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Benchmark Guidance Values for Microbiological Monitoring on Surfaces: A Literature Overview

Raffaella Giovinazzo,[1*] L. Caradonna,[1] G. Giaquinta,[1] E. Guerrera,[1] M. Mameli,[1] A. Mansi,[2] G. Marena,[1] T. Mastromartino,[1] D. Sarto,[1] P. Tomao[2]

[1] Inail – Technical Advisory Department for Risk Assessment and Prevention, Rome, Italy

[2] Inail – Department of Occupational and Environmental Medicine, Epidemiology and Hygiene, Rome, Italy.

Introduction

The Directive 2000/54/EC[1] of the European Parliament and of the Council of 18 September 2000 on the protection of workers from biological risks is geared toward workers exposed to microorganisms, cell cultures and human endoparasites during their professional activities. Environmental microbiological monitoring is not explicitly mentioned, but it is implicitly asserted in the Article 64 and in the Annex IV, point 1.3 of the Italian Legislative Decree 81/2008,[2] with the oblige of the employer to clean regularly the work areas, installations and mechanisms and to ensure "adequate" hygiene conditions. The lack of widely accepted quantitative thresholds for acceptable levels of microbiological contaminants prevents adequate assessment of the hygienic quality of indoor environments.

The aim of this paper is to present a literature overview of the main microbiological environmental monitoring techniques and related benchmarks proposed for the assessment of the hygienic status of surfaces in different workplaces. The bibliographical research has been carried out mainly using the following key words: *surface, (micro) biological contamination, surface sampling, surface contamination, hospital surface contamination, indoor surface microbiological pollution.* Articles in Italian, English and French, from 1981 until now, have been examined, including those regarding the surfaces of Personal Protective Equipment (PPE).

Literature Review

Procedures for the assessment of the hygienic status of surfaces

For microbiological monitoring of surfaces there are classic methods, such as contact plates, sponge-bag, swabs, as well as technical biochemistry tests like ATP bioluminescence. Among the microbiological techniques, the sterile contact plates (RODAC – *Replicate Organism Direct Agar Contact*) are often used as a single technique or in combination with others. This method seems to be more sensitive to detect Gram-positive bacteria compared to Gram-negative ones on hospital environment surfaces but it cannot be used for uneven surfaces or awkward areas e.g. door handles, curved surfaces and rough surfaces. The contact plate technique is used to monitor the surfaces of "clean room" of the pharmaceutical sector,[39] of sanitary rooms and domestic environments,[23] and for the evaluation of the microbiological contamination of antique books and manuscripts.[27] This technique has been used, also for the evaluation of the sterility of personal protection system.[40]

The swabs allow the microbiological sampling of hard surfaces, for example, behind the sinks[41] and the pipes[42] or along the bedrails.[43] This method is chosen to sample smooth, non-porous surfaces, like steel, painted walls, floor tiles, laminated wood etc. Sterile swabs are made of several materials: cotton, rayon,[44] nylon[45] and polyurethane foam.[46,47,48] The nylon swabs allow a greater efficiency of recovery of the microbial cells, because the microorganisms do not penetrate the nylon matrix, as occurs in the cotton swabs, and they remain on the external surface. The nylon swabs, compared to rayon, have a greater sensibility and a greater ability to recover *S. aureus* cells from the clinical patient's skin[49,50] and greater sensibility also when used in environmental sampling.[51] The use of swabs is widely diffused in the food sector, in Good Manufacturing Practices (GMPs) and Hazard Analysis Critical Control Point (HACCP) programs, in healthcare sector[52] for detection of fungi and Gram-negative bacteria[53] and in hospital kitchens during food manipulation.[54] The sponge-bag method uses a sponge composed of an absorbent sterile material contained in a sterile bag that can easily be closed. The method is widely used for the evaluation of the hygienic status of the surfaces in the food sector. Compared to the swab method, this method has the advantage of allowing the collection on wider surfaces and to ensure a greater collection efficiency in the presence of biofilm or cracks, because it is possible to make more pressure. This method is not suitable for small surfaces. The use of the sponge has been also validated for the detection, recovery and quantification of vital spores of *Bacillus anthracis* inoculated on steel surfaces, in environmental contamination simulations.[55] RODAC plates, swabs and sponge-bag are used to obtain both qualitative and quantitative analyses. The bioluminescence ATP technique is used as initial screening method to monitor the level of cleanliness of the surfaces in several workplaces, in particular in the field of HACCP. This technique does not allow the differentiation between species of bacteria and/or molds, but it gives a rapid detection of the contamination level.[56,57] It must be integrated with classical microbiological tests.[58-59] In hospitals, this technique may provide additional information of cleaning efficacy and allow identification of environmental surfaces that require additional cleaning. This technique is often applied in the pharmaceutical industry[60] and in cosmetic industry.[61]

