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A survey of exposure to enzymes in cleaning solutions used to clean endoscopes

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Research Report

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A survey of exposure to enzymes in cleaning solutions used to clean endoscopes

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Proteolytic enzymes are a recognised risk for respiratory and dermal allergy. Cases of asthma have been identified in health care workers using cleaning solutions containing these enzymes to decontaminate endoscopes and surgical equipment. An assessment was made of three hospitals using enzyme products to clean endoscopes. Air samples showed that approximately a third of the personal and a half of the static air samples contained protease activity at levels that may pose risk for allergic sensitisation. Wipe samples demonstrated protease on surfaces where manual pre-cleaning of endoscopes was undertaken but lower levels were present elsewhere in these rooms. A risk factor for increased levels of surface and air contamination was a lack of awareness that enzymes were present in the cleaning solutions and posed a risk for respiratory sensitisation. A contributory factor to the lack of awareness was that the enzymes are not required to be identified on material safety data sheets because the concentration of enzymes were less than 1%. As a result there were deficiencies in the application of control measures although the surface contamination levels were much lower at one hospital where regular cleaning of surfaces was undertaken throughout the day.

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KEY MESSAGES

1) Enzyme based solutions used to decontaminate endoscopy and surgical equipment may pose a risk to the health of workers. Exposure by inhalation to enzymes derived from microorganisms (bacteria and fungi) is a recognised risk factor for development of respiratory allergy (asthma) and in contact with the skin some enzymes (proteases) can cause dermatitis.
2) This study examined exposure of health workers to enzyme solutions used to clean endoscopes and site visits were undertaken to collect air samples and surface wipe samples at three different hospitals and a total of 7 different endoscopy units.
3) Different types of enzyme solution were used in these endoscopy units, and the selection of the product varied within hospitals and between units undertaking similar clinical work. The staff had limited knowledge about the constituents of these cleaning solutions and were generally unaware of the risk of respiratory allergy and skin damage associated with concentrated microbial enzymes.
4) The results demonstrated levels of protease in 4 out of 14 personal air samples and 6 out of 14 static air samples that may pose risk for allergic sensitisation. In contrast surface levels of enzyme were very high in all but one of the endoscopy units. The highest levels of enzyme deposits were concentrated around areas used to hand wash the endoscopes, but lower levels of contamination were also found throughout these rooms.
5) Staff were provided with PPE but generally this equipment was not used effectively especially where it could have provided better protection to exposed skin. Staff were generally not aware how to remove contaminated gloves or how to avoid skin contamination.
6) Much lower levels of surface enzyme were found at one endoscopy unit where regular wet surface cleaning was undertaken throughout the working day.
7) The endoscopy cleaning rooms examined were typically small and filled with equipment, making it difficult to implement good cleaning regimes.

EXECUTIVE SUMMARY

This pilot study was carried out to investigate whether hospital staff were exposed to enzymes in endoscope cleaning solutions by inhalation or through contact with the skin and, if so, to identify the causal factors and potential controls.

Site visits were undertaken at three different hospitals where different enzyme cleaning products were being used. A protease activity assay was used to monitor potential exposure to the enzymes in the cleaning solutions and the values measured were compared to a protease standard (i.e, subtilisin). Personal and static air samples were collected to assess the risks of inhalation of these enzymes and wipe sampling of work surfaces, clothing, and skin was used to assess the spread of enzyme contamination.

Endoscopes are cleaned by machine but it is necessary to manually clean them by scrubbing with enzyme containing solutions before they are machine cleaned. The results of the survey showed that those staff undertaking manual cleaning of the endoscopes were most at risk of contact exposure due in some cases to inadequate hygiene and control practice.

The study has demonstrated that concentrated enzyme was found on surfaces close to where manual cleaning of endoscopes was carried out with lower levels of enzyme in other areas of these rooms. Not all of the staff wore disposable gloves and many had bare arms and there was therefore a risk of contaminating skin. One hospital had adopted improved practices that reduced the risk of contamination whereas others used procedures that were more likely to result in spread of contamination.

About a third of the personal air samples and about half of the static air samples contained measurable enzyme activity that may pose risk for allergic sensitisation. Overall the air sampling results did not suggest that inhalation exposure was the most likely route of exposure but the likelihood for exposure of skin was greater (via spread of droplets and through contact with contaminated surfaces). Investigation at one site also showed contamination of work clothes and hands providing further evidence of the risk for personal exposure by contact. Whilst hospital staff were provided with suitable personal protective equipment they were uncertain when to use respiratory protective equipment or about the need to fit test it. The air sampling data obtained in this study suggested that use of RPE as a control measure was questionable and that other controls might suffice to minimise exposure by inhalation.

