

ZENTRAL STERILISATION 1

Keywords
- cleaning performance
- oxidative cleaning
- washer-disinfectors
- radionuclide method
- OPA method

Investigation of Cleaning Performance Following the Standard prEN/ISO 15883-1

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The radionuclide method was used to investigate comparatively the cleaning performance of automatic washing/disinfection processes on the critical joint areas of artery clips. The defined initial soil was radioactively marked coagulable blood. The results show the thorough effect of cleaning, which takes place in the presence of both an oxidative process stage, as well as a suitably composed detergent/hydrogen peroxide solution. Additionally, following elution of the joint areas, the direct measurements of the radionuclide method were correlated with the indirect determination of residual contamination in the eluate using the OPA method.

Introduction

Blood is the most common soil found on surgical instruments. Therefore the process used for cleaning instruments has to be able to deal with the protein content of blood, and in particular the water-insoluble fibrin polymers formed when blood clots. Blood is also a particularly relevant soil from the point of view of hygiene, because it can transmit facultative pathogens. In circulating blood the blood proteins present are in water-soluble form. An insoluble fibrin mesh, formed from a comparatively small proportion of the blood, containing aggregated thrombocytes and other embedded blood components, first forms when the process of blood clotting occurs. But even after clotting, over 90% of the proteins are still water-soluble, and do not pose a real cleaning problem. During cleaning they are often literally swept out of the fibrin mesh.

Proteins are however very sensitive to external influences that are able to cause a change in their structure. Even temperatures above 55–60 °C or contact with chemicals such as disinfectants lead to denaturation of proteins with a concomitant loss of water-solubility, so that soil is much more difficult to remove from instruments.

The cleaning process has to meet different requirements depending on the type of instrument and its design. Whereas simple instruments with directly accessible surfaces can be rid of blood to a considerable extent using a cold pre-wash, residual blood lodged in the crevices of joints or connectors is much more difficult to remove (1). The way a washer-disinfector (WD) is loaded can also directly affect the cleaning result because of possible shielding of the wash jets (rinse shadow). This is why it is always necessary to load the appliance in the same balanced way. Dirty instruments are put into standardised sieve baskets that are suitable for washing in a WD. The sieve baskets must not be overloaded, or effective washing of instruments will not be achieved. Instruments with joints must be cleaned in the open position, so that reciprocally overlapping areas are minimised, and the removal of residues in the joint is achieved (2).

Because most blood proteins are water-soluble, over 90% of the soil can be removed using a simple pre-rinse with cold water. Another reason for including the cold pre-rinse is because of possible increased foam formation in the WD due to too much blood on the instruments (3). On the other hand, problem areas such as fibrin and blood residues in the fissures of joints, cogs, screw holes etc. must be effectively washed off during the main wash, which should be appropriately designed to be capable of this task.

The aim of these experiments was to compare the attainable result quality determined by the radionuclide method, using the Oxivario process, both with and without additional oxidative effect, under simulated conditions (4, 5). At the same time the effect of various detergents and detergent/hydrogen peroxide combinations is compared.

A further aim of the experiments was to establish a test method to determine cleaning performance which is fast, simple and precise, and which delivers correct results. The practical relevance of the test soil is a decisive factor for the validity of the results, a wide consensus of opinion agreeing that use of heparinised sheep blood reactivated with protamine sulphate meets the criteria (6, 7). A method for testing the cleaning performance of washer-disinfector appliances (WDs) is described in the standard prEN/ISO 15883-1 in Appendix B, chapter 2.6.2, using 40 defined clamps for type testing (6.10 and following). The criterion of acceptance is that at least 95% of the clamps should be clean after washing (8). Following the standard, evaluation is carried out visually as well as by protein analysis, using for example the modified OPA method. This is why we conducted an additional test, to determine the correlation between the radionuclide and OPA methods.

Materials and methods

The radionuclide method (RNM)

The principle of the radionuclide method is the radioactive marking of the test soil used to contaminate the instruments (5).

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Here macroalbumins are incubated with radioactive Technetium^{99m} (Tc-99m) and are then mixed with coagulable blood. After contamination of the inner and/or outer surfaces of the instruments, the amount and distribution before and after washing can be determined quantitatively as well as noting its regional distribution, by measuring the emitted gamma radiation. Conclusions about the quality of washing can be reached after measuring the residual radioactivity on the instruments. An instrument is considered successfully cleaned if the residual contamination is less than 5 counts/second (emitted gamma quanta per second).

