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Keywords
- prEN ISO 15883
- testing cleaning processes
- test soil

Test Soils for Investigating the Cleaning Performance in Washer-Disinfectors as per prEN ISO 15883-1[1]

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Introduction

It has been stipulated that in European hospitals medical device processing be carried out with validated methods (5), preferably in washer-disinfectors. Manual processing tasks can only be standardised. The standard prEN ISO 15883-1 (Washer-disinfectors, general requirements, definitions and tests) (1) features a technical speciation listing the national or standardised soils of various countries (previously Annex B). Within the prescribed period of time it was no longer possible to develop between one and a maximum of three generally binding test soils. It was thus interesting to examine the different characteristics of the various test soils. Two studies conducted in 2002 and 2003 give a good overview of the situation.

It is difficult to verify the cleaning performance since it is determined by a plethora of parameters: mechanical action, temperature, exposure time, water quality, type and concentration of detergent. As already borne out by other studies (2), the intensity of the spray mechanical action within the chamber of a washer-disinfector can also vary greatly among the different levels and at the various locations. The spray pattern is also affected by the load, generating more or less spray shadowing. To circumvent the mechanical effects mediated by a specific machine, both studies were conducted as in vitro investigations, while endeavouring to standardise as far as possible the experimental conditions.

The test soil(s) is (are) intended by the Standard to simulate worst-case conditions so that removal of this test soil will support the conclusion that all other residues that can possibly be encountered in practice will also be removed from the medical devices. Examples of such soils include e.g. native blood dried or denatured to different degrees; but mucus and fats often also make more exacting demands on the cleaning process.

In the first study we first of all made a note of the properties of the different test soils at the time of preparation and quantitative distribution. We then investigated how easy it was to remove the dried test soil using a mildly alkaline detergent solution at two different temperatures and exposure times, while largely avoiding any mechanical action. In the second study we used three detergents with different pH values and with longer exposure times because of the greater degree of drying.

Country	Relevant Standard	Composition	Area of Application
Netherlands	B 3	1% bovine albumin, 1% mucin, 0,6% fibrinogen dissolved in 0.05 M phosphate buffer solution	Surgical instruments
Germany	B 5	100 ml sheep blood and 0.1 ml heparin; before use, 9.5 ml heparinised blood was mixed with 0.15 ml protamine hydrochloride; instead of 0.35 ml bacterial suspension distilled water was added	Flexible endoscopes
Germany	B 6	0.6% bovine serum albumin, 1% mucin, 3% corn starch	Bedpans
United Kingdom	B 9	100 ml fresh egg yolk, 100 ml defibrinated sheep blood, 2 g dehydrated porcine mucin	Surgical instruments
Austria*	B 17	700 g with 1.077% oatmeal solution, 0.077% nigrosin, 3 eggs (white and yolk of egg), 100 g dried potato flakes (KMNE[1] as per Koller)	Surgical instruments
Germany	B 19a*	100 g with 10 g skimmed milk mixed with tap water (study 15–19° dH), 5 g sugar, 5 g butter, 4 g durum wheat	Surgical instruments
Germany	B 19b*	Defibrinated sheep blood	Surgical instruments
Germany	B 19c*,+	Yolk of fresh eggs	Surgical instruments

Table 1: Composition and area of application of the test soils

* in these studies without a test organism
\+ in Study 1, with egg emulsion additionally (Oxoid)
* In Austria, this test soil has been replaced in the meantime by coagulable blood, see B 5
[1] KMNE is the German acronym for a test soil consisting of potato starch, flour paste, nigrosin and egg

1 based on a lecture given at the 4th EFHSS Conference, Winterthur, 3–5 July 2003

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Materials and Methods

Table 1 lists the composition of the test soils used based on draft standard 2002 (Annex B) as well as where they were used.

To begin with, in Study 1 we used six of the total of seven test soils listed in the Standard for processing surgical instruments. In Study 2 we used the test soils specified for flexible endoscopes (B 5) and bedpans (B 6) in addition. Furthermore, we investigated the test soil used for the TOSI cleaning indicators (Pereg GmbH, Waldkraiburg), consisting of haemoglobin, albumin and fibrin, using only the test soil without the ready-to-use gap process challenge device.

The test soil ingredients were selected exactly as specified in the Standard. In the interests of standardisation, we had to deviate from the prescribed drying methods in one instance. Furthermore, aliquots of 0.1 g were quantitatively applied to a surface measuring 10 x 1 cm and distributed with a Drigalski spatula. Weighing was done for control purposes.

