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Title:	CONTROLLED CONTAMINATION COMPACT SYSTEM FOR TREATMENT OF CELL LINES	
Documents Submitted:	BIO11BRWO10-appb-000001.pdf (BIO11BRWO10-Description.pdf)	56435
	BIO11BRWO10-appb-000002.pdf (BIO11BRWO10-Claims.pdf)	12240
	BIO11BRWO10-appb-000003.pdf (BIO11BRWO10-Abstract.pdf)	6994
	BIO11BRWO10-appb-000004.pdf (BIO11BRW010-Drawings.pdf)	36653
	BIO11BRWO10-appb.xml	883
	BIO11BRWO10-fees.xml	2246
	BIO11BRWO10-poat-000001.pdf (BIO11BRWO10-POA.pdf)	44334
	BIO11BRWO10-requ.xml	5231
	BIO11BRWO10-resr-000001.pdf (102018000000749-Earlier-Search-Report.pdf)	306235
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PCT REQUEST

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0	**For receiving Office use only**	
0-1	International Application No.	**PCT/IB2019/050055**
0-2	International Filing Date	**03 January 2019 (03.01.2019)**
0-3	Name of receiving Office and "PCT International Application"	**RO/IB**

0-4	**Form PCT/RO/101 PCT Request**	
0-4-1	Prepared Using	**ePCT-Filing Version 4.4.005 MT/FOP 20181226/1.1**
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	**International Bureau of the World Intellectual Property Organization (RO/IB)**
0-7	**Applicant's or agent's file reference**	**BIO11BRWO10**
I	**Title of Invention**	**CONTROLLED CONTAMINATION COMPACT SYSTEM FOR TREATMENT OF CELL LINES**
II	**Applicant**	
II-1	This person is	**Applicant only**
II-2	Applicant for	**All designated States**
II-4	Name	**BIOSCIENCE SERVICES S.R.L.**
II-5	Address	**Strada Rovereta, 42 47891 Falciano San Marino**
II-6	State of nationality	**SM**
II-7	State of residence	**SM**
II-10	e-mail	**g.mucci@bioinst.com**
II-10(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	**exclusively in electronic form (no paper notifications will be sent)**

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III-1	**Applicant and/or inventor**	
III-1-1	This person is	**Inventor only**
III-1-3	Inventor for	**All designated States**
III-1-4	Name (LAST, First)	**MUCCI, Giuseppe**
III-1-5	Address	**Strada Rovereta, 42 47891 Falciano San Marino**
III-1-10	e-mail	**g.mucci@bioinst.com**
IV-1	**Agent or common representative; or address for correspondence**	
	The person identified below is hereby/ has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	**Agent**
IV-1-1	Name (LAST, First)	**RUZZU, Giammario**
IV-1-2	Address	**Via Gulli, 5 40068 San Lazzaro di Savena Italy**
IV-1-3	Telephone No.	**+390516257522**
IV-1-4	Facsimile No.	**+390519515759**
IV-1-5	e-mail	**wipo@studioingruzzu.eu**
IV-1-5(a)	E-mail authorization The receiving Office, the International Searching Authority, the International Bureau and the International Preliminary Examining Authority are authorized to use this e-mail address, if the Office or Authority so wishes, to send notifications issued in respect of this international application:	**exclusively in electronic form (no paper notifications will be sent)**
V	**DESIGNATIONS**	
V-1	**The filing of this request constitutes under Rule 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents.**	
VI-1	**Priority claim of earlier national application**	
VI-1-1	Filing date	**11 January 2018 (11.01.2018)**
VI-1-2	Number	**102018000000749**
VI-1-3	Country or Member of WTO	**IT**

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VI-2	**Incorporation by reference :** where an element of the international application referred to in Article 11(1)(iii)(d) or (e) or a part of the description, claims or drawings referred to in Rule 20.5(a) is not otherwise contained in this international application but is completely contained in an earlier application whose priority is claimed on the date on which one or more elements referred to in Article 11(1)(iii) were first received by the receiving Office, that element or part is, subject to confirmation under Rule 20.6, incorporated by reference in this international application for the purposes of Rule 20.6.		
VII-1	**International Searching Authority Chosen**	**European Patent Office (EPO) (ISA/EP)**	
VII-2	**Request to use results of earlier search; reference to that search**		
VII-2-1	Filing date	**11 January 2018 (11.01.2018)**	
VII-2-2	Application Number	**102018000000749**	
VII-2-3	Country (or regional Office)	**IT**	
VII-2-4	Statement (Rule 4.12(ii)):	**This international application is the same, or substantially the same, as the application in respect of which the earlier search was carried out except, where applicable, that it is filed in a different language.**	
VIII	**Declarations**	Number of declarations	
VIII-1	Declaration as to the identity of the inventor	-	
VIII-2	Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent	-	
VIII-3	Declaration as to the applicant's entitlement, as at the international filing date, to claim the priority of the earlier application	-	
VIII-4	Declaration of inventorship (only for the purposes of the designation of the United States of America)	-	
VIII-5	Declaration as to non-prejudicial disclosures or exceptions to lack of novelty	-	
IX	**Check list**	Number of sheets	Electronic file(s) attached
IX-1	Request (including declaration sheets)	**4**	✓
IX-2	Description	**23**	✓
IX-3	Claims	**4**	✓
IX-4	Abstract	**1**	✓
IX-5	Drawings	**4**	✓
IX-7	TOTAL	**36**	

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	Accompanying Items	Paper document(s) attached	Electronic file(s) attached
IX-8	Fee calculation sheet	-	✓
IX-9	Original separate power of attorney	-	✓
IX-17	Copy of the results of earlier search(es)	-	✓
IX-20	**Figure of the drawings which should accompany the abstract**	**Figure 2**	
IX-21	**Language of filing of the international application**	**English**	
X-1	**Signature of applicant, agent or common representative**	**/Giammario RUZZU/**	
X-1-1	Name (LAST, First)	**RUZZU, Giammario**	
X-1-3	Capacity (if such capacity is not obvious from reading the request)	**Agent**	

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10-1	**Date of actual receipt of the purported international application**	**03 January 2019 (03.01.2019)**
10-2	**Drawings:**	
10-2-1	Received	
10-2-2	Not received	
10-3	**Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application**	
10-4	**Date of timely receipt of the required corrections under PCT Article 11(2)**	
10-5	**International Searching Authority**	**ISA/EP**
10-6	**Transmittal of search copy delayed until search fee is paid**	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	**Date of receipt of the record copy by the International Bureau**	

CONTROLLED CONTAMINATION COMPACT SYSTEM FOR TREATMENT OF CELL LINES

TECHNICAL FIELD

The present invention relates to the technical field concerned with extraction, preparation and colture of cell lines, specifically stem cell lines.

In particular, the present invention relates to a controlled contamination system for the obtainment and complete treatment of cell lines, more specifically stem cell lines, starting from a biopsy sample taken from a donor up to obtainment of a desired quantity of purified cells.

BACKGROUND ART

Over the last few years, techniques of isolation, culture and engineering of different stem cell lines have found space in an ever increasing number of applications, either for therapeutic purposes or in advanced cosmetic surgery treatments. Particularly, for these last applications there is a potential market that is extremely promising also from an economic point of view, for those operators in the fiels who wish to exploit the results of their research and know-how in the improvement of cell engineering techniques and in the creation of "cell factories".