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Legislation

Technical regulations or guidelines on microbiological control of surfaces are not available. However, several regulations and documents of national and international scientific organizations carried out microbiological tests on surfaces in the pharmaceutical,[28-30] health-care[31-33] and food/feed for animals[34,35,36] sectors**.** Some documents set the requirements for the environments of sterile products manufacture, and define specific classes of microbiological contamination, according to the level of environmental cleanliness required.[28,30,37] General indications or operative criteria for the environmental microbiological survey can be found in all these documents,[33,38] as well as contamination intervals useful for the classification of specific work environments and microbial contamination indicators to be used as references, for the assessment of the hygienic level of the surfaces in relation to the work context.[31,32]

Microbial contamination in workplace

In the assessment of biological agents in workplaces, the monitoring of airborne and surface microbiological contamination is an important step. Airborne biological agents in the workplaces can be deposited on the surfaces that act as substrate for the proliferation and the diffusion of microorganisms in the environment.

Recently, many studies have re-evaluated the role of the inanimate environment in the epidemiology of infections caused by antibiotic resistant bacteria, for example Methicillin Resistant *Staphylococcus aureus* (MRSA), Vancomycin Resistant *Enterococcus* spp. (VRE), *Clostridium difficile* and *Acinetobacter* spp. that can survive for long periods of time on the surfaces of hospital rooms,[3] operating rooms,[4] autopsy rooms[5] and intensive care units.[6,7] Although the transmission of pathogens from one patient to another often occurs through the hands of the health care staff,[8] water, air and surfaces can be involved, directly or indirectly. Like bacteria, some airborne virus (influenza viruses, respiratory syncytial virus, Adenovirus, Rhinovirus, Coronavirus, measles virus, rubella virus, mumps virus and human parvovirus B19) are transmitted by droplets that can be directly inhaled or deposited on the surfaces. Viral agents are also transmitted by oral-fecal route like the Rotavirus, human Adenovirus and Norovirus, that are frequently responsible of infections due to their presence in the air and on environmental surfaces.[9] Adenovirus responsible for keratoconjunctivitis and gastroenteritis have been isolated from contaminated surfaces and instruments in various healthcare settings;[10] the presence of viral nucleic acids such as Adenovirus and Norovirus, have been found in 16% of the total samples collected in hospital air and surfaces.[11] Epidemics of Norovirus have also occurred in non-hospital environments, such as schools,[12] military training centers,[13] cruise ships[14] and hotels.[15] In these environments, the virus has been isolated from many different surfaces such as door handles, stair railings, rest rooms, toys, telephones, cups, materials, etc.[9] Other studies have focused their attention on the role of the environmental surfaces in the transmission of bacteria from animals to humans and vice versa.[16] The infections caused by human Papillomavirus (HPV type 7) are very common among workers in the meat slaughtering sector[17] and among poultry farm workers.[18] High concentrations of mesophilic bacteria (*Bacillus cereus*) and their components (endotoxins), fungi (*Cladosporium* spp., *Mucor* spp., *Rhizopus oryzae*, etc.) such as *Aspergillus fumigatus*, toxins, metabolites (microbial organic volatile compounds, MVOC) present in bioaerosol and on work surfaces in composting systems have been recognized as being responsible for several pathologies (chronic respiratory diseases, allergies, mucous membrane irritation, *etc*.) in the workers in this field.[19] The risk of exposure to various pathogenic agents (*Enterococcus* spp, *Escherichia coli*, *Klebsiella pneumoniae*, *Leptospira* spp, *Pseudomonas* spp, *Salmonella typhi, Shigella* spp, Enterovirus, Rotavirus, Hepatitis viruses, *Entamoeba histolitica, Giardia lamblia, Ascaris lumbricoides,* etc.) present on work surfaces has been documented also among the workers of the waste water processing plants[20,21] and among workers of the solid waste treatment sector.[19,22] Finally, other studies[23,24,25] have estimated the bacterial and fungal loads on different indoor environment surfaces (houses, stores, nurseries, offices, gymnasiums, restaurants, etc.). Elsergany et al. (2015) have found that 80% of the total of the 224 samples collected from the surfaces of 4 different shopping malls in Sharjah (United Arab Emirates) showed bacterial concentrations with high medium values (range between 500 and 1500 CFU/cm^2).[26] In libraries and archives,[27] the fungal species most frequently isolated from books, manuscripts, documents, etc. have been *Cladosporium herbarum, Cladosporium cladosporioides, Penicillium corylophilum, Aspergillus fumigatus, Penicillium spp., Aspergillus sydowii, Rhizopus nigricans.*