In Study 1 we used as carriers stainless steel test coupons of defined roughness (DIN 10510) and the test soils were left to dry at room temperature (22 – 24 °C, 50 – 70% rF) for 4 h. Only after this time were all test soils optically dry.

Two test coupons were used for each test. These were immersed at 20 °C and 50 °C into a solution composed of a commercially available detergent (0.5%; pH 9.2; distilled water). The solution was shaken gently on a magnetic stirrer that could be heated, using contact times of 1 and 2 min as well as in some cases 5 min.

The Standard stipulates that no residues should be visible when subjecting the test coupons (process challenge devices – PCDs) to visual scrutiny. Hence we added nigrosin to the colourless, or practically colourless, soil to enhance visibility of residues during visual inspection.

On inspection we noted after what contact time the test coupons were deemed to be clean. The coupons deemed to be clean were subjected to a semi-quantitative check for protein residues or to a qualitative check for starch residues.

We used the biuret method to identify protein residues. We took the Konica "N" Swab Test to swab the contaminated surfaces. The detection limit is set at 25 µg protein. When using starch-based test soils, the optically clean test coupons were immersed in a 1% iodine/potassium-iodide solution. Starch residues assume a deep blue colour. This reaction is very delicate (3).

Test Soil	Prepared without laboratory equipment	Laboratory equipment needed, e.g. Stomacher	Consistency	Colour
B 3		X	Watery	Colourless
B 5		X	Thin – then precipitations	Crimson
B 6		X	Creamy	White/colourless
B 9		X	Creamy	Crimson
B 17	X		Mushy	Black
B 19 a	X		Mushy	Bright yellow
B 19 b	X (Lab. specialist dealers)		Watery	Crimson
B 19 c	X		Viscous	Yellow (egglike)
Egg emulsion	X (Lab. specialist dealers)		Thin	Bright yellow

Table 2: Test soils as per prEN ISO 15883-1, Annex B

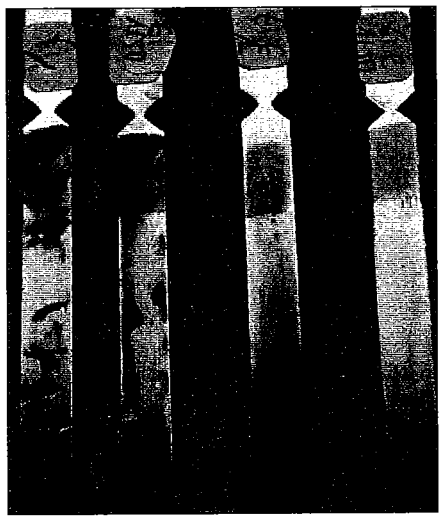

Fig. 1: Process challenge device (PCD) with egg yolk and egg emulsion after testing; the upper region of the PCD has not been treated

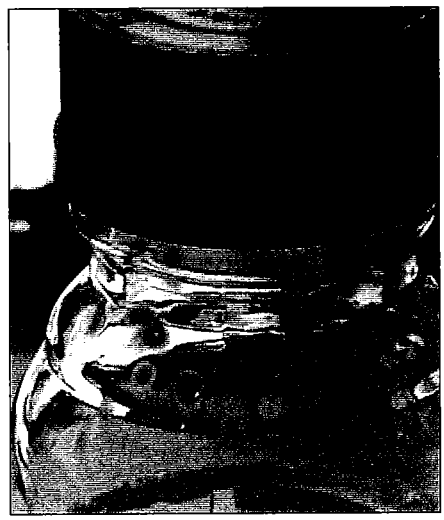

Fig. 2: Undissolved mucin components of test soil B 6

For Study 2 we used smooth stainless steel test coupons, onto which aliquots of 0.1 g test soil were quantitatively applied. The coupons were left to dry for 4 h at 36 °C ± 1 °C. For the solution we used three commercially available detergents with different pH values as well as demineralised water. In each case, 0.5% solutions of the detergents were prepared with demineralised water and the pH value was ascertained with an INTEC pH meter (set with Merck buffer solutions to pH 7and pH 9):

Detergent solution 0.5%	pH
Detergent A	9.2
Detergent B	10.8
Detergent C	12.6

Twenty test coupons (PCDs) were used for each test. Since it was revealed already in the preliminary tests that the test soils are more difficult to remove due to drying at higher temperatures, contact times of 5, 10, 15 and 20 min were selected.