The radionuclide method is a non-destructive physical test method, which allows a quantitative investigation of cleaning behaviour, especially for lumened instruments and instruments with hidden surfaces. It is important to note that there is no need to determine the recovery rate, as is necessary for microbiological methods, because conclusions about cleaning performance can be drawn from the residual soil on the instruments. At the same time the quality of individual wash steps e.g. the pre-wash, can be evaluated in the same experiment, as is the washing success of the overall process.

Contamination of the test instruments

Stainless steel Crile artery clamps were used as test objects, as described in the standard prEN/ISO 15883-1 (Fig. 1). The joints of these relatively simply constructed instruments are difficult to clean. The publications of the Cleaning Project Group have already shown that fissure width is a secondary criterion for washing when compared with other construction features (1).

Heparinised sheep blood rendered coagulable by protamine sulphate was used as the test contaminant. The sheep blood used should not be more than one week old, and must have been kept cool until it is used. It was radioactively marked before contamination. Here a marking set Pulmocis (IKS No. 42 553; Schering AG; Baar; Switzerland) was marked with a dose of 400 MBq Technetium^{99m}, and topped up to 5ml with a common salt solution. The solution was incubated for at least 10 minutes so that the Albumin-Technetium complex could form. 100 MBq (approx. 1.2ml) were removed and added to 10ml sheep blood (Acila GMN, Mörfelden). The sheep blood was rendered coagulable again with 1.5 I.U. protamine sulphate per 1ml blood.

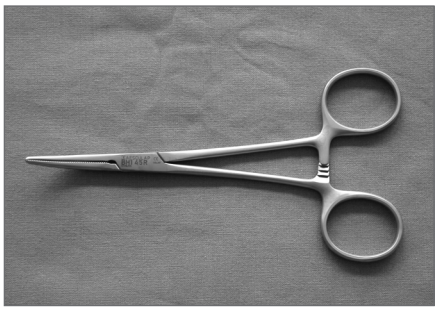

Fig. 1: Surgical clamp used as a test instrument in this experiment

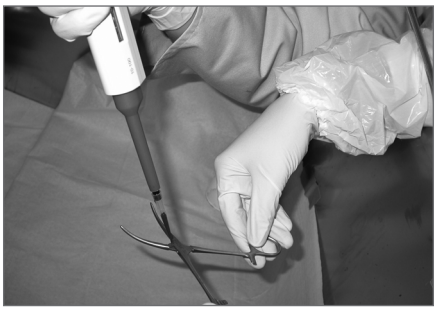

Fig. 2: Contamination of clamp joints using a pipette

Forty clamps of the type BH425R and BH145R were used, their serial numbers being noted for individual identification. 100µl of the test contaminant was dripped into the joint of each instrument using a pipette. Each clamp was opened and closed 5 times in order to distribute the contaminant solution evenly within the joint (Fig.2). This method of contaminating instruments is a modified form of the method described in the standard, used because it ensures that the starting contamination is defined and reproducible.

After contamination the clamps are laid in a sieve basket. It is important that the sieve baskets are positioned on a non-absorbent mat, preferably a little above the working surface. Otherwise the mat could absorb part of the test soil leading to variable and imprecise contamination of the clamps. Twenty clamps in the open position were put in each sieve basket and dried in a drying cabinet for 1 hour at 45 °C (Fig. 3).

Cleaning and determination of residual contamination

Before washing, the total contamination of the instruments in each sieve basket was measured using the gamma camera. After this the sieve baskets were put into an instrument mobile unit for 4 sieve baskets, one on each level (Fig.4) and loaded into the WD (Miele G 7735 CD). To ensure optimal use of the appliance's capacity, two additional sieve baskets containing a typical selection of instruments were placed in the vacant positions of the instrument mobile unit.

At the beginning of the experiments the original Oxivario programme with a two-phase main wash, but without thermal disinfection was used. In order to achieve a better differentiation of results, the instruments were given various chemical or physical treatments to increase the tenacity of the soil. The two washing phases were also shortened by two minutes.

