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Evaluation of the sanitization of equipment for Transport, Storage and Management of Medical Devices
Materials - Surface treatments - Geometry

Customer: *LUCINI SURGICAL CONCEPT Ltd*

Working Group: *Prof. Riccardo Guidetti (DiSAA), Dr. Laura Franzetti e Dr. Mauro Scarpellini (DeFENS).*

ABSTRACT

FOREWORD/PURPOSE:

The hospital environment is an important source of microbial contamination. Despite the progress made in recent years concerning the identification of risk factors and the development of techniques for prevention, infections continue to be a big problem for Healthcare (MeMo 6, 2011), in terms of mortality, morbidity and social costs.

The purpose of this study is: to identify the most suitable raw material for the realization of systems of transport, storage and management of medical and/or generic devices (trolleys, cabinets, equipment, etc.) to be used preferably in a hospitals, in order to allow more efficient and easy operational and environmental sanitization.

MATERIALS AND METHODS:

- *The raw materials compared in this study are:*
  Austenitic Stainless Steel, Anodized Aluminium, Generic Steel (Iron) painted, Generic Steel (Iron) chrome-plated, Corian®, Baydur® , Polystyrene.
- *The micro-organisms (infectious agents) used for testing are: Escherichia coli DSMZ 30083^{T}, Enterococcus faecalis DSMZ 20478.*
- *The disinfectant used is: disinfectant based on quaternary ammonium , widely used in hospital environments.*
- *Microbial count:*
  For the recovery of micro-organisms from contaminated surfaces, the technique of tampons and pads were adopted at the same time, both of which are deemed appropriate for ISO standards.. For the enumeration of live and vital microbial forms, both specific selective media for the micro-organism in question and generic media were used.

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RESULTS:

Austenitic Stainless Steels are the most resistant materials to microbial attack since they feature a reduced microbial adherence of both micro-organisms.

Anodized aluminium and the two types of Generic Steel (Iron) are placed in an intermediate position.

Polystyrene, Corian®, Baydur®, are the materials to which micro-organisms most easily adhere.

CONCLUSIONS:

The most suitable raw material for the realization of systems of transport and storage and management of medical and/or generic devices (trolleys, cabinets, stands, etc.) is AUSTENITIC STAINLESS STEEL.

Its use is recommended by the following evidence:

- *Best cleanability*
- *Lower tendency of organic dirt (micro-organisms) to remain adherent.*
- *Lower microbial affinity which facilitates cleaning and removal of dirt even after prolonged use and increased roughness (Vasone 2011).*
- *Lower tendency to develop cavities, scratches, cracks from use. In other materials,in particular plastic-polymeric materials, those coated with layers of paint, chrome or oxides; scratches, cavities and cracks create sites within which micro-organisms grow and proliferate, and where they remain protected from the action of disinfectants. Also in layered materials (painted, chrome-plated) the possible exfoliation of the surface layer generates macro-impurities.*

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1. INTRODUCTION

The hospital environment is an important source of microbial contamination. Despite the progress made in recent years concerning the identification of risk factors and the development of techniques for prevention, infections continue to be a big problem for Healthcare (MeMo 6, 2011).

Hospital-acquired infections are all those diseases that occur during hospitalization or soon after discharge of the patient: they are infections of varying degrees of intensity and can be lethal.

The main causes of transmission of HAIs can be (from www.epicentro.iss.it):

1. Direct contact between a healthy person and an infected person (contact via hands)
2. Transmission through body fluids (exudates, etc.) between an infected person and a susceptible person;
3. Indirect contact via contaminated devices (endoscopes, etc.)
4. Spread of infection through shared means (food, blood, etc.)
5. Through air carrying micro-organisms transmitted from a distance.

At the base of their prevention is firstly the adoption of good practices of sanitization on part of the staff and of the management of flows in the hospital (Cases 1, 2 and 4); however, the cleaning and disinfection of the environment and equipment must not be overlooked, whatever their use (Cases 3 and 5). Regarding the devices present in hospitals, based on the EBM (Evidence Based Medicine) literature, it is possible to identify three types of devices (MoMa, 6):

1. Devices intended to come into contact with the patient only if sterile (surgical instruments, catheters, etc.): are considered critical items as they can generate infections if they transmit unwanted micro-organisms. For this reason they are subject to sterilization treatments;
2. Devices intended for contact with mucous membranes and non-intact skin such as probes or endoscopes: these are considered semi-critical items because, usually, a non-sterile level is required since the tissues in question are able to resist the possible presence of spores but not of other micro-organisms, which can be eliminated with a pasteurization treatment or by using chemical detergents.
3. Devices intended for contact with intact skin but not mucous membranes: they are considered non-critical devices and so it is necessary only to eliminate/reduce the microbial

load as intact skin is a barrier that preserves the health of the individual. They may be a factor of secondary transmission by contaminating the hands of the operators who then come in to contact with patients with injured skin.

Therefore, the sanitization of the surfaces must be one of the main activities of the hospital staff to ensure an appropriate level of hygiene depending on the situation and to eliminate one of the possible causes of contamination (WHO, 2002).

Thus defining sanitization as an applied science that aims to design, develop, implement, maintain, restore and/or improve sanitary procedures and conditions (Mariott and Gravani, 2008) it is obvious that the best result is obtained by acting on the two main factors (taking into account that the phenomenon of pollution is not completely under control and in a hygienic risk assessment they should not be restricted), i.e. the type/shape of the material and the type of detergent with its degree of reactivity against the main organisms found.

Hence the importance of using, for the design, materials and geometry that allow easy cleaning from side to side and that do not constitute a substrate that favours microbial adherence. (Arnold, 2001). Austenitic stainless steels, for their properties, are the most logical choice among the materials used for hospital furniture; however, polymeric materials are often preferred to Austenitic Stainless Steels especially for their low cost and lower weight.

1.1 Roughness

Any surface, examined with a magnifying instrument, reveals roughness formed by grooves and ridges. The degree of finish of surfaces is certainly an important parameter which must be taken into account. It is not sufficient to search for materials with improved mechanical properties, or through the adoption of large dimensional tolerances, if this requirement is not accompanied by a good level of surface finish.

According to the guidelines of the European Hygienic Engineering & Design Group (EHEDG, 2004), surfaces in contact with biological materials should have a finish with an acceptable value of roughness average (*Ra*) and present no imperfections such as grooves, folds and crevices. Large

areas of contact surface should not exceed a *Ra* of 0.8 µm, although the cleanability depends largely on the technology of surface finish applied, as it can affect the topography of the surface.

In addition to being essential for the hygienic characteristics of the surfaces, the degree of surface finish is important for the correct operation of mechanical components for coupling, in particular the degree of filling of the profile affects the wear resistance. Surfaces with a high degree of surface finish have better corrosion resistance than areas with high roughness. However, it is not said that a low roughness value is always the best solution, in fact in some circumstances a minimum degree of roughness is essential, as is the case for example of components that require lubrication.

However, despite the discrepancies in the results found in the literature regarding bacterial adhesion and cleanability of stainless steel, most authors agree in stating that a reduced *Ra* is related to better hygiene (Jullien , 2002).

1.2 Hygienic design of equipment and devices.

Hospital furniture must be obtained through a hygienic design that prevents contamination and microbial growth. Inadequate hygienic design will make cleaning more difficult. In the cracks and dead spaces which can form with time, residues of dirt may remain trapped, which allow the micro-organisms present, and also those from the environment, to survive and to multiply up to unacceptable levels.

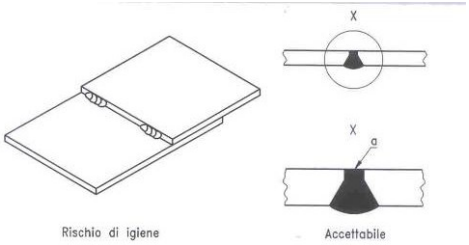
One of the primary goals of hygienic design is to ensure that structures are able to perform their function; however the hygiene requirements sometimes conflict with this objective. In search of an acceptable compromise, we must however remember that the safety of patients must not ever be put at risk.

