ORIGINAL ARTICLE

Environmental monitoring programme in the cell therapy facility of a research centre: preliminary investigation

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Key words

Cell therapy • Environmental monitoring • Good Manufacturing Practice (GMP)

Summary

Introduction. *Recent discoveries in cell therapy research present new opportunities for cellular products to be used to treat severe, and as yet incurable, diseases. It is therefore essential to implement a quality control programme in order to ensure that safe cells and tissues are provided.*
Methods. *In a preliminary phase of the setting up of a the cell factory, monitoring was carried out monthly over a 6-month period in one out of three cell therapy laboratories and filter rooms in order to evaluate the microbial contamination of air and surfaces and the presence of airborne particulates.*
Results. *The mean total bacterial and fungal loads measured in the air in the centre of the filter room were 20.7 ± 28.9 colony-forming units (cfu)/m^3 and 9.2 ± 15.4 cfu/m^3, respectively, and 5.2 ± 4.1 cfu/m^3 and 6.8 ± 13.4 cfu/m^3, respectively, in the laboratory. The mean fungal load values recorded on the surfaces sampled in the laboratory were in 6 out of 18 cases higher than the reference values (5 cfu/plate). As to the results of particulate monitoring, with regard to the 0.5 μm particles, about 83% of the samples revealed values below the limit of 350.000 particles per cubic metre.*
Conclusions. *In this set-up phase, monitoring was able to pick out structural and organisational flaws acceptable in a laboratory compliant with Good Manufacturing Practices class C (Annex 1), but not in a class B facility. Thanks to this preliminary monitoring phase, and by correcting these flaws, the clean room facility could achieve compliance to class B.*

Introduction

The transplantation of human cells shows promising opportunities for the treatment of a number of diseases, some of which are as yet incurable. To ensure the provision of safe and reliable cells and tissues for clinical use, national and international guidelines and regulations have been issued to regulate the procurement, processing, testing, preservation, storage and distribution of all cells aimed for application in the human body [1-7]. The implementation of a quality assurance (QA) programme including the principles of Good Manufacturing Practice (GMP) and a quality control system is a major requirement, as shown previously [8-10]. Like drugs, cells and tissues for therapeutic use are regulated in Europe by the Rules Governing Medicinal Products [2]. GMP regulations apply to all phases of cell collection, processing and storage, as well as documentation, training of personnel, and the laboratory facility [9, 11-13]. In Italy, all laboratories involved in the production of cells and tissues for therapeutic use must undergo inspection and authorisation in conformity with a Ministry of Health Decree [14]. Because of the specificity of cells and tissues, regulations have changed in recent years, and are still evolving: Annex 1 of European Commission (EC) Guide to GMP [15-17] in particular, sets the requirements for the level of airborne particulate classification.

The processing of cell cultures for use in human therapy protocols requires a physical environment in which air quality is controlled, in order to minimise the risk of contamination. The cell factory should be constructed and operated as to minimise the introduction and retention of particles and micro-organisms [18-20], and a formal programme of environmental monitoring should be set up within the QA programme implementation, in accordance with the GMP principles [21-23]. The main objective is to maintain a controlled environment that minimise the risk of infecting patients. The environmental monitoring programme should include a series of physical controls (concentration of particles in the air, flow of air, integrity of HEPA filters, differential pressure, temperature, relative humidity) and microbiological controls (microbial status of air quality by particle counting and microbiological culture, microbial surface contamination). Other aspects should also be determined: frequency, location and duration of sampling, establishment of "alert" and "action" levels, monitoring of the personnel.
There are two methods of measuring the microbial contamination of air: active air sampling, in which microbial air contamination can be measured by counting the number of colony-forming units (cfu) per cubic metre, and passive air sampling by counting cfu on settle plates [24, 25]. The advantages and disadvantages of each method have been described previously [26, 27].

The microbial monitoring of surfaces can provide a valuable estimate of the level of contamination on surfaces such as walls, work surfaces, floors and equipment. Various methods utilise contact plates, swabbing and bioluminescence techniques [26, 28, 29]. The most common methods use contact plates and follow the recommendations of the European and the US Pharmacopoeia [30, 31]; micro-organisms being visualised as colonies after incubation and the results being expressed as the number of cfu per plate.
This paper presents the results of the environmental monitoring programme carried out during the set-up phase of the three-laboratory cell therapy facility of the National Institute of cancer Research in Genoa. The laboratories were originally built to be compliant with class C, and had to be subsequently upgraded to class B. Each laboratory has its own dedicated equipment, so that individual patients cell manipulations and/or cultures can be physically isolated. Cross-contamination is minimised by standard operating procedures and an electronic door opening system. All operations requiring open manipulation of patient tissues are carried out in class A biological safety cabinets. Facility access is restricted to authorised, trained personnel. Fully redundant air-handling systems are used, and the laboratories are routinely monitored for viable and nonviable air particulate counts and surface microbial contamination.

