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Fluorescence-assay on traces of protein on re-usable medical devices: cleaning efficiency

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Abstract

The cleaning of re-usable medical devices before disinfection or sterilization is recognized as being an essential phase. Detection of residual proteins can be used to validate the process, provided a sufficiently sensitive method is employed. A fluorescent method is presented, using orthophtalaldehyde (OPA) bound to *N*,*N* dimethyl-2-mercaptoethylammonium, to demonstrate the presence of amino acids on a medical device following cleaning. The sensitivity of this method (10^{-5} g/l) was assessed and the applicability of this detection technique is verified, using three types of carriers (steel blades, glass tubes or ceramic penicylinders), three types of contaminants (yeast extract, bovine albumin with native sheep's blood and formaldehyde fixed fibrin). In this context, studies involving formaldehyde-fixed fibrin are more sensitive and are to be recommended.

Keywords: Cleaning; Disinfection; Medical devices; Orthophtalaldehyde; Protein; Sterilization

1. Introduction

The cleaning of re-usable medical devices before disinfection or sterilization must be effective, since organic matter is known to carry an inhibitory effect by means of protection of microorganisms from denaturing agents (Van Klingeren, 1995; Spicher and Peters, 1997). Validation of these cleaning processes would be of value, especially given that counts of viable microorganisms before sterilization remains difficult. The emergence of Creutzfeldt–Jakob Disease (CJD) has added a new risk factor with a new proteic infectious agent for which few sterilization methods are effective (Budka et al., 1995; Taylor, 1996), and appears to justify the chemical detection of proteins. The orthophtalaldehyde (OPA) method described by Roth for the detection of amino acids (Roth, 1971) was modified by Frister

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et al. (1988) and has been applied by Michels et al. (1996) in the evaluation of the cleanliness of medical and surgical equipment following machine cleaning, with spectrophotometric absorbance analysis. We propose a more sensitive fluorescence assay, using a fluorescent derivative after reactions between free alpha-amino acids, OPA and a thiolated derivative (dimethyl-2-mercaptoethylammonium chloride).

2. Methods

2.1. Carriers

We used 5 ml haemolysis glass tubes, surgical steel blades (Swann–Morton, ref 0208, Labo Moderne, Paris, France) and 5×10 mm ceramic penicylinders (AOAC, Arlington, VA) as protein carriers, and three protein solutions as soils. These solutions were: (1) yeast extract (Difco, Detroit, MI) 1 g/l in sterile distilled water; (2) bovine albumin (Sigma-BioSciences, St Quentin, France) 3 g/l in sterile distilled water + 15% native sheep's blood; (3) fibrin (Sigma-BioSciences, St. Quentin, France) 0.1 g/l, Tween 80 (Merck, Darmstadt, Germany) 0.5 g/l in sterile distilled water.

To determine the limit of detection, we first tested the method using human albumin solution (1 g/l). To evaluate the efficacy of treatments, each carrier (blades and tubes) received 50 μl of one of the protein solutions, or were soaked in the protein solution for 5 min (ceramic penicylinders). The carriers were then put into a 37°C incubator with ventilation, until visible dry. With the fibrin, add formaldehyde solution (3%, v/v) before drying to fix the soil.

2.2. Treatment and recovery of proteins on carriers

The treatment processes for the soiled carriers were selected because of their known efficacies in the prevention of CJD (Taylor, 1991; WHO, 1996). We used: (1) Javel bleach containing 2% free chlorine, soaking for 7.5 min; (2) a sodium hydroxide 1 N solution, soaking for 30 min; (3) a pure detergent, Salvanios (Anios, Lille, France), 0.8 ‰ (v/v), soaking for 15 min. Complementary, we performed washing machine complete cycles, for haemolysis tubes only (Techmil, Gütersloh, Germany), as the other supporting media were too small to be machine treated.

After the cleaning processes, each carrier was treated by ultrasound in 1 ml of sterile distilled water. The eluents were the 'test preparations'. At the same time, positive controls with untreated carriers were prepared in the same conditions. Detections of residual proteins were then performed.

2.3. Detection of residual proteins

2.3.1. Protein hydrolysis protocol

Add 100 μl of one of the protein solution to 2 ml of 6 N hydrochloric acid, then heat at 110°C over a 24-h period in a sand bath. Neutralize using 2 ml of 6 N sodium hydroxide.

2.3.2. Preparation of the OPA solution

Dissolve 40 mg of orthophtalaldehyde (Fluka, Buchs, Switzerland) in 1 ml of methanol (Sigma-BioSciences, St Quentin, France), then add 50 ml of 0.02 g/l disodium tetraborate buffer (Fluka, Buchs, Switzerland) and 100 mg of *N*,*N*-dimethyl-2-mercaptoethylammonium chloride (Touzart and Matignon, Courtaboeuf, France).

2.3.3. OPA reaction

A total of 100 μl of the protein solution with 100 μl of sterile distilled water added (validation), or 200 μl of the 'test preparation' (detection on carriers) were mixed with 1 ml of the OPA solution. Measurements were performed at room temperature (23 ± 2°C), with a Perkin Elmer LS-5 spectrofluorometer (Touzart and Matignon, Courtaboeuf, France), equiped with 10 mm optical path reduced quartz cuvettes. Excitation and emission were performed at 340 and 455 nm, respectively. Fluorescence intensities (FI) are expressed with arbitrary units (scale: 0.1 to 80). Each assay was performed five times and a relative standard deviation (RSD) was calculated for each set of data.