For the design it is important to follow some basic criteria: particularly with respect to the surfaces and their geometry, these must be cleanable. The surfaces must be resistant to all cleaning agents and disinfectants in the full range of operating conditions (conditions of use). The industry, however, has not yet formulated objective and common criteria: contrary to the food industry, where legislation in terms of equipment hygiene has produced a series of well established technical documents, the industry of hospital equipment has not yet formulated precise standards. Even the

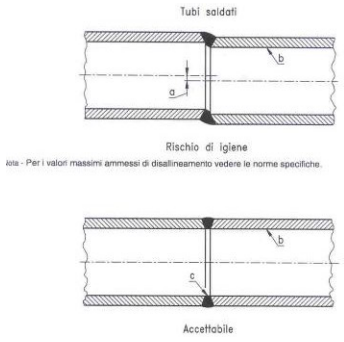
literature of hygienists has focused on the definition of diseases and on the identification of their causes: the non-critical devices (see below) are not yet the subject of specific criteria which aim to reduce pollution by related pathogens. Being related, however, to hygienic aspects concerning the removal of micro-organisms, it has been decided to highlight the principles of hygienic design that must inspire the correct design of systems even in hospitals.

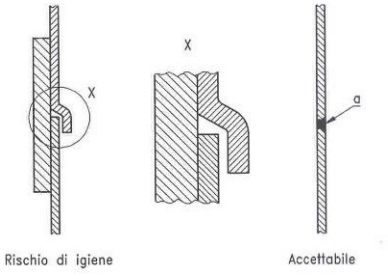
Some rules for the construction of surfaces according to hygienic criteria are (EHEDG, 1997; EN 1672-2:2009)

- Avoid direct metal/metal joints, except for welded joints (metal to metal contact can hold dirt and micro-organisms). In the case of equipment designed for aseptic processing, there is also the danger that the metal/metal seals do not prevent the entry of bacteria.
- Avoid steps caused by a misalignment of the connections of equipment and piping.



- In the case of the use of seals or gaskets, their design must not feature any cracks in which dirt may remain trapped and bacteria can accumulate and multiply.





- The corners should preferably have a radius equal to or greater than 6 mm, the minimum radius is 3 mm. Sharp corners must be avoided. In general, however, the connections must be as large as possible to facilitate cleaning.

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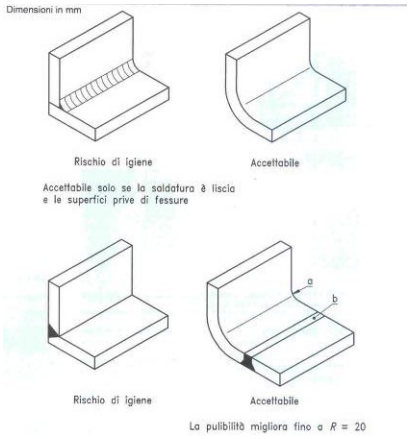
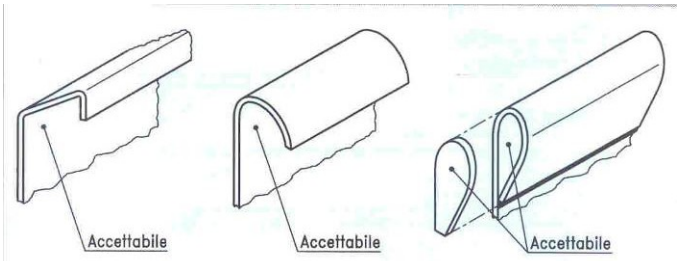


- When used as a seal, the corners should be as "sharp edged" as possible in order to form a watertight seal at the point closest to the interface product/seal. In this situation, you may need a small bevel or a connection of 0.2 mm to avoid damage to elastomeric seals during thermal cycles.
- The final part of a surface must prevent the accumulation of dirt/micro-organisms in areas designed to avoid safety problems for operators.

Benché tutte le versioni illustrate per la finitura del piano di lavoro conferiscano una certa rigidità alla costruzione, esse formano spazi in cui possono annidarsi residui di prodotto che risultano difficili da pulire.



Quando viene impiegato questo metodo di costruzione, le estremità aperte dovrebbero essere "chiuse" al fine di evitare che possano rimanervi intrappolati residui di prodotti. La saldatura deve essere levigata e lucidata al fine di garantire una superficie liscia.

If for technical and functional reasons one or more of these criteria cannot be met, the loss of cleanability must be compensated and the effectiveness of the adopted solution has to be proved by means of experimental tests.

- All surfaces in contact with the product must be easily accessible for visual inspection and manual cleaning.
- You should, as far as possible, avoid the formation of condensate: humidity favours microbial adherence.
- Equipment and support structures must be sealed to the support surface (floors, walls, columns, ceiling) so that there are no empty spaces. The areas between the equipment and the building must be suitable for cleaning and inspection (EHEDG, 1996).
- The permanent metal/metal joints must be continuously welded and have no imperfections (EHEDG, 1993)

1.3 Bacterial adhesion

Bacterial adhesion is a general phenomenon which occurs whatever the means, the micro-organisms or the nature of the surface. Bacteria adhere very rapidly to surfaces they come into contact with, whether animal or plant tissue, or inert supports. Regarding the inert supports, the number of micro-organisms that adhere to a surface is relatable to the charge of the surface and to its hydrophobic nature. A greater number of micro-organisms adhere to hydrophobic surfaces (Teflon, polystyrene and polypropylene), fewer adhere to with positively charged or neutral metal surfaces and even less to negatively charged hydrophilic substrates such as glass, mica and oxidized plastic (3).

Bacterial adhesion to surfaces follows three basic stages (Figure 1): adsorption, fixation and colonization (Cerf , 1986).

Adsorption is a very fast phenomenon that occurs in a few tens of seconds and it is partially reversible (figure 2a, 2b).

According to the theory of Derjaguine and Landau (1941), Vervey and Overbeek (1948), a micro-organism adheres to a surface when the free energy of interaction between the organism and the surface is negative; this energy is the result of electrostatic and electrodynamic interactions. Electrostatic interactions, caused by the charges of the ionic double layer, according to Gouy-

Chapman, present on the bacterial cell surface and on the substrate, generate phenomena of repulsion if they are of the same sign and of attraction if they are of different signs. To facilitate attraction, some electrodynamic interactions are also involved, that is the van der Waals forces, caused by the coupling of the electromagnetic fluctuations of charged molecules which constitute the surface and the micro-organism. According to this theory there is a strong attraction at a short distance (2 nm), an area of repulsion from 2 to 6 nm and a weak attraction in the area between 6-8 nm.

The limit is represented by the fact that this theory does not take into account the surface tension of the affected systems, deformation of the bacterial cells or the non-uniform distribution of charges (Isoard and Gauthier, 1989).

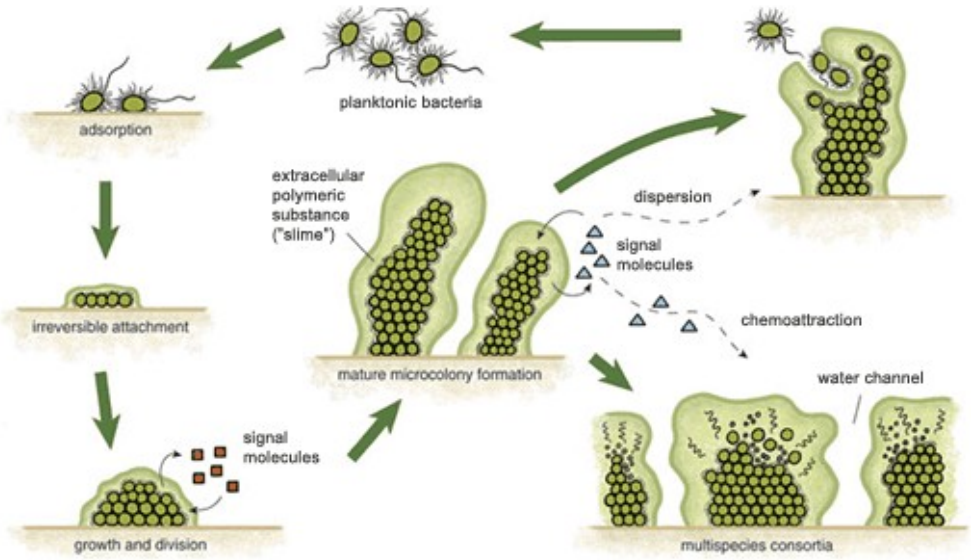


Figure 1 - Mechanism of bacterial adhesion

Fixation (figure 2c), is an irreversible step which is accomplished by macromolecules such as polysaccharides produced by certain micro-organisms; it is a slower phase than the previous one as it is linked to the metabolism of the microbial cell that can be in conditions of nutritional deficiencies. The polysaccharides excreted form a viscous layer which favours microbial anchoring, but also survival in a competitive environment of those bacteria that are able to produce it. Alternatively, microbial adherence is linked to the production of molecules such as lecithins or adhesins (Bazaka *et al*., 2011).

