ZENTRAL STERILISATION 6

**Keywords**
- test soil
- standardisation
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- cleaning

# Multicentre Trial on Standardisation of a Test Soil of Practical Relevance for Comparative and Quantitative Evaluation of Cleaning Pursuant to EN ISO 15883

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**The cleaning performance of washer-disinfectors can be verified pursuant to ISO 15883-1and ISO/TS 15883-5 using various country-specific test soils. Hence comparability of the results achieved is questionable. The Ad Hoc Group "Test Soil and Methods" was founded by the DIN standardisation committee NA Med 063-04-09 in order to standardise the test soils. To that effect, the Ad Hoc Group aimed to develop an in vitro reference system with a test soil suited to everyday practice, which could then be used to study different variables, such as detergents or the behaviour exhibited by other test soils compared to the reference soil.**

**Comparative testing was conducted by six experienced laboratories. In several experimental series it was possible to gradually optimise, first, standardisation of soiling the test objects for process challenge devices, second, the test procedure and, third, analysis of residual soils.**

**On the basis of the series of tests carried out, a system was devised and defined for characterisation of the detachment kinetics, providing for quantification of the cleaning performance relevant to the practical situation.**

**This meant that there was now a reference standard that also permitted quantifiable comparison of the various test systems available, including those cleaning indicators already commercially available.**

## Introduction

In 2005, Technical Specification ISO/TS 15883-5 was published (1) to furnish proof of the cleaning performance of washer-disinfectors (WDs) and has been used to that effect since. Table 1 of the Technical Specification (TS) lists the different test soils used in various countries of the European Union (EU) for different types of loads:

- Surgical instruments,
- Dishes, bowls and collection bottles,
- Anaesthesia accessories,
- Baby feeding bottles
- Suction bottles,
- Bedpans
- Urine bottles,
- Flexible endoscopes,
- Stainless steel utensils.

The composition and application of the test soils are described in detail in the normative annexes A – S of TS 15883-5.

It is not only the test soils specific to each type of load that are listed, but also a number of country-specific soils are outlined for each type of load. For example, for the load type "surgical instruments", six test soils from six countries are listed (Table 1). Only in the case of one test soil is quantitative proof of (residual) protein given, while the remaining five rely on visual inspection and assessment of residual soils.

In addition, tests are carried out in practice using Crile clamps harbouring test soils based on the German or Austrian Guidelines (2, 3) as well as with other commercially available indicators.

In the case of these indicator systems, there is a lack of transparency as regards, first, standardisation of the preparations used and, second, in respect of the different demands the indicators make on the cleaning performance and on specific WD parameters. Comparison of cleaning of smooth surfaces with cleaning of instrument lumens or joints is one example of the issues involved here.

In 2005, the Ad Hoc Group "Test Soil and Methods" was founded by the DIN standardisation committee NA Med 063-04-09 in order to standardise the methods used for verification of the cleaning performance. The Ad Hoc Group decided to have experienced laboratories carry out comparative tests. The participating laboratories are as follows[1]:

- Chemische Fabrik Dr. Weigert GmbH & Co. KG
- Ecolab GmbH & Co. OHG
- HygCen GmbH
- Miele & Cie. KG
- Schülke & Mayr GmbH
- SMP GmbH

*1 The order in which the laboratories are listed here differs from that given in the figures*

The main focus here must be on practical relevance. For example, indicators featuring a test soil composed primarily of starch must not be used when checking the cleaning performance of a WD used to decontaminate medical devices, since these harbour mainly blood or organic contamination. Ultimately, these manifold indicator systems gave rise to a situation whereby at the time of commissioning WDs, the processes were often brought

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into line with the indicator systems and no attention was paid to the instruments soiled by normal use.

The aim of the Ad Hoc Group was not to develop a new indicator system but rather to devise a reference system that quantitatively measures the test soil or the reduction in the test soil, thus providing for comparative evaluation on the basis of the detachment kinetics.

The aim of the comparative tests was to devise a system for quantification of the cleaning performance. The following had to be standardised when analysing the cleaning performance: 1. The type of test object (TO) and contamination used; 2. cleaning; 3. proof of the residual contamination. Hence the test series focused on specification of TOs, test soils, standardised cleaning, elution of residual protein and protein analysis. On that basis, complex processes with different variables can be evaluated.