There was a general lack of awareness that enzymes were present in these cleaning solutions and that these pose a risk for respiratory allergy. The low content of enzyme in these solutions does not require their identification on material safety data sheets which was the case with the products examined. However, the absence of this information may contribute to a lack of awareness that sensitisation and allergy can result from exposure to microbial enzymes at very low levels of exposure (e.g., less than $100ng/m^{-3}$).

The conclusion is that exposure to these enzymes during manual cleaning is mainly by skin contact but the risk of this occurring can be minimised by practical control measures. The effective use of these controls is dependent on providing end users with better information about the potential hazardous properties of microbial enzymes and advice about how to work safely with them.

LIST OF ABBREVIATIONS

Below limit of detection	BLD
Control of Substances Hazardous to health	CoSHH
Exploratory Work to Inform Enforcement Decisions	EXTEND
Gastrointestinal	GI
Health and Safety Executive	HSE
Hours	hrs
Material Safety Data Sheet	MSDS
Maximum exposure limit	MEL
National Health Services	NHS
Not Detected	ND
Operation Theatre	OT
Respiratory protective equipment	RPE
Standard operating procedure	SOP
Time weighted average	TWA
Workplace exposure limit	WEL

CONTENTS

1 INTRODUCTION

With societal pressure to reduce the use of hazardous chemicals increasing use is being made of biological agents for industrial and medical applications. The use of endoscopes in non-invasive and invasive surgical procedures has increased in the last ten years. Decontamination and sterilisation of endoscopes is critical for their safe use with patients, particularly to control the risk from hospital acquired infection (bacteria, viruses and prions). Glutaraldehyde was used to disinfect endoscopes but evidence that its use was associated with occupational asthma and dermatitis led the health sector to look for a replacement. Industrially manufactured microbial enzymes are now increasingly used to decontaminate endoscopes. They are considered more effective at removing biological soilant and infectious agents and are regarded as biodegradable and environmentally friendly compared to some chemicals cleaning agents.

Biological contaminants on surgical instruments include blood, mucin, and fats and usually require a mixture of enzymes to ensure complete remove of residues. These enzymes are typically microbial in origin (extracted from e.g., *Bacillus subtilis* and the fungus *Aspergillus oryzae)* and the mixtures may be prepared by purification or enrichment. Recombinant DNA technology may be used to produce genetically modified organisms, which secrete enzymes able to operate under specific conditions of pH or temperature. Endoscopy cleaning products, in addition to enzymes, often contain anionic or ionic surfactants to accelerate the release and degradation of biological soilant but it is the microbial enzymes that are potential asthmagens and skin irritants. Inhalation exposure to microbial enzymes is a recognised cause of occupational asthma in the baking industry [2], and of asthma and allergic dermatitis in the manufacture of biological washing powders [3]. The incidence of asthma and dermatitis in the manufacture of biological washing powders has been reduced in recent years by the application of tighter controls on exposure to these enzymes and the implementation of health surveillance programmes [4]. The introduction of enzymes in other types of cleaning products is a more recent innovation and raises concern that their regular use may give rise to asthma and dermatitis in other sectors of industry. Several cases of asthma attributed to enzyme cleaning solutions have been reported amongst health sector staff cleaning endoscopes [1].

HSE authorises HSL's Occupational Hygiene Unit to undertake brief investigations in areas where there is limited corporate knowledge about potential health risks arising from a particular activity or process. This pilot study was therefore carried out with the **aim** of investigating whether hospital staff were exposed to enzymes via inhalation or through contact with the skin and, if so, to identify the causal factors and potential improvements to controls.

The **objectives** were to undertake hygiene assessments of the methods used to clean endoscopes with enzyme-containing cleaning solutions i.e,

- to quantify exposure by collecting air samples
- to map the distribution of enzyme contamination on surfaces by the collection of wipe samples, and
- to identify (where practical) the sources of exposure by assessing the consequences of the working practices observed

2 METHODOLOGY

Exposure assessment visits were undertaken at seven endoscopy-cleaning units in three different hospitals. The units cleaned endoscopes used for gastroenterology, spinal injury, urology and quick early diagnosis units (QED) procedures.