Colonization once they have adhered to the surface, the bacteria form micro colonies that join and grow larger until they produce an extended mass that forms the biofilm (figures 2d and 2e). The presence of polysaccharides, besides favouring the fixing to the support, can also serve as a food reserve for the bacteria and as a protection against the disinfectant solutions.

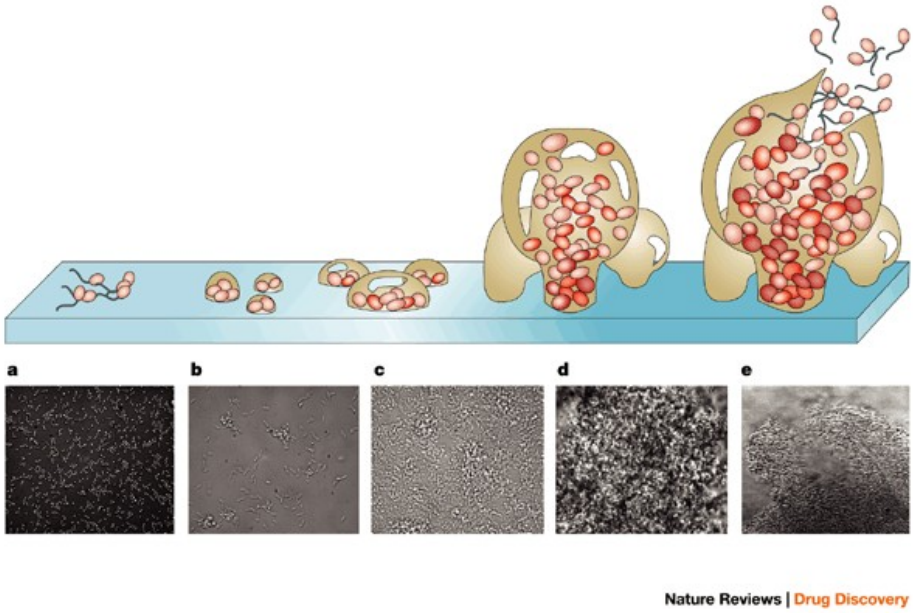


Figure 2 - Phases of formation of the Biofilm

The formation of the biofilm is a major limitation to the subsequent cleaning operations since the micro-organisms that form it and that are trapped in it are protected from the action of disinfectants, the action of which is greatly reduced.

Adhesion is influenced not only by the characteristics of the substrate but also by the pH, temperature, the increase of which generally results in an increase of the phenomenon, and the duration of the contact with the substrate.

(Dunsmore *et al.*, 1982). In agreement with Fletcher 1977, bacterial adhesion is greater when the cells are in the exponential phase at 20°C than in the stationary phase at 3°C (Old and Galli, 1990).

The resistance of micro-organisms to disinfectants is different if these are suspended in a liquid or adhere to a solid surface; in fact a micro-organism in suspension presents a greater surface area of contact with the disinfectant, and the diffusion of the disinfectant is more rapid in a liquid that in a capsule mucosa (Cerf , 1986). In some studies (Chevalier *et al* 1988) it was observed that even

taking into account other factors such as nutritional deficiencies, adhesion to surfaces made the resistance to chlorine of some bacteria 150 times greater (Old and Galli, 1990).

1.4 Sanitization of surfaces

The surfaces, of transport and storage, with which the various hospital instruments and equipment come into contact, can become a significant source of microbial contamination, if not cleaned properly and regularly. The micro-organisms come from the equipment itself, from the air, from the staff and also from the sanitizing solutions. (Leveau, 1988; Snyder jr, 1986). It is therefore essential to correctly place the furniture, divide the departments and design the characteristics of the premises, including floors, walls and ceilings, air quality, staff hygiene, and of course the materials for construction.

Poor attention and superficiality in the cleaning procedures are the leading cause of occurrence of hospital-acquired infections, the consequences of which can be severe (Tood, 1985).

In general, cleaning consists of two different and consecutive steps: cleansing and disinfection.

The first consists of the removal of coarse residues often visually evident, through the use of detergents. The disinfection has the purpose of reducing, and eventually eliminating, the micro-organisms present. The effectiveness of disinfection depends on how the area was previously cleaned, but it is also strongly influenced by factors such as the concentration of use, the contact time, temperature and pH, water hardness and the type of surface to treat.

When choosing the disinfectant, it is essential to assess the presence of organic matter or residues of detergents, and the type of micro-organisms to eliminate. You should also consider the toxicity, corrosive effects, the residual activity, the polluting effect once discharged and of course the cost.

To assess how efficient a sanitation system is, consider the amount of residual dirt, the number of surviving micro-organisms and the degree of corrosion (Old and Galli, 1990).

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1.5 Evaluation of the hygienic condition of surfaces

The methods for evaluating the germicidal power of detergents and disinfectants can be divided into three categories according to Scheusner (1982):

- standard laboratory tests
- tests carried out on work surfaces and surfaces of equipment
- tests that simulate the process conditions in a laboratory

The laboratory tests applicable to a disinfectant miscible with water, establish:

- the number and type of microbial strains that are to be used
- the preparation of the inoculum
- the contact conditions between micro-organisms and disinfectant
- the concentration of the disinfectant and the contact time
- the pH , the presence of electrolytes or organic substance which can inactivate the disinfectants
- the procedures for disposal of residual disinfectant (Cremieux and Fleurette, 1983).

The methods of bacteriological control of the equipment do not have to be expensive, do not necessarily require trained personnel and particularly sophisticated equipment.

The are called direct methods when there is direct contact between micro-organisms and the culture medium and indirect when the micro-organisms, collected using various techniques, are then transferred to the culture medium; among the first is the use of agar plates for contact, among the second is the method using cotton swabs or calcium alginate and cellulose sponges. Each of these has advantages and disadvantages (Marenzi, 1983; Cousin, 1982; McGoldrick *et al.* 1986).

For closed equipment you can perform microbiological analyses of the water used for the final rinse after sanitization (von Bockelmann et al, 1985). Some methods mentioned can also be used for the skin surface of the operators (Goldsmith et al., 1988).

The tests of the third category consist in using suitable devices to sediment and remove dirt on test strips of the same material of the equipment. In this way it is possible to reconstruct in a laboratory, under controlled conditions, what happens in real situations.

The mechanisms of removal of dirt from the surfaces are very complex (Jennings, 1980; Koopal, 1985), and it is also difficult to obtain an objective assessment of the degree of cleanliness of a surface, and the quantification of the dirt deposited.

Tamplin (1980) defines a surface clean, both wet and dry, when it shows no visible signs of contamination under good lighting conditions, it should not emanate odours, feel greasy or rough when touched with clean fingers, change the colour of a piece of white paper when rubbed several times and it must not show any signs of breakage of water while it is drying.

In truth, the problem is much more complex, because the visual assessment of the conditions of cleanliness is extremely subjective and is influenced by the intensity of the light, by the fact that films of residues may be invisible if the surface is wet or difficult to identify, such as proteins, even when they are dry.