Evaluation was conducted only visually. We elucidated the number of optically clean coupons for each contact time. However, we did not investigate further the quantity or type of residues on the coupons but, instead, carried out spot

checks for optically clean PCDs using the biuret method or the iodine/potassium-iodine test.

Results

Table 2 describes the test soils, how they were prepared and their properties.

Some test soils are easier to prepare than others; some cannot be prepared without special laboratory equipment.

The consistency of the test soils varies greatly, ranging from watery to mushy.

Some test soils are colourless or have a white/yellowish colour, while others have a strong colour because of the added nigrosin stain or of the haemoglobin contained in blood. It is easier to optically detect residues of intensely coloured test soils.

Some of the ingredients needed for preparation of test soils as well as the sheep blood (without test organisms) can be obtained from specialist laboratory dealers, while those less precisely specified ingredients can be purchased from a supermarket.

The findings of Study 1 are summarised on the basis of the principle ingredients and given in Figures 3 – 5.

Figure 1, for example, illustrates PCDs with egg yolk or egg emulsion before and after the test.

Undissolved mucin components can be detected in Figure 2 (test soil B 6).

The findings of Study 2 at 50 °C are presented in Figures 6 – 13. Only for defibrinated sheep blood (B 19 b) are the results shown for both 23 °C and 50 °C. For the other test soils the results are only shown for 50°C, since at 23°C the test soil was not at all or only partially removed. The various bars show how many test coupons were deemed to be optically clean after the exposure times of 5, 10, 15 and 20 min. In the right column is summarised how many PCDs were not clean after a 20 min contact time with the cleaning solution (R). Noteworthy is that not all 20 test coupons evinced the same behaviour but, despite standardised conditions, were cleaned more or less quickly.

The "coagulable blood" (B5) consisted of sheep blood with heparin, to which a stochiometric amount of protamine had been added as antagonists before use. This provides for fibrin precipitation within the space of one hour. While the coagulation factors might differ from one sheep to the next, achievement of reproducible results could be demonstrated (10).

The test soil used for the TOSI cleaning indicators is composed of haemoglobin, albumin and fibrin, and is thus similar to blood.

Under the selected conditions, it was not possible to remove the test soils "coagulable blood" (B 5) or the test soil used for the TOSI cleaning indicators, or these could be removed only to an extent that all 20 coupons had to be declared as not being optically clean and were thus classified under "R", except one. Fibrin residues, in particular, could be detected.

Discussion

As the first step of the processing cycle, increasingly more importance is being ascribed to cleaning, especially also because

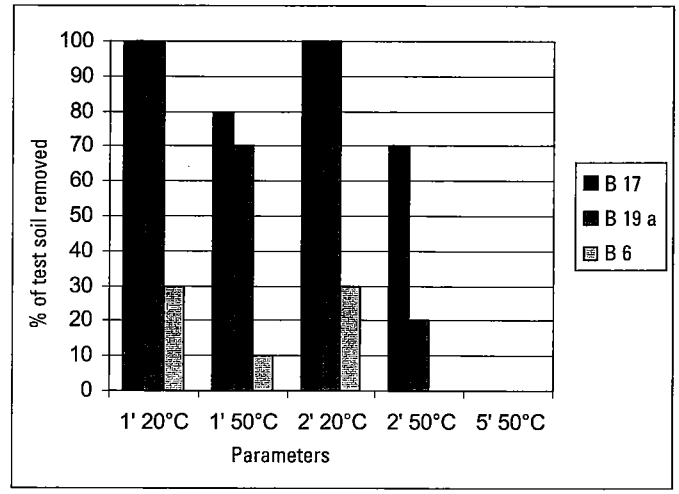

Fig. 3: Starch-based test soils (B 17, B 19a, B 6), showing the parameters tested (time, temperature) and % of test soil removed (Study 1)

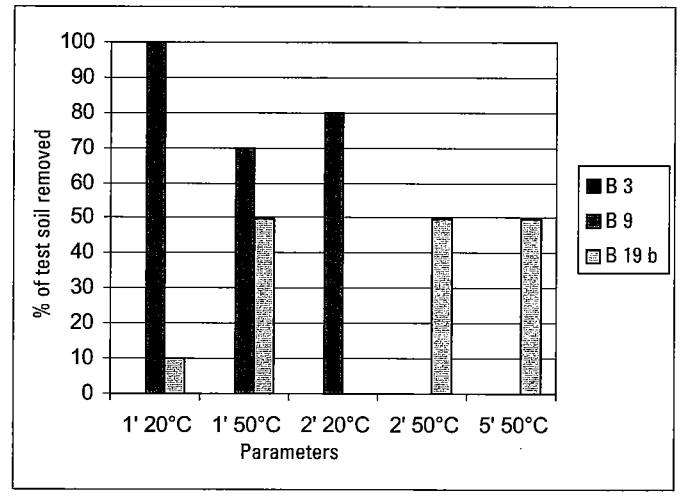

Fig. 4: Protein-based test soils (B 3, B 9, B 19a), showing the parameters tested (time, temperature) and % of test soil removed (Study 1)