Index/benchmark for occupational sectors

The literature on the quantitative evaluation of the levels of microbial contamination of the surfaces does not report standards or legislative references.[62,63] The reported values are mostly finalized to estimate the cleaning efficacy of sanitation actions or to quantify and provide a general measure of bacterial load. Table 1 summarizes the benchmark values proposed by several authors in Healthcare, Pharmaceutical and Food work environments.

In the hospitals, the bacterial load considered as microbiological standard for the surfaces, is generally indicated between < 2,5 CFU/cm^2 and < 5 CFU/cm^2. The values of ATP bioluminescence indicating a clean surface ranged from < 250 RLU to 100 RLU. The index organisms that must be absent or <1 CFU/cm^2 are mostly *Staphylococcus aureus* (including MRSA and MSSA), *Aspergillus* spp., *Pseudomonas* spp. and Enterobacteriaceae.

The *Guide du Bionettoyage* (Biocleaning Guide) n. 5670 ARECLIN and the ISPESL 2009 Guide Lines, divide the hospital workplaces into two\six zones characterized by different values of acceptability according with increasing risk. Different limits of acceptability occur for the pharmaceutical sector.[37,31] For dentistry studies, a limit of acceptability of ≤ 1 CFU/cm^2 had been proposed in 2008, while in 2012 an Italian multicenter study proposed threshold values based on the mean levels of the analyzed microbial accumulation.[71,70]

Microbiological contamination of foods can be ascribed to contaminated raw materials or cross-contamination events, caused by microorganisms originating from various sources, air, water, human or animal faeces, mucus, hair, infected wounds, dirt, dust. Mainly surface sampling is assessed with contact plates or swabs and further viable cell counting. A more rapid method, as the ATP bioluminescence, is used for the real-time evaluation of the cleanliness of food contact surfaces.