Therefore other methods have been proposed; among these is the accurate weighing of test strips contaminated with food, before and after cleaning, the evaluation of the light transmitted through a glass surface, or the light deviated by dirt when a light ray hits the test surface with constant intensity, the evaluation with turbidimetric measurements of the presence of milk in mixtures of milk, detergent and water or the monitoring of milk residues during rinsing using electrical conductivity.

The use of radioactive tracers using food labelled with ^{32}P, ^{14}C and ^{45}Ca has also been suggested to study the removal of dirt (Jackson, 1984).

Anderson *et al.* (1986) developed two methods, the Lowry method modified and the "Chemstrips GH" method, generally applied in the field of clinical analyses, to highlight residues of proteins on the surfaces of contact with food.

More recent is the introduction of the use of the bioluminometer; bioluminometric detections (light emitted by cellular ATP that reacts with the luciferin/luciferase system) are a valid support to the monitoring as they give a real-time measurement of the presence of residual ATP, index of contamination, which may be of microbial and/or organic origin.

The use of the bioluminometer does not replace traditional microbiological control, because this tool is not designed to measure the number of micro-organisms, but it completes and makes objective and verifiable the condition of the surfaces after washing.

A good sign of cleanliness of the surfaces where protein-based products are processed or packaged is the search for residual proteins on the surfaces where the processing took place. The tests on the market give a positive result for the presence of proteins if the buffer has detected at least 50 µg of residual protein, the limit under which these tests do not work.

Of course, these tests are valid if the contamination contains protein (processing of meat, cheese, milk-based products, delicatessen products, tinned meat, fish, etc.), whereas they are useless on surfaces contaminated by non-protein substances (mineral water, soft drinks, wine, etc.) or with a very low protein content.

2. MATERIALS AND METHODS

2.1 Materials used

	Material	Description
1	Austenitic Stainless Steel (Glossy Surface Finish)	AISI 304 - X5CrNi1810 BA surface finish
2	Austenitic Stainless Steel (Scotch-Brite Surface Finish)	AISI 304 - X5CrNi1810 SB surface finish
3	Anodized Aluminium	5005H24 Oxidation for Silver Anodizing
4	Chrome-plated Iron (Chrome-plated Generic Steel)	S235JR Fe 360 Chrome-plated surface
5	Painted Iron (Painted Generic Steel)	S235JR Fe 360 Epoxy Coating
6	Corian®	Generic for hospital use
7	Baydur®	Generic for hospital use
8	Polystyrene	Generic for hospital use

2.2 Micro-organism test

Two micro-organisms with different origins and behaviour were used. International collections were used for both. This choice allows us to examine the two main species of micro-organisms found in hospitals (www.epicentro.iss.it).

a) *Escherichia coli* DSMZ 30083^T, Gram-negative, facultatively anaerobic, mesophilic, non-motile, non sporulated. Faecal micro-organism, the discovery of which, especially in a hospital environment, is definitely a sign of organic contamination. In general , however, it includes pathogenic strains responsible of severe gastrointestinal forms (intoxications and infections).
b) *Enterococcus faecalis* DSMZ 20478. Aerobic, Gram-positive Cocci, thermotolerant, belonging to the group of lactic acid bacteria (lactic acid fermentation). Micro-organism found in numerous environments such as the intestines of mammals including man. This too is generally non-pathogenic, its discovery in large quantities in a hospital setting indicates a lack of attention to hygiene to the point of being considered one of the most important causes of nosocomial infections. Its clinical importance is also connected to the ability of certain strains to be resistant to treatment with antibiotics (vancomycin -resistant strains).

2.3 Disinfectants

A disinfectant based on quaternary ammonium, widely used in hospitals, was considered.

2.4 Microbial Count

For the recovery of micro-organisms from contaminated surfaces, the technique of tampons and pads were adopted at the same time, both of which are deemed appropriate for ISO standards.. For the enumeration of live and vital microbial forms, both specific selective media for the micro-organism in question and generic media were used.
For the count of *Enterococcus faecalis*, the selective medium used was Kanamicina Esculina Azide Agar (KEA VWR International) whereas for the count of *Escherichia coli* the medium used was TBX (TBX, VWR International); both were incubated at 37°C for 48 and 24 hours respectively.
The generic medium used was Tryptic Soy Agar (TSA, VWR International); incubation under the same conditions.
All counts were carried out twice and the interpretation of the results, expressed as ufc/cm^2, was carried out in accordance with ISO 18593 (2004), using the following formula:

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$$N_s = (N \times F/A) \times D$$

where

N_s = number of ufc counted

F = number of millilitres of diluent added to the swab test-tube or bag for homogenization

A = sampled area in cm^2

D = dilution factor

3. RESULTS

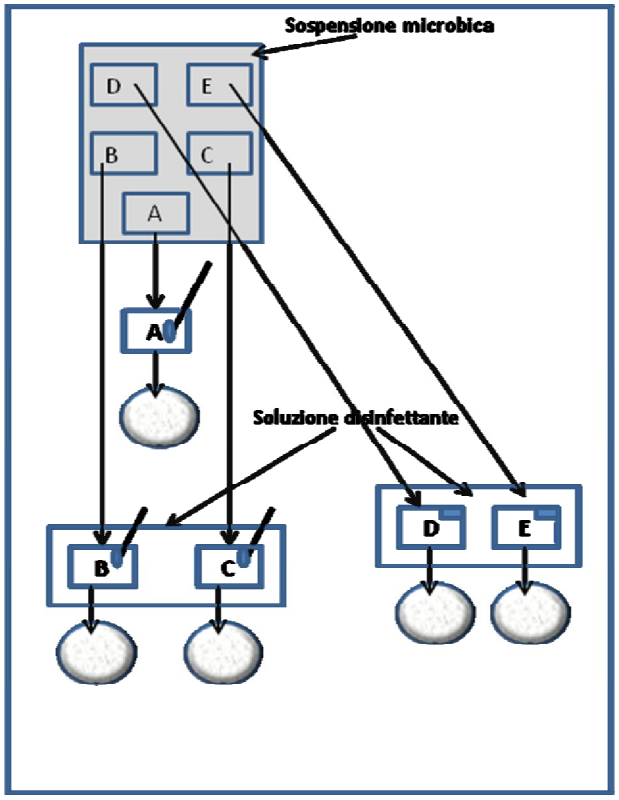
3.1 Development of the experimental protocol

The first part of the work consisted in the development of the experimental protocol and provided for the setting up of tests characterized by different steps and operations. In all the tests the micro-organism *Escherichia coli* DSMZ 30083^T was used. Three different modes of operation were developed.

Protocol 1 (figure 3).

- **Contamination***:* the materials were placed in a microbial suspension at a known concentration, for 1 hour (contamination by immersion)
- Samples extracted, drained and dried under a hood for 10 minutes
- **Sample A**: smeared with a dry swab and analysed through decimal dilutions
- **Disinfection:** Samples B, C, D and E were left immersed in a 5% disinfectant solution; the contact time was 1 min (B and D) and 5 min (C and E).
- **Samples B and D:** smeared with a dry swab and analysed through decimal dilutions
- **Samples C and E:** padded with sterile sponges soaked with tryptone salt and analysed through decimal dilutions