A detailed standard operating procedure was drafted so that all participating laboratories could conduct the experiments as accurately as possible.

Comparative testing was conducted in a series of six experiments, with the participants meeting between each test series to discuss the results and define / agree the methodology.

| Country | Composition of the test soil | Evaluation |
|---|---|---|
| Austria | Heparinised sheep blood, coagulated with protamine | Visual inspection, no quantitative protein analysis |
| Germany | – Sheep blood,<br>– Egg yolk, semolina, butter, sugar, milk powder | Visual inspection, no quantitative protein analysis |
| Netherlands | Bovine serum albumin fraction 5, Porcine stomach mucin type 3, Bovine fibrinogen fraction 1, Bovine thrombin | Qualitative and quantitative proof of protein |
| Sweden | Bovine citrate blood, coagulated with calcium chloride | Visual inspection, no quantitative protein analysis |
| United Kingdom | Defibrinated horse/sheep blood, egg yolk, dehydrated porcine mucin | Visual inspection, no quantitative protein analysis |
| USA | Organic- or protein-based test soil (optional), *B. atrophaeus* endospores | Visual inspection, no quantitative protein analysis |

**Table 1:** Test soils for surgical instruments pursuant to ISO/TS 15883-5

| Parameter combinations | PCD | Blood | Temperature | Exposure time |
|---|---|---|---|---|
| 1 | Stainless steel | Hep. blood | 45 °C | 1, 2, 3, 4, 5 min |
| 2 | | Citrate blood | | |
| 3 | Frosted glass | Hep. blood | 45 °C | |
| 4 | | Citrate blood | | |
| 5 | Stainless steel | Hep. blood | 60 °C | |
| 6 | | Citrate blood | | |
| 7 | Frosted glass | Hep. blood | 60 °C | |
| 8 | | Citrate blood | | |

**Table 2:** Overview of the test parameters for experimental series 1 – 4 (test medium: highly purified water)

## Materials and Methods[2]

2 *A detailed description of the tests can be obtained from the authors and is to be published in a subsequent issue of this journal.*

### Process challenge devices

To test two different surfaces, test objects (TO) made of frosted glass (15 × 60 × 1 mm) and stainless steel (15 × 50 × 1 mm) were used.

### Test soils

The TOs were each contaminated with 100 µl reactivated heparinised sheep blood or reactivated (sheep) citrate blood on a defined surface.

The blood had to be less than a week old and was obtained from the same supplier.

### Test parameters

The test parameters are given in Table 2.

### Experimental design

An example of an experimental design is given in Figure 1.

### Test procedure

A glass beaker containing 100 ml water was brought to a specified temperature in a water bath. To assure uniform mixing of the water and the test soil, now undergoing detachment, the mixture was stirred with a magnetic stirrer at 350 rpm. This slight movement was used not to simulate a mechanical effect but rather to ensure that the detached soil would be carried away. Once the target temperature was reached, the TO has hung in each case in the beaker and withdrawn again on expiry of the exposure time (1, 2, 3, 4 and 5 min). Then the residual protein on the TO as well as the protein content of the liquid in the beaker was quantitatively analysed.

### Elution of residual protein from the TO

The TOs were placed in screw-top test tubes (15 ml) with 5 ml 1% sodium dodecylsulphate (SDS) and glass beads and agitated for 20 min at 300 rpm on a horizontal agitator. The TOs were removed again and the protein content of one aliquot of the eluate was measured.

### Protein analysis

Baseline contamination of the TOs, residual protein on the TOs after exposure and detached protein in the beaker liquid were measured using the modified ortho-phthaldialdehyde (OPA) method (details of the methodology given in Reference No. 4).

### Calculation

The reduction in the test soil [%] was calculated on the basis of the protein content on TOs that had been contaminated but not used in the tests.

Reduction [%] = 100 –
(residual contamination/baseline load ∗ 100)

In addition, the sum was calculated from the residual protein on the TO and the dissolved protein in the liquid. As expected, this sum should correspond to the baseline value and serves as proof of the suitability of the test methodology, in particular of protein recovery through elution.

## Results

Several tests were carried out during this comparative series of tests. The various parameter combinations used are listed in Table 2. Below are given by way of example only the results for the parameter combination comprising 45 °C, test soil reactivated heparinised sheep blood on a frosted glass TO.

