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EDITORIAL

Prof. Dr. Peter Heeg
Schriftleiter

Wir leben in einem Zeitalter des immer schnelleren und umfassenderen Informationsaustausches. Die Wahrnehmungs- und Speicherfähigkeit des menschlichen Gehirns scheint sich indessen nicht mit der gleichen Dynamik weiter zu entwickeln, weshalb wir angesichts der täglichen Informationsflut zum einen nach Neuigkeitswert selektieren, zum anderen unseren begrenzten „Speicherplatz" ständig wieder für Neues frei machen müssen. So kommt es, dass Dinge, die heute die Welt bewegen, morgen in Vergessenheit geraten sind oder nur noch wenige Spezialisten sich mit diesen Themen beschäftigen. Es gibt dafür zahllose Beispiele, eines davon sind die Ereignisse, die vor kurzem noch als BSE-Krise die Gemüter erregten. Heute ist dieses Thema und seine langfristigen Auswirkungen bei vielen in fast in Vergessenheit geraten. Die Arbeit von Rosenberg greift es im Zusammenhang mit der Reinigung von Medizinprodukten wieder auf. Dies sollte uns unter Anderem auch daran erinnern, dass Ursache und Wirkung nicht unbedingt in einem zeitlich leicht überschaubaren Zusammenhang stehen müssen, gerade deshalb aber nicht weniger Risiko bergen und wir auf der Hut sein sollten – auch in Zeiten, in denen das Thema BSE und CJK aus den Schlagzeilen verschwunden ist.

Fragen der Sterilisation haben in den letzten Jahren in der Fachwelt, verglichen mit der Problematik der Reinigung, weniger Interesse erfahren. Umso erfreulicher ist es, dass mit der Arbeit von Overthrow und White wieder ein Thema aus dem Bereich der Sterilisation präsentiert wird. Sie haben sich mit der Lagerdauer – hier definiert als Integrität der Verpackung – beschäftigt, und mit Hilfe eines einfachen Testaufbaus die Barrierewirkung verschiedener Sterilisationsverpackungen untersucht.

Sommerzeit steht für viele von uns für Urlaub, Erholung, Zeit für Familie und Freunde. Auch wenn der Sommer in Deutschland in diesem Jahr viele Erwartungen offen lässt, so wünschen Ihnen, liebe Leserinnen und Leser, Verlag, Schriftleitung und Redaktion, dennoch Zeit und Muße zum Ausruhen, Seele baumeln lassen und Kraft schöpfen. Denken Sie daran: Infektionserreger machen keinen Urlaub und fordern immer wieder unsere Aufmerksamkeit und unseren vollen Einsatz. ◀

We are living in an age where information is exchanged at an increasingly faster pace and in greater volumes. But in appears that the capacity of the human brain to process and store information is not developing at the same rate, which explains why faced with a daily avalanche of information we have to, first, adopt a selective approach to new data and, second, constantly ensure that we keep some of our limited "storage capacity" free for this new information. This in turn means that issues that are of global interest one day are forgotten the next day or that only a few specialists will now deal with these topics. There are numerous examples of such cases, one being the findings relating to the BSE crisis which recently elicited widespread interest. Today, this topic and its long-term implications are practically forgotten by most people. Rosenberg's paper addresses this problem once again with respect to the cleaning of medical devices. This should bring to mind that, among other things, cause and effect need not necessarily be assignable to the same short period of time and that, precisely for that reason, the risk posed is no less and that, as such, we must remain vigilant – even in times when the topic of BSE and CJD is no longer in the headlines.

In recent years, sterilisation issues have generated less interest in specialist circles than have the problems relating to cleaning. It is thus all the more opportune that the paper presented by Overthrow and White now deals with the subject of sterilisation. Here the authors focus on the storage duration – defined in this paper as the pack integrity – and, using a simple test design, they have investigated the barrier effect of various forms of sterilisation packaging.

For many of us, summer time means holidays, relaxation, time for family and friends. Even if the weather has proved somewhat disappointing this summer in Germany, the editors and editorial staff wish you, dear readers, a relaxing time to replenish body and soul. Don't forget: infectious organisms take no holidays and always call for vigilance and complete preparedness. ◀

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Keywords
- Washer-disinfectors (WDs)
- Alkaline cleaning
- Enzymatic cleaning
- 2-component cleaning systems
- Cleaning performance
- Radionuclide method
- Prions
- Western blot

Effective Cleaning Processes and "Efficacy against Prions"

U. Rosenberg

Using different experimental approaches, this paper endeavours to show that alkaline detergents do not assure optimal performance at 55 °C but that, instead, the best results are achieved at temperatures above 70 °C. Alkaline cleaning at 90 °C is at least as effective as, what is known as, the "OxiVario process". Using two newly developed 2-component cleaning systems with an enzymatic component – one in the alkaline, the other in the neutral range – a cleaning performance comparable to that of a high-temperature alkaline process was achieved already at 55 °C. Cleaning experiments must reflect everyday practice, whether carried out in a washer-disinfector (WD) or using an immersion procedure, as otherwise misleading conclusions could be drawn from the results. The conclusion from the findings concerning the influence of the temperature on the efficacy of alkaline cleaning processes can be extended to the destabilisation of infectious prion proteins. The effectiveness of this destabilisation mechanism, for which an alkalinity with a pH >11 is needed, also increases in line with the rising temperature.

Introduction

Already for many years now instrument processing has been carried out in washer-disinfectors (WDs). The standard cleaning process used for a long time in Germany and neighbouring countries was the "epidemic" or "BGA programme" devised by the former Federal Health Office, now known as the Robert Koch Institute (RKI). This process was based on highly alkaline detergents, with the cleaning solution being heated to 93 °C, without a preliminary rinse step, and cleaning continued for 10 minutes at this temperature. The cleaning results were excellent provided that no foam was generated within the WD. However, problems emanating from material incompatibility became more widespread in view of the fact that increasingly more delicate instruments, especially for minimally invasive surgery (MIS), were being used. The need for milder processes led to the introduction of the "Vario programme", followed by the advent of neutral and neutral-enzymatic detergents. While this solved the problem of material incompatibility, the cleaning results were now often unsatisfactory, with discoloration of instruments and WDs seen additionally. This meant that manual pre- and post-cleaning tasks had increasingly to be resorted to.

In recent times the cleaning component of the decontamination process has been catapulted into the spotlight for two reasons: first, the drafting of standard prEN ISO 15883 dealing with the requirements for WDs and processes carried out in these; second, the emergence of variant Creutzfeldt-Jakob disease (vCJD) in the wake of bovine spongiform encephalopathy (BSE), commonly known as "mad cow disease". The aetiological agent of this disease is an infectious prion protein that cannot be inactivated with the customary sterilisation methods. The implications of the latter now mean that it is definitely no longer possible to invoke the following excuse: "It doesn't matter if the instrument is not 100% clean as it will be sterilised in any case".

With the publication of a memorandum by the Robert Koch Institute (RKI), alkaline cleaning was set for a comeback as a consequence of the prion issue (1). On the basis of various studies investigating the effect of alkaline detergents on cleaning (2, 3, 4, 5, 6, 7), we know today that what was advocated by the RKI was correct in principle. But it is rather unfortunate that the RKI Memorandum had specified a lower pH value limit, i. e. pH 10. Attention was drawn to this in a series of reader's letters (8, 9). Can the cleaning performance and "efficacy against prions" be defined on the basis of the pH value? Are there other important factors to be considered? Is it at all possible to achieve really good results using a cleaning process within the neutral range? Queries of this nature served as the impetus for this present study. What is at issue here can be categorised into three topics:

– Which process sequence assures the best cleaning results on using an alkaline cleaning method?
– How can one enhance the effectiveness of neutral or "mild' cleaning processes?
– Is it possible to achieve "efficacy against prions" under routine process conditions? Does a good cleaning performance also mean good "efficacy against prions"?

As regards the first question, different opinions are found in the relevant literature. On the one hand, different experiments have shown that on using alkaline detergents the best results are achieved at 55 °C (10, 11, 12). The investigations conducted by Dogs and Pfeifer (13) as well as our own experiences demonstrate, on the other hand, that alkaline cleaning produces the best results at high temperatures (70 – 90 °C). Earlier experiences gleaned with the "BGA programme" (see above) pointed in the same direction. This present study was thus carried out in order to cast, finally, some light on this matter.