Only by way of example anti-aging treatments, body remodeling by using various fillers, filling of wrinkles and the advanced antibaldness treatments are to be mentioned.

TECHNICAL PROBLEM

Treatment of biological material for the extraction of stem cells and the subsequent obtainment of cellular material with good

quantitative and qualitative characteristics, in terms of purity and sterility, such as to allow implantation in a patient, require execution of a well-defined sequence of operations, aimed at isolating, purifying and expanding the cell lines, until a sufficient quantity is obtained for the treatment of the patient.

The processes of acquisition and preparation of the biopsy sample, isolation and purification of stem cells, their subsequent expansion and possible differentiation, may be different according to the type of sample and cellular material and the preparation of the expanded cell culture for subsequent use (for example, transfer onto three-dimensional "scaffolds", generation of fabric portions for implantation by association with a natural support, such as a fibrin matrix, a gauze of connective tissue or other known support, etc.).

However, in general, the biopsy sample, once taken from the donor, is transported to the treatment site in a special isothermal container, which ensures that optimal conditions of safety, temperature and pressure are maintained to keep the full functionality of the biological material. The sample is then processed by a biologist, in roder to isolate and purify the type (or types) of stem cells that are being cultivated.

The cells so obtained are subsequently expanded in a suitable culture medium, inside incubators, until the desired population is obtained. Finally the expanded culture (or cultures) cells are prepared for the system, or anyway for aesthetic or therapeutic application for which they have been produced.

The entire sequence of biological material processing takes place within "white rooms", highly controlled environments, both for sterility conditions and due to concentration of contaminated particulate matter carried by the air. The control and the consequent removal also concern vapors that may originate inside the

environments, which are generated by the treatment processes or by the operators themselves.

All the equipment and work stations necessary for the operators to perform the different steps of the treatment protocols are included in these environments. In particular, at least one or more laminar flow insulators are required for the manipulation operations of the biopsy sample and of the cellular material, as well as microscopes, centrifuges for the separation of the cell fraction and incubators for expansion of the cultures.

Cleanrooms, in their conventional conformation, are made up of a series of intercommunicating compartments that are insulated from the outside and in which conditions of pressure, temperature and contamination, both bacteriological and particulate, are obtained and rigorously maintained. In particular, contamination is maintained within parameters whose maximum value defines the class of cleanroom or of each of its compartments.

As a pure example, according to the GGMP standard currently applied by pharmaceutical companies, a compartment is defined as Class "A" when concentration of particles > 0.5 micron is less than 3.500/m3, and is zero for particulates > 5 microns, both when non operational and when in operative conditions. It is defined as Class B if the non operational conditions of Class A are maintained in non operating conditions, while concentrations of particulates > 0.5 micron lower than 350.00/m3 and 2.000/m3 are permitted for particulates > 5 microns and under operating conditions.

A strict access control is also provided both for operators and for materials in order to ensure absolute compliance with the contamination conditions. Cleanrooms therefore provide access spaces with double interlocked entrance, inside which decontamination of operators and objects takes place before they

enter the controlled areas.

It is also important to maintain a positive atmospheric pressure difference between compartment classified with lower contamination and those with higher contamination, as well as between these last compartments and the environment outside the cleanroom.

The contamination conditions of each Class for the various environments are obtained and maintained by means of complex systems for filtration and thermohygrometric treatment of the air that is introduced into each compartment, and for forced circulation of the same with turbulent or laminar flow, according to the particular dispersion requirements of the residual particulate.

Purification systems necessary to maintain correct conditions in a conventional cleanroom, which are made using HEPA or ULPA absolute filters, can be particularly complex and expensive to be installed and maintained.

In operating conditions, air included in the cleanroom compartments is almost entirely re-conveyed to the HEPA or ULPA filters, and only a small part is conveyed to the external environment, to obtain the strictly necessary exchange. An equal amount of air is then taken from outside and purified, so as to maintain the overpressure required by the operating specifications. This requires complex and difficult-to-maintain air conveyor ducting systems, and affects the duration of production off-times - and therefore the related costs - required for periodic maintenance of controlled contamination compartments.

In general, design, installation, on-site validation and maintenance of a cleanroom of the type described above require a particularly demanding investment, both from the economic and construction time point of view. As a pure example, the scale of investments can be defined as some millions of dollars, and

implementation times, including system validation and staff training, can take even several years.

However, the financial and time investments required by the implementation of conventional production systems in a clean environment do not allow the already mentioned needs of the stem market to be met for therapeutic or aesthetic uses.

The success of an activity intended for exploiting the potential of such applications is in fact particularly linked to the presence of production facilities operating on site and to how fast these facilities can be made operational.

It is also of fundamental importance that productivity and installation and start up costs of the facilities be adjusted to the immediate potential demand of each territory, and that thay can subsequently be reconfigured in accordance with the trend in demand. Finally, the market response in the sector mentioned above to date in most of the areas potentially including new installations appears not perfectly stable and predictable, and it is therefore also important providing facilities that, in case of non-profitable installations, can be easily moved and installed in more promising sites.

It is therefore evident that the characteristics of the conventional falicities as described above cannot satisfy the requirements deriving from new market opportunities, both in terms of costs and time for their installation, start-up and maintenance.

OBJECTS OF THE INVENTION

Therefore, it is an object of the present invention to propose a compact system for the treatment of cell lines, and in particular of stem cells or stem derived cells, that is self-contained and self-supporting, ie including all the equipment and workstations necessary

for treatment, and therefore adapted to be assembled, tested and validated in factory, and that can be easily installed and made operational in short time on the production site.

A further object of the invention is to propose a controlled contamination compact system for the treatment of cell lines that is capable of ensuring times necessary for obtaining cleaning conditions, as well as periodic or extraordinary maintenance performance times, and also time for restoration from eventual failure situations that are much shorter than those of other conventional controlled contamination systems.

Another object of the invention is to propose a compact system for treatment of stem cell lines, whose total cost for production, installation and maintenance is much lower than that of a traditional cleanroom system.

Another object of the invention is to propose a compact system for treatment of stem cell lines capable of being moved in its operative configuration up to a different production site.

SUMMARY OF THE INVENTION

The aforementioned and other objects are all obtained in accordance with the content of the claims, by means of a controlled contamination compact system for treatment of cell lines, in particolar stem cell lines, and in particular stem cell lines, that includes:

– a supporting structure, designed to form lateral faces of a closed cabinet;

a floor, extended over the entire surface of the cabinet and airtight connected to the supporting structure and to the lateral faces;

- a controlled access, forming an interface between the cabinet and an external environment, provided on a lateral face of said cabinet and adapted to allow access by an operator;

- an absolute filtering HEPA module with laminar flow, mounted in the upper part of the cabinet, to form, or cooperate to form, the ceiling of the same, and designed to generate a downwardly directed laminar flow of overpressured filtered air.

The system also comprises a plurality of internal partition walls, supported by the supporting structure and/or by the lateral faces in order to define operating compartments within the cabinet, which communicate with each other and are adapted to be brought and maintained at different levels of contamination. The operating compartments comprise at least one working compartment, an intermediate compartment and an access compartment, which are set in communication with one another.