### Results of the first experimental series

The results of the first experimental series highlight the differences manifested in the detachment kinetics, as measured by the participating laboratories (Fig. 2). For example, detachment in Laboratories A and C was continuous, whereas at Laboratories D and E more than 80% of the test soil was already detached already after 1 min. Conversely, no detachment took place at Laboratory F.

To verify reproducibility of the tests conducted by the participating laboratories, each test was repeated twice after 1 min and 5 min (Table 3).

Major differences were seen in some cases in the test soil detachment patterns, ranging from continuous detachment through fixation of the soil to abrupt detachment of the soil from the TOs.

The reasons for the divergent results obtained by the laboratories were due to variations in TO conditioning (basic cleaning, contamination, drying and storage). For the 2nd experimental series, these conditions were investigated in a series of preliminary tests, and then modified, more accurately defined and thus standardised.

### Results of the 2nd experimental series

Following modification and enhanced standardisation of TO conditioning, there was a marked improvement in the uniformity of the results obtained by the different laboratories (Fig. 3).

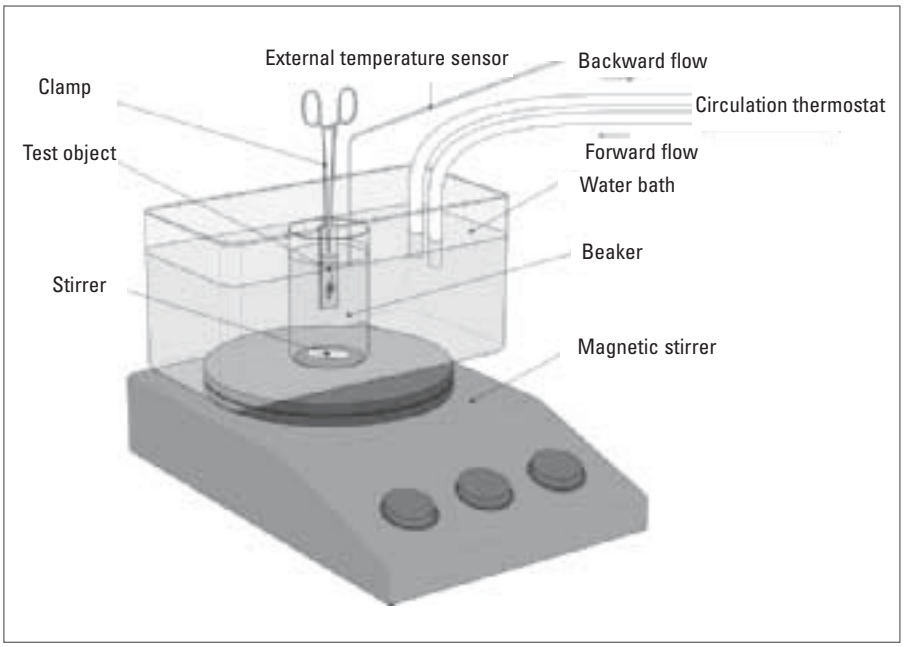


**Fig. 1:** Example of an experimental design

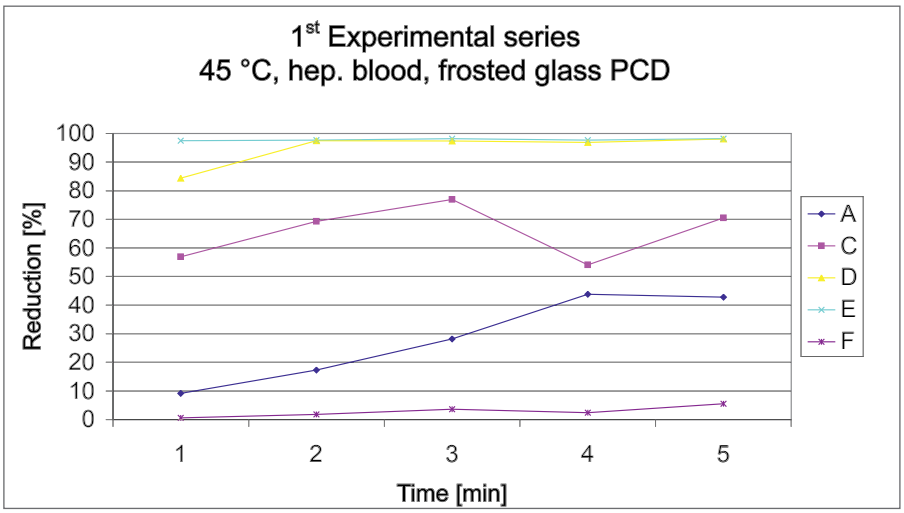


**Fig. 2:** Reduction in the reactivated heparinised blood test soil (1st experimental series)

### Results of the 3rd experimental series

Next 30-sec intervals were used so as to provide for more precise depiction of the detachment kinetics (Fig. 4). To verify reproducibility of the methodology, these tests were repeated with various blood loads and total recovery from the TOs and from the liquid was measured (Fig. 5).

