

Process parameters for optimal automatic instrument reprocessing

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Summary

When reprocessing surgical instruments, process design is significant, in particular the choice of relevant process parameters for the effective removal of blood, the most common soil present. Cleaning performance is highly dependent, among other factors, on the alkalinity of the cleaning solution at appropriate temperatures to ensure that relevant specifications are fully complied with on site. Here, the denaturation of protein, which impairs the final cleaning result, must be ruled out. A new concept using an oxidising detergency enhancer leads to a significant improvement in cleaning performance.

Key words

Washer-disinfector, process optimisation, alkalinity, temperature, oxidation.

Introduction

The introduction in 1994 of the Vario process, which improved cleaning in automatic decontamination processes, also represented a first step towards compliance with the then imminent European standardisation process on performance requirements for washer-disinfectors (1,2). This achieved a measure of convergence among European countries on basic requirements regarding the design of processes offering adequate cleaning performance. However, opinions still differ regarding detailed specifications. These opinions are partly based on knowledge and experience gained from automatic dishwashing, and unfortunately only seldomly on a scientific knowledge of specific instrument soils in practice (3,4).

For some time now it has been generally known that only a pre-wash with cold water actually standardises cleaning results to at least a certain degree. This is not only because the soil is dissolved and softened, and most of it removed, before the actual cleaning phase, at least reducing soiling levels to a calculable level. Too often in the past, the use of alkaline detergents led to the saponification of blood fats which in turn resulted in excessive foaming, drastically reducing mechanical spray impact and culminating in poor cleaning results (5).

When first introduced, the Vario process limited the temperature in the main wash to 45°C. This was because pertinent literature indicated that protein denaturation sets in at 45°C, causing proteins to become fixated on instruments (6). At the time, it was unclear whether detergents could compensate for this negative effect on cleaning performance. It is now known that a level of denaturation sufficient to cause cleaning

problems only starts to occur above 55°C, and is not compensated for by simple alkaline detergents. Up to this temperature, the solubilisation of blood is also improved by the fact that sulphur bonds, which stabilise proteins, are severed in this temperature range. Temperatures above 55°C are detrimental to cleaning because of the denaturation which occurs (7). In addition to all this, and considering the recommendations of the vCJD task force of the Robert Koch Institute, the question arises as to how alkalinity and temperature interact, and whether commencing hydrolysis could compensate for denaturation, leading to better cleaning performance.