The filtering module is provided with filtered air laminar emission outlets made in all the operating compartments. Discharge openings are made in the floor and act as air intakes aimed at channelling the flow of filtered air coming from the outlets and at conveying it to the outside of the cabinet, thus reducing the turbulence in the same cabinet to a minimum.

The system further comprises:

a laminar flow hood, placed inside an operation compartment of the cabinet in a region close to an outer side thereof;

- a plurality of laboratory apparatuses and pieces of equipment for the treatment and the culture of cellular material, arranged inside the laminar flow hood or the operation compartment, or directly accessible by the same.

BRIEF DESCRIPTION OF THE DRAWINGS

The characteristics of the invention, as will result from the claims, are highlighted in the following detailed description, with reference to the attached drawings, in which:

- Fig. 1 illustrates a schematic plan view of an embodiment of the controlled contamination compact system according to the invention;
- Fig. 2 shows a three quarter lateral perspective view of the compact system of Figure 1;
- Fig. 3 shows a view of the inside of the compact system according to the section line III-III of figure 1;
- Fig. 4 shows a front view of the compact system of Figures 1 and 2, with some features highlighted.

DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

With reference to Figure 1, and to a preferred, but not exclusive embodiment of the invention, 100 indicates, as a whole, a controlled contamination compact system, suitable for the treatment of cell lines, made according to a preferred embodiment of the invention.

The compact system 100, in the illustrated configuration, is conceived to carry out all the operations for treatment of cell lines, and in particular of stem cell lines, in a controlled environment; for example, manipulation of a biopsy sample taken from a donor, separation of cellular material, purification and expansion of the same, up to obtaining a quantity of cellular material that is undifferentiated or already differentiated, inserted or not in a two-dimensional or three-dimensional support necessary to carry out the implantation in the donor itself or in another subject.

With the term "controlled environment" we mean an environment, inside the system 100, in which conditions of temperature, pressure, humidity and particle and biological contamination, necessary to qualify the environment as "cleanroom",

and specifically to identify it according to one of the "cleaning" classes of the same, are obtained and kept under control.

In essence, the compact implant 100 of the invention is capable of replacing in all respects, considering productive volumes compatible with its dimensions and the equipment and the instrumentation contained in it, a laboratory for conventional cell cultures as previously described.

Basically, the compact system 100 comprises a supporting structure 10, intended to constitute the side faces of a cabinet 1 closed on four sides. With reference to Figures 2, 3 and 4, the supporting structure 10 comprises a bearing frame 11, made of channel or box-like elements, and a plurality of cladding panels 12, which are so shake as to cover and seal the walls of the cabinet 1.

The vertical uprights of the frame 11 have feet 13 provided to rest on or be fixed to the ground.

The system 100 also provides a raised floor 8, which is airtight fixed to the frame 11 and to the side panels 12. The floor 8 is raised above the ground level at which the system 100 is installed, so that underneath the floor 8 a discharge chamber 9 is defined, closed laterally by grid panels 9a.

At one of the side faces of the cabinet 1 a controlled access 2 is provided (figure 1) for allowing access to the inside of the cabinet 1 by operators and cleaning material. In the embodiment shown herein, the controlled access 2 is formed by a sliding door with controlled opening and closing motion, and provided with suitable sealings to ensure air tightness when closed.

The ceiling of the cabinet 1 is provided by a laminar flow filtering module (20), or by a series of such modules according to their size, provided with absolute filters HEPA or ULPA, motor-driven fans aimed at generating a continuous flow of filtered air towards the

inside of the cabinet 1, and devices for control and regulation of temperature and humidity of the filtered air. The air flow is generated by the motor-driven fans in a plenum above the ceiling, and from latter this conveyed to the absolute filters HEPA or ULPA.

A series of laminar emission outlets 20a is provided in the ceiling of the cabinet 1, as exits from the aforementioned absolute filters, and are designed to generate a laminar flow F of overpressured filtered air directed downwards, inside the cabinet 1.

Once that a condition of particle contamination below a predefined threshold is obtained inside the cabinet 1 (for example, such as to classify the cabinet 1 in Class B of the GMP standard), the laminar flow coming from the absolute filters allows maintainance of this condition indefinitely, and, in addition, an optimal adjustment of temperature and humidity conditions of the environment.

According to a feature of the invention, air discharge openings 30 are provided in the floor 8 for continuously discharging the flow of air coming from the filtering module 20 to the environment below the floor. In particular, the discharge openings 30 are arranged regularly inside the cabinet 1, so as to maximize interception of the laminar flow F coming from above and keep perturbation of the laminar flow itself at a minimum.

The air flow is then conveyed to the discharge chamber 9 and from there to the external environment through the grid panels 9a, due to the overpressure generated inside the cabinet 1 by the laminar flow.

The cabinet 1 is divided into a plurality of internal operating compartments 21,22,23 by means of suitably mounted airtight partition walls and controlled curtain-type accesses. Each operating compartment is crossed by a laminar flow, which can be adjusted in accordance with different flow rates in order to obtain different levels

of contamination thereinside, so that it can be classified in different GMP classes according to its task and the flow adapted thereto.

The operating compartments are arranged according to a preset path for the operator, which corresponds to the different procedural steps that the same performs during a work session.

In particolar, (see Figures 1 and 2), an access or entry/exit compartment 21 is located immediately downstream of the controlled access 2 and is generally maintained at a class D contamination level. The entry/exit compartment 21 communicates, via a first curtain-type access 17, with an intermediate or dressing room compartment 22, which is maintained at a level of contamination in class C, or is already in class B, in which the operator takes uncontaminated clothing and puts it on. The locker room ocmpartment 22 communicates, in turn and again via a second curtain-type access 18, with a work compartment 23, maintained in contamination class B. In the latter compartment all the operations requiring handling of the biological material are carried out.

A class A laminar flow hood 40 is located in the work compartment 23 (Figures 1, 3 and 4), which constitutes the effective work station of the cabinet 1, in which an operator performs all the handling operations on the biopsy sample and the cellular material needed during the treatment process.

In the embodiment shown herein, the hood 40 is on the rear face of the cabinet 1 (see Figure 4). Since pre-assembled and certified laminar flow hoods are available on the market, it is conveniently mounted on a rear extension 10a of the supporting structure 10.

The hood 40 comprises a working surface 41, which extends along the entire length of the same, and a front access for the operator's arms. Moreover, an HEPA absolute filtering system is

provided to certify the degree of contamination inside the class A hood. According to a construction technique used in the invention, the hood 40 comprises a front glass wall, not shown, manually operated or motor-driven. The front wall can slide between a raised or operative position, in which the operator can introduce the arms inside the hood 40, and a lowered or non-opertive position, in which the hood is closed and substantially isolated in respect of the work compartment 23.

According to a particular aspect of the invention, the laminar flow hood 40 is mounted in the cabinet 1 in such a way that it can be extracted. Basically, the entire self-bearing block of the hood 40 is mounted slidingly on supporting guides suitably provided in the rear extension 10a of the structure of the cabinet 1. In this way the hood 40 can be extracted, totally or only in part, and subsequently reassembled in the cabinet 1 for maintenance, repair or replacement needs (see dash line section in figure 4). In any case, this makes it possible to operate on the laminar flow hood 40 without having to dismantle important parts of the cabinet 1, and therefore without having to repeat the decontamination and certification procedure of the entire cabinet.