Methods

Monitoring was carried out monthly over a 6-month period in order to evaluate the microbial contamination of the air and surfaces and the presence of airborne particulates in one out of three cell therapy laboratories and the corresponding filter rooms. The microbiological and particulate samplings were carried out, in at rest conditions, by trained personnel wearing sterile protective clothing for clean room; the surface of all the instruments used was accurately disinfected with 70% isopropyl alcohol.

Microbiological monitoring

The total microbial and fungal count was carried out on representative surfaces (work bench, upper horizontal surface of the refrigerator and surface of the cable conduit in the laboratory; surface of the washbasin in the filter room), in the air in the centre of the room and in the air emerging from the outlet ports of the air-conditioning system. RODAC plates (diameter: 55 mm, surface area: 24 cm^2) containing respectively γ-irradiated TSA medium for total microbial count and Sabouraud medium for fungal count were utilized.
Active sampling of the air in the centre of the room and of the air emerging from the air-conditioning outlets was carried out by means of a SAS super 100 aspirator (International Pbi S.p.A.). The volumes of air aspirated were 500 litres in the centre of the room and 1000 litres from the outlets (2 outlet ports in the laboratory and 1 in the filter room). In order to prevent contamination, after sampling every plate was sealed and carried to the quality control laboratory in a biocarrier.
For the total bacterial count, the plates were incubated at 37°C for 48 hours; for the total fungal count, the plates were incubated at 25°C for at least five days.
The results were expressed as cfu/plate for the surface bacterial and fungal counts and as cfu/m^3 for the airborne bacterial and fungal counts. In the statistical analysis, the value " < 1" was considered equal to "0".

Particulate monitoring

Particulate monitoring was carried out in conformity with the UNI EN ISO 14644-1 [19] and 14644-2 [32] norm. In accordance with this norm, 2 sampling points in the filter room (5,40 m^2) and 4 sampling points in the laboratory (12,69 m^2) were identified, uniformly distributed through the room area. The apparatus used was the particle counter MET ONE Mod. 227A Pacific Scientific Instruments (sampling load: 2.83 L/minute). Sampling was performed at working bench level, and the probe was pointed vertically upwards.
The results were expressed as the number of particles per cubic metre.

Results

Air microbiological monitoring

Results of total bacterial and fungal load, measured in the air in the center of the room and in the air emerging from the air-conditioning outlet ports in each room, are described in Tables I and II respectively.
The mean total bacterial and fungal loads measured in the air in the centre of the filter room were 20.7 ± 28.9 cfu/m^3 and 9.2 ± 15.4 cfu/m^3, respectively, and 5.2 ± 4.1

Tab. I. Total bacterial and fungal load measured in the air in the center of the room (cfu/m^3); mean values and standard deviations (SD). Recommended limits: 10 cfu/m^3, according to EU-GMP Annex 1, 2003.

	Bacterial and fungal load (cfu/m^3)						
	JUL	AUG	SEP	OCT	NOV	DEC	Mean ± SD
Filter room							
Bacteria	< 1	2	34	8	74	6	20.7 ± 28.9
Fungi	< 1	2	7	< 1	40	6	9.2 ± 15.4
Laboratory							
Bacteria	8	1	4	10	8	< 1	5.2 ± 4.1
Fungi	< 1	2	< 1	4	34	1	6.8 ± 13.4

Tab. II. Total bacterial load (BL) (cfu/m³), fungal load (FL) (cfu/m³) measured in the air emerging from the air-conditioning outlet ports; mean values and standard deviations (SD). Recommended limits: 10 cfu/m³, according to EU-GMP Annex 1, 2003.

	Laboratory				Filter Room	
	OUTLET 1		OUTLET 2		OUTLET 3	
Month	BL (cfu/m3)	FL (cfu/m³)	BL (cfu/m³)	FL (cfu/m³)	BL (cfu/m³)	FL (cfu/m³)
Jul	< 1	< 1	1	< 1	1	< 1
Aug	1	< 1	1	< 1	< 1	< 1
Sep	< 1	< 1	1	< 1	< 1	< 1
Oct	< 1	< 1	< 1	< 1	1	< 1
Nov	80	20	57	22	29	43
Dec	< 1	< 1	3	1	1	2
Mean ± SD	13.5 ± 32.6	3.3 ± 8.2	10.5 ± 22.8	3.8 ± 8.9	5.3 ± 11.6	7.5 ± 17.4