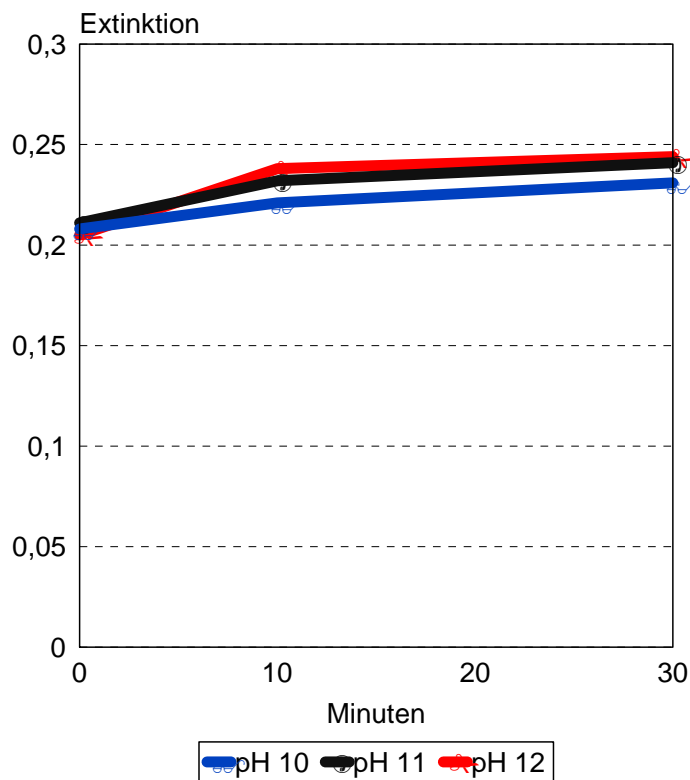
Alkaline hydrolysis

Proteins are polymers of some of the 20 amino acids, and are connected to each other by peptide bonds. Here the α -carboxyl group of one amino acid bonds with the α -amino group of a second amino acid. The partial breakdown of peptide bonds by hydrolysis reduces the proteins into smaller and also more polarised fragments, thus rendering them more soluble. Theoretically at least, this action should improve the removal of protein residues from surgical instruments.

Peptide bonds are however relatively stable, and chemical digestion under laboratory conditions is usually carried out at high temperatures using 6-molar hydrochloric acid or 4-molar caustic soda (160 g/l). Under these circumstances, the objective is complete hydrolysis in order to identify the amino acids released. Even if only partial, hydrolysis would still play a supporting role in instrument cleaning. The question is whether this also applies to automatic cleaning processes at a temperature of up to 60°C and at a pH value of up to 12. Obviously, more severe conditions would result in material compatibility problems (8).

To gain clarity on this issue, we tested a blood solution for hydrolysis at 60°C at varying pH values. Free amino groups should be formed by hydrolysis, and it should be possible to detect these by chemical analysis using the modified OPA method (8). Sheep's blood (at a concentration of 1 ml per litre of distilled water) was set to a pH of 10, 11, or 12 by adding caustic soda, and the temperature of each solution was raised to 60°C. After 10 and then 30 minutes, 400 μ l of each were removed and added to 2 ml OPA reagent. After three minutes, extinction was measured photometrically at 340 nm, using a UV photometer (Kontron Instruments, Uvikon). Figure 1 shows a minimal rise in extinction after treatment, in comparison to room temperature. In contrast to the normally expected free α - and ϵ -terminal amino groups, hydrolysis should liberate many times the amount of free amino groups, and the extinction value should rise steeply. One would also expect hydrolysis to increase with a rise in the pH value, but surprisingly the measurements at pH 10, 11, and 12 show that there is practically no significant increase in amino groups released as alkalinity rises. Consequently, there is no hydrolysis of proteins under the given conditions.

Extinction



Minutes

Figure 1: OPA extinction of blood solutions after 0, 10, or 30 minutes under the influence of various pH values, at 60°C.

Denaturation of blood and pH value

In order to investigate the cleaning results on surgical instruments, using chemical analysis to detect the presence of proteins, sampling was carried out by eluting protein with 1% sodium dodecylsulphate (SDS) solution. In order to improve the recovery of blood proteins, we carried out optimising experiments, recording the recovery as a function of the pH value of the SDS solution. For this sort of experiment, designed to show how well a chemically conditioned solution can dissolve dried-on blood, we used the easily reproducible filter paper method (7). Here a test object was prepared by pipetting 25 µl of a 1:1 mixture of reactivated, heparinised sheep's blood and double-distilled water, onto a 4 cm² piece of filter paper. This was then dried for 30 minutes at room temperature, followed by further drying for an hour at 40°C. The test objects were finally agitated in 20 ml of SDS solution for 5 minutes at 200 rpm using a magnetic stirrer. After this the test objects were removed, dried, extracted with 5 ml 1% SDS solution, and the extract subjected to OPA analysis. The results are very easily reproducible in the non-denaturing temperature band with an accuracy of +/- 1.5%. In a similar way borosilicate test objects were contaminated using 500 µl of a 1:1 mixture of blood and distilled water (see above), and dried for 16 hours in a dessicator (2). After this they were treated on the laboratory shaker at 500 rpm for 5 minutes in 60 ml sodium hydroxide solution at a defined pH value. After drying, the test objects were ground in a mortar, the powder extracted with 1% SDS-solution, and the protein residue determined using the OPA method. The results are shown in Fig. 2.