A chair 44 can optionally be provided inside the work space 23, in front of the work surface 41 of the hood 40.

According to a feature of the invention, inside the laminar flow hood 40, inside the work compartment 23, or in any case in locations directly accessible therefrom, there are also provided laboratory equipment and apparatuses sufficient to allow treatment and culture of cellular material, in particolar sufficient to perform the entire procedure of treating a stem cell line.

In the herein described embodiment a microscope 42 is provided and positioned resting on the work surface 41. A compact

centrifuge 43 for the mechanical separation of cellular material is placed inside a fluxed niche 24 made next to the aforesaid dressing room compartment 22 and communicates with the work compartment 23. This niche 24 is provided with a raised support surface, designed to allow easy access to the centrifuge 43 by the operator and to a propre laminar flow generation system, with an filtered air outlet and a grilled discharge opening 30 for the outflow of the same. In this regard, the supporting surface can conveniently be constituted by a grid, so as to minimize the hindrance caused to the air flow coming out of the niche 24.

One or more incubators 45 are also provided in the work compartment 23, for the expansion of the cultures obtained in the hood 40. Depending on production requirements, however, two or more incubators 45 can be installed in the same way, stacking them on the first one and/or positioning them on the wall opposite thereto.

According to a feature of the invention, shown in Figures 1 and 2, the incubators 45 are mounted in the outer part of the cabinet 1, on extensions of the supporting frame 11, in such a way that the respective access doors are facing the inside of the cabinet 1, while the bodies of the incubators occupy the space outside it. The interface between each incubator 45 and the wall of the cabinet 1 is properly sealed, to prevent any contamination from the outside. An incubator 45 visible in the above figures is mounted, for comfort and operational efficiency, in such a way as to make it directly accessible to the operator from inside the laminar flow hood 40. Of course, the same can alternately be mounted on the wall of the cabinet 1 facing the work compartment 23, with its own door that can be opened from this last mentioned compartment.

In this way, the space inside the cabinet can be optimized obtaining a minimum total volume of the same, and, above all,

reducing obstacles for the laminar airflow F inside it, and therefore the turbulence, to a minimum.

The access doors of each incubator 45 are conveniently arranged at such a height as to be easily accessible by the operator and are located in the upper part of the work space 23, in which the airflow F is less disturbed and retains all the laminariness characteristics.

Depending on production requirements and size of the cabinet 1, more incubators 45 can be mounted side by side or stacked, keeping anyway their respective bodies outside the cabinet 1 and the access doors thereinside, flush with its external wall, in the region of the work space 23.

According to a feature of the invention, the compact system 100 also advantageously comprises a pair of first 60 and second 63 fluxed "pass-box", designed to allow safe introduction, from the outside of the cabinet 1, of biological material, reagents and other into the operative area defined by the work space 23, and also to allow exit of waste material from the same work space 23 without risk of contamination of the same compartment. In this regard, both pass-boxes 60, 63 are conveniently provided with a double interlocked access 61, 62.

In particolar, (see Figure 1), the first pass-box 60 is installed outside the cabinet 1, in the region of the work space 22. and allows interchange between the latter and the outside. The second pass-box 63 is installed between the access space 22 and the work space 23, and allows interchange between them.

The presence of the double fluxed and interlocked pass-box is provided to fully meet the most strict requirements for contamination of working environments in the highest purity classes. Basically, from an operational point of view, all incoming material is packaged within

a double wrapper (double- or triple-wrapped material). The first wrapper is removed by an operator who picks it up from the first pass-box 60 inside the access space 22, and which subsequently introduces it into the second pass-box 63. The operator inside the work compartment 23 then proceeds to withdraw it and remove the second wrapper.

The aforementioned pass-boxes 60, 63 each comprise a HEPA absolute laminar flow filter module, located in the upper part of the same pass-box 60, 63 and a grid floor, set in communication with the drainage compartment 9, intended for keepig perturbation of the laminar flow at a minimum.

In the embodiment shown herein of the system 100, a third fluxed pass-box 15 is also provided in the wall of the cabinet 1 which faces one side of the laminar flow hood 40, substantially made as the previously described first pass-box 60, and also equipped with double doors with interlocked access. This is intended for the direct exit of biological material for which the working cycle has been completed, from the work station formed by the hood 40 towards the external environment.

The third pass-box 15, according to an aspect of the invention, is provided with protection means aimed at ensuring a high level of safety from contamination coming from the outside for the cabinet 1. In particular, in order to minimize the probability of a communication to be established between the outside and the work space 23, enablement measn are provided for the outer door of the pass-box 15 to open only and exclusively when the aforementioned front wall of the hood 40 is totally lowered. From an operational point of view this means that the work on the biological material has been completed and the finished product has been introduced in the third pass-box 15.

The structure of the protection means and the above-mentioned enablement means, which usually comprises mechanical or electromagnetic locking devices, actuators and various sensors, can be physically realized according to different construction techniques, within the reach of the medium-experienced designer technician.

According to the invention, one or more fluxed cabins 65 can be provided, for example installed on a wall of the locker room compartment 22 or of the access space 21, and accessible both from the aforementioned compartments and from the external environment. These cabins 65 are designed to accommodate sterile and uncontaminated clothing, process waste and other, which have to be stored or transferred from the external environment inside the cabinet 1 and vice versa.

The main procedures for using the compact system 100 will be briefly described in the following, as optimized according to the structure of the same system.

EXAMPLE PROCEDURE FOR THE USE OF THE SYSTEM

The compact system with controlled contamination for the treatment of cell lines is a work station able to meet the requirements dictated by the processing of biological material in a controlled contamination environment. The compartments have been designed with the aim of combining the portability of the structure and the minimization of risks for the biological sample, in terms of possible biological contamination and exposure to particulate material of any nature.

The standard operating procedures, already conceived and certified to be applied in a standard controlled contamination environment, must be able to find the same application in the mobile

and compact system of the invention, with some indispensable and obvious differences, due to the structure itself of the installation and the positioning of the compartments.

In particular, the standard operating procedures that must necessarily be applied in order to comply with the processing requirements of biological material, concern the passage (entry and exit) of personnel and material (biological and non biological) and the processing of the biological product which, in all its phases, it must take place under controlled contamination conditions.

Material handling

The material entering/leaving the controlled contamination compact system is divided into biological and non-biological material.

In particolar, incoming biological raw materials include:

- Lipoaspirate
- Frozen intermediates
- Finished product

Incoming non-biological materials are:

- Reagents, solutions and media for production and quality control
- Primary disposable materials for production and quality control (falcon tubes, tips, flasks, cryovials, syringes, etc)
- Clothing for Clean Room
- Materials for clearing for Clean Room

The outgoing material, of a biological nature, includes:

- Finished and intermediate product to be frozen
- Samples for quality control
- Solid/liquid waste (exhausted land, reagents and waste solutions, used disposable materials, containers of biological material, reagent/solution empty containers, used clothing, used cleaning materials).

While outgoing non-biological material includes:

- non-hazardous waste (enclosures of disposable material).

The handling of the material in the controlled contamination system is carried out using the aforementioned pass boxes first 60, second 63 and third 15, both in and out.