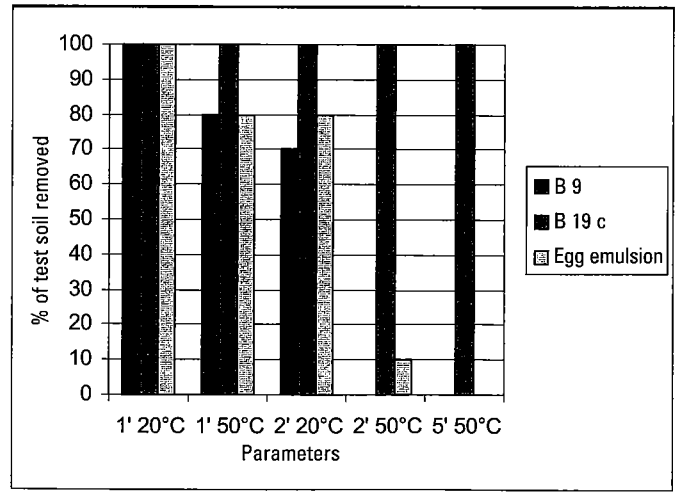

Fig. 5: Egg-based test soils (B 9, B 19c, egg emulsion) showing the parameters tested (time, temperature) and % of test soil removed (Study 1)

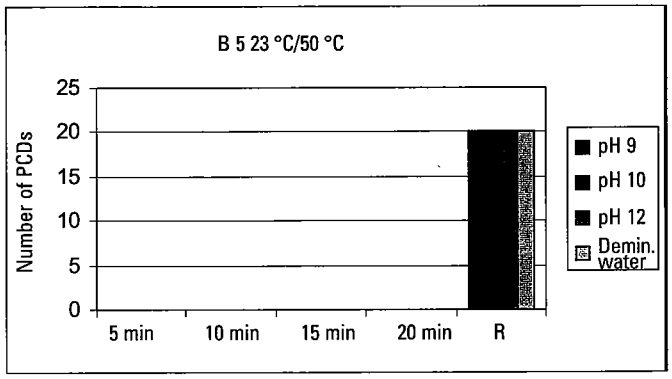

Fig. 6: Behaviour evinced by test soil B 5 at 23 and 50°C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

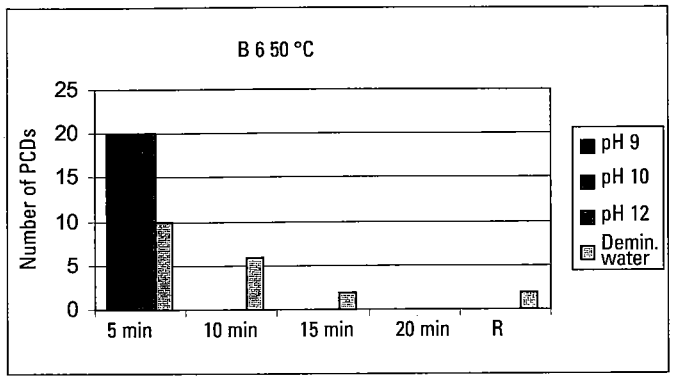

Fig. 7: Behaviour evinced by test soil B 6 at 50°C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

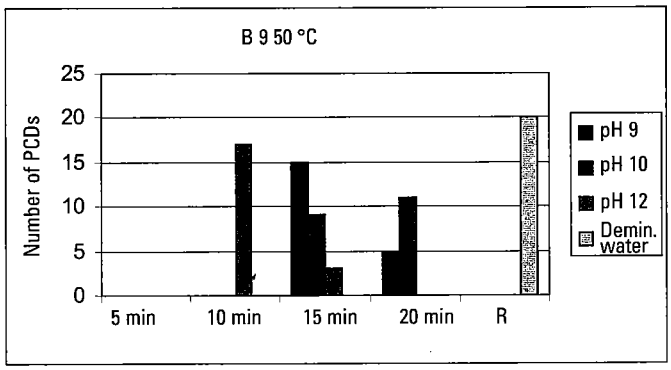

Fig. 8: Behaviour evinced by test soil B 9 at 50°C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