The effectiveness of cleaning and disinfection practices is often monitored by reductions of bacteria such as *Salmonella*, *Salmonella, E. coli, Listeria monocytogenes* and for total bacterial load, total coliform load. Proposed reference values for bacterial contamination in the food sector show a high variability compared to the sanitary sector. Risk management, reflected in the HACCP principle used by the food industry, encompasses the view that relevant pathogens are widespread, occurring with wide variation in time and space. This reasoning could be ap-

Table 1. Healthcare, Pharmaceutical and Food Work Environments

	Sampling technique (microbiological parameter)	Benchmark Guidance Value	Microbiological indicator	References
Hospitals	Dipslide (ACC 30°C/48h)	≤ 2,5 CFU/cm^2	*Staphylococcus aureus*; MRSA; MSSA	[64]
	Contact plates (ACC 37°C/48h)	≤ 2,5 CFU/cm^2		[65]
	ACC	< 5 CFU/cm^2	*Staphylococcus aureus*; MRSA; *Clostridium difficile*, *Salmonella*; *Aspergillus* Vancomycin-resistant enterococci	[66]
	ATP-bioluminescence	< 100 RLU		[65, 64]
	ATP-bioluminescence	< 250 RLU	*Staphylococcus aureus*; MRSA	[56]
	Contact plates (TBC)	From ≤ 5 CFU/plate (operating room and other critical environment) to ≤ 50 CFU/plate (ICU-Intensive Care Unit, neonatology)	*Staphylococcus aureus*; *Aspergillus* spp.; *Pseudomonas* spp.; *Enterobacteria*	[31]
	Contact plates (TBC 37°C)	Low risk area (office) < 5 CFU/cm^2 Medium risk area (waiting room, lifts, counselling etc.) < 2 CFU/cm^2 High and very high risk area (ICU, operating room, neonatology, emergency, etc.) < 0,2 CFU/cm^2	*Aspergillus fumigatus*	[37]
	Swabs (ACC 37°C/48h)	< 2,5 CFU/cm^2	MRSA	[56]
	Swabs (ACC 48h)	< 2,5 CFU/cm^2	*S. aureus*; *E. coli*; *P. aeruginosa*; *A. baumannii*	[67]
Ambulances	Swabs (TBC 37°C/48h)	< 5 CFU/cm^2		[68]
Surgery practices	Swabs (TBC 37°C/72h; Total Fungal Count: TFC 28°C/72h)	0 CFU/cm^2 (acceptable) 1-5 CFU/cm^2 (doubtful) > 5 CFU/cm^2 (not acceptable)	Enterobacteria; *P. aeruginosa*	[69]
Dental practices	Contact plates (TBC 36°C/48h)	≤ 0,64 CFU/cm^2 (acceptable) 1,48 CFU/cm^2 (alert)		[70]
	Swabs (TBC 36°C/48h)	≤ 1 CFU/cm^2		[71]
Pharmaceutical Clean rooms	RODAC plates (TBC 30-35°C/48h, than 20-25°C/72h)	Class A1: < 1 CFU/plate (walls and benches) Class B1 and B2: < 5 CFU/plate (walls); < 10 CFU/plate (benches) Class C: < 25 CFU/plate (walls); < 50 CFU/plate (floor) Class D1 and D2: < 50 CFU/plate (floor and walls)	*Staphylococcus* spp.; *Micrococcus* spp.; *Bacillus* spp.; *Candida* spp.	[36]
	Swabs	Class A2: < 1 CFU/swab		
	Contact plates	Surfaces: from < 1 CFU/plate (sterility level A) to < 50 CFU/plate (sterility level D) Gloves: from < 1 CFU/glove (zone A) to < 5 CFU/glove (zone B)		[30]