The biological material and any other necessary material for every single work session, all with at least double wrapper, are transferred up to class B of the system (work compartment 23) through the first 60 and second 63 pass boxes, according to what has already been described.

The clothing used in the work space, through the pass box, is stored in class C lockers 65, and remains available for each entry of the operators.

Cleaning materials used in rooms classified D or C (inlet compartment 21 and possibly the intermediate compartment 22), are introduced into the appropriate areas by the cleaning operators entering the module carrying said materials; the material required in the class B room is introduced through the aforementioned first 61 and second 63 pass boxes.

At the end of processing, any outgoing biological product (intermediate to be frozen or finished product to be released) is transferred to the outside through the already described third pass box 15, integral with the Class A hood 40, whose opening, inside the hood 40, it only takes place at the end of processing, while it is carried out from the outside only when the work session is completely finished, with the laminar flow hood 40 completely closed. The biological product, before being placed in the third exit pass box 15, must be closed inside the sterile primary container properly identified, in turn positioned, always in class A, inside a secondary container.

The waste coming from the processing in the hood of each

single batch (for example: pipettes, flasks, filters, test tubes and packaging wrappers) are placed in a "biohazard" waste bag, together with the liquid waste (sterile "waste" labelled bottle). At the end of the batch processing, the bag is closed under the hood and placed in the appropriate steel container for waste that can be accessed directly from the class A hood.

At the end of each working day, all the clothing used is stored inside the waste bins in the locker room compartments; the cleaning operator at the end of the working day eliminates the bag transporting it through the dressing room compartments to be disposed of as biohazardous waste.

At the end of the cleaning operations after the work session, all the material used to be removed is placed in the biological waste bag and eliminated.

The materials passing through the pass box will be divided into three categories:

- Triple-wrapped materials

For this type of material the operator, wearing gloves, opens the first pass box 60 at the "dirty side", opens the outer envelope and drops the stuffed material contained thereinside on the perforated surface of the pass box 60.

The operator shall take care not to touch the material with his bare hands but always with gloves, and thaking care not to touch the inner edges and the inner envelope of the stuffed material, rather always and only the outer edges of the same.

- Double-mono packaged or unwrapped materials

For this type of material a sanitization with IPA is required before the introduction in the pass box. Using gloves, the operator sprays the sanitizer on the entire surface of the incoming material and places it on the floor of the pass box.

- Material being defrosted or cold

For this type of material a sanitization with IPA is required before the introduction in the pass box. Using gloves, the operator sprays the sanitizer on the entire surface of the incoming material and places it on the work surface of the pass box, in the appropriate container (rack or basin). Staff movement

Personnel entering/exiting the controlled contamination system include:

- Clean Room operators;
- Cleaning staff;
- Appropriate trained external personnel (whose access is limited to cases of real necessity).

Personnel access the controlled contamination system along a path through locker room compartments and areas intended to increase the degree of cleaning and therefore the classification. In particolar, the operator enters the Class D first compartment 21 (subdivided into dirty and clean areas), then passes into the intermediate compartment or locker room Class C compartment 22 and, finally, into the Class B work compartment 23, where the Class A laminar flow hood is present. The input/output of the operators is regulated in such a way as to prevent multiple operators from passing through the same room at the same time.

Access to the first locker room is allowed only through the access code. Once in Class D, the operator must get rid of the work clothes by placing them in the appropriate locker 65, remove the boots and wear a pair of single-use socks passing the line that delimits the "dirty" area from the "clean" one, wearing a cap, mask and a beard cover (if necessary) which are presente in the cabinet 65, sanitize the hands with an appropriate alcohol solution.

Once these procedures have been completed, the operator

can access the second locker room compartment 22 through the curtain (Class C or B). In this area the operator must wear a pair of gloves after sanitizing them with an appropriate alcohol solution, wear an undersuit taking care that it does not touch the floor, and finally wear the shoe covers while going beyond the line that delimits the "dirty" area from the "clean" one. Passing in the Class C or B locker room compartment 22 allows the operator to open an envelope, taken from the cabinet 65, containing the disposable sterile coverall and to wear it, followed afterwords by a package including sterile single-use gloves.

At this point the operator has the possibility to enter the last class B compartment, which represents the area where it is located during the sample processing step in class A.

At the end of the processing, the operator follows the same path backwards, taking the garments off in the opposite order with respect to the one followed while wearing them. Disposal of this material follows what is described in the paragraph on material handling.

Biological sample treatment

The biological samples are received in unclassified environments, following specific operative procedures. the treatment in classified environment takes place in special cases and only when the sample, if it meets all the required specifications, is brought into the controlled contamination system, directly in a Class A compartment (hood 40).

Each treatment step takes place inside the laminar flow hood 40 present in the system; this is the only environment in which the operator can work on the sample, handling it and opening the container(s) containing the sample.

During the processing period, when the sample is not

manipulated by the operator, it is incubated inside the appropriate incubators, whose only access is from the Class B environment (work compartment 23). This ensures that the sample never comes out of a Class B environment.

At the end of the processing, the sample is taken out of the system by means of the third dedicated pass-box 15, integral with the laminar flow hood 40, as previously described.

The controlled contamination compact system 100 described above has peculiar characteristics that make it particularly advantageous in applications for the treatment of biological materials, extraction and purification of cellular material and in particular of stem cells, expansion and preparation thereof, compared to all the controlled contamination systems ("Cleanrooms") currently foreseen for the execution of the aforementioned operations.

In particular, the entire system 100 can be advantageously assembled and tested at the factory, then transported to the site of use and simply installed on site. Also all the operations necessary for the first activation of the system 100, including the decontamination operations of cabinet 1 and the equipment contained therein, are extremely simplified, by virtue of the simplicity and linearity of the internal structure of the cabinet 1 and its dimensions. In this regard, it has been verified that times for obtaining low contamination conditions to make the system operational can be even less than an hour.

assembly, testing and validation operations of the system can be easily standardized, as the dimensions, structural simplicity and overall cost allow production in series thereof. It has been verified that the whole procedure requires no more than 30-40 days compared to several months, or even a few years, of a conventional "cleanroom".

Also for these reasons, the total cost of the compact system

according to the invention can be contained in a fraction of that of a conventional "cleanroom", (some hundreds of thousands of euro compared to a few millions).

Furthermore, the production capacity of a site can be easily sized according to needs, by installing (and possibly uninstalling) one or more compact systems 100. In fact, in the case of reduced production needs, or failure of the initiative, the compact system 100 can be immediately transported, without any disassembly and removal operation being necessary, and installed in a different operating site.

Finally, it should be noted that, in the compact system 100, the set of laboratory equipment and apparatuses for the treatment and the culture of cellular material can, in case of particular needs, be divided between two or more cabinets 1, which can be installed in the same site, in proximity or in communication with each other, without however leaving the scope of the present invention.

It is understood that the above has been described purely by way of non-limiting example. Therefore, possible changes and different variations of the invention are considered within the protective scope granted to the present technical solution, as described above and claimed below.