* Dr. Urs B. Rosenberg, Scientific Affairs, Borer Chemie AG, Gewerbestr. 13, 4528 Zuchwil, Switzerland; E-mail: urs.rosenberg@borer.ch

The neutral detergents referred to in the second query are largely based on surfactants (tensides). The effectiveness of neutral detergents against protein-based soils, as encountered when processing instruments, can (at least theoretically) be greatly enhanced with enzymes, especially with proteases. But one must bear in mind that a detergent must also perform other functions in addition to this detergent function. Such functions include e. g. binding of water hardness and prevention of deposition of silicates and of other undesirable compounds on the items to be cleaned and on the WD walls. It is virtually impossible to combine all the desirable or necessary functions as constituents in a single product concentrate, endowed with the necessary shelf-life properties, such that they can later give rise to a maximum performance. For example, the stability of enzymes, and hence also their efficacy, is greatly reduced by complexing agents that bind water hardness, to mention but one of the major problems encountered. This problem with getting the right formulation for conventional detergents can be overcome by physical separation of mutually disruptive constituents into two separate components. These two components, which are manufactured, transported and stored as concentrates, are unified only at the time of use in the washer-disinfector, i. e. when added to one and the same cleaning solution. In the present study we investigated the performance of two 2-component cleaning systems with a formulation based on this principle. One of these was in a pH neutral range and the other in the alkaline range.

We know that prions, the topic of the third query, can be destroyed in highly alkaline solutions (pH 14). Taylor gives a summary of the relevant literature (14). The latest literature reveals that alkaline detergents with pH values clearly below 14 lead to destabilisation of the prion protein, as measured by the elimination of proteinase-K resistance (3, 4, 5), as well as to a reduction in, or elimination of, infectiousness in animal experiments (2, 4, 5, 6). The present study set about elucidating the conditions implicated in the efficacy of a process against prions as well as their relationship to the cleaning performance.

Materials and Methods

Detergents Used

Three detergents were used:

- deconex 28 ALKA ONE, an alkaline detergent based on potassium metasilicate (without KOH), designated here as "28AO"
- deconex TWIN BASIC, a weakly alkaline detergent based on phosphate, known here as "TB"
- deconex TWIN ZYME, a concentrate with proteases, amylase and surfactants, known here as "TZ"

28AO was used alone as well as in combination with TZ. TB was always used with TZ. The concentrations used and the resulting pH values are given in the Results' section.

Process Challenge Devices and Test Soils

TOSI process challenge devices (PCDs), manufactured by PEREG GmbH, without an acryl glass cover were used for the immersion tests. Preparation and loading of the stainless steel sintered PCDs for the washer-disinfector tests and ensuing gravimetric evaluation were conducted as described (15), the only difference being that the prepared PCDs were subjected to denaturation treatment with 30% ethanol for 15 minutes before use and then dried again at 50 °C. Other stainless steel as well as additional borosilicate sintered PCDs (16) were each contaminated with 100 µl reactivated, coagulated sheep blood that had been marked with radioactive technetium (Tc 90). These PCDs were dried at 45 °C for 30 min. Crile clamps were each contaminated with 100 µl coagulated sheep blood that had been marked with radioactive technetium as described (17).

Methods Used To Verify the Cleaning Performance

Automated processes were carried out in washer-disinfectors (WDs) manufactured by Miele (Miele G 7735) and Hamo (Hamo LS-850). Both WDs were electrically heated. The heating rate of the Miele machine was around 6 °C/min, and that of the Hamo machine around 5 °C/min. All experiments using the radionuclide method were conducted on behalf of Borer Chemie AG on the premises of SMP GmbH in Tübingen, where the Miele washer-disinfector had been installed. All other cleaning tests were carried out in the laboratories of Borer Chemie AG. Dataloggers from ebro Electronic GmbH were used to record the process temperatures. Details of the automated processes can be consulted in the Results' section.

For the immersion tests the TOSI PCDs were incubated in a 50 ml beaker in a standing position and without use of any mechanical intervention (stirring). The beaker, for its part, had been placed in a water bath (Julabo Paratherm II) whose thermal capacity (measured in the beaker in the immediate vicinity of the TOSI) had been increased to around 5.5 °C/min by using an additional immersion heater. This value corresponds to somewhat the thermal capacity of a WD. The temperature course was measured and recorded with a sensor measuring device of type ECOLOG TN4-L manufactured by Elpro-Buchs AG.

Tests were performed in the following order:

- The water bath was filled with 3.5 litre fresh demineralised water.
- A 50 ml beaker, which had been filled with 50 ml demineralised water, was placed in the water bath
- The TOSIs and temperature sensor were immersed in the beaker and measurement of the time commenced
- After 2.5 minutes (simulation of precleaning) both heating sources were switched on
- On reaching 30 °C the cleaning detergent was added (deconex 28 ALKA ONE and deconex TWIN BASIC: 150 µl; deconex TWIN ZYME: 100 µl)
- After 16:20 min (Test 1); 12:48 min (Test 2); 10:36 min (Test 3); 9:23 min (Test 4); 8:37 min (Test 5); 7:44 min (Test 6); 7:57 min (Tests 7a and 7b) both heating sources were switched off
- After 17:20 min the PCD was withdrawn from the solution and immersed twice, briefly and vertically, in a beaker filled with demineralised water and then set aside to dry
- A fresh TOSI PCD was placed for 5 minutes in the hot solution, then withdrawn, immersed twice in demineralised water and set aside to dry

Destabilisation/Degradation Experiments of the Infectious Prion Protein

The experiments were carried out on behalf of Borer Chemie AG by SMP GmbH at the Friedrich Loeffler Institute (FLI), formerly known as the Federal Research Centre for Viral Diseases in Animals (Bundesforschungsanstalt für Viruskrankheiten der Tiere - BFAV) in Tübingen. A 10% brain extract fromtwo hamsters that had been infected with scrapie 263K was used as basic infectious material (6). The test protocol for the *in vitro* experiments (Western blot experiments) was as follows:

- The 10% brain extract was diluted with phosphate buffer (PBS) 1:1
- Using municipal water, the detergents were diluted to twofold the final concentration (to a 4-fold concentration on using two components)
- Aliquots of 50 µl of the diluted brain extract (5%) were pipetted to 50 µl diluted detergent (mixture)
- The test tube with the mixture was incubated for 10 minutes in the water bath that had been heated to the target temperature
- The solution was then neutralised by addition of 50 µl 100 mM Tris-HCl, pH 7.5, and then well mixed
- 30 µl aliquots of this neutralised mixture were pipetted to two new test tubes
- 3 µl of a diluted proteinase K solution (1:40 dilution of a 20 mg/ml stock solution (Qiagen)) was pipetted into one of the two test tubes
- Both test tubes were incubated for 60 min at 37 °C
- Then 10 µl of a 4-fold concentrated gel loading buffer was added in each case, followed by incubation at 70 °C for 10 min
- Following subsequent, 5-minute cooling on ice, electrophoresis and Western blot were performed on an Invitrogen NuPAGE Gel System.
- Prion protein bands were rendered visible using a WesternBreeze Chemiluminescent Kit from Invitrogen, based on the monoclonal antibody 3F4 (DAKO, dilution 1:2000).

The pH values listed in the Results' section were calculated by mixing the detergent dilutions with phosphate buffer instead of with brain extract; this was done using a volume that provided for measurement with a pH electrode.

Results

Experiments in the Washer-Disinfector

Crile clamps contaminated with coagulated sheep blood – radionuclide method used to verify cleaning results

Studies have been recently published on experiments carried out with other cleaning detergents/systems, in particular with the OxiVario system, while using this cleaning model (17). The experiments described in this present paper were performed by the same experimenters, at the same site and in the same WD. Hence the results can easily be compared with each other. The results of two experiments from the Draghici et al. publication have therefore also been included in Figure 1 here (17).

Our experiments aimed, on the one hand, to elucidate the cleaning performance of the new 2-component cleaning systems and, on the other hand, to identify the role of the cleaning temperature in alkaline cleaning. To find an answer to the latter, the plateau times (hold times) were selected to match the WD thermal capacity such that the overall cleaning time was roughly the same for the selected temperatures of 55, 70 and 90 °C. The corresponding temperature curves are depicted in Figure 1a. Figure 1b shows the radioactive residual contamination per tray (laden with 20 Crile clamps) after cleaning. As can be seen from the publication by Draghici et al (17) the cleaning performance in the WD used was poorer on the upper level than on the lower. Figure 1c shows for each test, as a block diagram, those clamps that harboured more than 5 counts/s at the time of individual measurements. This limit value, which has also been used in the publication cited above, was defined by Heeg et al. (18) and Roth et al. (19) on the basis of an earlier publication (20).

The results demonstrate the following:

- The alkaline detergent achieved the worst performance at 55 °C and the best at 90 °C.
- With the two 2-component cleaning systems – the alkaline and the neutral – a similarly good performance can be achieved as with the alkaline detergent at a high temperature.
- With the alkaline detergent a performance that is at least as good as that of the OxiVario process, introduced by Michels und Pieper (21), can be achieved at 90 °C.

Stainless steel sintered PCDs and borosilicate glass sintered PCDs contaminated with coagulated sheep blood – radionuclide method used to verify cleaning results

The use of borosilicate glass sintered PCDs as a cleaning model for WD processes was first described by Frister and Michels (16). At that time, the PCDs were contaminated with native human blood or with citrate bovine blood. The cleaning performance was verified by measuring the residual protein after cleaning. This was done by crushing the sintered PCDs in a mortar, using SDS for protein extraction and the OPA method for protein detection, hence a very onerous test method.