cfu/m³ and 6.8 ± 13.4 cfu/m³, respectively, in the laboratory, as reported in Table I.
Microbiological analysis of the air revealed mean values above the reference values (10 cfu/m³) only with regard to the bacterial load in the filter room. The reference standards were respected in 83% of observations with regard to both the bacterial and fungal loads. The highest level of contamination was recorded in the month of November (Tab. I).
As shown in Table II, all the data concerning the air supplied by the outlet ports of the air-conditioning system conformed to the standards, with the exception of the November analysis. In approximately 47% of the observations carried out, the air proved to be microbiologically pure; in about 47% of samples revealed no bacterial contamination, and in approximately 87% of cases no fungal load was recorded.

Surfaces microbiological monitoring

Results of total bacterial and fungal counts on surfaces in each sampling are reported in Table III. The results obtained, expressed as cfu/plate, revealed that the mean fungal load values recorded on the surfaces sampled in the laboratory were in 6 out of 18 cases higher than the reference values (5 cfu/plate); this was particularly true for the surface of the cable conduit. In all other cases, the mean values recorded were below the reference values; all the measurements of surface bacterial loads, and 71% of those of surface fungal loads, proved to be lower than the standards considered.

Particulate monitoring

The results of the particle counts at the individual sampling points during each sampling in the laboratory and in the filter room are reported in Tables IV and V respectively.
With regard to the small-diameter (0.5 μm) particles, about 83% of the samples taken, both in the laboratory and in the filter room, revealed values below the reference limit of 350.000 particles per cubic metre. On considering the large-diameter (5 μm) particles and the corresponding limit of 2.000 particles per cubic metre, the percentages were 100% and about 92% in the laboratory and filter room, respectively.

Discussion and conclusion

The present paper describes the initial phase of a programme of environmental monitoring, drawn up in accordance with the EC Guide to Good Manufacturing Practice, Annex 1 [15] prior to its 2008 revision [16]. The initial objective was to meet the requirements laid down for class C environments, with a view subsequently to fulfilling those prescribed for the higher class B.
The results obtained at the end of this initial phase yielded useful information on which to assess the current situation as to both microbial contamination of air and surfaces and particulate status of the air.
The norm does not indicate limits on microbial contamination in at-rest conditions; with regard to par-

Tab. III. Total bacterial load (BL), fungal load (FL) measured on some surfaces (cfu/plate); mean values and standard deviations (SD). Recommended limits: 5 cfu/plate, according to EU-GMP Annex 1, 2003.

Laboratory	(cfu/plate)	Jul	Aug	Sep	Oct	Nov	Dec	Mean ± SD
Upper surface of work bench	BL	< 1	< 1	< 1	1	1	1	0.5 ± 0.5
	FL	< 1	3	< 1	27	13	2	7.5 ± 10.7
Upper surface of cable conduit	BL	< 1	< 1	< 1	3	< 1	< 1	0.5 ± 1.2
	FL	< 1	4	159	46	< 1	2	35.2 ± 63.2
Upper surface of refrigerator	BL	< 1	< 1	< 1	2	< 1	< 1	0.3 ± 0.8
	FL	< 1	16	1	52	< 1	1	11.7 ± 20.7
Filter Room	**(cfu/plate)**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Mean ± SD**
Washbasin	BL	< 1	< 1	< 1	< 1	< 1	< 1	0 ± 0
	FL	< 1	6	< 1	< 1	1	< 1	1.2 ± 2.4

Tab. IV. Laboratory – Number of particles (0.5-5 μm) per cubic meter measured at each point in each sampling; mean values and standard deviations (SD). Maximum permitted number of particles/m³: 350 000 (0.5 μm) and 2000 (5 μm), according to EU-GMP Annex 1, 2003.

Laboratory						
Month	Particle size (μm)	Point 1	Point 2	Point 3	Point 4	Mean ± SD
Jul	0.5	16 254	27 208	19 435	33 216	24 028 ± 7661
	5	0	0	0	0	0 ± 0
Aug	0.5	114 134	86 926	83 746	89 399	93 551 ± 13 916
	5	0	353	0	0	88 ± 177
Sep	0.5	94 700	98 233	106 714	140 283	109 983 ± 20 820
	5	353	0	353	353	265 ± 177
Oct	0.5	251 237	232 862	307 420	290 106	270 406 ± 34 327
	5	0	0	353	0	88 ± 177
Nov	0.5	263 251	258 304	296 820	292 933	277 827 ± 19 854
	5	353	0	707	353	353 ± 289
Dec	0.5	962 898	867 138	590 459	693 286	778 445 ± 167 810
	5	0	0	0	0	0 ± 0
Mean	0.5	283 746	261 779	234 099	256 537	
	5	118	59	236	118	
SD	0.5	346 059	309 726	210 341	238 547	
	5	182	144	289	182	

Tab. V. Filter room– Number of particles (0.5 – 5 μm) per cubic meter measured at each point in each sampling; mean values and standard deviations (SD). Maximum permitted number of particles/m³: 350 000 (0.5 μm) and 2000 (5 μm), according to EU-GMP Annex 1, 2003.