CLAIMS

1. Controlled contamination compact system for treatment of cell lines, and in particolar stem cell lines, comprising: – a supporting structure (10), designed to form lateral faces of a closed cabinet (1); a floor, extended over the entire surface of said cabinet (1) and airtight connected to said supporting structure (10) and to said lateral faces; - at least one controlled access (2), forming an interface between said cabinet (1) and an external environment, provided on a lateral face of said cabinet (1) and adapted to allow access by an operator; - at least one absolute filtering module (20) with laminar flow, mounted in the upper part of said cabinet (1), to form, or cooperate to form, the ceiling of the same, and designed to generate a downwardly directed laminar flow of overpressured filtered air, said system (100) being characterized by further comprising: a plurality of internal partition walls, supported by said supporting structure and/or by said lateral faces in order to define operating compartments (21,22,23) within said cabinet (1), which communicate with each other and are adapted to be brought and maintained at different levels of contamination, said operating compartments comprising at least one operation compartment (23), an intermediate compartment (22) and an access compartment (21), which communicate with each other through controlled accesses; that said at least one filtering module (20) is provided with filtered air laminar emission outlets (20a) made in all the above mentioned operating compartments (21,22,23); with a plurality of discharge openings (30) being provided in said floor and designed to act as air intakes aimed at channelling the flow of filtered air coming from said openings (20a) and at conveying

it to the outside of said cabinet (1), thus reducing the turbulence in the same cabinet to a minimum; and by further comprising: a laminar flow hood (40), placed inside an operation compartment (23) of said cabinet (1) In a region close to an outer side thereof;

- a plurality of laboratory apparatuses and pieces of equipment (42,43,45) for the treatment and the culture of cellular material, arranged inside said laminar flow hood (40) or said operation compartment (23), or directly accessible by the same.

2. Compact system according to claim 1, characterized in that, in said floor, the exhaust openings (30) are arranged in axis with corresponding outlet ports (20a) for the exit of filtered air.

3. Compact system according to claim 1, characterized in that said floor is arranged above ground level with respect to the plane in which the system is installed, and in that the aforementioned discharge openings (30) lead to a discharge chamber (9), in turn provided with side openings (9a) in order to distribute the air outlet in overpressure.

4. Compact system according to claim 1, characterised in that said laboratory apparatuses and pieces of equipment comprise: a microscope (42) mounted inside said laminar flow hood (40); a centrifuge (43) for mechanical separation of cellular material, also mounted in said operating station (40); at least one cell cultures incubator (45), mounted on an external wall of the aforesaid cabinet (1),near to said laminar flow hood (40) and having its own access door accessible from inside the cabinet (1).

5. Compact system according to claim 1, characterised in that said laminar flow hood (40) is self-supporting and can be extracted from said cabinet (1).

6. Compact system according to claim 5, characterised in that

sliding support guides are provided in said cabinet (1), and in that said laminar flow hood (40) is mounted slidingly on said support guides.

7. Compact system according to claim 1, characterised in that said discharge openings (30) are made in the entire floor of said cabinet (1).

8. Compact system according to claim 4, characterised in that said at least one incubator (45) is fastened to a side face of said cabinet (1), with its own body (46) outside the same, and with the corresponding access door facing the inside of the same cabinet (1).

9. Compact system according to claim 1, characterized by further comprising: a first fluxed "pass-box" (60), made between said operation (23) and access (21) compartments, and designed to set said operation compartment (23) in communication with said access compartment (21), said first pass-box (60) being provided with with an interlocked double access (61, 62) designed to allow exchange of objects between said two compartments; a second fluxed "pass-box" (63), made between the external environment and said access compartment (21), designed to set this latter in communication with the outside, said second pass-box (60) being provided with an interlocked double access in order to allow the exchange of objects between the outside and said cabinet (1).

10. Compact system according to claim 1, characterized by further comprising a third fluxed "pass-box" device (15), mounted in said cabinet (1) in a region close to a side of said laminar flow hood (40), with the body outside the same said cabinet (1) and with an access door facing the inside of the same substantially flush with its inner wall, said third fluxed pass-box (15) being

provided with an interlocked double access in order to allow the output of the processed product from inside the aforesaid hood (40) to the environment outside the system (100), safety means being provided to allow the output of the processed product only in a situation of complete closure of said laminar flow hood (40).

ABSTRACT

The controlled contamination compact system for treatment of cell lines comprises:

- a supporting structure (10);
- a floor;
- a controlled access (2);
- an absolute filtering HEPA module (20) with laminar flow.

The system (100) also comprises a plurality of internal partition walls, defining operating compartments (21,22,23) within said cabinet (1). The operating compartments comprise at least one operation compartment (23), an intermediate compartment (22) and an access compartment (21), which are set in communication with one another.

The filtering module (20) is provided with filtered air laminar emission outlets (20a) made in all the operating compartments (21,22,23).

The system 100 further comprises:

a laminar flow hood (40), placed inside an operation compartment (21) of the cabinet (1);

- a plurality of laboratory apparatuses and pieces of equipment (42,43,45) for the treatment and the culture of cellular material.

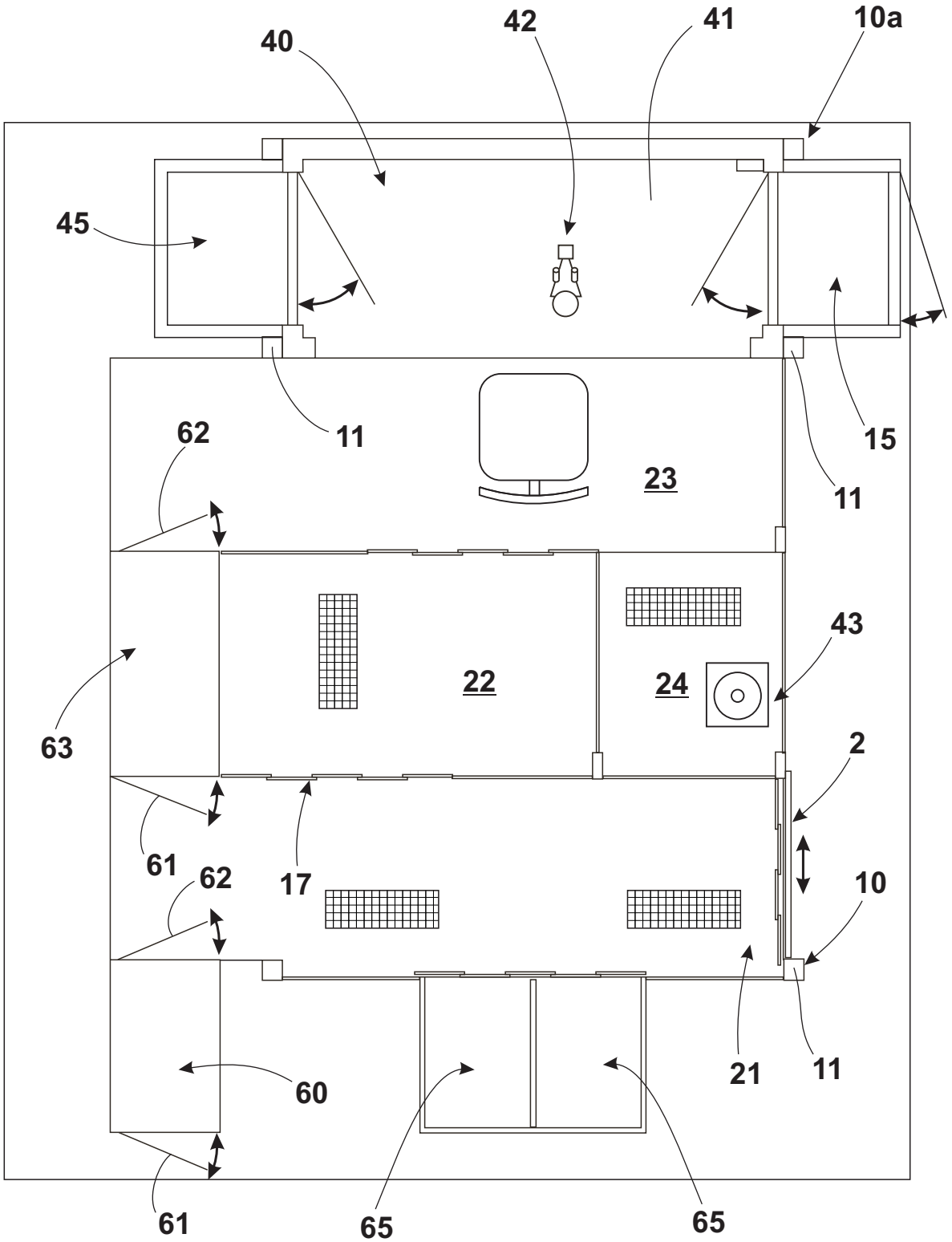
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FIG. 1