Sequence of the unaltered Oxivario programme

- 2 min. cold pre-wash using tap water
- Drainage
- 5 min. wash at 55 °C using 0.5% alkaline detergent
- Drainage
- 5 min. wash at 55 °C using 0.5% alkaline detergent and an oxidative additive 0.35%* (see table 1)
- 1 min. neutralisation at 38°C using an acid additive (0.1%) based on citric acid
- Drainage
- 1 min. intermediate rinse

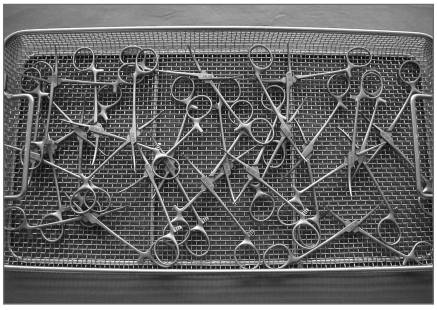

Fig. 3: Stainless steel clamps, placement in sieve basket

– Drainage

– 1 min. intermediate rinse

* 35ml Hydrogen peroxide solution (0.35%) was manually squirted by syringe into the wash cabinet via a hose fixed to the door, during programme step 20

After washing, scintillation measurements were taken from the full sieves and from the instruments individually, using the gamma camera. The assessment of residual contamination was refined by establishing ROIs (regions of interest). Via this selectively detectable activity of residual contamination, a very sensitive and reliable proof of residual soil can be obtained.

In order to gain a direct comparison of the intensive Vario TD programme and the Oxivario programme, the Oxivario programme was run with and without oxidative additives. Further to this, we wanted to document the improvement in cleaning attained by the addition of hydrogen peroxide in an alkaline environment, in comparison to a purely alkaline wash, and to this end varying concentrations of hydrogen peroxide were added.

In order to document the differences between the two programmes more accurately, experiments on the Oxivario programme were conducted, shortening the wash stages to 3 minutes from 5 minutes. Because thorough cleaning under these conditions is more difficult to attain, the remaining soil allows conclusions about the quality of the individual reprocessing programmes and the extent of spare performance capacity, to be made more easily.

Two experiments were conducted per programme tested, each using 40 clamps. The following varying combinations of detergent/hydrogen peroxide were tested:

– Alkaline detergent A (phosphate, disodium- and potassium-metasilicate, tenside-free, with and without an oxidative additive B (hydrogen peroxide solution somewhat less than 30%) in the second wash step; also the oxidative additive was tested in different concentrations (0.175% and 0.35%).

– Alkaline detergent C (sodium phosphate, sodium silicate, potassium hydroxide, tenside-free) with and without an oxidative additive D (hydrogen peroxide solution somewhat more than 30%) in the second wash step at a concentration of 0.35%.

– Mildly alkaline detergent E (enzymes, tenside, caustic potash solution) with and without oxidative additive B.

The pH of the applied concentration was determined compensating for temperature (Table 1).

Comparison of the radionuclide method with the OPA method

In order to correlate the experimental results from the RNM with those from the protein-analytic method described in the standard prEN ISO 15883-1, 16 test instruments out of the Cleaning Project Group (1) were contaminated with radioactively marked sheep blood, and after washing were tested for residual soil using the radionuclide method. When the radioactivity had decayed, these instruments were additionally investigated using the modified OPA method. To obtain samples, the instrument joints were thoroughly eluated by swirling in a beaker with 2ml 1% sodium dodecylsulphate solution (pH 11) and UV-spectrometric measurements were made of 400µl of each eluate with 2 ml OPA reagent at 340nm.

Results

Pre-experiments had shown that the Oxivario programme delivers very good results even without the oxidative additive proscribed in the design of the experiment. These good results are achieved because of its two wash phases. To determine what improves the possibility of better differentiation, experiments with variously treated test soil were carried out first of all.

Improved differentiation is not attained even if fat (in the form of coffee whitener – 10% fat content) or a disinfectant (Betaisodona solution) is added before reactivation of the sheep blood. The required denaturation of protein and fixation via Betaisodona solution was expected, but did not occur. Even putting the instruments in Betaisodona solution for 10 minutes after coagulation and drying had taken place did not deliver the expected success.