Figure 3 - Protocol 1

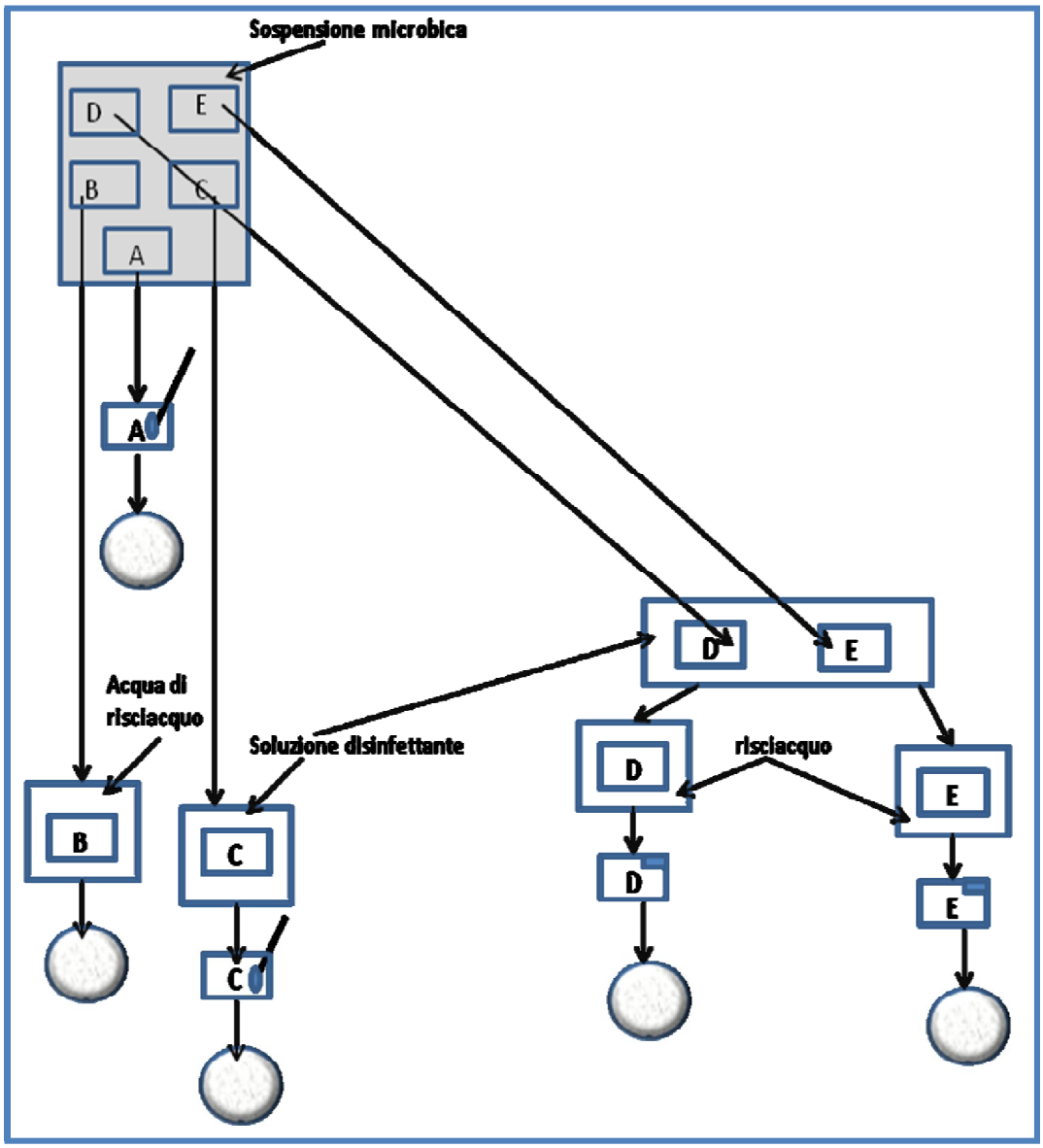


Protocol 2 (figure 4).

- **<u>Contamination:</u>** the materials were placed in a microbial suspension at a known concentration, for 1 hour (contamination by immersion)
- Samples extracted, drained and dried under a hood for 10 minutes
- **Sample A**: smeared with a dry swab and analysed through decimal dilutions
- **Sample B**: rinsed in sterile water for 5 min, analysed through decimal dilutions
- **<u>Disinfection:</u>** Samples C, D and E were left immersed in a 5% disinfectant solution; the contact time was 1 min
- **Sample C:** smeared with a dry swab and analysed through decimal dilutions
- **Sample D:** rinsed in sterile water and padded with sterile sponges soaked with tryptone salt and analysed through decimal dilutions

- **Sample E:** rinsed in LPT and padded with sterile sponges soaked with tryptone salt and analysed through decimal dilutions

Figure 4 - Protocol 2



Protocol 3 (figure 5)

- Contamination of the materials by means of microbial suspension at a know concentration (0) with a sprayer, contact time 1 hour

- Dried under a hood for 10 min
- **Sample A:** smeared with a dry swab and analysed through decimal dilutions
- **Disinfection**: sprayed with 5% disinfectant suspension for 2 min for samples B, C, E and F
- Dried under a hood for 10 min
- **Sample B**: smeared with a dry swab and analysed through decimal dilutions
- **Sample C**: taken with sterile sponges soaked with tryptone salt and analysed through decimal dilutions
- **Sample D**: smeared with a dry swab and analysed through decimal dilutions of sample C
- Samples E and F immersed in a neutralizing solution (LPT) for 1 min.
- Air dried for 10 min.
- **Sample F**: smeared with sponge and analysed through decimal dilutions (F)
- **Sample G**: sample F smeared with a dry swab and analysed through decimal dilutions

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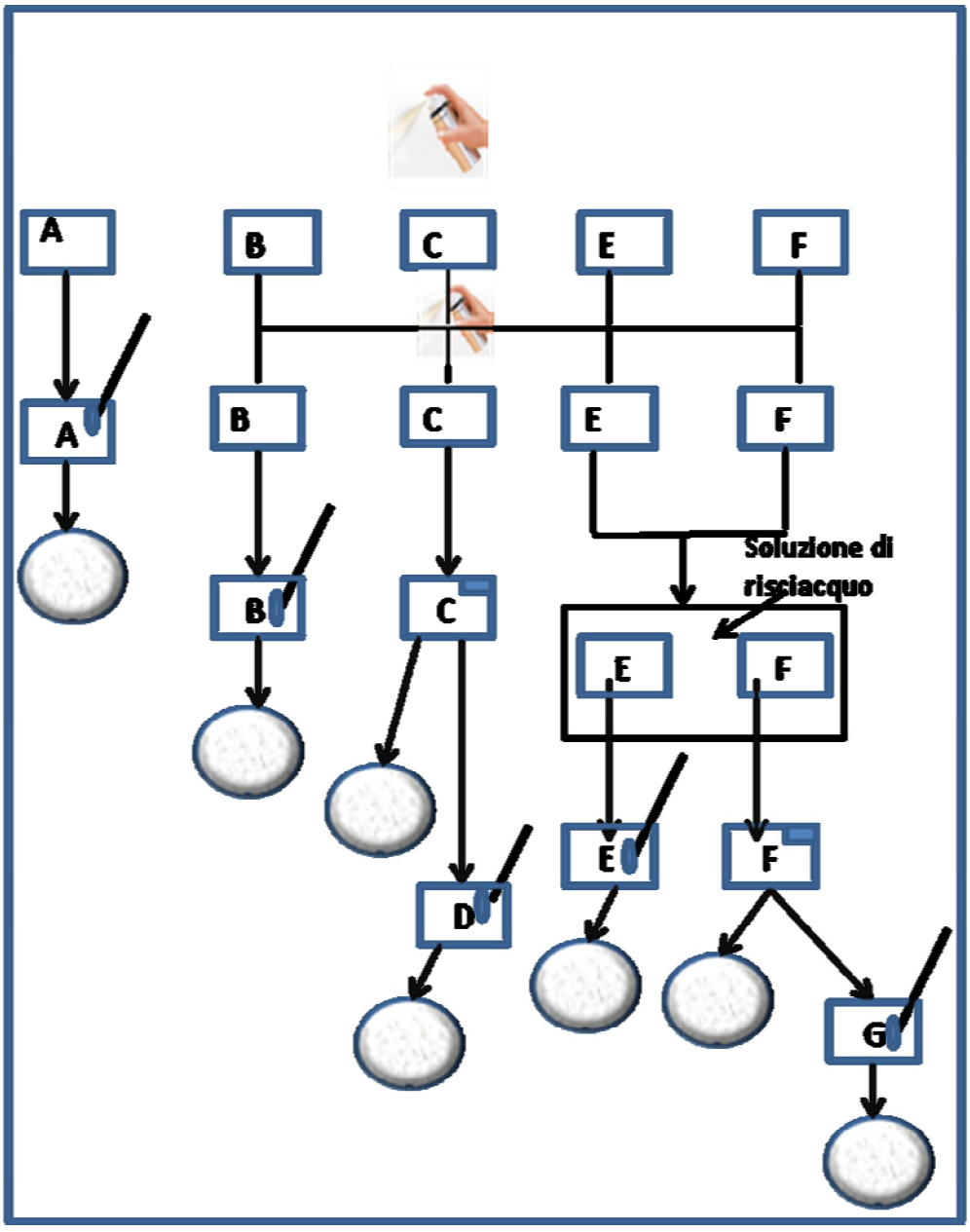
Figure 5 - Protocol 3



The results obtained with the first operational protocol show that, regardless of the materials and the contact time adopted, the disinfectant eliminates all microbial forms, so it was discarded. Probably

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the presence of traces of disinfectant, taken with a swab and/or sponge, not neutralized, affects the analysis.