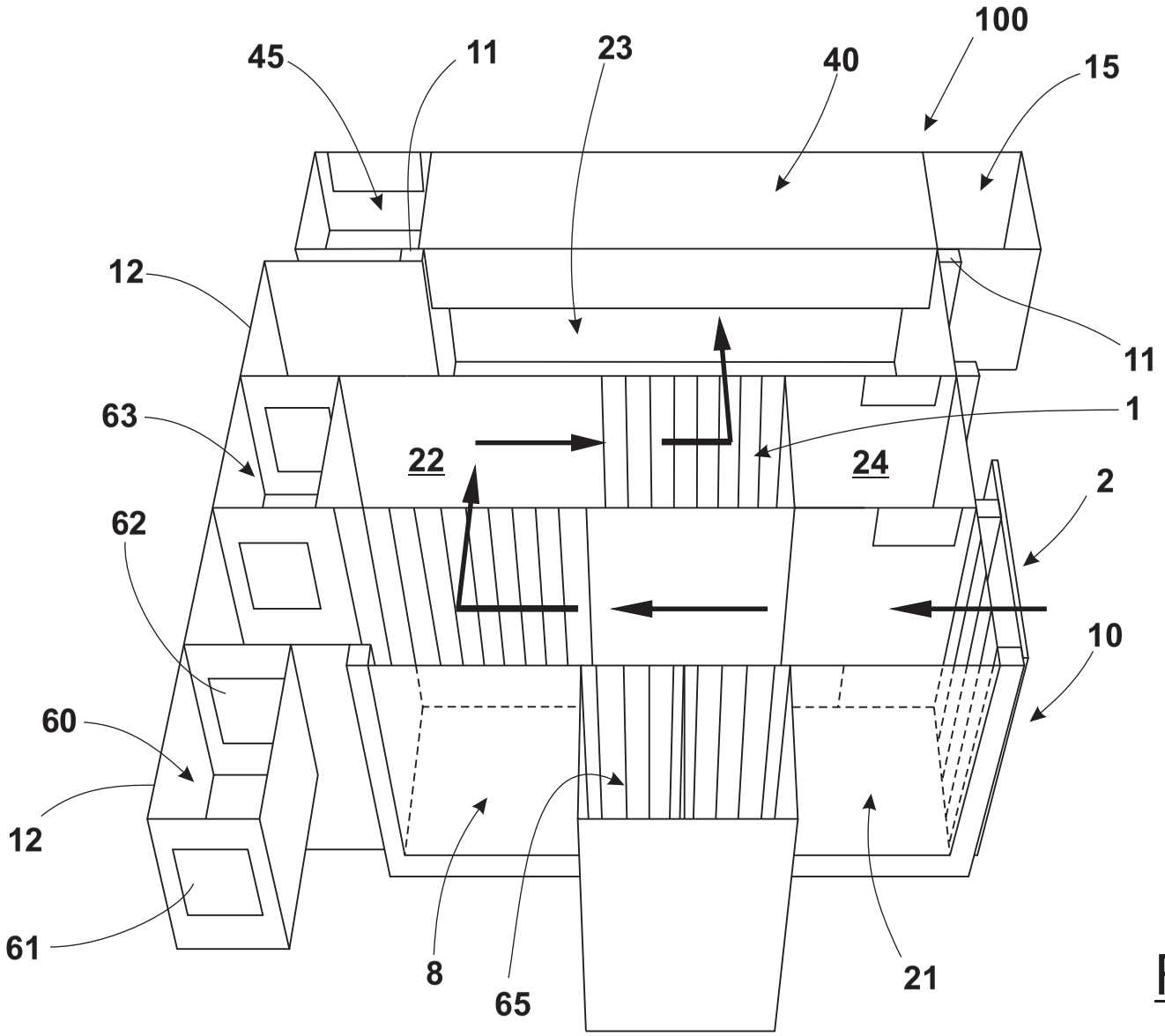

FIG. 2

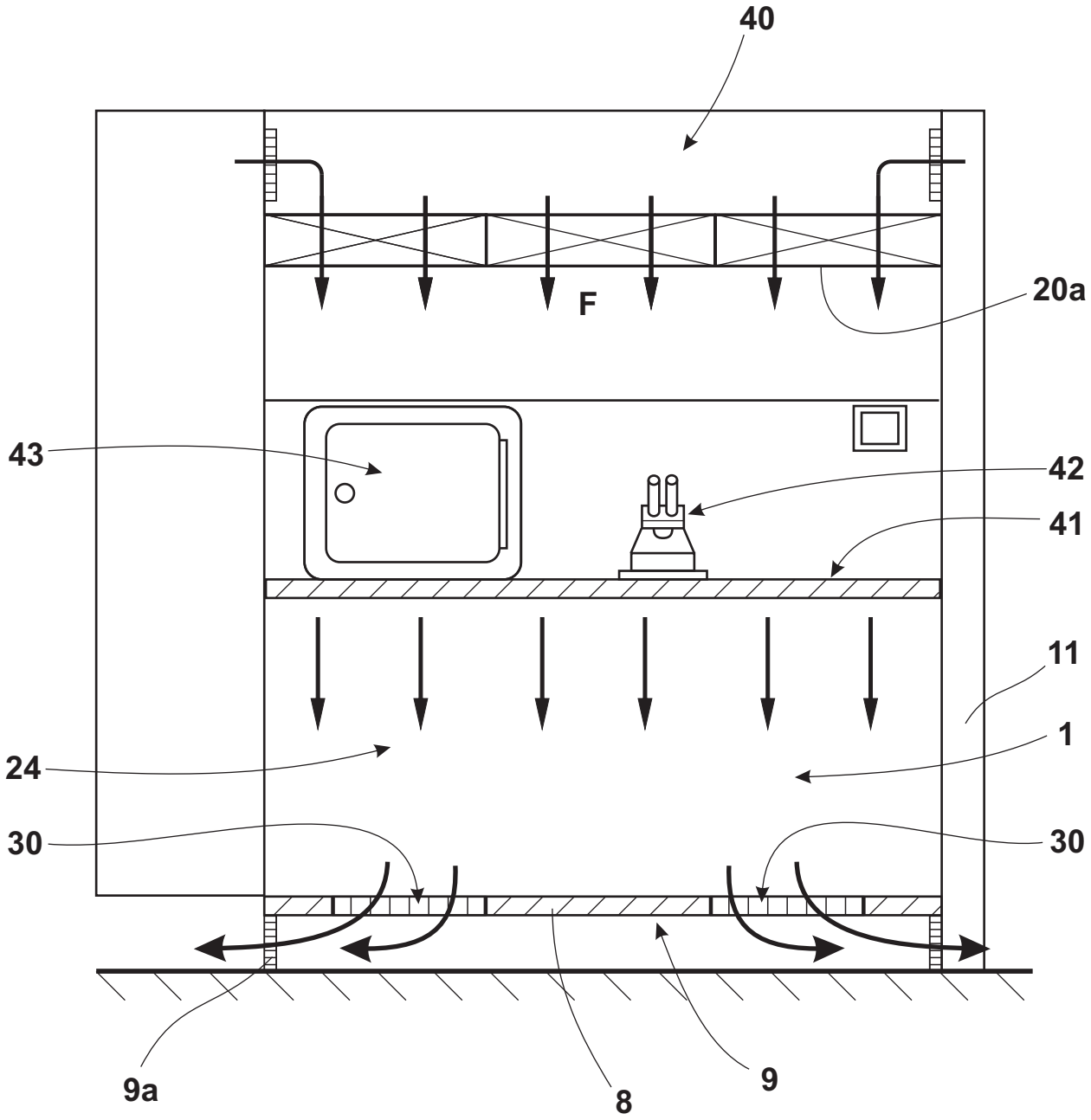

FIG. 3

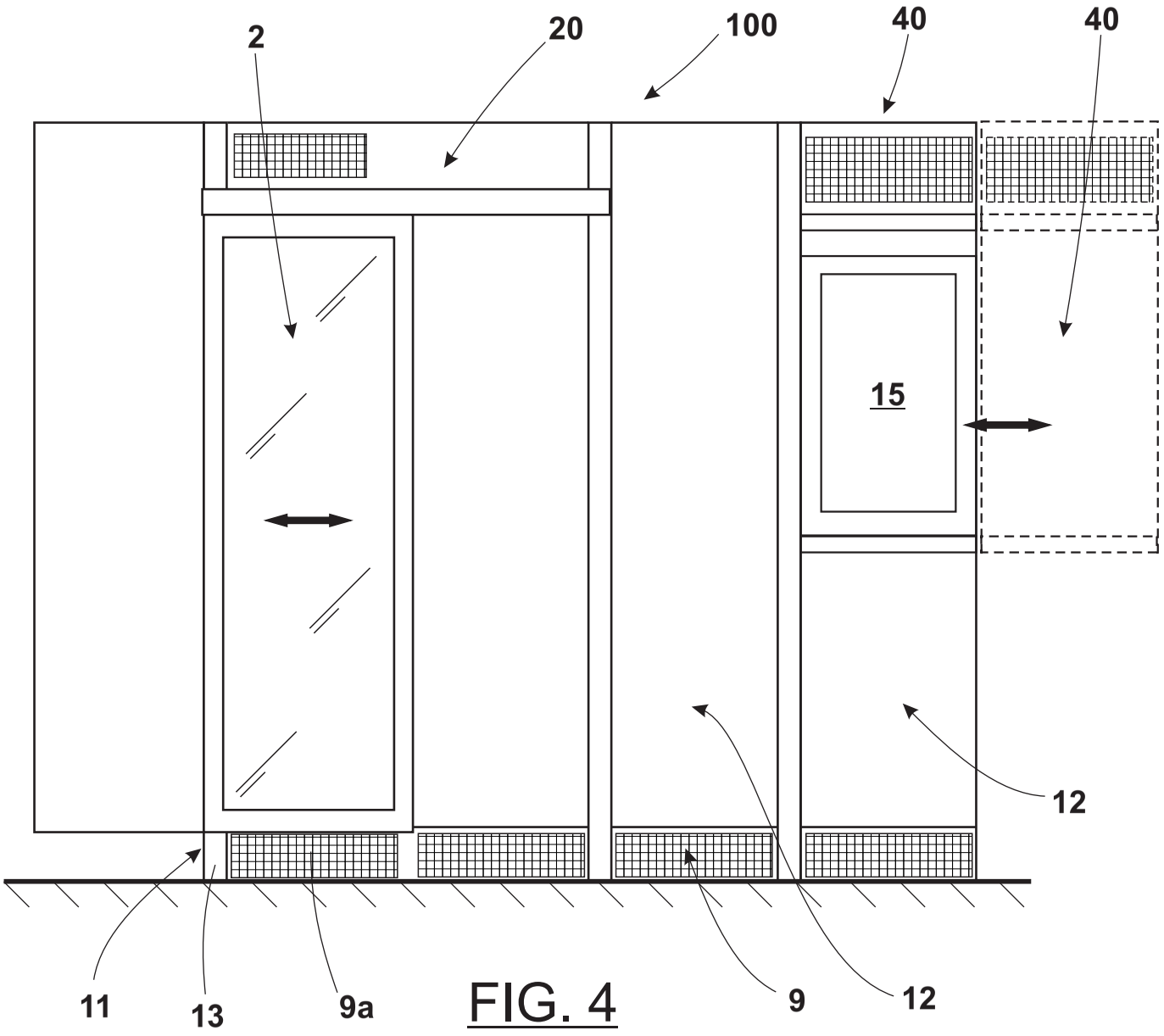

FIG. 4

PCT

POWER OF ATTORNEY

(for an international application filed under the Patent Cooperation Treaty)

(PCT Rule 90.4)

The undersigned applicant(s) *(Names should be indicated as they appear in the Request Form (PCT/RO/101))*:

BIOSCIENCE SERVICES S.r.l.
Strada Rovereta, 42
47891 FALCIANO
REPUBBLICA DI SAN MARINO

hereby appoints (appoint) the following person as: ☒ agent ☐ common representative

Name and address
(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

RUZZU, Giammario
Via Gulli, 5
40068 SAN LAZZARO DI SAVENA (BO)
ITALY

to represent the undersigned before

☒ all the competent International Authorities

☐ the International Searching Authority only

☐ the Authority specified for supplementary search only: _____
(please indicate the Authority(ies) specified for supplementary search)

☐ the International Preliminary Examining Authority only

in connection with the international application identified below:

Title of the invention: Controlled contamination compact system for treatment of cell lines

Applicant's or agent's file reference: BIO11BRWO10

International application number (if already available):

filed with the following Office INTERNATIONAL BUREAU as receiving Office
and to make or receive payments on behalf of the undersigned.

Signature of the applicant(s) *(where there are several applicants, each of them must sign; next to each signature, indicate the name of the person signing and the capacity in which the person signs, if such capacity is not obvious from reading the request or this power)*:

MUCCI, Giuseppe
Sole Administrator of BIOSCIENCE SERVICES S.r.l.

Date: 24.10.2018