Step onto the sticky mattress and take off the regular shoes and change to clean air room shoes right there.

3. Disinfect hands with 70% alcohol.

4. Tight hair and put on hair net.

5. Disinfect hands, put on hand gloves, and sterilize again.

6. Wear the hair cap over the hair net. Hair must be completely covered!

7. Put on clean room gown.

8. Put on mouth mask and eye glasses (when not wearing glasses).

9. Disinfect hands.

10. The material, which should be brought into the clean room, should be disinfected and put into the basket to carry in.

***In air shower-airlock*:**

11. Enter the airlock and close the door to the preparation room.

12. Stay at least 5 min in the air shower-airlock and turn around regularly.

13. Meanwhile carry out one measurement using the particle-counter and one measurement using the air-collector.

14. Put on the clean room gown hanging on the door to the clean air room.

15. Stand on the sticky mattress, then enter the clean air room.

In clean air room (HEPA filter 1, mobile):

16. Run through once the particle counter and once the air collector.

17. Before entering the softwall clean air space (HEPA-Filter 2, hanging), step on the sticky mattress.

In softwall cleanroom (HEPA-Filter 2, hanging):

18. Before starting to work, carry out once the particle count and once the air collection.

Figure 8. S.O.P. -2. Out of clean air room

G.M.P./S.O.P.2 J.B. J.B. / AG Chcn written on: 16.08.01

valid until: 16.08.03

Titel: Out of the cleanair room

At:_____ Until:_____hr

1. The total surface area inside the soft-wall clean room should be sprayed with 70% alcohol and wait a few minutes to let it absorb.

2. Turn on UV light with the key provided.

3. Turn off clean bench.

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4. Record the data in the log book (Hera safe) and put the log book back to the book shelf.

5. Bring the garbage and agar plates to the entrance-preparation room. (Let agar plates incubate and check for growth).

6. Hang the clean air coat back on the door hook.

7. Take off all other clothes in the entrance preparation room and hang on the hooks or dispose. (Dirty clothes should be autoclaved).

8. Register in the log-book.

9. Turn off HEPA filters.

10. Turn off light.

Name 1 _____

Name 2 _____

Figure 9. S.O.P.-3. Routine clean air room control

G.M.P./S.O.P.3 J.B.	J.B. / AG Chcn	written on: 15.03.01
		valid until: 15.03.03
	Performed & filled by:_____	on:_____
	Controlled by:_____	on:_____

Important: Turn on both HEPA filters in Room 130 and Room 131 at least one hour before the Monday S.O.P. routine control.

Change to clean room gown.

Room 130 (HEPA-Filter 2, i.e. inside the soft wall)

-Read and record the temperature and CO_2-content on a data sheet attached to the CO_2 incubator front door.

-Place one agar plate each (from the refrigerator located in the entrance preparation room) under the Flow/clean bench, on the desk, and in the CO_2 incubator.

-One hour later, bring the agar plates to the incubator in Rm 108 (research lab) and let it incubate overnight (and count the bacteria colonies).

- Afterwards store the agar plates (inside one plastic bag) for four weeks on the wood shelf located in Rm 108 at room temperature, (then count the bacteria/fungi colonies again).

-Clean the flow/clean bench and working desk

-Afterwards, turn on UV light inside the flow/clean bench for one hour

-After that, place one more agar plate under the Flow for one hour, and proceed to incubation and colony counts similar to the above procedure

-Carry out once particle count and once air collection under the Flow and in clean room (see G.M.P./S.O.P.13)

Room 130: (HEPA-Filter 1, i.e. mobile one outside the soft wall)

-Clean the working surface.

-Perform one particle count and one air collection (see G.M.P./S.O.P.13).

-Place one agar plate in the cabinet next to the door to Rm 131 and proceed as of above.

Air lock:

-Perform each time one air collection and one particle count when coming in and going out (see G.M.P./S.O.P.13).

-Place one agar plate on top of the mobile HEPA filter and proceed as of above.

Entrance preparation room

-Read and record the temperature of the refrigerator and -20°C deep freezer in the log book.

-Clean the working surface.

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