The cleaning model based on stainless steel sintered PCDs contaminated with artificial blood has been described by Rosenberg (15). Here the cleaning performance was measured gravimetrically on a weighing balance.

In this present study both types of PCDs were used in combination with the radionuclide method to elucidate the cleaning performance of the new 2-component cleaning systems. The shorter 5-minute plateau time was used for cleaning and the lower concentration of 1 ml/l deconex TWIN ZYME. A process using the alkaline detergent (5ml/l) at 55 °C was used as a control. Figure 2a shows the results as average counts/s per PCD. The block diagram in Figure 2b shows those PCDs that harboured more than 5 counts/s after cleaning.

The results demonstrate the following:

- Borosilicate glass and stainless steel sintered PCDs yielded very similar results.
- The performance of the 2-component cleaning systems was once again much better than that of the alkaline detergent at a low temperature.

– The neutral 2-component system appeared to be slightly better than the alkaline system.

Stainless steel sintered PCDs contaminated with coagulated artificial blood – gravimetric method used to verify cleaning results

These tests aimed at investigating the comparability of two methods, one of which, as described, used real instruments (Crile clamps) as PCDs and the other model PCDs (stainless steel sintered PCDs). The test soil – artificial blood – was additionally denatured with ethanol for these experiments (see Methods' section) to highlight the differences in performance between the different processes. The same cleaning processes with the same parameters were used as had been in the tests series using the contaminated Crile clamps. Since the WD (Hamo LS-850) used here had a lower thermal capacity than that of the Miele WD used for the clamps, the overall cleaning times for the plateau temperatures of 55, 70 and 90 °C were no longer quite the same (Figure 3a). The pH values of the cleaning solutions also differed from those measured for the experiments in Tübingen (see legends to Figures 1 and 3). This is because the municipal water in Zuchwil is endowed with a greater buffering capacity/hardness than the municipal water in Tübingen. The results obtained for gravimetric evaluation of the tests conducted with the stainless steel sintered PCDs are shown in Figure 3b. In each case these are shown as mean values (residue as % of the initial contamination) for 6 PCDs. The standard deviation is also given. In Figure 4 these results are given in combination with the results obtained with the Crile clamps (counts/s/clamp).

The results demonstrate the following:

– The alkaline detergent achieved the worst performance at 55 °C and the best at 90 °C.

– With the two 2-component cleaning systems – the alkaline and the neutral – at a temperature of 55 °C a similarly good or better performance can be achieved as with the alkaline detergent at a high temperature.

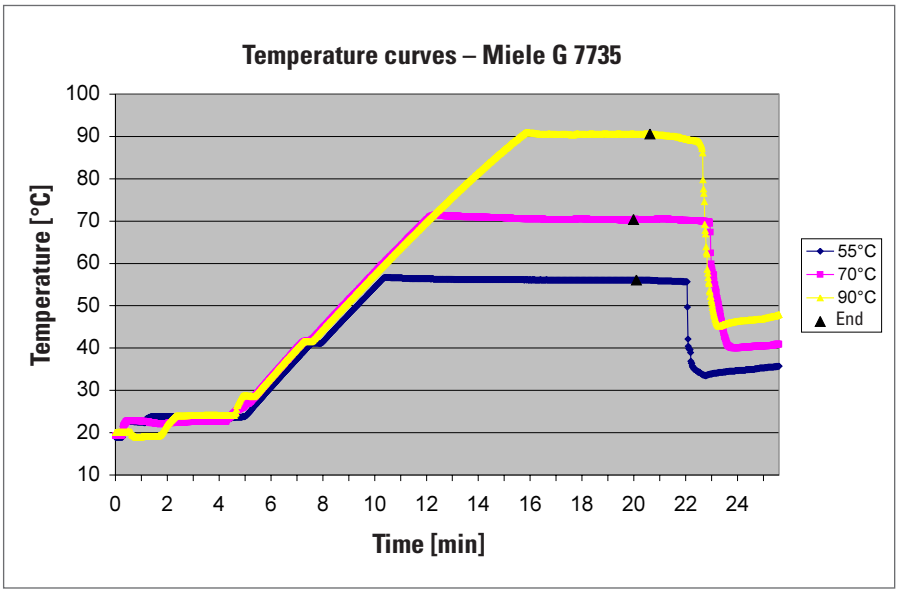

Fig. 1a–c: Cleaning of Crile clamps in a Miele G 7735 WD. The PCDs were contaminated with reactivated (coagulating) sheep blood that had been marked with radioactive Technetium.

Figure 1a: shows the temperature curves for Tests A, F and G (55 °C/10 min), for Test B (70 °C/8 min) and for Test C (90 °C/5 min). The plateau time for Tests D and E was 5 minutes shorter than shown for the 55 °C curve.

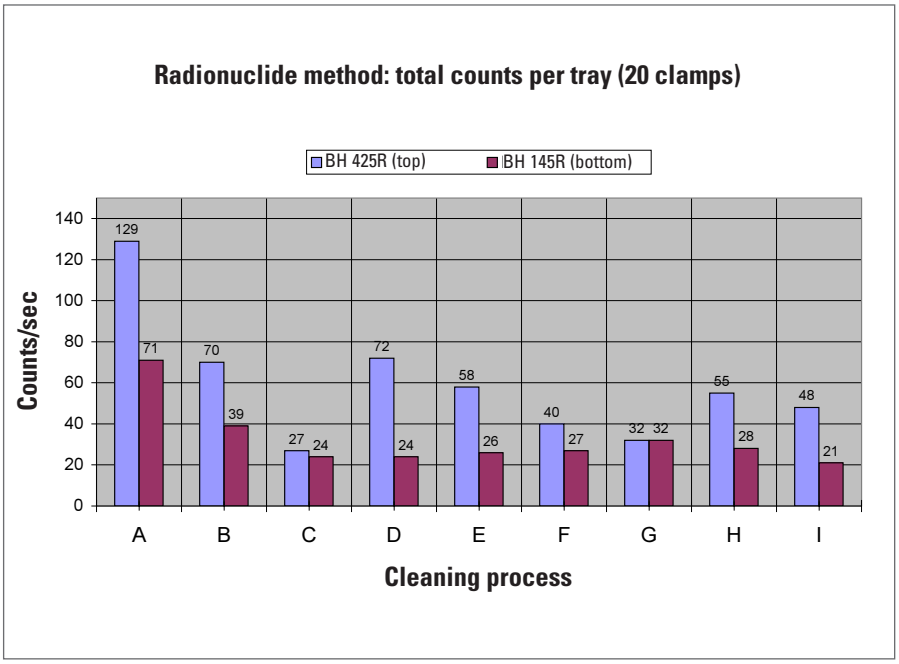

Fig. 1b: shows blood residues after cleaning in radioactive counts per tray, above (left bar) and below (right bar).

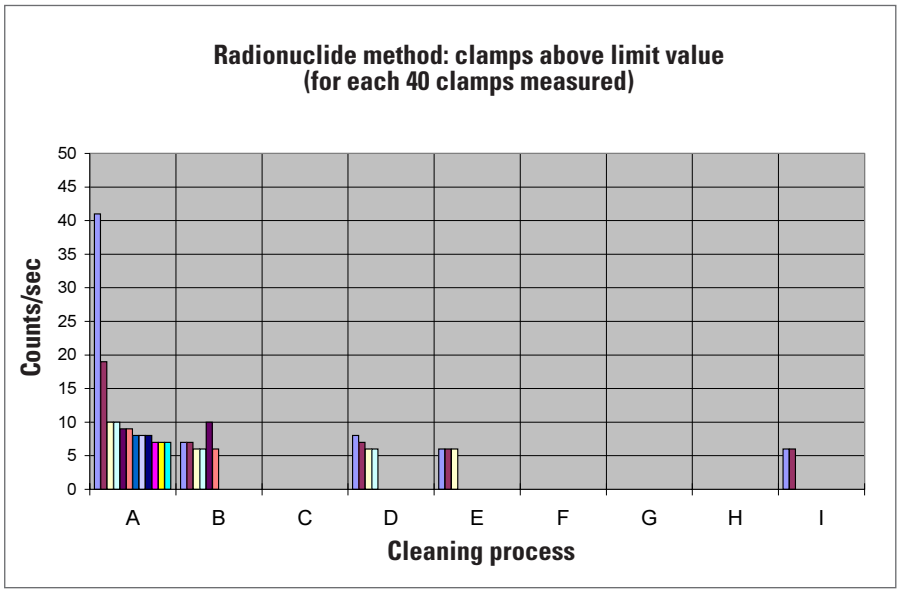

Fig. 1c shows those clamps from the upper and lower tray that had harboured more than 5 counts/sec after cleaning.