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Pharmaceuticals	Contact plates (TBC 37°C/24h, than RT/48h)	From < 0,2 CFU/cm2 (clean rooms) to < 5 CFU/cm2 (offices, stairs, etc.)	*Staphylococcus* spp.; *Micrococcus* spp.; *Bacillus* spp.; *Candida* spp.	[37]
Food preparation areas (surfaces and tools)	Contact plates (25,8 cm^2 20-25°C/48h)	< 150 CFU/plate	*Pseudomonas fluorescens*; *Escherichia coli*; *S. aureus*	[72]
	Contact plates (TBC)	≤ 10 CFU/cm^2 (after sanitization)	*Salmonella* spp.*; Listeria monocytogenes*; Total coliforms ≤ 1 CFU/cm^2	[73, 74]
	Contact plates (TBC)	Satisfactory sanitization: TBC ≤ 10 CFU/cm^2	Total coliforms ≤ 1 CFU/cm^2 Absence *Salmonella* and *Listeria monocytogenes*	[75]
	Contact plates (TBC 30°C/48h; TFC 25°C/120h)	0 ≤ CFU/plate ≤ 2 (very good) 3 ≤ CFU/plate ≤ 9 (good) 10 ≤ CFU/plate ≤ 29 (satisfactory)	Coliforms; *S. aureus*	[36, 76]
	Swabs (TBC)	0 ≤ CFU/100 cm^2 ≤ 8 (very good) 12 ≤ CFU/100 cm^2 ≤ 36 (good) 37 ≤ CFU/100 cm^2 ≤ 116 (satisfactory)	Coliforms; *S. aureus*	[36, 76]
Catering	Contact plates (TBC)	≤ 4 CFU/cm^2	Total microorganisms, coliforms, *S. aureus*, *E. coli*, *Salmonella* spp. and *Listeria monocytogenes*	[77]
Restaurants	ATP-bioluminescence	50 RLU	*Escherichia coli*	[78]
	Swabs (TBC 30°C/24-48h)	80 CFU/cm^2		
Butcheries and supermarkets	Contact plates (TBC)	≤ 4 CFU/cm2	Total coliforms < 1 CFU/cm^2; *E. coli*	[79]
Area microbiologically checked (cooked dishes)	Contact plates (TBC 37°C/24h and room temperature/48h)	Area 1 < 5 CFU/cm^2 Area 2 < 2 CFU/cm^2 Area 3 < 0,2 CFU/cm^2 Area 4 < 0,2 CFU/cm^2	Coliforms and *E.coli*	[37]

Legends - TBC: Total Bacterial Count; ACC: Aerobic Colony Count; RLU: Relative Light Units

plied to surface level cleanliness in hospitals. Widespread adoption of standards would allow risk assessment and evaluation of infection risks to patients (and staff) in hospitals. Many papers propose acceptable values of mold contamination, lower than the bacterial ones for workplaces and houses.

Discussion

In the scientific literature and technical regulations there are many papers about the surface contamination in "Pharmaceutical-Sanitary" and "Food and Animal Feed" workplaces. In hospitals, surface contamination is an important source of potential pathogen microorganism. Correct cleaning systems and efficient disinfection of the surfaces reduces the incidence of the infections related to healthcare assistance, because the surfaces contamination has a principal role in the transmission of pathogenic microorganisms. Microbiological studies about air, water, (hydro-sanitary systems and air conditioning systems) and surfaces in various hospital environments are generally carried out for risk assessment and to establish monitoring actions. The total bacterial load and/or the pathogenic species responsible of nosocomial infections represent the microbial contamination indicators.

In ISPESL Guidelines[31] and Annex I of EU GMP Guide[30] are reported the reference values for the estimation of microbiological monitoring results respectively in the operating units and in the medicine industry.

Microbiological samplings are carried out mainly using the contact plates method, swabs, *sponge-bag* method and bioluminescence technique. Each single method shows both the advantages and limits of its use and the choice of the method to adopt is only conditioned by the surfaces to be examined.

Conclusion

This review shows the absence of standard operating procedure applicable to every workplace and the lack of threshold values for surface microbiological monitoring. We can only refer to index/benchmark proposed in literature, that are summarized in this paper. However, many different methods are indicated by the authors. This makes the comparison between the analytical results difficult because the protocols are characterized by different analytical parameters. Moreover, in the scientific literature there are not threshold limit for the microorganism indicators of the indoor air quality[62,63] and the only found references are linked to the effectiveness of the sanitation actions. One adoptable proposal of procedures is present in the Inail Manual on microbiological Monitoring of the working environments.[80]

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