During each visit staff activities (i.e. cleaning endoscopes) were recorded and representative photographs taken. Staff provided information about the frequency and type of endoscope cleaning work undertaken.

Exposure and potential skin contact with the enzymes was monitored using two methods. Personal and static samples of airborne contamination were collected using IOM samplers with pumps operated at 2 l/min. In addition to air samples, wipe samples were recovered from surfaces adjacent to the endoscope washing activity (in both manual & machine cleaning areas) to which the enzyme might have transferred (e.g., work surfaces, floors and door handles). Wipe sampling was used to assess the level of enzymes that staff might be exposed to via skin contact from work surfaces or handling endoscopes; This involved sampling a uniform (100cm2) grid on all flat surfaces, and on other objects a uniform length (e.g., of an endoscope tube) was wiped. The wipe samples provided a measure of surface contamination, and therefore of general hygiene, and should not be taken as a measure of respiratory exposure.

The air and wipe samples were extracted and the level of proteolytic activity quantified using an enzyme substrate activity assay. This was based on a fluorescent quenched Bodipy™ casein substrate at pH 7.8 using a fluorescent multifunctional platereader. Levels of enzyme in the samples were quantified and expressed in relation to a purified subtilisin protease standard. Bulk samples of the cleaning solutions were also analysed for their proteolytic activity. Altogether five different types of enzyme cleaning solution were being used (see Table 1 in Annex). Two hospital units had recently introduced cleaning agent A, whilst another site used three other products (cleaning agents B, C and D) and one used cleaning agent E. It should be noted that the assay for proteolytic activity was calibrated against a subtilisin standard, the protease for which a UK WEL has been set. Other proteases will differ in the rate at which they degrade the substrate used in this assay and so the results presented here provide comparison in terms of relative levels of contamination for the same product. They do not allow direct comparison between different products.

Fourteen personal air samples were collected with a further eleven static air samples taken close to the breathing zone. Wipe sampling was undertaken at sites close to the washing of the endoscopes, on the automated washing machines, on floors and at sites away from the main cleaning activity. These were taken at the start and at the end of the endoscope cleaning. At one site additional wipe sampling of personal clothing was undertaken before and at the end of the work period.

3 FINDINGS

3.1 PROCESS

The complete cycle of the endoscope cleaning process involves manual pre-cleaning, machine cleaning and for some applications drying the endoscope before use, and for invasive surgical procedures a further instrument sterilisation process is required. At the manual cleaning stage the main surface contamination is removed from the outer and inner surfaces of the endoscope. A variety of methods are used including the use of wipes, small scrubbing brushes, and injection of fluid into the endoscope lumen under pressure. Enzyme cleaning solutions are used in all three of these processes. The critical steps in manual cleaning of endoscopes were as follows. At some units, the used endoscopes were first wiped with tissues soaked in the cleaning solution, and concentrated enzyme was then injected into the lumen of the endoscope. The manual pre-cleaning was then undertaken in a sink. In some units, a fixed volume (~50ml) of the concentrate was poured into the sink and then the water taps turned on to mix and dilute the enzyme.

Once the manual cleaning was completed the endoscopes were transferred to automated wash machines in which the enzyme cleaning solutions were fully contained except when the stock solutions were replaced.

In this study it was noted that the manual cleaning techniques were carried out differently by these hospitals and even between units within the same hospital. For example, at some units once an examination had finished the endoscopes were wiped with an enzyme cleaning solution, at other units they were immediately soaked in a bucket containing enzyme cleaner, and in some cases the equipment was cleaned with enzyme solutions when transferred to the washing facility. Some units used small brushes to clean the outer surfaces of the endoscope holding the endoscope at eye level, other units only carried out surface cleaning with the instrument submerged in a sink beneath the level of the cleaning solution. The procedures for cleaning the inner surfaces also varied with some units using syringes to flush the solution through under pressure.

It was also noted that at the large and busier units the first stage of cleaning work was undertaken by dedicated staff, at smaller units this responsibility was shared amongst different staff.

The automated machines typically used combinations of chlorine dioxide, ortho-phthaladehyde, peracetic acid or a combination of chemical cleaners and enzymes to clean the endoscopes. The only potential for exposure to enzymes occurred when the stock solutions in these wash machines were changed. Work to repair or service these machines was carried out either by dedicated engineering service staff or by external contractors. Their exposure was likely to be limited to contact with dried enzyme solution when servicing the equipment or replacing the enzyme stocks.