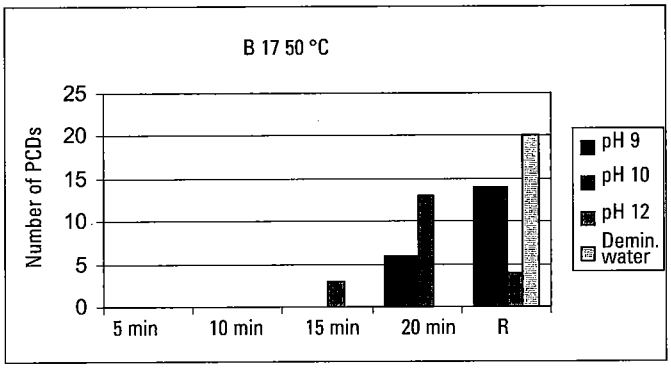

Fig. 9: Behaviour evinced by test soil B 17 at 50°C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

of the risk of transmission of the causative agent of vCJD. During the cleaning process, body fluids, tissue residues, pharmacological substances and also microorganisms must be removed. Being able to furnish proof of optical cleanliness following cleaning is of paramount importance because any lingering residues can detract from the effectiveness of disinfection and sterilisation (6, 7).

As already presumed, the various test soils make different demands on the cleaning process. Contaminants are removed from medical devices through the optimised interaction between chemical products, temperature, exposure time and mechanical action. The mechanical effects, which greatly contribute to detachment of the test soils, was deliberately suppressed in the present two studies, so as to better highlight the effects mediated by the detergent, temperature and exposure.

Table 2 shows the pronounced differences in the properties of the different test soils. Depending on their composition, they can be classified into predominantly starch-, protein- and egg-based test soils. Table 2 and Figures 3 – 4 show that there are also differences among the test soils of a particular group.

Starch-based test soils such as KMNE (B 17 - potato starch, flour paste, nigrosin and egg) and semolina (B 19 a) are very difficult to remove at 20 °C, and even at 50 °C 2 minutes' contact time is not enough. Conversely, the test soil BAMS (bovine albumin, mucin and corn starch – B 6) is easier to remove at both 20 °C and 50 °C because of the lower starch content.

As regards the water-soluble and protein-based test soil, it was possible to remove this mixture of three proteins (B 3) at both 20 °C and 50 °C already after 1 min. Defibrinated sheep blood (B 19 b) is also easy to remove at 20 °C. At 50 °C the mildly alkaline detergent did not prevent blood coagulation, hence it was no longer possible to remove the test soil. Noteworthy was also the marked difference in the cleaning performance when using fresh egg yolk and commercially available egg yolk emulsion. It was essentially easier to remove the latter.

In Study 2 it was possible to demonstrate that the cleaning performance also depended on the type of detergent used. The 0.5% detergent solution with a pH value of 12.6 showed a superior detachment profile in respect of both starch- and protein-based test soils compared with that of a detergent solution

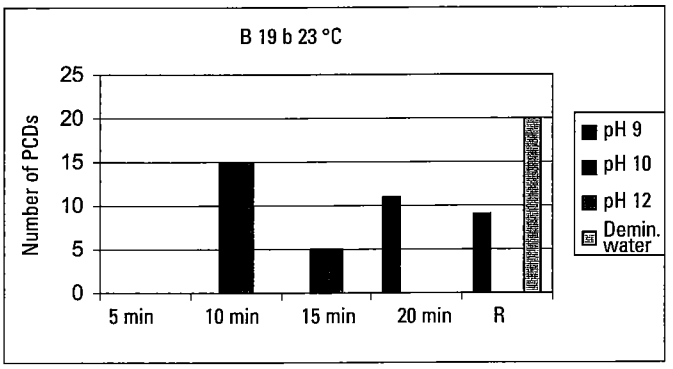

Fig. 10: Behaviour evinced by test soil B 19b at 23 °C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

