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

Description of Test Procedure

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The ad hoc group "Test soils and methods", part of the standards committee NA Med 063-04-09 of the DIN, had reported in a previous edition of this journal initial results of a multicentre trial with an "in vitro reference system" for the comparative evaluation of test soils for testing the cleaning performance of washer-disinfector appliances (WD) according to EN ISO 15883-1 and ISO/TS 15883-5. Exploratory information about materials and methods was given there. This contribution now gives detailed information on the execution of the tests.

Introduction

The test soils for proving cleaning performance of washer-disinfector appliances (WD), published since 2005 in the "Technical specification ISO/TS 15883-5" (1), are not all particularly relevant to the practical situation, when subjectively evaluated. Ultimately the relevance must be substantiated using quantitative tests, so that tests, for example in the frame of a Type Test, yield information with a sufficiently high probability that the WD and its processes are capable of fulfilling successfully relevant tasks in the practical situation.

The test soils for surgical instruments should simulate native human blood, as this is the chief actual soil in practice. The point of testing cleaning performance in the frame of the Type Test is to see if the WD and its processes fulfil what are known as the basic requirements. The characteristics that considerably influence cleaning results are for example coagulation, polymerisation (fibrin), denaturation/fixing (temperature/chemistry), foam formation (cleaning pressure) etc., which are reflected in the characteristic removal of soils under various conditions. Kinetic examination of the detachment behaviour of test soils compared to actual soils can yield information about the practical relevance, or about the quality of simulation of the practical situation.

The establishment of detachment kinetics under various conditions relevant to automatic cleaning processes has already been reported on (2). This is possible with the test execution described in detail below.

Materials and methods

Experimental set-up

The test apparatus used to establish detachment kinetics can be put together from appliances normally found in laboratories. A spray test rig is difficult to standardise and removal of soil in an immersion bath simulates the unfavourable case of dirty instrument surfaces to be cleaned in a WD, that are not directly impacted by jets of cleaning fluid and which the cleaning solution really only runs over. To simulate this in a laboratory test a moderate but uniform exchange of liquid is necessary!

The uniform geometries and the movement of liquid in the small immersion bath are of crucial significance. For this a 100 ml glass beaker, low form (VWR, Darmstadt, Order No. 213-1122) was chosen. To agitate the liquid, magnetic stirrers (Heidolph, Schwabach or IKA, Staufen) were used with the fine adjustment at 350 rpm (+/– 20) as well as magnetic stirrer rods 25 mm in length and 6 mm in diameter (VWR, Darmstadt, Order No. 442-4524). First of all a larger container was placed on the magnetic stirrer, and in this was placed the glass beaker containing 100 ml of the solution to be tested and a magnetic stirrer rod was added. The container was filled with water up to the 80 ml mark on the glass beaker and recirculated using a circulation thermostat so that the predetermined test temperature was kept to with an accuracy of +/–1 °C. This was monitored by calibrated temperature sensors in the glass beaker as well as in the water bath. The test objects were exposed to the solution by being held in the centre of the glass beaker by a Crile clamp fixed to a tripod clamp.

Test objects

In order to test two different surfaces, test objects made of matt glass (15× 60×1 mm, Menzel-Gläser, Braunschweig, Germany) and stainless steel (15×50×1 mm, Pereg, Waldkraiburg, Germany) were chosen. The

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test objects were thoroughly cleaned before use. This entailed removing coarse dirt with a sponge under running water, then boiling in 5% Decon90 solution (VWR, Darmstadt, Order No. 148-0324) for 10 minutes, then pouring off the solution. The test objects were then held with forceps and rinsed for 5 seconds under running fully demineralised water and then briefly dipped in 70% ethanol. Drying followed on filter paper exposed to ambient air.

Test soils and soiling of test objects

For the test soils we chose at first to use heparinised sheep's blood (Order No. 31400500) and citrated sheep's blood (Order No. 31200100), both from Fiebig Nährstofftechnik, Idstein-Niederauhoff. (*Note:* the blood should be as fresh as possible and not older than one week. The blood is kept at 4 °C in the fridge and before use a test portion is removed and warmed to room temperature.)

1 ml of HPLC water (Merck Chemicals, Darmstadt, Order No. 1153332500) was added to 9 ml of heparinised blood, mixed thoroughly and coagulation was reactivated by addition of 150 µl protamine hydrochloride (50 mg in 5 ml = 1000 I.E./ml, ICN Pharmaceuticals, Frankfurt). For 10 ml citrated sheep's blood reactivation was effected by adding 300 µl of a 250 mmol $CaCl_2$ solution.

The test objects were soiled using 100 µl blood evenly spread with a pipette on a surface of 1 × 2 cm on the lower end of the test object. For the later measurement of the initial amount as protein, two test objects were also put into screw tubes pre-filled with 1% sodium dodecyl sulphate (SDS, always adjusted to pH 11 using a 0.1 molar sodium hydroxide solution) and glass microspheres (details under "Elution of residual protein") were added. Additionally the protein content of the blood was determined by pipetting two 100 µl blood samples into screw tubes each pre-filled with 5 ml 1% SDS.