The following processes were investigated:

A. 28AO (0.3%, 55 °C, 10 min),	pH 11.1	B. 28AO (0.3%, 70 °C, 8 min),	pH 11.1
C. 28AO (0.3%, 90 °C, 5 min),	pH 11.1	D. TB/TZ (0.3/0.1%, 55 °C, 5 min),	pH 7.9
E. 28AO/TZ (0.3/0.1%, 55 °C, 5 min),	pH 11.0	F. TB/TZ (0.3/0.2%, 55 °C, 10 min),	pH 7.9
G. 28AO/TZ (0.3/0.2%, 55 °C, 10 min),	pH 11.0	H. OxiVario process with detergent C*	
I. OxiVario process with detergent A*			

*) The results in H and I (as well as the naming "A" and "C" of the detergents have been taken from the Draghici et al. (17) publication for comparative purposes.

The following programme sequence was used in the WD:
- 4 min precleaning with cold municipal water
- Cleaning with municipal water using the parameters outlined above
- 5 min rinse with demineralised water

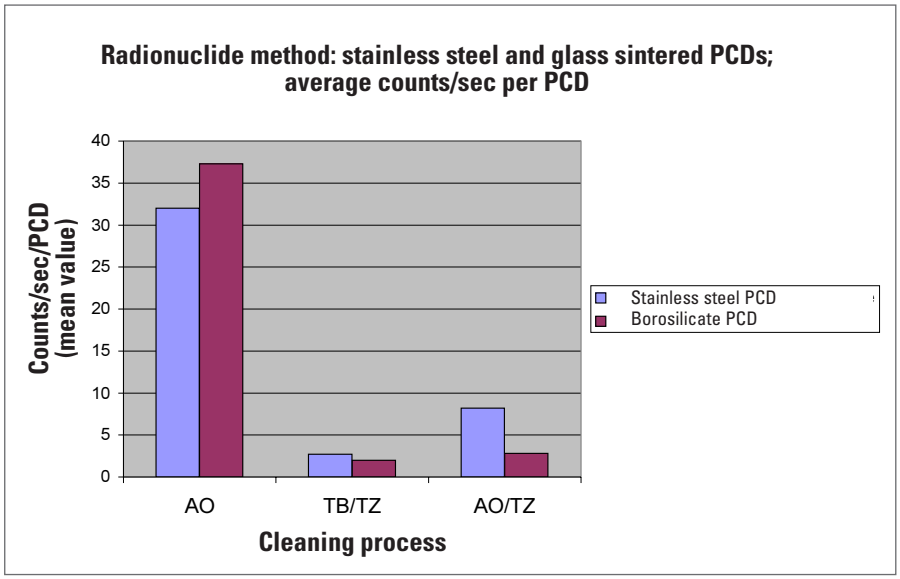

Fig. 2a–b: Cleaning of stainless steel and borosilicate sintered PCDs in a Miele G 7735 WD. The PCDs were contaminated with reactivated (coagulating) sheep blood that had been marked with radioactive Technetium.

Figure 2a: shows the average blood residues in radioactive counts/secs per PCD after cleaning (for each 6 PCDs). Left bar: stainless steel PCDs; right bar: borosilicate PCDs).

– Despite a number of deviations from a few parameters (overall cleaning time, pH values) both test methods produced largely comparable results.

Immersion Tests without any Mechanical Intervention

The same authors (10, 11, 12) have repeatedly expressed the view that blood is best removed with an alkaline detergent at a temperature of 55 °C. The experiments conducted to prove this – both immersion tests and cleaning experiments in the WD – were always performed at a constant temperature and without precleaning, i. e. in a manner that has nothing to do with the real conditions of a WD process, as also pointed out in a reader's letter (22).

In the immersion experiments described here, using TOSI PCDs, it was thus attempted to reflect as far as possible the real conditions prevailing for a WD process. As described in the Methods' section, this was achieved by means of a simulated precleaning step in combination with a temperature course for the cleaning cycle, similar to that in the WD. As in the case of the immersion tests described by Michels and Pieper (11), the alkaline detergent cited here was used at a concentration of 0.3 % in demineralised water. The resulting pH value was 11.6 compared with 11.7 in the case of Michels and Pieper (11). In addition to the tests with the alkaline detergent with target temperatures between 55 and 80 °C, the 2-components cleaning systems with a target temperature of 55 °C were used again by way of comparison. The overall cleaning duration for all tests was identical, i. e. the plateau times were correspondingly shorter for higher temperatures (1 min at 80 °C) or longer for lower temperatures (approx. 7 min at 55 °C).

Figure 5a depicts the temperature courses for all tests, and Figure 5b gives the results. The PCDs shown in Row A had been exposed to the processes whose temperature courses are shown in Figure 5a. The PCDs shown in Row B had been immersed in the respective hot solutions for 5 minutes after removal of the first PCDs. The latter somewhat corresponds to the procedure used by Michels and Pieper for their experiments conducted with blood-contaminated paper filters (11).

The results demonstrate the following:

- The best cleaning results for the experiments reflecting real conditions using an alkaline detergent were achieved at a target temperature of 80 °C rather than at 55 °C.
- Even in situations that did not reflect real conditions the results obtained for the alkaline detergent were best at the highest temperature.
- At a target temperature of 70 °C the PCD was almost perfectly clean at the end of the real-conditions' test, but marked protein fixation was noted for those experiments that did not reflect real conditions.
- At the lowest temperature, the best performance in the real-conditions test series was achieved by the 2-component cleaning systems (completely clean PCDs).
- The real-conditions' immersion experiments produced results that concorded with the WD tests described above.

In vitro experiments on destabilisation or on disintegration of infectious prion protein (PrP^{Sc})

A memorandum by the Robert Koch Institute focusing on minimisation of the risk of iatrogenic transmission of vCJD recommends to use as far as possible always an alkaline detergent with a pH value > 10 for instrument processing (1). It goes on to stipulate that suitable tests be conducted to demonstrate the destabilising effect of such detergents on PrP^{Sc}. This destabilising effect has been defined in another publication, also by the Robert Koch Institute, as the effect rendering the proteinase K-resistant infectious prion protein sensitive to proteinase K (3). The same publication draws attention to the experimentally corroborated close correlation between infectiousness and PrP^{Sc} proteinase K resistance. Testing proteinase K resistance or sensitivity was deemed to be a suitable and quick screening method for identification of potential decontamination agents. Indeed, this was the approach taken in the experiments outlined below, using the same detergents whose cleaning performance has been investigated in the present study.

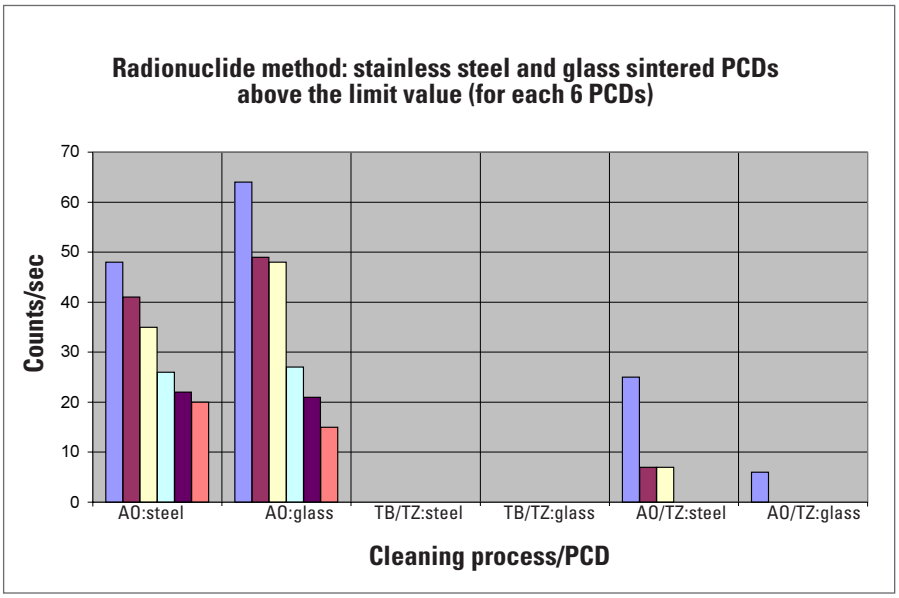

Fig. 2b: shows those PCDs harbouring more than 5 counts/sec after cleaning.
The following processes were investigated:
A0: 28A0 (0.5%, 55 °C, 5 min), pH 11.4
TB/TZ: TB/TZ (0.3/0.1%, 55 °C, 5 min), pH 7.9
A0/TZ: 28A0/TZ (0.3/0.1%, 55 °C, 5 min), pH 11.0