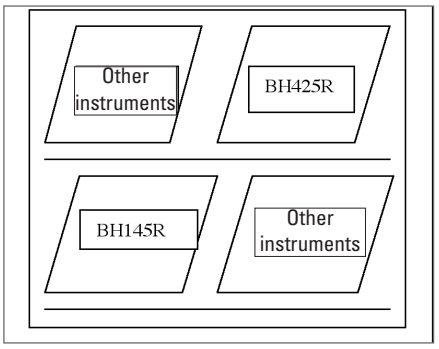

Fig. 4: Positioning of the instrument sieves on the mobile unit

It took the increased drying-on of test soil achieved in a drying cabinet at 45°C for at least half an hour to attain any significant increase in difficulty of cleaning. The effect of warm drying air caused increased tenacity of the test soil (blood/Technetium) on an instrument. (Results not shown)

To investigate the contribution of the pre-wash on washing, the Oxivario programme using detergent A was interrupted after the pre-wash, and the clamps were removed for interim testing. Afterwards the clamps were returned to their places in the WD and washing was allowed to continue, although no hydrogen peroxide was added to this second wash step. After neutralisation and the interim rinse the instruments were tested again.

The results are shown in Fig. 5. The starting contamination of the instruments

Detergent and concentration	pH value
Alkaline detergent A 0,5%	11.2
Alkaline detergent A 0,5% & H_2O_2 0,175% (oxidative additive B)	10.9
Alkaline detergent A 0,5% & H_2O_2 0,35% (oxidative additive B)	10.4
Alkaline detergent B 0,5% & H_2O_2 0,35% (oxidative additive D)	11.1
Alkaline detergent E 0,5%	9.8
Alkaline detergent E 0,5% & H_2O_2 0,35% (oxidative additive B)	9.2

Table 1: pH of the applied concentrations of detergents

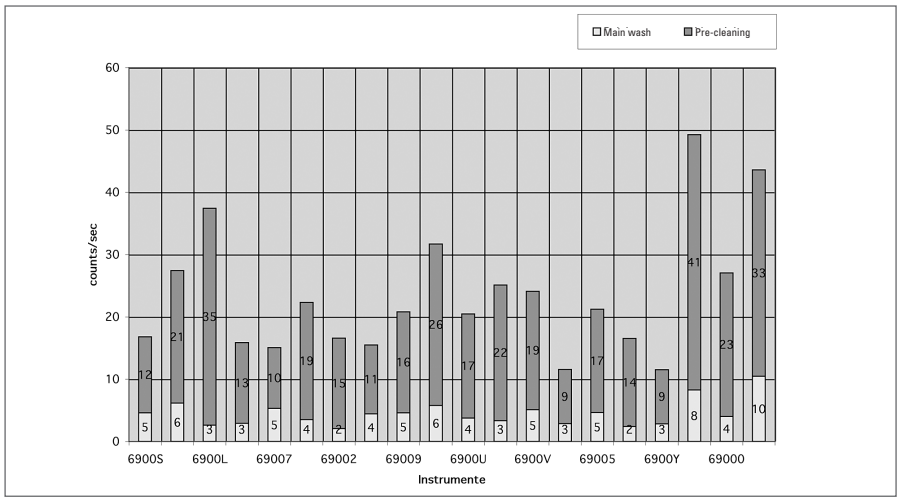

Fig. 5: Comparison: pre-cleaning/main wash

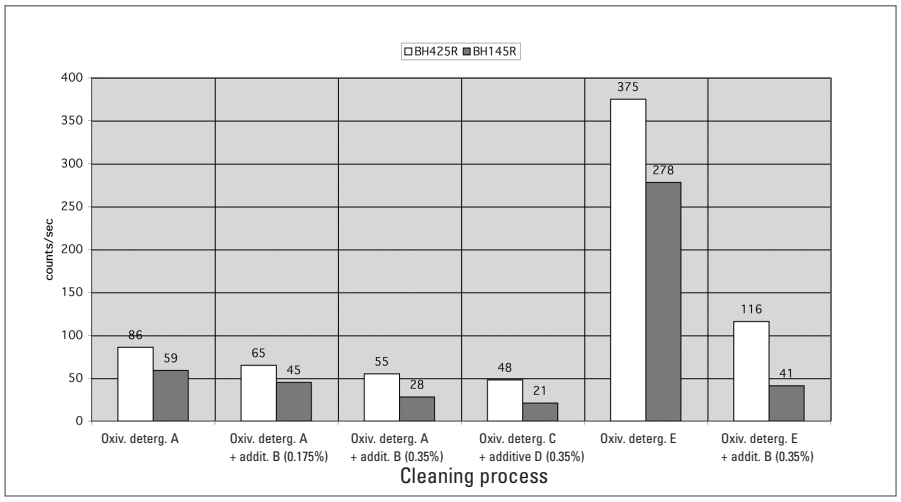

Fig. 6: Summary of the various wash results when using the radionuclide method. The blue bars represent the upper sieve; the red bars the lower sieve

was over 120 counts /sec. for each and every instrument. Even the two-minute cold pre-wash alone caused an average reduction in contamination down to 19 counts/sec. After washing, 4 out of 20 instruments (20%) still had counts over the limiting value of 5 counts/sec.