3.2 EXPOSURE CONTROLS

The three hospitals visited provided different personal protective equipment (PPE) but even between units within the same hospital the equipment varied. Most staff involved in manual cleaning processes were provided with disposable gloves, aprons, and overalls and only some were provided with (or wore) protective visors and longer length gloves (or covers) to protect skin on the lower arms. Respiratory protective equipment (RPE) was provided by some of the hospitals but for the specific purpose of changing the stocks of chemical cleaning agents. The types of RPE provided included fluid resistant surgical masks with face shields, half masks and full face masks, FFP2D particulate disposable respirators, and organic vapour particulate respirators. Face fit testing was not routinely undertaken.

3.3 OBSERVATIONS ON WORK METHOD AND ROUTES OF EXPOSURE

Endoscope cleaning: For all the sites the time that staff worked with the enzyme cleaning solutions varied from a few minutes to several hours. This work was shared between nurses, charge hand nurses, and some staff dedicated to the washing of endoscopes. At one hospital theatre unit staff wiped the instruments over with enzyme solution before passing them to the cleaning staff. Typically the number of staff involved in the cleaning of the endoscopes was small (~2 staff per unit). Where many instruments

were cleaned the work was shared with each individual working with the solutions for half a day. Here the complete cycle of manual pre-clean, machine cleaning and storage for re-use occurred entirely within the unit. At the other two units within the same hospital, enzyme solutions were used to pre-clean endoscopes in a room adjacent to the procedure room. After this they were transferred to a central sterilisation unit and machine cleaned using peracetic acid.

Six different enzyme-based cleaning solutions were used but none of the material safety data sheets for these products detailed the type of enzyme or provided hazard and risk phrases appropriate to microbial enzymes (as respiratory allergens and skin irritants). Staff were unaware that the cleaning solutions contained enzymes and that microbial enzymes are a potential causes of respiratory and skin disease. Written operating procedures were available to staff and for staff trainers, but staff were generally unfamiliar with their content.

During these activities splashing was observed when the enzyme concentrate was poured into the sink (which was sometimes done before water was added in some locations) and spray droplets were released when the taps were turned on. At other units the supplier had provided pumps and fluid delivery lines so that the enzyme concentrate was added beneath the level of the water once the sink had been filled to a predetermined level. Using this second method droplet sprays were not observed.

Some of the endoscopy units used small brushes to manually clean the endoscopes which were held at head height; during this stage fine sprays were observed close to the operators head. Other units had been advised by the cleaning solution supplier to keep the endoscope under the water surface whilst carrying out this work.

The insides of the endoscopes were usually cleaned by injecting enzyme solution into the lumen whilst the endoscope was lying flat on a work surface. Alternatively the body of the endoscope was submerged beneath water in the sink whilst the solution was injected in the lumen and this minimised the risk of spray falling on the operator.

General contamination: In all but one endoscopy unit, the enzyme cleaning solution had spread to work surfaces and the floor. High levels were measured on the floor closest to the sinks and where pre-cleaned endoscopes were carried to the automatic wash machines. To reduce this contamination the suppliers of one product had advised that endoscopes should be placed onto a disposable absorbent pad placed at the side of the sink before transferring them to the washing machines.

Other factors that contributed to the spread of the enzyme included staff wearing contaminated gloves and touching equipment and door handles. Stock bottles were not cleaned and consequently work surfaces beneath them became heavily contaminated.

Within the automated washing machines the stock solutions were generally well contained and the concentrate was drawn into the machine along closed lines. However, engineers whose job included cleaning out dried enzyme residues may have been exposed.

3.2 MEASURED EXPOSURE TO ENZYME CLEANING SOLUTIONS:

The detailed results for all three hospital sites are presented in the separate reports which contain summary tables of data, room plans with results of the wipe sampling mapped to specific locations, and graphs which show the levels of enzyme contamination close to and further away from the main endoscope cleaning area. The data below (ng/m^3 (for air samples) or ng/wipe) represent measures of enzyme activity relative to a subtilisin enzyme standard.