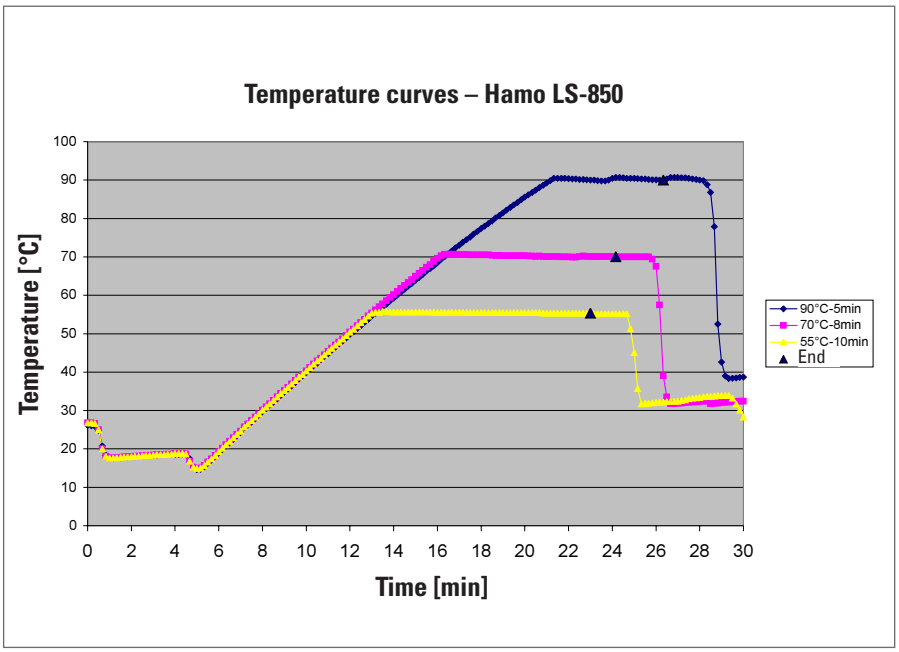

Fig. 3a–b: Cleaning stainless steel sintered PCDs in a Hamo LS-850 WD. The PCDs were contaminated with artificial blood that had been denatured by treating it with ethanol.

Figure 3a : shows the temperature curves for Tests A, F and G (55 °C/10 min), for Test B (70 °C/8 min) and Test C (90° C/5 min). The plateau time for Tests D and E were 5 minutes shorter than shown for the 55 °C curve.

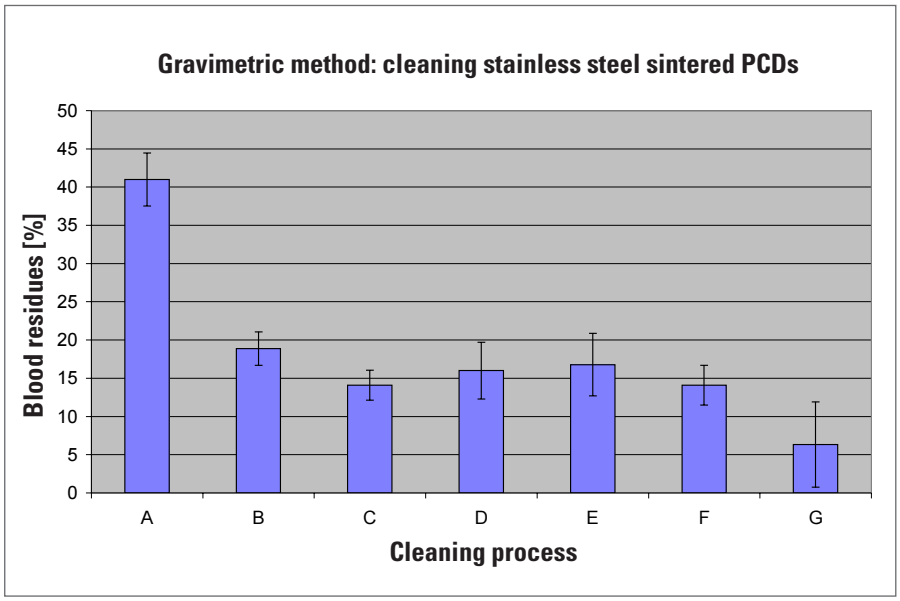

Fig. 3b: shows blood residues in % of initial soil after cleaning (mean value for each 6 PCDs).
The following processes were investigated:

A. 28AO (0.3%, 55°C, 10 min),	pH 10.6	B. 28AO (0,3%, 70°C, 8 min),	pH 10.6
C. 28AO (0.3%, 90°C, 5 min),	pH 10.6	D. TB/TZ (0,3/0,1%, 55°C, 5 min),	pH 8.0
E. 28AO/TZ (0.3/0,1%, 55°C, 5 min),	pH 10.5	F. TB/TZ (0,3/0,2%, 55°C, 10 min),	pH 8.0
G. 28AO/TZ (0.3/0,2%, 55°C, 10 min),	pH 10.5		

The following programme sequence was used in the WD:

- 2 min precleaning with cold municipal water
- Cleaning with municipal water using the parameters outlined above
- 2 min rinse with municipal water
- 2 min rinse with demineralised water

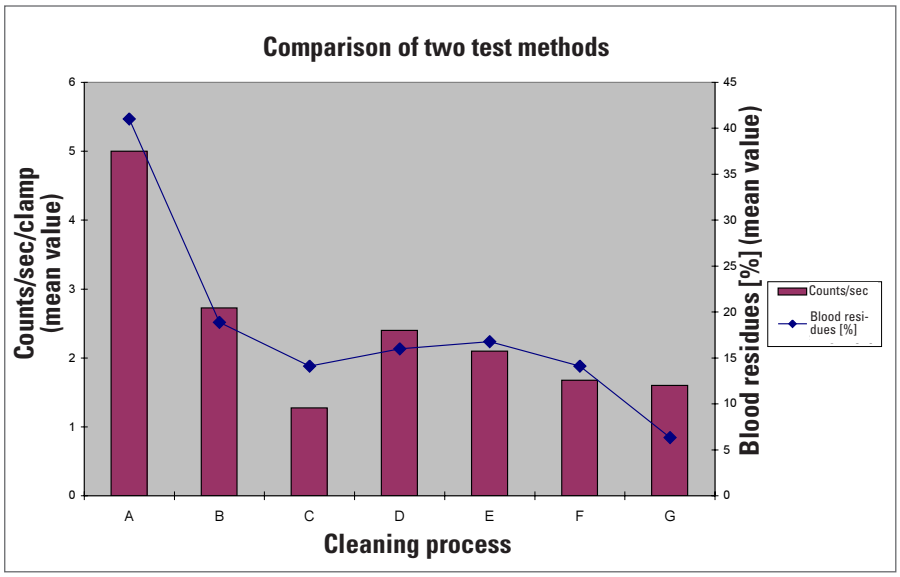

Fig. 4 Comparison of two test methods: cleaning of Crile clamps in the Miele WD: average residual radioactivity per clamp (bar). Cleaning of stainless steel sintered PCDs in the Hamo WD: residual blood soil as % of initial quantity. Cleaning Tests A–G as described above.

The effect of deconex 28 ALKA ONE alone or in combination with deconex TWIN ZYME, on the proteinase K-resistant prion protein was tested under different conditions in the suspension test. The experimental procedure is described in the Methods' section. The results are given in the form of a qualitative Western blot in Figure 6. A black band on a track that had not been treated with proteinase K (–) attests to the presence of prion protein. If this black band has disappeared on the right neighbouring track following treatment with proteinase K (+) this attests to the fact that the infectious prion protein has been rendered sensitive to proteinase K by the cleaning agent used, i. e. it has been destabilised and can thus be enzymatically cleaved (disintegrated). If a band is still visible after treatment with proteinase K this means that no, or only a limited, destabilising effect has been generated by the cleaning agent employed. Conversely, if the band already disappears in the (–) track this means that the agent used has not only destabilised the PrP^{Sc}, but has also removed (disintegrated) it.

The results demonstrate the following:

- As expected, treatment of the brain extract with phosphate buffer (negative control) did not produce any destabilising effect.
- Treatment with a caustic potash solution with pH value 12 at 70 °C showed only a limited effect.
- Treatment with 0.5% 28AO at 70 °C as well as treatment with 1.0% 28AO at 55 °C generated a pronounced destabilising effect.
- Treatment with 28AO at a low concentration and low temperature (in the presence of 0.15% TZ) was not enough to destabilise the prion protein.
- The combination 1.0% 28AO/0.3% TZ at 55 °C did not only lead to destabilisation of the prion protein but also to its degradation.
- The destabilising effect of the alkaline detergent increased as the temperature rose.

Discussion

This Discussion section is divided into three parts to reflect the questions posed in the Introduction.

Which process sequence assures the best cleaning results on using an alkaline cleaning method?

Blood is best removed from textiles with cold water. This is something that every housewife knows. Likewise, today every CSSD staff member should know that a cleaning process in the WD should always be commenced with a cold-water rinse. By no means should the soiled instruments be exposed first of all to a hot cleaning solution. Therefore it makes no sense to seek the optimal parameters in experiments that have little to do with the real cleaning process. Such experiments have been described by Michels and Pieper (11). Here, blood was applied to pieces of filter paper and these filters were then immersed at temperatures of 40, 50, 60, 70 and 80 °C for five minutes in a preheated detergent solution with a pH value of 11.7 Following this, the residual protein on the filters as well as dissolved protein was measured. It was noted that protein detachment declined already at 60 °C. An earlier study described in addition to filter-paper tests also cleaning experiments in a WD during which blood-contaminated borosilicate glass sintered PCDs were subjected to isothermic cleaning (i. e. with a preheated detergent solution) at various temperatures, without a precleaning step (10). From these experiments the author concluded: "Since alkaline detergents do not change the thermal denaturation pattern (compared with water), they should not be used above 60 °C."