## Discussion

Four series of experiments were required to assure widespread standardisation in the three problem areas related to verification of the cleaning performance listed below.

1. Procedure used to prepare the test soil and condition the TOs

2. Methodology used for test soil detachment
3. Quantitative protein analysis

These problems had to be solved in order to assure reproducible testing of detachment patterns.

For example, the type and applicability of the blood load were important.

In the first experimental series the drying specifications were a drying time of 1.5 h in a drying cabinet at 30 °C. The atmospheric humidity varied according to the respective laboratory, giving rise to differing degrees of adherence of the test soil to the TOs after expiry of the drying time. In Figure 2 (1st experimental series), the reduction is expressed in % with reference to the exposure time. The variance in reduction [%] after 1 min ranged between 0.6% and 97.5% and after 5 min between 5.5% and 98.2% .

The test procedures at 60 °C using stainless steel TOs gave rise to similar results (data not shown).

To improve standardisation of drying the soiling on TOs, for the 2nd experimental series drying, enhanced conditioning, was carried out for a period of 24 h at 30 °C in a desiccator over a saturated potassium carbonate solution.

It was revealed that drying is the most important factor for assuring a gradual reduction in the test soil. Likewise, the composition of the TO surface, its pretreatment and the type of soil influenced the results. These factors were also investigated and standardised, but are not described in the present publication.

In Figure 3, the reduction [%] is depicted as occurring over the course of time. It was revealed that thanks to improved standardisation, there was greater concordance between the results obtained by the different laboratories. The interlaboratory variation coefficient was ≤ 10.0%.

To achieve better scattering of the detachment kinetics, the time intervals were reduced to 30 sec for the 3rd experimental series. The interlaboratory variation coefficient now ranged between 1.9% (120 sec values) and 17.7% (60 sec values).

The detection sensitivity of the OPA method assured comparability of the absolute protein concentration on TOs and in the liquid. Figure 5 shows for each laboratory the protein concentration of the

| Time [min] | | A | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 min | 1st series | 9.1 | 56.9 | 84.3 | 97.5 | 0.6 |
| 1 min | 1st reproduction | 11.0 | 32.7 | 78.9 | 98.0 | 1.3 |
| 1 min | 2nd reproduction | 20.1 | 34.3 | 76.4 | n.c. | n.c. |
| Mean value | 13.4 | 41.3 | 79.9 | 97.8 | 0.9 | |
| Standard dev. | 5.9 | 13.6 | 4.0 | 0.3 | 0.5 | |
| VC [%] | 43.8 | 32.8 | 5.1 | 0.3 | 48.7 | |
| **Time [min]** | | **A** | **C** | **D** | **E** | **F** |
| 5 min | 1st series | 42.8 | 70.4 | 98.1 | 98.2 | 5.5 |
| 5 min | 1st reproduction | 53.9 | 90.3 | 97.3 | 98.0 | 0.6 |
| 5 min | 2nd reproduction | 37.0 | 91.6 | 98.4 | n.c. | n.c. |
| Mean value | 44.6 | 84.1 | 97.9 | 98.1 | 3.1 | |
| Standard dev. | 8.6 | 11.9 | 0.6 | 0.1 | 3.4 | |
| VC [%] | 19.2 | 14.1 | 0.6 | 0.1 | 112.3 | |