Figure 6 shows the results of the tests on the modified Oxivario programme using various detergents with and without hydrogen peroxide in varying concentrations. Here the blue bars show the total activity of the instruments, which had been placed in the sieve baskets on the upper level. The red bars show the total activity of the instruments in the sieve baskets on the lower levels. It can be seen that increasing the hydrogen peroxide concentration causes a decrease in contamination after washing.

For each wash process 2 test series were conducted. There were 20 instruments in the upper and 20 in the lower sieve basket. The results shown are the median of the detected counts/sec. from both procedures. Besides the positive effect of the addition of hydrogen peroxide, it can easily be seen that the mechanical washing effect in the lower part of the machine is greater than that in the upper half.
When washing is carried out using detergent C plus the oxidative additive D (0.35% hydrogen peroxide concentration), the results from the sieves holding 20 instruments are somewhat worse than when using detergent A and the oxidative additive B (0.35% hydrogen peroxide concentration). On the right hand side of Fig.6 one can see the results from using detergent E with and without the oxidative additive B. Detergent E has a pH of about 10 in tap water, whereas detergents A and C have a pH of slightly over 11, which delivers better results. The results are better for detergent E when using an oxidative additive, but nowhere near the results for the other combinations, because the alkalinity required for efficient active oxygen release is not attained.

Figure 7 shows the evaluation of the individual instruments, taking into account the number of instruments that lie over the upper limit of 5 counts per second, when using the reduced Oxivario programme, with the various detergents and additive combinations. These data come from 40 measurements, taken during 2 test series. Because of the obvious results with detergent E (with and without oxidative additive) data from only one test series were given.

If one takes the acceptance criterion from the standard prEN/15883 of 95% as a basis, then only the example with the hydrogen peroxide concentration of 0.35% (oxidative additive D) attained a really good result, where all the instruments were ideally cleaned.

The results of the determination of residual contamination using the radionuclide and OPA method are shown in Figure 8. The radioactive decay correlates well with the extinction values, but it is obvious that the radionuclide method is considerably more sensitive. However, the RNM can so far only be used in the laboratory on artificially contaminated instruments, whereas the modified OPA method can be used to test real contamination on instruments used in clinical practice.

This was the reason that a rinsing solution was chosen at the first clinical Multicentre Study on residual soil with proteins, which could be used in the different analysis methods (9).

Discussion

The test method described in the standard prEN/ISO 15883 when combined with the RNM, proves to be a suitable way of comparing the different wash

processes with one another. The question is then whether the principle of using a partial or half cycle of wash steps to gain improved differentiation, should be used for WDs - as it has already been used to validate sterilisation. At the moment wash tests are carried out without any safety net, which means that no performance reserves are taken into account to deal with fluctuations that may occur in the practical situation, for example by drying-on of soil.

Because the clamps were contaminated in a defined way with a pipette, a standardised starting contamination could be assured. Thus it can be shown that the results of washing with the Oxivario programme without an oxidative effect are definitely inferior, and also that the statistical deviation increases.

Even at a low hydrogen peroxide concentration there was more constant washing with fewer differences in washing results. Another result of the Oxivario process with added hydrogen peroxide is the proven cleaning effectiveness, even in areas that are difficult to reach. This special deep cleaning makes the process superior in comparison to the alkaline process run without an oxidative additive.

Washing using the Miele Oxivario programme with the addition of hydrogen peroxide achieves an increased cleaning performance compared to that from the alkaline detergent alone, and also attains better results in critical areas, so that the cleaning performance is standardised at a high level. The alkaline activation of release of active oxygen from hydrogen peroxide is certainly an important process step. The oxidative effect of active oxygen splits proteins, thus rendering them water soluble and easier to remove by washing. Because the favourable cleaning results shown here were attained even with the foreshortened cycle, it can be presumed that there is a generous performance reserve when using the full cycle. Only with secure results such as these is it relevant to consider a preventive effect on iatrogenic transmission of vCJD.