In Figure 5b, Row B, of this present study one can see the results of an experiment very similar to that conducted by Michels and Pieper (in respect of detergent concentration, pH value, temperatures) (11). Here the TOSI PCDs had been immersed for five minutes at temperatures between 50 and 80 °C in the hot detergent solution. These results are endowed with sufficient power, even without a quantitative analysis. They are not unlike those of the filter-paper experiment, the only difference being that at 80 °C the detergent managed despite non-real conditions, to remove almost the entire blood on and from the PCD (PCD on far left of Row B).

The real-conditions' immersion tests whose results are given in Figure 5b, Row A, clearly show that the two processes with the highest temperatures produced

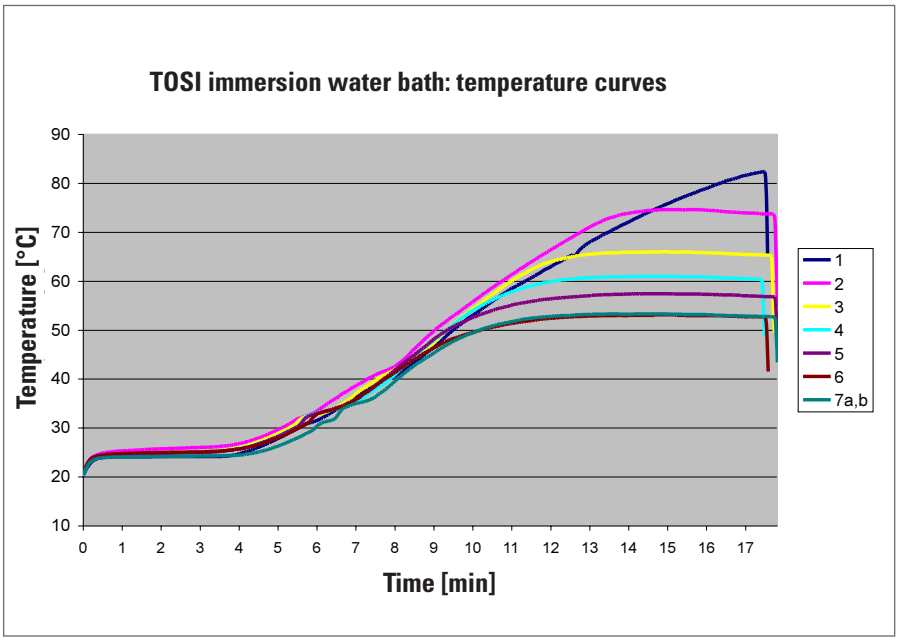

Fig. 5a–b: Immersion test with TOSI PCDs (test detergents in beaker placed in water bath.

Figure 5a: shows the temperature curves (measured in the beaker in the immediate vicinity of the TOSI) for the different tests whose results are shown in Figure 5b, Row A.

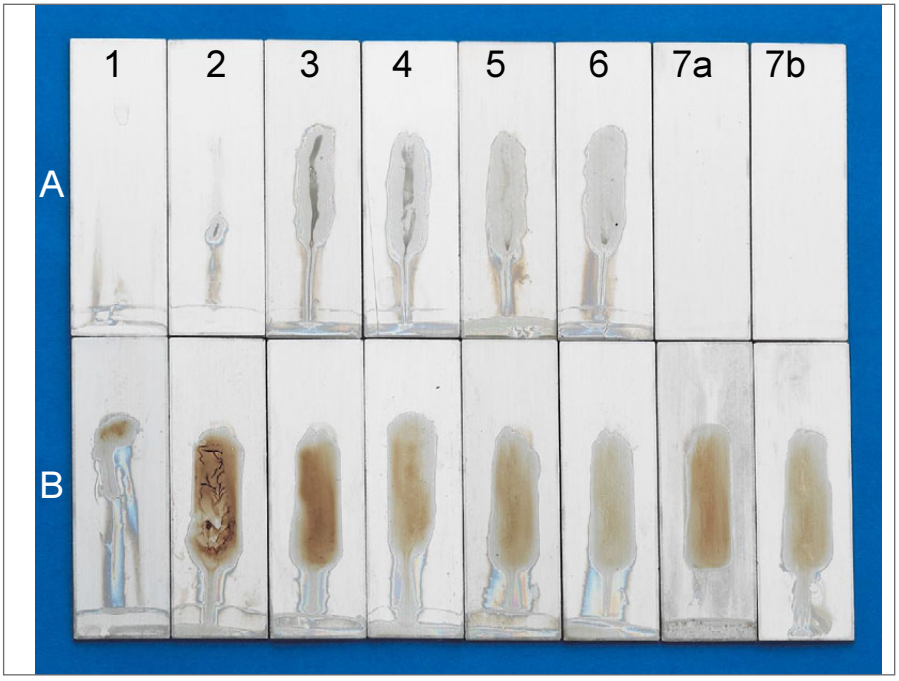

Fig. 5b Photographs of the TOSI PCDs at the end of the experiment. Row A: These PCDs were placed in a beaker containing demineralised water at timepoint 0.The detergents were added in each case once a temperature of 30 °C had been reached.Row B: PCDs that had been immersed in the hot solution for 5 minutes with the heat switched off, once incubation of the upper PCDs had been completed (comparable with the procedure used by Michels and Pieper for their immersion tests with blood-contaminated filter papers (11)).
The detergent concentrations and solution pH values were as follows:
Test 1–6: 0.3% 28AO in demineralised water: pH = 11.62
Test 7a: 0.3% TB/0.2% TZ in demineralised water: pH = 10.19
Test 7b: 0.3% 28AO/0.2% TZ in demineralised water: pH = 11.60

ZENTRAL STERILISATION 4

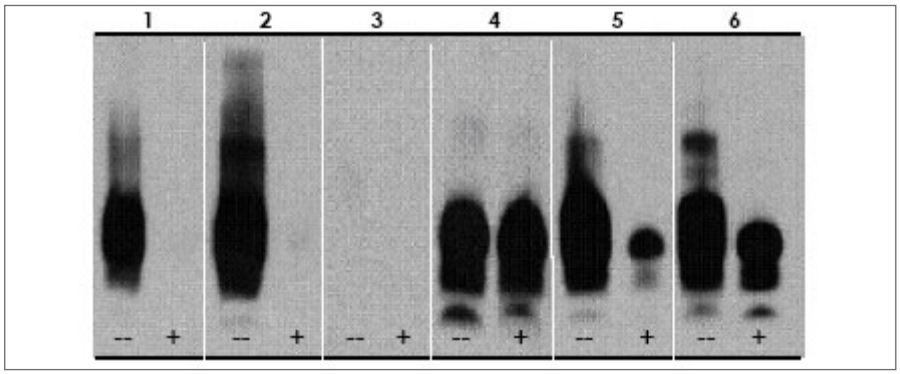

Fig. 6 In vitro treatment of hamster brain extracts with infectious prion protein PrP^{sc}.
1. 28AO (0.5%, 70 °C, 10 min), pH 11.1
2. 28AO (1.0%, 55 °C, 10 min), pH 11.5
3. 28AO/TZ (1.0/0.3%, 55 °C, 10 min), pH 11.5
4. 28AO/TZ (0.5/0.15%, 55 °C, 10 min), pH 11.1
5. KOH (0.056%, 70 °C, 10 min), pH 12.0
6. Phosphate buffer (PBS) (70 °C, 10 min), pH 7.4
The extracts thus treated were separated on an acrylamide gel after undergoing no further treatment -- and after additional treatment with proteinase K (+). Western blot was then performed for immunological detection of prion protein (black "bands").

the best cleaning results (PCD 1 and 2). Partial dark discoloration of residues on both TOSIs, which were treated at a final temperature of around 60 or 65 °C, point to denaturation (PCDs 3 and 4).

That alkaline cleaning at 70 and 90 °C is better than at 55 °C was also demonstrated with the two other cleaning models used here (Figures 1 and 3). The model that best reflects everyday practice, i. e. the Crile clamps contaminated with reactivated sheep blood, was proposed in the German Guideline (23), which deals with implementation of standard Norm (pr)EN ISO 15883, for use in process validation. However, the cleaning performance or the blood residues cannot then be measured with the highly sensitive radionuclide method used here. The protein detection methods listed in Annex C of Part 1 of the standard or the peroxidase test from Annex J of Part 5 are used.

In the present study it was possible to demonstrate with three completely different cleaning models, which, however, as far as possible reflected real practice conditions, that alkaline cleaning produces the best results not at 55 °C but rather at temperatures above 70 °C. Dogs and Pfeifer (13) produced similar findings. Using two alkaline detergents, they were able to achieve residue-free TOSI as well as "TOSI Gold" PCDs and arterial clamps after cleaning them in the WD at 90 °C, whereas this was not possible at 55 °C. For these tests, the clamps were examined for residual blood using a peroxidase reaction. "TOSI Gold" PCDs contaminated with a soil comprising heat-denatured albumin are essentially more difficult to clean than the TOSIs using native, artificial blood as a soil.