3. Results

3.1. Validation

We performed a determination of the limit of detection, using dilutions of the hydrolysed soil solutions: yeast extract, human albumin, bovine albumine with sheep's blood and fibrin. For each compound, five different measurements at different concentration level were performed on successive dilutions (10^{-3}–10^{-6}) of the preparations. Negative controls (distilled water) were used under exactly the same conditions, with the same added reagents, in order to evaluate any contamination arising from reagents or handling. Results are shown in Table 1. The detection limit signals for hydrolysed human albumin and yeast extract allow us to validate the 10^{-5} dilutions, which correspond to a concentration of 10^{-5} g/l of proteins (RSD = 4.6%). We observed similar results for the bovine albumine and fibrin solutions with an acceptable sensitivity using the 10^{-6} dilutions, which correspond respectively to 3.10^{-6} g/l of bovine albumin and 10^{-5} g/l of fibrin.

3.2. Detection of experimental contaminants on treated carriers

We performed determination on every hydrolysed test preparation. The best results were obtained where tubes are machine washed. In this case, we never found traces of protein, after treatment and analysis of 81 haemolysis tubes exposed to nine cycles with the three types of soils (FI < 4). This confirms the greater efficacy of the cleaning process itself and the benefits of rendering this cleaning process automatic when preparing re-usable medical devices for disinfection or sterilization (Miles, 1991). For the three processes of cleaning by soaking, and for the three carriers, we evaluated the efficiency of treatments (nine experiments for each soil). The results are shown in Table 2. A cleaning process was considered as effective when the mean from FI values obtained on the eluent of the treated carriers was lower than the FI value obtained at the 10^{-6} dilution of soil solutions, during the validation study, as described in Table 1. Taking into account the 1:10 dilution ratio of the protein soil from the carrier to the eluent (Section 2.2, paragraph 2), it was then considered that a reduction of at least five logarithms was reached, or that the treated carriers were able to retain less than 10^{-5} g/l of residual proteins.

Table 1
Fluorescence intensity (FI) obtained for dilutions of the protein solutions used as soils[a]

	Fluorescence intensity (FI)			
Dilutions of protein solutions	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Human albumin	61	50	25	4
Yeast extract	32	27	15	1
Bovine albumine + sheep's blood	77	33	55	10
Fibrin	>80	76	45	25

[a] Yeast extract (1 g/l), human albumin (1 g/l), bovine albumine (3 g/l) with native sheep's blood added (10 ml/l) and fibrin solution (10 g/l). Negative controls (distilled water) were always equal or under 1 FI (RSD < 5%).

4. Discussion and conclusion

In this study, we wanted to evaluate the modified orthophtalaldehyde (OPA) method, and to identify the role of several procedures in the elimination of protein contaminants from re-usable medical devices. When the detection itself is considered, the modified OPA method could be applied with an acceptable sensitivity. Numerous other methods have been proposed in order to detect traces of proteins. Some of these methods are less sensitive (Silvestre et al., 1994; Ma et al., 1996, 1997), while others, such as sensitized europium luminescence (Lim et al., 1997) or high-performance liquid chromatography (HPLC) following a precolumn derivatization with OPA (Cooper et al., 1984) are difficult to use. Considering the cleaning processes, it was seen in our experiments that the automatic machine was efficient in every case. The use of a 1 N sodium hydroxide solution allows us to validate by soaking eight effective treatments out of nine. The fibrin contaminant fixed using formaldehyde was

Table 2
Evaluation of the soaking treatment efficiencies, for sodium hypochlorite (2% free chlorine, 7.5 min), sodium hydroxide (1 N, 30 min) and commercial detergent (Steranios, 0.8‰, 15 min)[a]

Treatment	Sodium hypochlorite			Sodium hydroxide			Detergent		
Carriers	Ceramic cylinders	Steel blades	Glass tubes	Ceramic cylinders	Steel blades	Glass tubes	Ceramic cylinders	Steel blades	Glass tubes
Yeast extract	8	2 (E)	6	4 (E)	3 (E)	<1 (E)	2 (E)	1 (E)	1 (E)
Bovine albumine	6 (E)	7 (E)	12	4 (E)	7 (E)	9 (E)	2 (E)	<1 (E)	18
+ sheep's blood									
Fixed fibrin	28	41	34	18 (E)	11 (E)	37	12	29	32

[a] Detection of residual proteins after treatment expressed in FI units, means from ($n = 3$) carriers for yeast extract and bovine albumine, means from ($n = 6$) for fixed fibrin. (E) = Effective treatment, or less than 10^{-5} g/l of residual proteins.

the more resistant soil in all types of solutions for soaking, with only three effective treatments out of nine. This finding could be disturbing, given that this hydrophobic protein is that which most closely resembles the protein which may be the infective agent in CJD. On the other hand, the nature of the carriers does not appear to exert a marked influence, although ceramic penicylinders and steel blades seemed to be easier to clean than glass tubes.

As a conclusion, we can recommend the OPA method which was found to be applicable in detecting traces of proteins, and the use of fibrin fixed by formaldehyde as experiment soil in the validation of cleaning procedure for re-usable medical devices.

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