*n. c. = not conducted*

**Table 3:** Reproducibility among the laboratories (data given as % of test soil reduction)

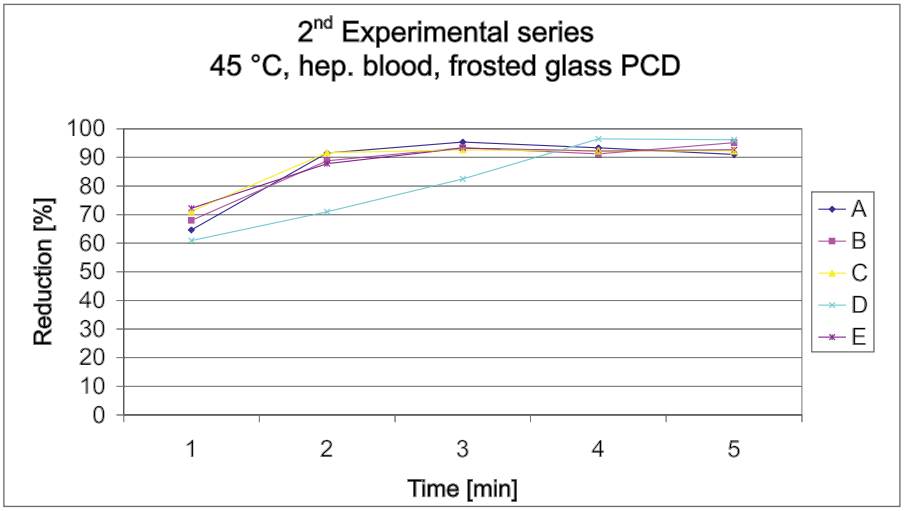


**Fig. 3:** Reduction in the reactivated heparinised blood test soil (2nd experimental series)

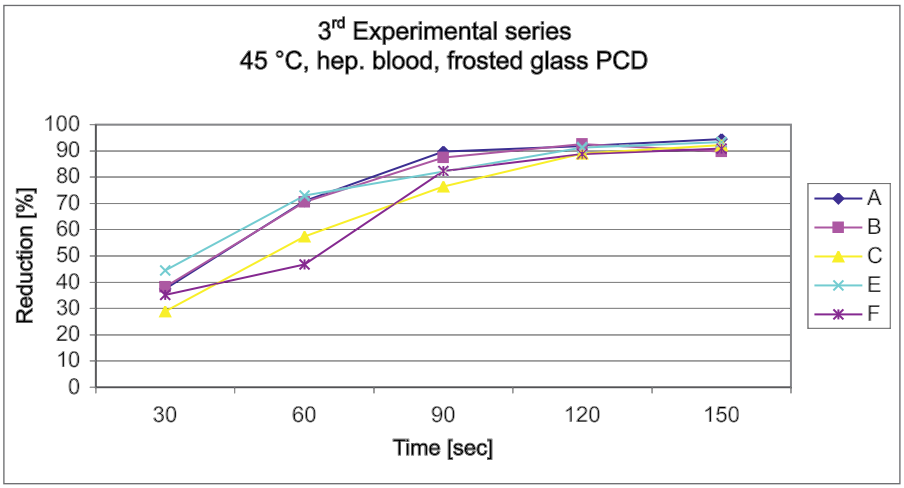


**Fig. 4:** Reduction in the reactivated heparinised blood test soil (3rd experimental series)

positive controls as well as the concentration for TOs and liquid. This procedure provided for verification of the methodology. Despite the high dilution of the test soil in the beaker liquid, residual protein recovery from the TOs and the protein content of the liquid ranged between 82.4% and 102.8% (Table 4). The variance between the absolute protein concentrations of the baseline load (controls) in the 3rd experimental series dropped to 14.9%, highlighting, inter alia, how important the standardisation of the test procedure and protein analysis is.

To record the fluctuations manifested by the different blood loads, test days and measurement time periods, two further reproduction tests were carried out in each case (data not presented).

Apart from isolated outliers, possibly attributable to the different blood loads, the trend towards greater concordance between the results obtained by the different laboratories continued.