Table 1 - Results of protocol 1

Samples	AISI 304	Painted iron
0	$3.0x10^6$	$6.0x10^6$
A	$2,4x\ 10^5$	$1.3X10^5$
B	<1	<1
C	<1	<1
D	<1	<1
E	<1	<1

Therefore, in the second protocol, after disinfection a rinsing phase, and optionally a neutralization phase, was introduced to eliminate every trace of disinfectant. In this case, the higher affinity to microbial adhesion of the material painted iron is confirmed compared to AISI 304 BA, which is easier to clean even when only rinsed with water. However, even this operational solution was abandoned because, again, after cleansing, no significant differences were detected.

Table 2 - Results of protocol 2

Samples	AISI 304	Painted iron
0	$1,1\ x10^6$	$9x10^6$
A	$6.0x10^5$	$7\ x\ 10^5$
B	$3.0x10^5$	$2,4x10^4$
C	<1	<1
D	<1	<1
E	<1	<1

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Table 3 - Results of protocol 3

Samples	AISI 304	Painted iron
0	2.0×10^{7}	5.0×10^{7}
A	4.7×10^{6}	1.0×10^{6}
B	<1	<1
C	<1	<1
D	<1	<1
E	<1	<1
F	<1	<1
G	<1	<1

A third operational protocol, closer to reality, was then developed: all operations (excluding neutralization) were carried out through spraying.

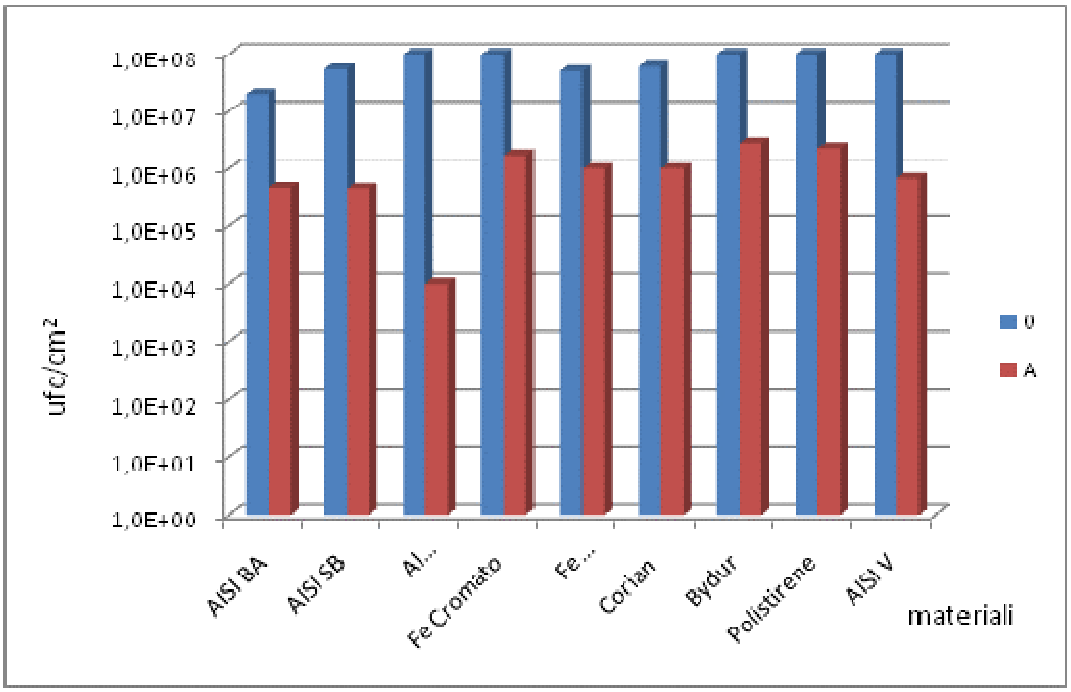
The results obtained are not very different from the previous ones: they highlight the difference in the adhesion of the micro-organism to the two surfaces, while the disinfection does not seem to have a different effect up to this point. This protocol was, however, one more suited to the objective of this paper, therefore, the next tests followed the same operating procedure.

In light of the fact that the sanitizing treatment appears more than effective, independently from the micro-organism and the surface considered, the next tests concentrated on the evaluation of the adhesion capacity of micro-organisms on surfaces, using protocol 3 (spraying) for contamination.

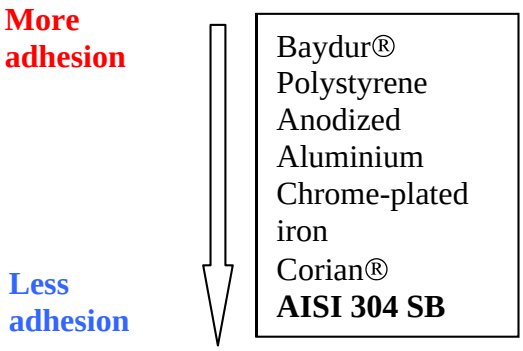
3.2 Tests with *Escherichia coli*

Against a high initial contamination (sample 0 blue column) cleaning operations always proved to be more than effective. Instead, more interesting is a comparison between columns 0 and A.

Figure 6 - Results of the tests carried out with *E. coli.*



Column 0 (the blue column) indicates the number of micro-organisms seeded on the sample and that have actually adhered to the surface (the higher the number the more micro-organisms adhered); column A (red column), on the other hand, indicates the amount of micro-organisms that were removed by cleaning with a sponge, therefore the higher the number (sample A red column) the more efficient the microbial removal and the material remains cleaner. It is therefore possible to say that the materials tested show a different affinity to microbial adhesion: the final result will have to consider both aspects (adhesion and removal). The materials the micro-organisms adhere to more are Bydur® and Polystyrene, followed by anodized Aluminium. There are no particular differences between Corian®, AISI 304 SB painted Iron. The material the micro-organisms adhere to less is AISI 304 BA. The scale of microbial affinity of the materials with regards to *E. coli* is therefore the following.



After calculating the ANOVA, the calculation of the value of Fisher's LSD (Least Significant Difference) showed that the materials with regards to the adhesion of *E. coli* are all significantly different from each other except for polystyrene and Baydur® which are similar to each other (AISI BA[a]; AISI SB[b]; painted Iron[c]; chrome-plated Iron[d]; anodized Aluminium[e] ;Corian[f]; Bydur[g]; Polystyrene[g])

3.3 Tests with *Enterococcus faecalis*

The results of the tests carried out using *Enterococcus faecalis* are shown in figure 7. Despite some differences due to the different nature of the micro-organism, as compared to the materials, the results obtained confirm what was observed for *E. coli* (see 3.2). In the presence of an initial contamination between 10^7ufc/ml and 10^8 cfu / ml , the difference between sample 0 (initial inoculum) and sample A (amount removed with a sponge), also in this case, is lower for the two materials AISI BA 304 and SB 304, compared to all the others;