Since the experiments conducted with the Crile clamps were identical in terms of design, WD, experimenters and location to those whose results were recently published by Draghici et al. (17), it was possible, and appeared advisable, to incorporate the results of two OxiVario processes from that publication into Figures 1b and 1c by way of comparison (Cleaning Process H and I). By doing so, it was possible to establish that on using an alkaline cleaning process at 90 °C a cleaning result that was at least as good as, or better than, the OxiVario process could be achieved using a similar process duration. The 0.3% detergent dosage in the 90 °C process was even lower than the detergent dosage in the OxiVario process (0.5%).

At this juncture, attention should also be drawn to the role played by the technical features of the machine. The WDs used for this study were both equipped with an electric heating facility. But there are also machines fitted with a quicker steam-heating facility as well as tunnel washers using preheated cleaning solutions. To prevent any denaturation problems, a plateau with a lower temperature (45 – 55 °C) can be interposed in the cleaning step in such systems.

How can one enhance the effectiveness of neutral or "mild" cleaning processes?

Cleaning of medical devices under comparatively mild conditions is and remains a topical issue. There continue to be instruments and utensils which are not able to withstand alkaline cleaning at a high temperature. It has been attempted to overcome this problem by emulating the example of the laundry detergent manufacturers and incorporating enzymes, in particular proteases into the surfactant-based, pH-neutral formulations. However, this comparison with laundry detergents has its limitations because the latter are primarily based on powder formulations so that compartmentalisation, i. e. physical separation of mutually interfering components is possible. Such compartmentalisation is not possible in the case of liquid products as required for automated, validable dosage in WDs. Investigations into the performance of liquid neutral-enzymatic detergents have revealed that there are very few products that produce acceptable results (24, 25). In a study of the cleaning performance and enzyme stability of enzymatic detergents for manual instrument processes, Cheetham and Berentsveig (26) demonstrated that in the case of practically all products it was not possible to detect the enzymes at all after artificial ageing at 40 °C over a 14-day period or that only a fraction of the initial quantity was present. If it is difficult to achieve adequate enzyme stability for manual products, this is all the more so the case for products intended for automated use, which have to combine even more functions at the same time (see Introduction).

An obvious choice would seem to be to incorporate the required, but mutually interfering formulation constituents into two separate components and to unite these only at the time of use. As opposed to a potential manual system comprising two components, the use of a 2-component cleaning system in the WD presents

no major difficulty as both components can be automatically dosed. The two systems investigated in the present study are based on a common enzymatic component, deconex TWIN ZYME (TZ), which still proved to be 100% active in the ageing test after 4 months at 25 °C followed by one month at 40 °C. This enzymatic component can be combined optionally with the alkaline detergent deconex 28 ALKA ONE (28AO) or the mildly alkaline detergent deconex TWIN BASIC (TB). The second system is designated as "neutral" since compatibility with anodised aluminium is assured even in demineralised water with a pH value of around 10. The pH value of this system can drop to around 8 if used in municipal water.

All the series of cleaning tests conducted in the course of this study also entailed experiments with the 2-component systems whose results could thus be directly compared with those obtained for the alkaline processes. In all experiments using a dosage of 2 ml/l TZ and the same overall cleaning duration, the performance of both the alkaline system 28AO/TZ and the neutral system TB/TZ was on a par with that of the best alkaline processes (Figures 1b, 1c, 3b and 4, Processes F and G; Figure 5b, Row A, Processes 7a and 7b). For these series of tests no traditional neutral, neutral-enzymatic or mildly alkaline detergent was used for comparative purposes. However, one can get an idea of what such a comparison would reveal from the results given in the publication by Draghici et al. (17). The detergent designated "E" in that study was a mildly alkaline cleaner which was added in a 0.5% concentration to Tübingen municipal water and had a pH value of 9.8 (used in demineralised water, the pH value would presumably be > 10). The cleaning results, shown in Figures 6 and 7, for that study were catastrophic. Even when combined with hydrogen peroxide in the second phase, this detergent was unable to achieve results that were anywhere close to those obtained with the 2-component cleaning systems.

The results of this present study clearly demonstrate that achieving an excellent cleaning performance with mild processes is not an illusion but can, indeed, be achieved with 2-component cleaning systems.

Is it possible to achieve "efficacy against prions" under routine process conditions? Does a good cleaning performance also mean good "efficacy against prions"?

The classic prion inactivation methods such as high concentrations of sodium hydroxide solution or sodium hypochlorite combined with long hold times (14) are generally "lethal" for medical instruments. Hence for a few years now, the quest has continued for less destructive, if possible routine, methods for decontamination of medical devices potentially contaminated with prions and for rendering them safe for reuse.

Käsermann and Kempf (7) have demonstrated with *in vitro* experiments that destabilisation of prion protein with sodium hydroxide solution is more efficient that had been hitherto thought. Their study has revealed that treatment with 25 mM NaOH (pH 12.4, calculated) at room temperature reduced the titre of the proteinase K (PK)-resistant prion protein within 15 minutes by almost 4 log levels. Admittedly, the concentration of the brain extract used in the suspension test, set at 0.125% (10% original extract in Tris-NaCl (TBS) diluted with water) was lower than that used by other experimenters. On exposing iron powder with adsorbed brain extract for 15 minutes to a 0.1 N NaOH solution (pH 13, calculated), a reduction of > 4 log levels was shown using quantitative Western blot.

Baier et al. (5) published a study investigating both the effect of sodium hydroxide solution as well as that of a commercially available alkaline cleaning agent in the suspension test, followed by a PK Western blot assay or *in vivo* in the hamster model. The *in vitro* tests investigated 0.5 N (pH, no information) or 0.1 N (pH 12.3) NaOH and 1.0% (pH 12.3) or 0.5% (pH 12) detergent at 55 °C and exposure times of 10 and 30 minutes. All treatment episodes led to destabilisation of the prion protein so that after PK digestion there was no longer any evidence of prion protein on the qualitative Western blot. Treatment with 0.5 N NaOH resulted in disappearance of the prion protein even without PK treatment, something that was attributed to alkaline hydrolysis. In the animal experiments, the infectiousness of brain extracts treated with either 0.1 N NaOH or a 1.0% detergent for 30 min at 55 °C was examined. 526 days after injection of the neutralised suspension into the brain of healthy experimental animals, none of the animals manifested any signs of infection. Nor were there any signs of disease in the animals after an incubation period spanning 260 days if treatment was performed at 20 °C instead of 55 °C. However, these experiments are questionable as the brain extracts used had been prepared while using surfactants (5% original brain extract in 0.5% Triton X-100, 0.05% SDS), which could also have had an effect on the stability of the PrP^{Sc}. The brain extract concentration in the treatment suspension was 2.5%.

A further study by Fichet et al. (4) focused on, inter alia, the effect of an alkaline detergent on the infectious prion protein, conducting once again both *in vitro* as well as *in vivo* experiments. However, in both cases the tests involved here were carrier tests. For the *in vitro* tests, glass slides were contaminated with brain extract, while stainless steel wires implanted after contamination and decontamination into the brain of healthy hamsters were used for the *in vivo* experiments, using a 10% brain extract in phosphate buffer NaCl (PBS). In the *in vitro* experiment it was possible to destabilise the prion protein by means of treatment with a 0.16% detergent at 43 °C for 15 minutes, whereas this could not be achieved at 25 °C. The alkalinity of the cleaning agent used here was comparable with that of 0.006 N NaOH, resulting in a pH value of 11.8. For the *in vivo* experiments the contaminated wires were treated with a 1.6% cleaning agent (pH 12.8) for 15 minutes at 43 °C (immersion), before being implanted into the brain of healthy hamsters. None of the hamsters showed any signs of disease after 365 days. The effectiveness of the detergent treatment described was calculated as being > 6 log levels on the basis of an *in vivo* experimental series during which wires contaminated with serially diluted extracts were implanted into healthy animals.

In a comprehensive study Lemmer et al. (3) conducted *in vitro* carrier tests during which stainless steel wires were contaminated with brain extract (10% in TBS) and were then treated with various agents, including an alkaline cleaner. Following this, both the wire surfaces and the treat-

ment agent solution were examined for the presence of prion protein by means of PK Western blot assay. The alkaline detergent (the same one used in the Baier et al. (5) study) was used in a concentration of 1.0% (pH 12.2) and 0.5% (pH 11.9). Treatment was carried out in each case at 55 °C for 5 or 10 minutes and at 23 °C for one hour. Without PK digestion, prion protein could be detected in minute amounts on all wires, and could not be detected at all after PK digestion. Conversely, a heavy prion protein load was detected in the cleaning solutions without PK digestion but, once again, this was no longer the case after PK digestion. From these results the authors conclude that the detergent had detached to a large extent the prion protein from the wire surfaces, while destabilising it at the same time. Virtually the same results were achieved under the same experimental conditions on using 0.1 N NaOH (pH 13). But on using 0.5 N NaOH (pH 13.4), prion protein could no longer be detected after treatment for 10 min at 55 °C without PK digestion (comparable with the result of the corresponding suspension test by Baier et al. (5)). However, on reducing the temperature to 23 °C while at the same time prolonging the treatment time to 30 and 60 minutes, prion protein could be detected in the detergent solution without PK digestion. The authors conclude that at a higher temperature the prion protein was not only destabilised but also degraded, i. e. broken down into its constituent amino acids, despite the short treatment time.