Drying/Conditioning

It was found that drying for 1.5 h in an incubator at 30 °C yielded very differing results from the various laboratories involved, so conditioning followed under strictly defined conditions. 140 g potassium carbonate was boiled in 100 ml fully demineralised water and then allowed to cool. This saturated solution was transferred to a large Petri dish and placed on the bottom of the dessicator. A humidity sensor was fixed in the dessicator (e.g. Almemo, Holzkirchen), which was then closed and placed in an incubator at 30 °C. After a few hours a relative humidity of 45% was reached. After the first coagulation in ambient air the test objects were placed (15 minutes after soiling) in the dessicator in open Petri dishes and conditioned thus for 24 (+/– 2) hours. After this the test objects were transferred to Petri dishes, sealed air tightly (e.g. Parafilm) and made available for the execution of the experiments.

Test procedure

Beakers of 100 ml HPLC water or the solution to be tested (with or without detergent) were pre-warmed in a water bath. For each individual experiment with a determined exposition time to establish the kinetics (60 or 30 sec. intervals), one beaker was fitted with a magnetic stirrer rod, placed in the warming bath on the magnetic stirrer, stirred at 350 rpm and the exact temperature e.g. 45 °C +/–1 °C was awaited. When the desired temperature was reached, during all of our test series a 5 ml sample was taken from the water or solution in the beaker for a blank value checkup. Then the test object was fixed centrally in the glass beaker using a Crile clamp on a tripod and simultaneously the stopwatch was started. Test objects were removed immediately after the exposition time was up. As a control of the method a portion of the solution was extracted from the glass beaker. At the end, residual protein on the test object, the blank value checkup, as well as the protein content of the liquid in the glass beaker was quantitatively analysed. Sampling from the test objects was carried out by elution.

Elution of residual protein from the test objects

The test objects were each transferred to screw tubes (15 ml, Sarstedt, Nümbrecht, Order No. 60/732.001). Glass microspheres (200 – 420 µm, Potters Europe, Kirchheimbolanden, cleaned in the same was as the test objects) had already been added to these up to the 1 cm mark, as well as 5 ml of a 1% sodium dodecyl sulphate (SDS) solution adjusted to pH 11. The screw tubes were then intensively shaken on a horizontal laboratory shaker (e.g. IKA MS2 from IKA, Staufen) for 20 mins. at 300 rpm. Finally the test objects were again removed with forceps and a portion of the eluate was transferred for protein determination. The test objects and glass microspheres were later subjected to thorough cleaning as described under "Test objects".

Protein analysis

The modified OPA method was used to determine the blood protein content, the initial contamination of the test objects, the blank value of the solution, the residual protein on the test objects after exposition and the dissolved protein in the liquid in the glass beaker. As the thiol component either N,N-dimethyl-2-mercapto-ethylammonium chloride or 2-mercaptoethane sulfonic acid were used, which both lead to the stable, fluorescent 1-alkylthio-2-alkylisoindoles whose absorption maximum can be detected at 340nm (3,4).

Each time fresh OPA solution was prepared with one of the thiol components. Here 80 mg o-phthaldialdehyde was dissolved in 2 ml methanol and added to 50ml 0.1 mol/l (2.0122 g/50 ml) disodium tetraborate buffer (pH 9.3). Then 200 mg N,N-dimethyl-2-mercapto-ethylammonium chloride or 234 mg 2-mercaptoethane sulfonic acid as a sodium salt was added and finally 2.5 ml aqueous 20% sodium dodecyl sulphate solution (Chemicals: Merck, Darmstadt; Serva, Heidelberg). The same relative amounts of sample solution to OPA solution were always maintained: e.g. 400µl sample to 2 ml OPA solution were placed in a quartz cuvette, or suitable disposable cuvette, stirred thoroughly and then the extinction read off after 3 minutes against pure OPA solution using a photometer (e.g. Varian, Darmstadt, Cary 100) at 340 nm. The volume relationship between sample and OPA solution may not exceed 1:1. From time to time it is necessary to check the pH value, as it may deviate from pH 9.3 for larger sample proportions. It is advantageous to use a double-beam photometer so that zero calibration takes place automatically against pure OPA solution. Measurement of intrinsic extinction was conducted using the same proportional amounts of sample so-

lution to a solution made up from 2.0122 g disodium tetraborate (pH 9.3), 2 ml methanol und 2.5 ml 20% SDS solution in 50 ml HPLC water, against the same solution without added sample. To calculate the amount of protein as an equivalent of bovine serum albumin, it makes sense to have carried out a dilution series (calibration line) to determine a reference point for samples, using pure bovine serum albumin (Fraktion V, Serva, Heidelberg) using the same mixing proportions

Future prospects

In the meantime multicentre tests using the ad hoc group's methods described here have been conducted by another group of laboratories, in order to work out a guideline for the validation of processes in washer-disinfectors for flexible endoscopes. The results showed good conformity (5). This shows that the method presented is suitable and easy for other laboratories to conduct without great investment of finances or time.

The ad hoc group is now working on establishing detachment kinetics with the test soils described in ISO/TS 15883-5 as well as native human blood, thus allowing comparative assessment. ❊

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