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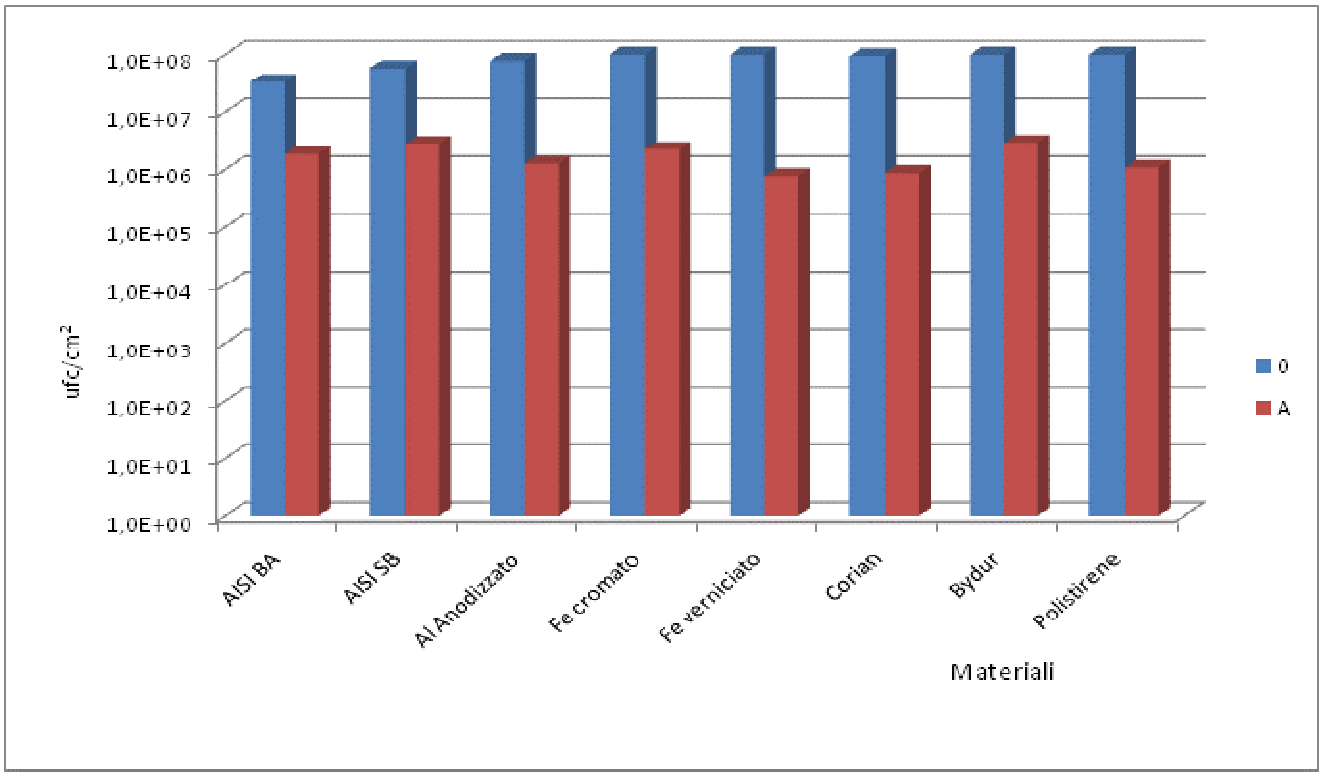
Figure 7 - Results of the tests carried out with *Enterococcus faecalis*



These results show that the micro-organism tested (*Enterococcus faecalis*) is less likely to adhere to the two types of Austenitic Stainless Steel.

The scale of adhesion to the materials tested with Enterococcus is, in ascending order, the following:

More adhesion

Chrome-plated iron
Painted iron
Polystyrene
Baydur®
Corian®
Anodized Aluminium
AISI 304 SB

Less adhesion

In this case the situation is more complex, the ANOVA calculation on the data obtained showed that there are significant differences between the materials (AISI BA[a]; AISI SB[ab]; anodized Aluminium[ab] ,Corian[bc]; Bydur[cd]; Polystyrene[cd], chrome-plated Iron[d]; painted Iron[d])

The calculation of the value of Fisher's LSD (Least Significant Difference) showed that the materials can be grouped into 4 groups

Group 1: AISI 304 BA, AISI 304SB anodized Aluminium

Group 2: AISI 304SB Anodized Aluminium, Corian®

Group 3: Corian®, Baydur®; Polystyrene

Group 4: Painted Iron, chrome-plated Iron, Baydur® , Polystyrene

It can be observed that the values of sample F+G, which represents the amount of micro-organisms removed from the sample after disinfection and neutralization, through the removal of residual micro-organisms using a sponge+pad is greater on AISI 304 BA and AISI 304 SB.

3.4 Influence of contact time

At this point we proceeded by contaminating the different materials through spraying, but by leaving the micro-organism in contact with the surface for 24 hours in order to verify whether the higher contact time would change the amount of micro-organisms adhered and to simulate a normal working day when the surface is cleaned only at the end of the day.

The micro-organisms spread through spraying of a suspension at a known concentration, were removed using a sponge in order to simulate the operations that are normally carried out with a cloth. The differences observed after 1 hour of contact tend to cancel themselves: the amount of micro-organisms removed is very similar between the various materials. This confirms how the phenomenon of microbial adhesion is a process which proceeds rapidly (within 30'), after which there is the formation of biofilm, difficult to remove, and the levels of microbial concentration tend to conform, irrespective of the material (Vasone, 2011). Cleaning is more effective the sooner it is done. However, AISI BA steel always proves to be the material to which the micro-organisms adhere less, while plastic materials, despite their particular smoothness, are those to which the

micro-organisms adhere more. Figures 8 and 9 confirm the scale of adhesion already extrapolated with the previous tests.

Figure 8 – Micro-organisms (*E. coli*) removed with a sponge after 24 hours of contact.