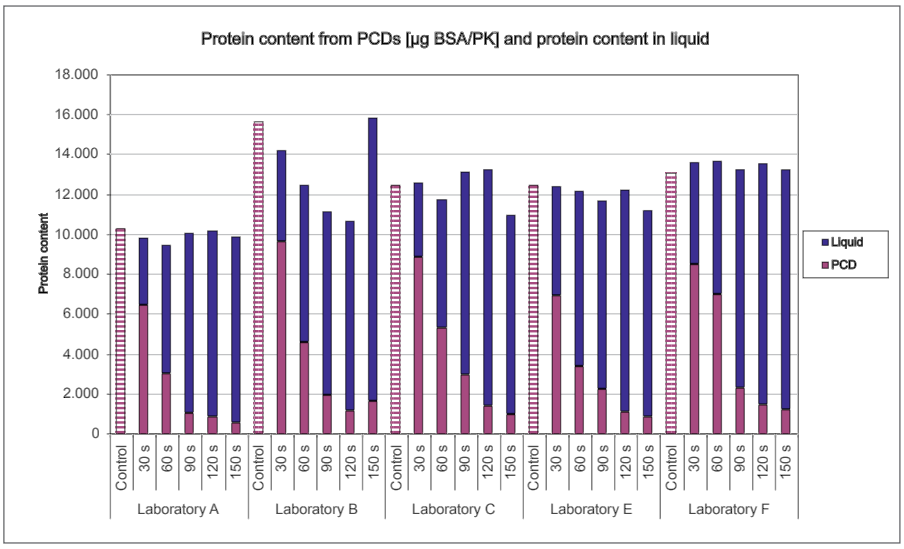


**Fig. 5:** Sum of the protein content recovered from the PCDs and the fluid in the beaker (3rd experimental series)

## Conclusion

The results of one single laboratory, which used a special test method based on ISO/TS 15883-5, are not necessarily comparable with those of other laboratories. Based on the experiences gathered from this multicentre trial, the methods described in ISO/TS do not lend themselves to assuring comparable results in different laboratories.

The details of the different test protocols must be harmonised and confirmed in a multicentre trial in order to assure reproducible results.

As demonstrated by the results of the 1st and 2nd series of experiments, during the different steps undertaken for standardisation of the test method it proved to be relatively difficult to achieve uniform and continuous detachment of the test soil.

Further development of these experiments could possibly demonstrate that standards will feature different everyday test soils that are not specific to any particular country but rather are determined by the respective application. This would ultimately mean that the different washer-disinfectors as well as processes would become more specific. However, due to the vital importance attached to medical device reprocessing, and as such to the prevention of infections, standardisation of the test concept and test methodology must be achieved.

The test soils and TOs used should meet the following criteria:

- Practical relevance
- Precise specification of the test soil and its typical ingredients

| Laboratory | A | | B | | C | | E | | F | |
|---|---|---|---|---|---|---|---|---|---|---|
| Protein concentration | µg BSA | Recovery [%] | µg BSA | Recovery [%] | µg BSA | Recovery [%] | µg BSA | Recovery [%] | µg BSA | Recovery [%] |
| Control value | 10288 | – | 15625 | – | 12475 | – | 12452 | – | 13083 | – |
| 30 sec | 9788 | 95.1 | 14210 | 90.9 | 12559 | 100.7 | 12385 | 99.5 | 13610 | 104.0 |
| 60 sec | 9475 | 92.1 | 12485 | 79.9 | 11728 | 94.0 | 12162 | 97.7 | 13655 | 104.4 |
| 90 sec | 10031 | 97.5 | 11165 | 71.5 | 13119 | 105.2 | 11694 | 93.9 | 13216 | 101.0 |
| 120 sec | 10149 | 98.6 | 10650 | 68.2 | 13218 | 106.0 | 12204 | 98.0 | 13518 | 103.3 |
| 150 sec | 9866 | 95.9 | 15850 | 101.4 | 10955 | 87.8 | 11180 | 89.8 | 13217 | 101.0 |
| Mean value | 9862 | 95.9 | 12872 | 82.4 | 12316 | 98.7 | 11925 | 95.8 | 13443 | 102.8 |
| MIN | 92.1 | 68.2 | 87.8 | 89.8 | 101.0 | | | | | |
| MAX | 98.6 | 101.4 | 106.0 | 99.5 | 104.4 | | | | | |

**Table 4:** Recovery of baseline contamination expressed as a percentage

- Coagulability
- Defined, standardised contamination of TOs
- Temperature stability as well as protein denaturation patterns
- Resistance to defined cleaning steps, e.g. immersion in water
- Sensitive quantitative analysis of baseline contamination and of residual contamination

In view of these insights, the proposals of the Ad Hoc Group should be forwarded to the DIN committee and to the ISO working group entrusted with revision of ISO/TS 15883-5, in order to create a reference system for evaluation of different test systems and thus introduce specifications for standardisation of test soils for quantification of the cleaning performance. Furthermore, the reference system can also be used for classification of commercially available indicator systems. ❊

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