Filter room				
Month	Particle size (μm)	Point 1	Point 2	Mean ± SD
Jul	0.5	18 375	28 622	23 499 ± 7246
	5	0	1767	884 ± 1249
Aug	0.5	74 205	72 438	73 322 ± 1249
	5	1767	353	1060 ± 1000
Sep	0.5	73 145	75 618	74 382 ± 1749
	5	0	353	177 ± 250
Oct	0.5	311 661	319 081	315 371 ± 5247
	5	0	0	0 ± 0
Nov	0.5	311 661	286 926	299 294 ± 17 490
	5	1767	3180	2474 ± 999
Dec	0.5	615 901	511 661	563 781 ± 73 709
	5	0	0	0 ± 0
Mean	0.5	234 158	215 724	
	5	589	942	
SD	0.5	226 192	188 944	
	5	912	1277	

ticulate contamination, however, it indicates limits for each class during operating conditions, equal to those applicable to the class below it in at-rest conditions. It was therefore decided that, as reference values for microbial contamination in at-rest conditions, the present study should utilise those prescribed for class B in operating conditions (10 cfu/m³ in air; 5 cfu/plate on surfaces).

The results of microbiological analysis of the air revealed mean values above the reference values only with regard to the bacterial load in the filter room and the highest level of contamination was recorded in November. This was confirmed by the data concerning the air supplied by the outlet ports of the air-conditioning system: the only data non complying with the standards were found in the November analysis (Tab. II). The high

values, observed simultaneously in both the rooms monitored, pointed to a malfunction of the air-conditioning system in the period between the October and November sampling. Indeed, the investigation revealed that the system had suffered a short breakdown during that period. Prompt intervention to repair the fault soon brought the microbiological parameters back in line with the values registered previously. This demonstrates the importance not only of having an air-conditioning system that can quickly recover its functional capacity, but also of establishing adequate routine maintenance procedures aimed at preventing emergencies and of creating an organisation that can ensure a rapid response to unexpected, and sometimes critical, problems.

In order to evaluate the bacterial and fungal contamination of surfaces, some representative surfaces potentially exposed to microbial contamination were identified. The results obtained, expressed as cfu/plate, revealed that the mean fungal load values in some cases were higher than the reference values; this was particularly true for the surface of the cable conduit in the laboratory. On the basis of this evidence it was decided that: a) the QA Responsible should reassess cleaning procedures; b) potential sites where microbes might settle and proliferate should be removed (i.e. electrical cables should be embedded in walls rather than running in external conduits).

The status of the air was assessed by considering particle diameters of 0.5 μm and 5 μm, as indicated in EU-GMP Annex 1. The mean values recorded at each sampling point did not allow us to pick out any consistently critical positions of contamination in either of the environments with regard to either of the particle sizes considered. The gradual increase in the number of 0.5 μm particles observed in both environments over the samplings may be regarded as indicating a progressive decline in the filtration efficiency of the air-conditioning system and in its ability to screen out the finer particles. This may also be interpreted as a useful early warning of a brief temporary breakdown of the ventilation system. These considerations underline the key role of particulate monitoring as a means of assessing the efficacy of air-conditioning systems and as an adjunct to the normal control and maintenance of system components, such as absolute filters. The above-limit values of 0.5 μm particles were all recorded during the December sampling, and the only above-limit value of 5 μm particles was observed in November. The explanation for this may be that the time interval between the repair work carried out on the air-conditioning system and the subsequent sampling was too short. While it may well have been sufficient to allow the 5 μm particles to be brought back within the proper limit, it does not seem to have been long enough to enable the number of finer (0.5 μm) more persistent airborne particles to be reduced sufficiently.

In conclusion, in this set-up phase, microbiological and particulate monitoring provided information able to contribute to the efforts to comply, from both a structural and procedural point of view, to GMP regulations. Once the critical points have been dealt with, validation of the environments, equipment and processes was carried out, at rest and in operation, including biological safety cabinets. The continuous monitoring yields an abundance of significant microbiological data that are used for the trend analysis program, to make sure that the operating conditions are good enough to allow the facility to be used for cell therapy activities.

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