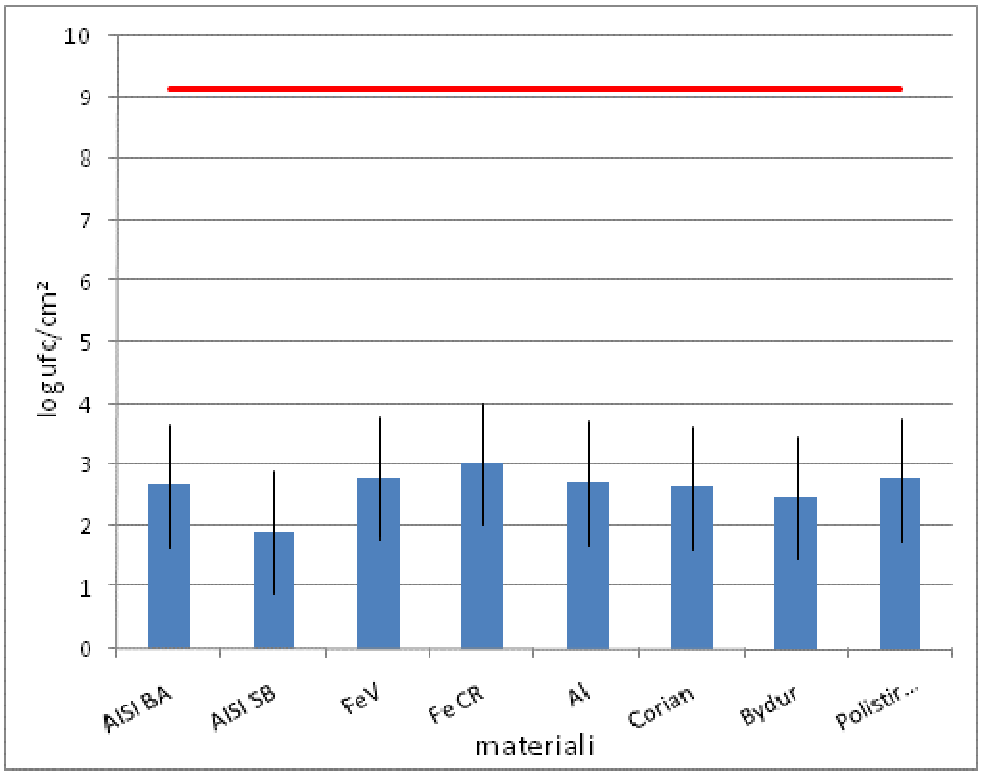


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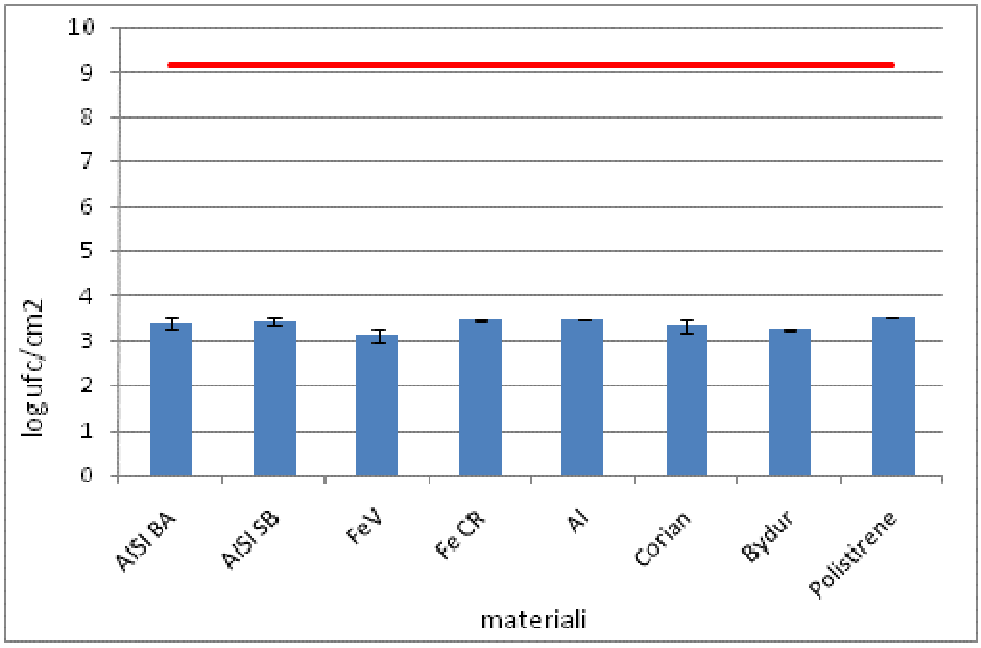
Figure 9 – Micro-organisms (*Enterococcus faecalis*) removed with a sponge after 24 hours of contact.



The different behaviour concerning microbial adherence of the various materials can be seen in the photographs that show the different distribution of the microbial suspension on the various surfaces. The microbial suspension, or dirt, is uniformly distributed on Corian and painted iron, but especially on Baydur and Polystyrene, on which it forms a homogeneous surface layer, thus showing a greater affinity towards these materials. Anodized Aluminium follows, where the microbial film is distributed fairly evenly. On chrome-plated iron the distribution appears as drops of different sizes, but spread all over the surface. Much less homogeneous is the distribution of microbial suspension on AISI 304 SB and especially on AISI BA on which the dirt tends not to stop. These materials are therefore confirmed as those having less affinity for microbial adhesion.

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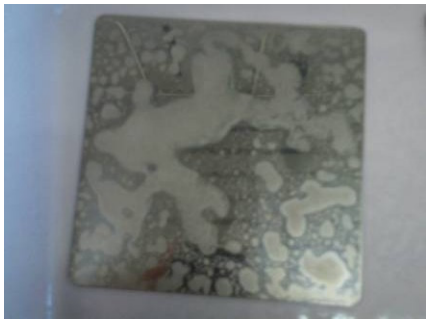
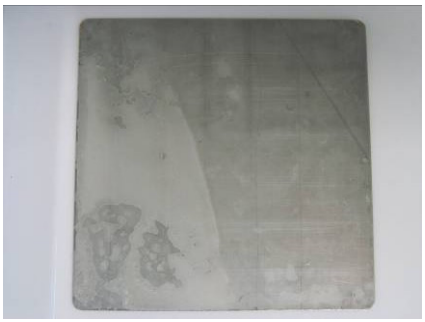
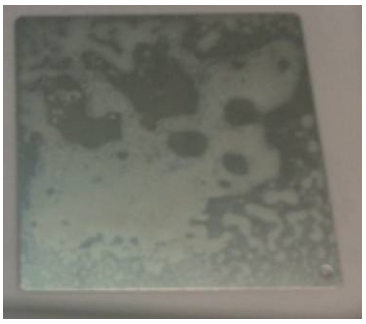
AISI 304 BA



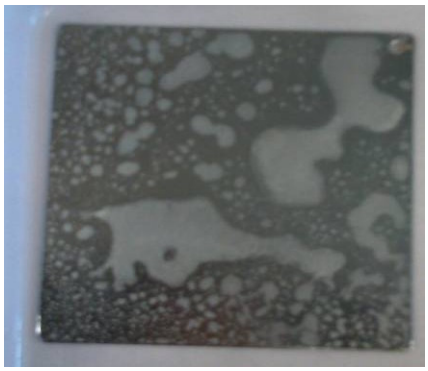
AISI 304 SB



anodized Aluminium



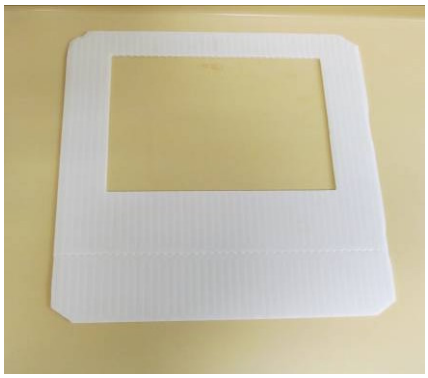
Chrome-plated iron



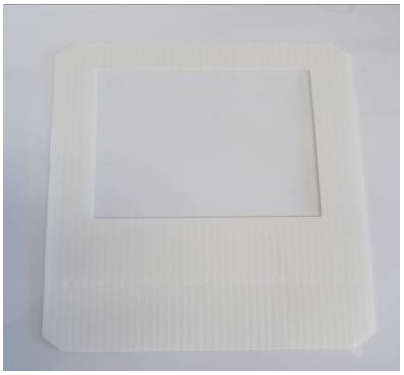
Painted iron

Corian®

Baydur®



Polystyrene



4. CONCLUSIONS

The tests carried out have shown how the materials analysed behave differently in terms of reaction to the presence of micro-organisms: the degree of affinity and the resulting bacterial retentivity of the materials turned out to be significantly different. In particular:

- **the two Austenitic Stainless Steels were the most resistant materials to the microbial attack for short contact times, in particular AISI 304 BA seems to be the best followed by AISI 304SB (the first material showed less microbial adhesion with both micro-organisms, see tests);**
- anodized Aluminium, chrome-plated iron and painted iron are placed in an intermediate position (mixed results depending on the micro-organism for iron, intermediate position always for aluminium);
- the materials that micro-organisms adhere to most easily are Polystyrene, Corian® and Baydur®.

These differences tend to be reduces if the contact times between micro-organisms and material increase (24h). Disinfection eliminates all microbial forms, but only if carried out on a regular basis and in accordance with the times and concentrations indicated on the label. To discourage the use of materials other than austenitic stainless steel is not so much the different cleanability but rather the lesser tendency of organic dirt (micro-organisms) to remain adherent. This is evident from the pictures where the dispersion of the solution can be taken as another sign of the affinity between materials and micro-organisms.

Another important aspect is related to the wear of the material: originally (that is new materials) plastic materials (Corian□, Baydur□ and polystyrene) are very smooth and, therefore, apparently easy to clean (unless there is higher retentivity, a phenomenon that has already been discussed); eventually, as a result of daily use in the hospital environment (opening drawers, doors, etc.) and of characteristics of the surface which such as greater aptitude towards abrasion, cracking, etc. compared to austenitic stainless steel, they allow the formation of an environment characterized by geometries and micro-geometries where micro-organisms can easily spread, protected by the action

of the disinfectant, and thus becoming niches for microbial growth. At this point their wear will make sanitization ineffective proportionally to their wear.

Instead, the lower microbial affinity observed for Austenitic Stainless Steel favours cleaning and removal of dirt (ecological niche that favours microbial growth) which are easier to perform even after a prolonged use of the detergents themselves, regardless of the roughness and wear of the materials (Vasone 2011) and the upkeep of a stable situation of the surface for longer (lower surface alteration).

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