%Residual contamination

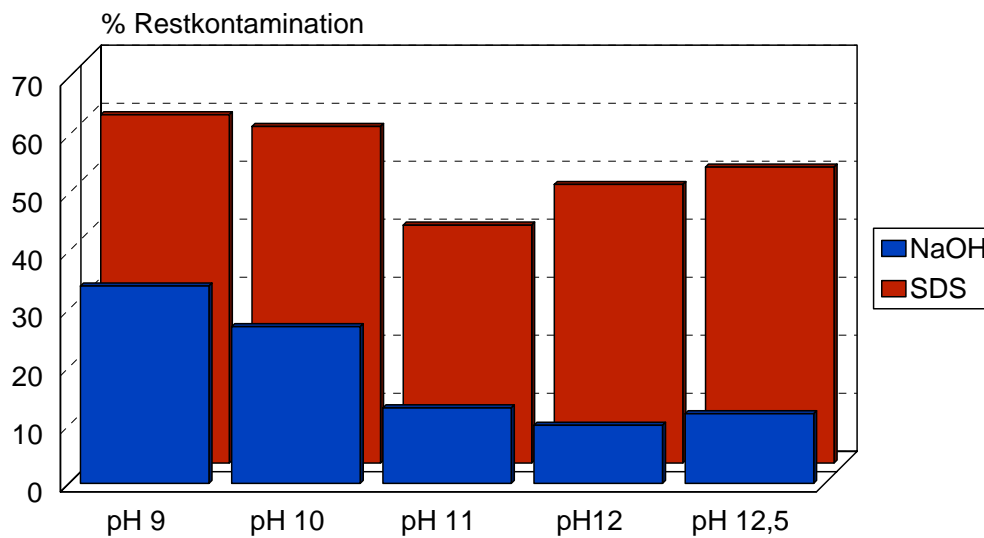


Figure 2: Removal of blood at room temperature from a filter paper with 1% SDS solution and from a borosilicate test object with sodium hydroxide solution at various pH values.

The results in Figure 2 are the mean of three measurements, and show optimum solubility at pH 11 for blood removed with SDS solution. The pure sodium hydroxide solution shows the optimum pH value to be 12. At a higher pH value the removal of blood is less good, and therefore alkalinity must have a denaturing and fixing effect, as hydrolytic reactions do not yet take place in this range. This also lowers recovery levels in these experiments so that the actual scale of this effect is not fully apparent. We were interested to see how removal of blood depended on pH value in the temperature band where denaturation commences, in other words above 55°C. For this we used the filter paper method again, as described above, agitating contaminated pieces of filter paper in sodium hydroxide solution at pH values of 11, 12, and 12,5 for 5 minutes, at temperatures of 55, 60, and 70°C. The residual amount of protein was then determined after extraction with SDS solution using the OPA method.