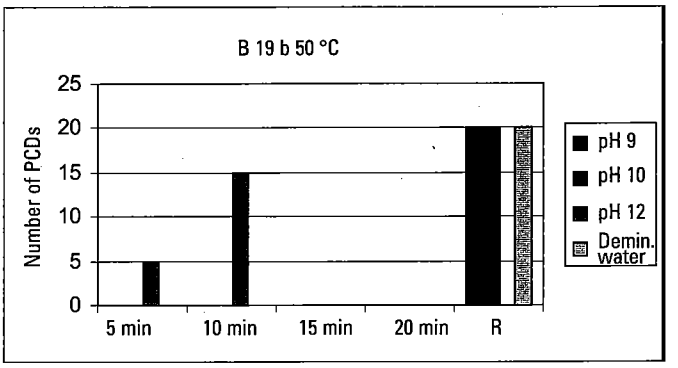

Fig. 11: Behaviour evinced by test soil B 19b at 50°C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

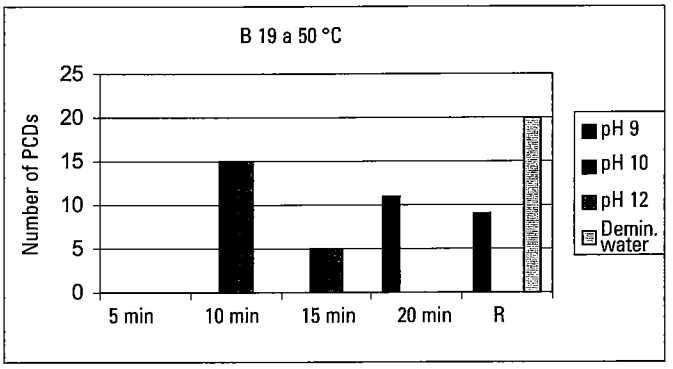

Fig. 12: Behaviour evinced by test soil B 19a at 50°C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

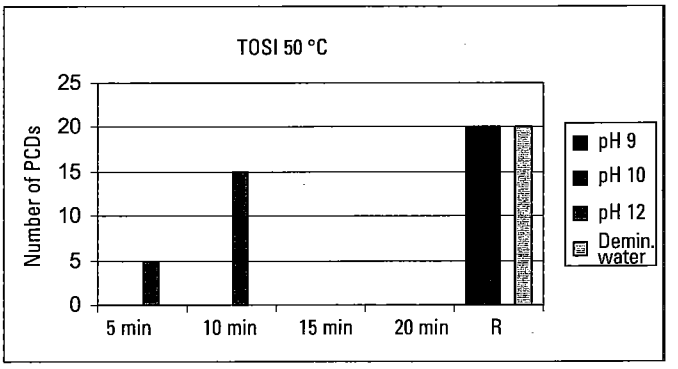

Fig. 13: Behaviour evinced by the TOSI test soil at 50°C in three 0.5% detergents and demineralised water, illustrated as PCDs deemed to be optically clean (n=20) as a function of time (5-20 min) or as R, i.e. PCDs not deemed clean after 20 min (Study 2)

with a pH value of 9.2. This has also been corroborated by other studies (8, 9).

The contact time selected for the test soils and detergent solutions also had vast implications for the cleaning performance. For example, both studies found a correlation between the degradative action/detachment profile and the duration of the exposure time, i.e. the longer the exposure time, the more powerful the degradative action and detachment profile. In the case of alkaline detergents, it was possible to expedite the detachment action by further increasing the temperature; this has also been demonstrated by other publications (4).

More strongly alkaline detergent solutions and higher temperatures promote the removal of starch- and fibrin-based test soils. Therefore further studies are to be conducted with test soils containing fibrin.

In the laboratory tests it was possible only to investigate in comparative terms the effects exerted on the test soils listed in the Standard. These tests revealed that some test soils are easier to remove than others. Therefore a requirement to be fulfilled by a suitable test soil is that it should evince the same behaviour as that of soils encountered in practice. Only if this is assured can a positive result furnished by a test soil or corresponding PCDs lend credence to the belief that a satisfactory cleaning result will also be achieved under typical everyday conditions. Furthermore, the tests should be designed such that their reproducibility is assured.

Summary

The studies give an overview of the ease with which removal of the test soils differs under the influence of temperature and exposure time as well as of different alkaline detergents in laboratory tests conducted while suppressing the mechanical action. They concord with the recommendation by the Robert Koch Institute, stating that preference be given to alkaline detergents with a pH value of at least 10, to temperatures > 55 °C and contact times of 10 min for the cleaning step (6). On the other hand, the studies show how the cleaning performance can be improved in the face of tenacious test soils and associated, everyday residues on medical devices. Further in-depth studies with blood soils are planned. ✻

For references, please refer to p. 235