The *in vitro* suspension tests used in the present study were carried out with PBS brain extract. The concentration of the brain extract in the treatment suspension was 2.5%, i. e. the same as that used in the Baier et al. (5) study. Experiments at room temperature were omitted since the test products involved were detergents intended for use in washer-disinfectors that generally use high temperatures. The results obtained for the alkaline cleaner 28AO attested to its "efficacy against prions" as well as to its limitations. Process 4 in Figure 6 (no attention should be paid here to the presence of 0.15% TZ) using a 0.5% 28AO (pH 11.1) dosage and an incubation temperature of 55 °C is not effective. But an effective process (Process 1) can be generated by increasing the temperature to 70 °C and retaining the same concentration and same pH value. Likewise, efficacy can be achieved at 55 °C by increasing the detergent concentration and hence the pH value. Fichet et al. (4) and Lemmer et al. (3) had identified similar relationships between "efficacy against prions" and temperature/alkalinity. The treatment environment had a pH value > 11 for each of the tests described in the literature using an alkaline cleaning agent and demonstrating either *in vitro* or *in vivo* efficacy. The following effective pH value/temperature/time combinations have been identified:

pH 12.0/55 °C/10 min (*in vitro*, (5))
pH 12.3/20 °C/30 min (*in vivo*, (5))
pH 11.8/43 °C/15 min (*in vitro*, (4))

pH 12.8/43 °C/15 min (*in vivo*, (4))
pH 11.9/55 °C/5 min (*in vitro*, (3))
pH 11.9/23 °C/60 min (*in vitro*, (3))

pH 11.5/55 °C/10 min (*in vitro*, present study
pH 11.1/70 °C/10 min (*in vitro*, present study)

In a lecture (2) given at the Decontamination Sciences Congress in April 2005 in London, K. Roth from Tübingen presented the results obtained from *in vitro* experiments on the effects exerted by caustic potash solution (KOH) on the prion protein. Whereas KOH was effective at pH 13 for a 70 °C/10 min treatment, KOH at pH 12 was effective only at 90 °C/10 min. Bearing in mind all discussed findings, a link can now definitely be established to the cleaning performance described above. Here and there one notes that on using a (real) alkaline detergent, a temperature increase is always accompanied by process acceleration or enhanced efficacy. This means that whatever is beneficial for alkaline cleaning, also underpins "efficacy against prions" and vice versa.

From Figure 6 one can see that KOH, despite being used at pH 12, produces a poorer result than the detergent at pH 11.1, and under otherwise identical experimental conditions. This poorer efficacy of a pure caustic solution compared with formulated detergents was also noted by Fichet et al. (4) and is not surprising as such differences have been known from time immemorial in respect of the cleaning performance. But the opposite also appears to be true in certain cases. In the Baier et al. (5) *in vitro* tests using both the alkaline detergent and NaOH at pH 12.3, while the prion protein had completely disappeared on the Western blot after PK digestion in both cases (Fig. 1b and 1d), the figure without PK digestion showed markedly weaker signals for 0.1 N NaOH than for the 1% test detergent (Fig. 1a and 1c), thus pointing to additional, partial degradation of the prion protein by the NaOH treatment, besides its destabilisation.

In the present study the 2-component cleaning systems were also investigated for their "efficacy against prions". It was noted that the enzymatic component TZ was devoid of efficacy if the alkalinity was not sufficiently high (Figure 6, Process 4). Even when increasing th TZ concentration to 0.3 and 0.6% respectively, no effect could be found if the AO concentration was left at 0.5% (not shown). But if alkalinity was increased by dosage of 1.0% 28AO, the prion protein disappeared on the Western blot, even without PK digestion (Figure 6, Process 3). As noted by Lemmer et al. (3) in respect of the effect generated by 0.5 N NaOH at 55 °C, the prion protein must have been not only destabilised but also degraded by the combined treatment. The logic underlying this phenomenon is clear: the alkalinity destabilises, the proteolytic enzymes degrade – the latter assume the same role as proteinase K in the assay. But what is new is that with 28AO/TZ this does not unfold successively but rather in a single step and that TZ is not a laboratory enzyme but rather a constituent of a commercially available cleaning system. In their quest for proteases capable of eliminating the prion protein, McLeod et al. (27) did indeed discover some. However, this degradation mechanism unfolded only if the proteases were used at pH 12. From this the authors conclude that the β-pleated sheet structure of the prion protein is partially or completely destabilised by the alkalinity, thus enabling the proteases to generate their effects on the protein. They go on to state: "Since detergent formulations with pH values of around 12 are already being used for instrument decontamination, one would only need to add proteases which would be effective under these alkaline conditions…". A different approach to

destabilisation of the prion protein as preparation for proteolytic degradation was taken by Langeveld et al. (28). The authors heated extracts in the presence of surfactants to 115 °C (in a pressure cooker) before these extracts were then digested by proteases. Lower temperatures and the absence of surfactants during heat treatment detracted from the efficacy of the proteases. Once again, sensitivity of the prion protein to proteinase was ascribed to denaturation of the β-pleated sheet structure, brought about in this case by heat treatment. However, Jackson et al. (29) report that they managed to inactivate prions through proteolytic treatment alone within a neutral pH range. Admittedly, unrealistically high enzyme and surfactant concentrations were used in these experiments. A use solution containing 20% of a protease concentrate and 4% SDS is far away from a commecialisable detergent.

The "efficacy against prions" of the alkaline detergent 28AO as well as of the combination of 28AO with the enzymatic component TZ is based on *in vitro* experiments. As is generally well known, *in vivo* tests are also needed to furnish proof of efficacy against prions. K. Roth (2) presented the results of *in vivo* carrier tests in which contaminated wires were treated in a specially designed spray system simulating a washer-disinfector. The agents used here included the 28AO detergent, used as a 1.0% concentration at a temperature of 55 °C with a 10-minute hold time. All hamsters, apart from two lost to cannibalism and two sacrificed for histological PET blot analysis after 210 days, continued to be symptom-free 447 days after implantation of the wires that had been treated with 28AO. The results of PET blot analysis were negative, i. e. there was no evidence of prion protein in the brain tissue. In two other *in vivo* carrier tests of a similar type, which are still underway and have not yet been published, contaminated wires were treated for 10 minutes with 0.5% 28AO at 70 °C or with 1.0% 28AO/0.3% TZ at 55 °C for 10 minutes. The hamsters continued to be healthy 277 days after implantation of these wires. PET blot analysis conducted in this case after 214 days was also negative. Based on these results obtained for survival time after implantation together with the results obtained by Fichet et al. (4) as regards the infectiousness of the wires contaminated with brain extract dilutions, it can be assumed that the cleaning processes using 28 AO or the 28AO/TZ combination had effected a > 6 log level reduction in the prion protein on the wire surfaces. From the *in vitro* experiments outlined here as well as from the Lemmer et al. (3) study it can be assumed that this effect can be ascribed to destabilisation of the prion protein with concomitant detachment from the wire surfaces. In the case of the 2-component cleaning system 28AO/TZ degradation of the prion protein can be assumed additionally.

Conclusions with Implications for Instrument Decontamination Practices

The question posed at the beginning of the preceding Discussion section, "Is it possible to achieve "efficacy against prions" under routine process conditions?", can definitively be answered with "Yes". A process with 0.5% 28AO and 10 min cleaning at 70 °C can be used as a routine measure. In addition to its "efficacy against prions", it also produces good cleaning results (even better than those of the process described above using 0.3% 28AO and 8 min hold time at 70 °C). The cleaning performance could have been further enhanced by increasing the cleaning temperature to 90 °C (5 min hold time), as demonstrated in this present study. This, as we now know, would have also enhanced "efficacy against prions". Both 70 °C and 90 °C cleaning processes have already been successfully implemented in many hospitals.

The second question: "Does a good cleaning performance also mean good "efficacy against prions"? can be answered only with a qualified "yes". As demonstrated by the present study, an excellent cleaning performance can be achieved with 2-component cleaning systems in the neutral pH range. However, no investigation has yet been able to show the "efficacy against prions" of a routine cleaning process at a neutral or mildly alkaline pH. Conversely, "efficacy against prions" could be expected from an alkaline process using a formulated detergent with a high pH value (> 11) and at a high temperature (> 70 °C). ❊

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