The comparison of detection of residual contamination using the RNM and the OPA method shows that the results correlate well. RNM measurements ≤ 5 counts/sec. correlate with an OPA extinction of < 0.02. However this OPA upper limit differs from that described in the standard prEN ISO 15883-1, because here the sampling was carried out using 2ml instead of 5ml 1% sodium dodecyl sulphate solution, and 400µl are added to 2ml OPA reagent instead of 100µl added to 1ml reagent. Here a solution was used that was more concentrated by a factor of 4.6 compared to that in the prEN, leading to a shifted upper limit of the prEN to 0.092. Using the RNM the threshold of 5 counts/sec. makes much greater demands on result quality. Experiments using the OPA method in the practical situation show that most of the processes generally used today are mostly not in a position to meet these demands. Validation of cleaning processes in practice will be forced to go hand in hand with a successive improvement in performance ability, because grave deficits are recognised here. Thus the current upper limit of the OPA method in the prEN 15883 does not over-challenge cleaning processes in practice. But in the future the aim should be to increase the level of cleaning performance whilst lowering the OPA upper limit, in order to produce more secure results. ❊

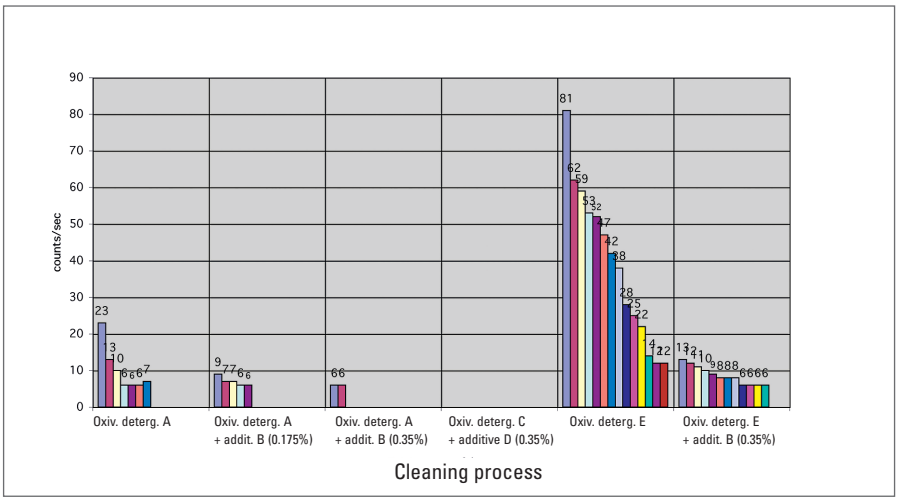

Fig. 7: Number of clamps over the allowed limit

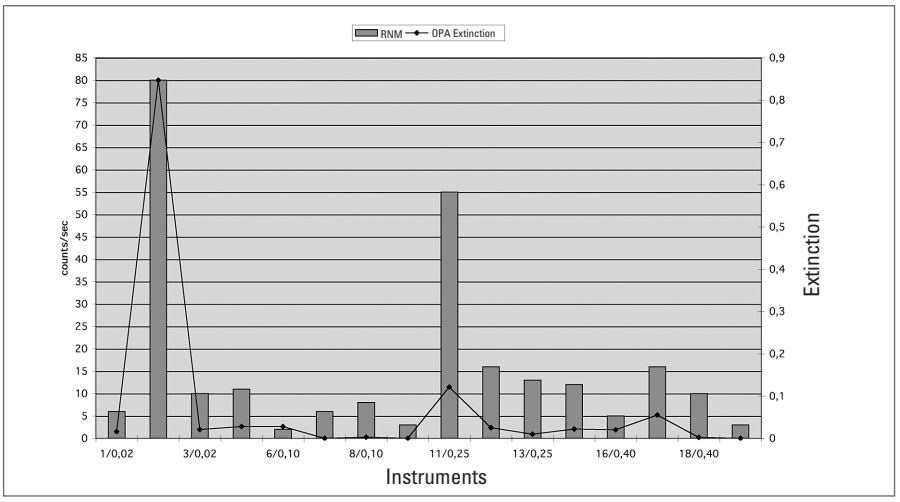

Fig. 8: Comparison of the values obtained with the radionuclide method with the results of the OPA method from 16 test instruments of the Cleaning Project Group

References see p. 39