% Residual protein

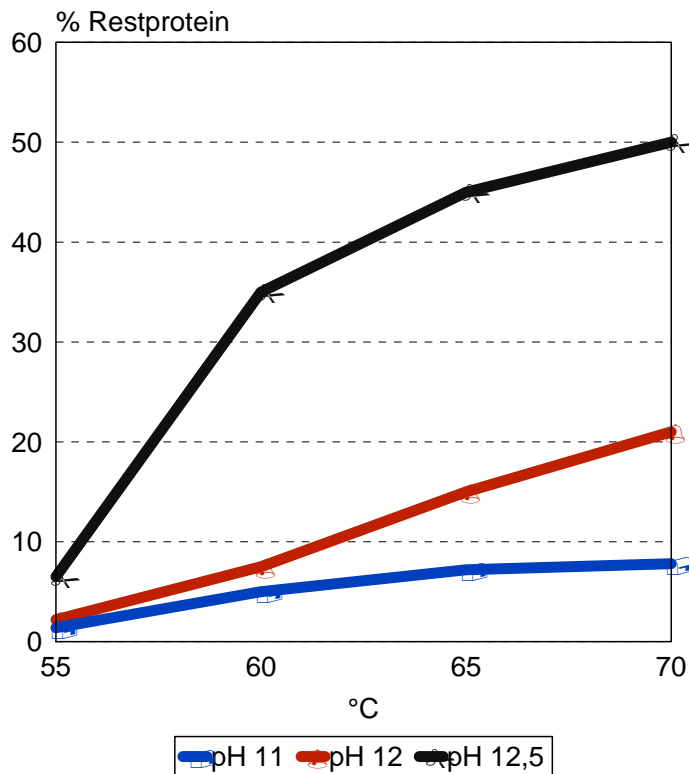


Figure 3: Removal of blood using the filter paper method, at various pH values and temperatures.

The results shown in Figure 3 show that pH values of 12 and higher have a strong denaturing effect, which is amplified as the temperature rises within the denaturing range. It must be taken into account that when extreme denaturation occurs the recovery of protein by extraction is reduced, and therefore amounts of residual protein are actually greater. This phenomenon was also observed when testing a number of commercial detergents. Hence, optimal conditions in the cleaning phase are a pH value of just under 12 and a temperature of 55°C. The effect of partial hydrolysis of proteins, which could improve cleaning, only commences at a temperature of 90°C and pH values of over 12. These values however are too severe and unacceptable for most instruments (8). Because the characteristics of heparinised sheep's blood vary slightly from batch to batch, depending on how old it is, it is only valid to compare experiments within one and the same series. The actual effects observed under practical applications, even when attempting to answer other questions, always supported the results obtained with this method. Testing cleaning phenomena using other test contaminants may represent conditions which are less than ideal. However the question is how relevant these are to practice, and whether their use really brings us nearer to the truth. This certainly applies to synthetic blood, which has far fewer components than natural blood, following Hegel's Law according to which 'the whole is greater than the sum of its parts'. So we are faced with the issue of whether to validate cleaning performance using contaminants found in practice or using contaminants far removed from those present in the real world.

New levels of cleaning optimisation

In order to further improve the cleaning performance of automatic reprocessing methods, the soil-suspension capacity and tenside content of detergents have been named as mitigating factors. Soil-suspension capacity has nothing to do with the ability of a detergent to dissolve soil, and tensides have not shown any significant improvements in removing blood residues in our experiments. Modest improvements were noted in the presence of mucus. The problem here is that anionic tensides, which are those tensides most effective in removing soil, are less suited to use in automatic processes on account of their propensity to foam.

In practice there are often cleaning problems with strongly incrustated, coagulated blood, especially on HF instruments, or on closer inspection, traces of residue have been found to be fibrin. In order to be sure of breaking down and removing these residues, other more precisely targeted ingredients are needed, for example, oxidising additives, without compromising the material compatibility of tried and tested alkaline detergents. We decided to test whether hydrogen peroxide led to significant improvements in the alkaline cleaning stage because in combination with alkalinity high concentrations of active oxygen are released from hydrogen peroxide. This active oxygen digests proteins, producing shorter and more polar fragments which are more soluble in water. Figure 4 shows the residual protein from a comparative experiment using the filter paper method, after one hour of drying at room temperature, having removed protein using either water, sodium hydroxide solution at pH 11, 0.1% hydrogen peroxide solution or the same peroxide solution at pH 11. The latter reduces blood proteins leaving only undetectable traces.