Atmospheric enzyme concentrations: Only 4 out of 14 personal air samples contained detectable enzyme (8.9, 14.5, 17.4 and 66.7 ng/m^3 of activity 8Hr TWA); these samples were all taken whilst staff manually cleaned the endoscopes in the sinks (involving wet wiping, scrubbing, and injecting enzyme cleaner). Six out of 14 static air samples contained detectable enzyme (0.6, 7.0, 9.3, 10.2, 14.4 and 45.1 ng/m^3 8Hr TWA); the air samplers were fixed at head height above the sinks and the samples taken whilst staff manually cleaned the endoscopes in the sinks (as per the personal air samples).

Wipe sampling surfaces: The highest levels of contamination were detected in those units cleaning most endoscopes and in those areas surrounding the manual washing (i.e., the sinks, surrounding surfaces and floors). The enzymes activities ranged from 4 to 267,997 ng/100cm^2. Some of these samples had enzyme activities close to that of the stock solution (e.g., wipe samples of 199,403ng/100cm^2 with the cleaning agent B stock containing 983,670ng/100cm^2 of activity). In other areas around the sinks the level of activity was consistent with diluted enzyme stocks (ie., ranging from 100 to 1,000ng/100cm^2). In the areas further away from the endoscope washing levels of enzyme were approximately 10-100 fold lower

At two units high concentrations of protease were present on work surfaces, on equipment, the floor, and handles on doors and levels increased at the end of the working day. However, surface cleaning in these units was undertaken only at the end of the day. In contrast, at one site wet surface cleaning was undertaken throughout the day and surface enzyme levels remained low throughout the day.

At two units, the endoscopes were 'wipe' sampled before and after the manual cleaning process with the pre-cleaned levels ranging from 19.6 -3082.8ng per wipe. The highest values may be due to endoscopes that had been wiped with cloth soaked in enzyme solution. Following manual cleaning the enzyme activities ranged from 18.5-1517.8ng/wipe. Endoscopes were also wiped before and after the automatic machine washing. After the endoscopes were machine washed only low levels of protease activity were detected (ranging from 28.7-152.8ng). Low level contamination was found on the outside of boxes used to transport cleaned endoscopes (3.30, 4.0, 217.08 ng/100cm^2) and at one hospital low level contamination was present on clothing (ranging from 10.0 - 47.6 ng/wipe) and hands (ranging from 39.8- 3692.8ng/ wipe).

4 DISCUSSION

This survey at three hospitals investigated exposure to industrial microbial (proteolytic) enzymes in endoscopes cleaning solutions and found evidence that skin exposure was more likely than respiratory exposure. At one site 3 personal air measurements showed exposure at 167%, 36% and 43% of the GB WEL for subtilisin (40ng/m^3 8Hr TWA), and at another site only one out of 8 personal air samples showed exposure (~22% of the WEL). Similar results were obtained when fixed air samples were collected close to the operator breathing zone above the wash sinks. All of these air samples were taken whilst staff undertook manual cleaning of the endoscopes which included the use of small brushes and injection under pressure of cleaning solutions into the endoscopes.

Surface protease contamination was present at all units not only the area used to wash the endoscopes. At two hospitals the level of contamination increased throughout the working day but cleaning was only undertaken at the end of the day. In contrast, at one unit wet surface cleaning was undertaken throughout the day and surface levels of enzyme were low throughout and at the end of the day.

Following manual washing of endoscopes some of the highest enzyme activities were measured on the surface of this equipment demonstrating a potential for transfer to skin and clothing; some staff handled the washed endoscopes without gloves. After automated washing of the endoscopes protease contamination was reduced to low but detectable levels; however, the significance of this observation may depend upon whether the equipment was then used for 'non-invasive procedures or was subject to further cleaning and sterilisation.

Wipe sampling at one site demonstrated potential transfer of protease to clothing and skin and this may suggest a risk of carrying this contamination elsewhere. Low levels of protease were found on the hands of staff carrying out this work but the relevance of this observation is less clear. The use of immunoassays to quantify specific enzymes (e.g., industrial subtilisin) will help to address this uncertainty

Enzyme contamination on work surfaces creates a direct risk for skin contact and general contamination on flooring may results in indirect risk of inhalation through contaminated dust. Simple well-established control techniques could eliminate these risks including:

- Carefully removing contaminated gloves before moving to undertake other activities
- Using absorbent pads next to the sinks to soak up splashes
- Dispensing enzyme stocks beneath the surface of the water bath using a gentle mixing action
- Taking steps to minimise the generation of aerosols in all activities, e.g., cleaning the endoscopes with brushes beneath the surface of the water
- Using disposable protective long length gloves when handling enzyme solutions including after their dilution
- Regular wet wiping of work surfaces and floors
- Keeping stock solutions in trays to contain leaks

These particular hospitals used several different enzyme-cleaning products containing different proteolytic activities (see Table 1 in Annex,) and mixtures of enzymes. However, the material safety data sheets for these products did not specify which types of enzymes were present (i.e., proteolytic, glycolytic or lipolytic). In this study, a general protease activity assay was used to monitor the contamination and exposure. This method included a subtilisin standard and the levels of enzyme activity in the survey samples were compared to this standard. The results therefore provide only a relative measure of protease contamination and cannot be used to compare the activities between different products.

Microbial enzymes are potential irritants and allergens capable of causing respiratory and skin disease at very small concentrations. Surface protease contamination at most of these units was up to ~200μg/100cm^2, and sufficient to cause adverse reactions. Levels of airborne enzyme were much lower

but published evidence suggests that for a range of microbial allergens, respiratory allergy may be caused (or existing allergy exacerbated) at very low levels of exposure (5).

5 CONCLUSIONS

1. When endoscopes are cleaned manually the main risk is that the skin of the operator will come into contact with microbial enzymes. The evidence reported in this study suggests that many of those employed to clean the endoscopes are unaware of these risks, or that practical control methods could allow safe use of these products. A simple method to demonstrate how enzymes can be spread elsewhere (e.g., using a non-toxic fluorescent tracer dye) would help to demonstrate control procedures that can minimise contamination and contact with enzymes.

2. Staff were provided with PPE but this equipment was generally not used effectively. Some staff wore disposable gloves but many used the enzymes solutions with no protection for their lower arms. Face visors had been provided to protect against splashes but were not always used. The use of protective visors and long length gloves should be standard practice.

3. The limited air sampling data obtained here indicate that airborne levels of enzyme in the breathing zone were not high; possibly because the manual washing procedures are releasing droplets not fine sprays. There may be a risk of breathing in enzymes in dust accumulated on work surfaces but regular wet cleaning throughout the working day can minimise this risk, as demonstrated at one of these hospitals. Regular wet cleaning of work areas should be undertaken and enzyme contamination monitored to demonstrate that contamination is controlled.

4. Many staff were unaware how to remove contaminated gloves to avoid skin contamination. This is a simple procedure that should be part of training staff using enzyme containing cleaning solutions.

5. The use of practical spill control procedures would help to reduce the contamination of work surfaces with enzymes.

6. The design of these endoscope-cleaning rooms was not ideal; some were small and cluttered with equipment, making it difficult to implement good cleaning regimes.

7. There was a general lack of awareness that enzymes were present in these cleaning solutions and that these pose a risk for respiratory allergy. The low content of enzyme in these solutions does not require their identification on material safety data sheets which was the case with the products examined use. However, the absence of this information may contribute to a lack of awareness that sensitisation and allergy can result from exposure to microbial enzymes at very low levels of exposure (e.g., less than $100ng/m^{-3}$).

8. The assay technique used in this study could provide a quick and practical means to monitor the controls on exposure to enzyme cleaning solutions.

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7 ANNEX

Table 1: List of enzyme products investigated

Name product	Enzymes & other ingredients	Protein concentration mg / ml	Proteolytic activity ng / ml
A	Non ionic surfactants, alcohol, solubiliser, with a combination of **enzymes**, fragrance, colouring agents, alcohols and polyethylene glycols at pH 7.0 (described as biodegradable)	5.8	2,587,450
B	Perfume, alcohol; **enzymes (**<3%) and water at pH: 6.0 to 7.0. (described as biodegradable)	184.4	983,670
C	Anionic surfactants, **enzymes,** preservatives (ethanols and parabens) pH 7.5-7.8. (described as biodegradable)	195.4	828,515
D	Anionic surfactants, **enzyme**, preservatives (ethanols and parabens) pH 7.5-7.8): (described as biodegradable)	3.7	314,268
E	Non-ionic surfactants, **enzymes**, perfumes, glycols, ethanol, glycerine and calcium salts. (described as biodegradable)	3.2	3,168,000
F	Non-ionic surfactants, ethanol, **enzymes** (~ph7.0) (described as biodegradable)	2.5	849,445

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