% Residual contamination

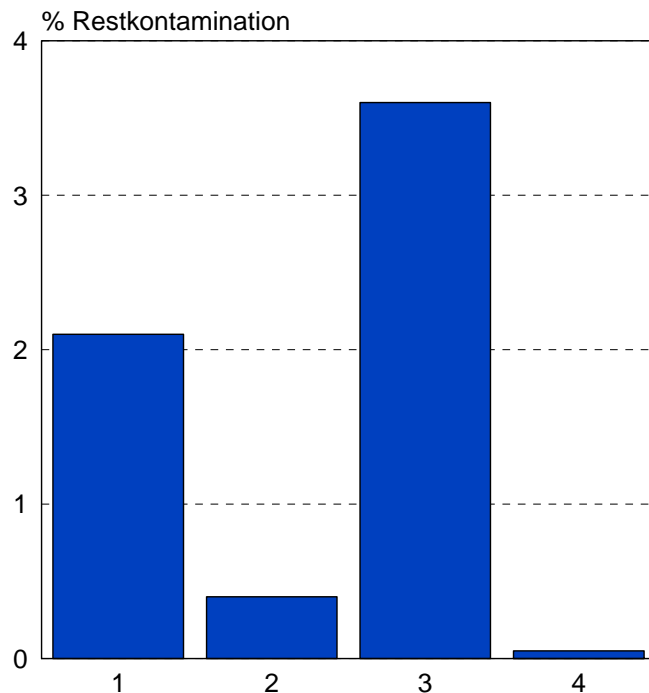


Figure 4: Residual contamination using the filter paper method after treatment with **1** = fully demineralised water, **2** = NaOH solution pH 11, **3** = 0.1% H₂O₂ solution **4** = 0.1% H₂O₂ solution at pH 11.

It makes most sense to integrate an oxidising stage after the pre-wash and the first main wash, by which time easily removable protein residues will already have been removed, so that the freshly produced active oxygen can target and break down the more stubborn residues. This way, cleaning is very efficient, even on inaccessible instrument surfaces as well as the stubborn residues on coagulation instruments, or where antiseptics have fixated soil. Successful cleaning, however, only occurs where alkaline cleaning take place in two separate phases, whereby hydrogen peroxide is dispensed in the second of the two stages.

Discussion

The cleaning results obtained here in automatic reprocessing are first of all dependent on the design and construction of the instruments and their cleanability, i.e. the extent to which the contaminated parts are exposed to mechanical cleaning action. As well as this, the following factors have considerable influence: the level of contamination; fixation by thermal influences or antiseptics; the time elapsed between use of the instruments in the operating theatre and instrument reprocessing. Many validating concepts, some of which use test contaminants not actually found in practice, cannot reflect these factors. Thus their conclusions are irrelevant and do not throw any light on the true situation occurring in practice (4). In some CSSD units,

instruments are subjected to pre-treatment before automatic reprocessing in order to obtain more or less acceptable results from a visual standpoint (10). Even so, some instruments often need follow-up cleaning. This is even the case when the detergents are used as recommended by the vCJD task force (3). It actually appears incongruous to speak of preventing the iatrogenic spread of vCJD pathogens when a percentage of instruments still shows visible residues! The differences between visual inspection and protein analysis results are even more remarkable (11). Only the non-visual measurement of the absence of protein has real relevance. A further problem is that of prions bound to stainless steel surfaces. Here cleaning methods based on anionic charges at moderate levels of alkalinity, or on reducing the surface tension of the solution with tensides, will accomplish nothing (12, 13). Discussion of the marginal differences around pH 10 seem to miss the truth. Whether the destruction of bound proteins by oxidation is conceivable or not, the level of cleaning performance achieved through the addition of hydrogen peroxide most certainly creates at the very least the ideal conditions for subsequent safe sterilisation.

Apart from the problems surrounding prions, the pre-conditions for automatic cleaning are different in each and every hospital. Therefore a suitable escalation policy involving different degrees of instrument reprocessing performance to meet the specific needs of the instruments used in hospitals is needed. Instruments used the previous day in the operating theatre have to be cleaned just as thoroughly as those which were used in the direct vicinity of the CSSD unit just an hour ago, or those with blood and mucus fixated by antiseptics. The oxidising process now offers further stages in such an escalation policy, ensuring that problems with alkaline-processable instruments can be solved.

Literature: