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**Standardised Test Soil Blood 1:**
Composition, Preparation, Application

In addition to the protein content, the behaviour evinced by a blood soil is essentially determined by the blood coagulation process. This process can be monitored in a controlled manner due to the reaction of fibrinogen with thrombin, with the high protein content being simulated by a matrix of albumin and haemoglobin. A two-component system based on freeze-dried protein fractions and solvents provides, before application, for separation of the coagulation factor fibrinogen from thrombin. This setup permits a stable test soil of standardised composition with reproducible behaviour and correlation with human blood.

Keywords: test soil, blood coagulation, cleaning process

1 Introduction
Test soils are an important fundament for the development of detergents and washer/disinfectors as well as for optimisation of existing cleaning processes. While simple starch and fatty soils are adequate for many domains, the requirements addressed to a test soil for verifying the reprocessing action of surgical instruments are infinitely more stringent: since blood is the most prevalent contaminant encountered in this domain, in addition to the general requirements - such as standardised composition, reproducible dissolution action or facilities for elucidation of residues - there are special requirements dictated by the process of blood coagulation.

The extremely complex sequence of blood coagulation gives rise to the formation of insoluble fibrin fibres thanks to an enzyme cascade (1). This process is initiated only by injury to blood vessels or by contact with unphysiologic surfaces, which explains why fibrin formation also takes place directly on the surface of the instruments. This means that in the case of a practice-oriented test soil which is correlated with human blood, not only the fibrin component, which is of special relevance to cleaning, but also its formation is to be implemented only at the time of application.

2 Materials and Methods

2.1 Composition of the Test Soil
To effect fibrin formation in a protein-based soil, a major part of the enzyme cascade, which is required in the blood circulation for assured wound closure, can be omitted. Of decisive importance is the enzymatic cleavage of fibrinogen to fibrin monomers, which automatically merge to thread-shaped polymers (1).

To integrate the important step of blood coagulation into the test soil, a two-component system, which contains fibrinogen separately from thrombin and calcium ions, was developed. Hence coagulation takes place only after mixing both components, thus permitting the formation of fibrin fibres on the surfaces of challenge devices being used for test purposes.

Stability of the sensitive protein fractions is assured by employing lyophilised products. By dissolving the two components in the respective solvents one obtains a coagulable test soil that is always of a similarly high quality. The composition of a test kit for preparation of 10 ml standardised test soil is described in the following:

- **Component A:** 400 mg albumin
  400 mg haemoglobin
  60 mg fibrinogen
- **Solvent A:** 5.0 ml 0.4% NaCl solution
- **Component B:** 400 mg albumin
400 mg haemoglobin  
12.5 NIH units thrombin  
- Solvent B: 5.0 ml 0.4% NaCl solution  
+ 8.0 mmol./l CaC12

2.2 Preparation of the Test Soil
Before application, the two solid components A and B are dissolved in the respective solvents A and B. To bring all protein fractions completely to solution, dissolution is effected for 1 hour at 36°C while shaking or agitating. Here temperatures above 40°C must absolutely be avoided. Temperatures that are 5-0°C lower reduce the speed of dissolution, but do not adversely affect the test soil. After dissolution, components A and B are stable for four hours when stored separately. After mixing both components, coagulation immediately begins with formation of insoluble fibrin fibres and is complete after 30 min. Analogous to this process, blood escaping from a blood vessel - and in this case the test soil - is transformed into a gelatinous mass.

2.3 Application of the Test Soil
If both components are separately pipetted onto challenge devices and immediately mixed a reproducible quantity can be spread out, coagulating only on the desired surface (Fig. 1). By adding test microbes to component A before use, microbiological investigations can also be conducted while having recourse to a standardised soil.

Of special importance for reproducible control of cleaning processes - in particular on recording the cleaning kinetics - is not only a standardised composition but also a reproducible layer thickness of the test soil. In the present case, this is achieved with a robot dosing system (Fig. 2). In this manner, e.g. soils can also be applied for minimally invasive surgery instruments.

By treating challenge devices for instance with heat, alcoholic or aldehyde solutions, cleaning problems emanating from denaturation or chemical changes can be investigated. After drying, challenge devices with the standardised test soil can be preserved for 6 months, when stored protectedly against humidity, light and temperature fluctuations.

3 Results
A blood soil can be compared with the standardized test soil by simply immersing it in water. Employed for this comparison were a high-grade steel challenge device with 20 mg (protein content) of a standardised test soil on 430 mm² and a challenge device with 75 uL human blood, spread on the same surface. After 10 minutes, in both cases only a white layer composed of fibrin can still be seen. The remaining components, in both cases primarily haemoglobin and albumin, are easily dissolved by water. If the detached protein content is continually recorded at 240 nm for this dissolution experiment by using a flow cuvette, the detachment kinetics can be concurrently recorded (Fig. 3). The curves rapidly assume a flat shape within 5 min. and in both cases there remains a residue, since the fibrin layer cannot be removed by water alone without a mechanical cleaning action.

Since the fibrinogen content in human blood is subject to fluctuations between 0.2-0.4% (2), this has been set at a higher value for the test soil compared with the illustrated blood sample. Thanks to standardisation, the cleaning performance of an automated reprocessing procedure as well as of detergents can be investigated in the immersion experiment (Fig. 4).

4 Discussion
The employment of easily soluble plasma proteins in conjunction with the enzymatic reaction for formation of fibrin fibres, and hence of coagulation, gives rise to a standardised test soil endowed with a very good correlation with a human blood soil. The high protein content of blood is simulated by the haemoglobin content in addition to the albumin. Hence investigations can also be conducted in respect of the chemical or thermal denaturation action.

In conjunction with standardised challenge devices and a reproducible layer formation, the test soil constitutes the fundament for scientific as well as practice oriented investigation and examination of the cleaning step for reprocessing surgical instruments. Concomitantly, microbiological tests can be conducted by virtue of the suitability or
the suspension medium.

**Reagents' Appendix**

- Calcium chloride hexahydrate, purity DAB (German Pharmacopoeia), Merck No. 102072
- Fibrinogen. From Bovine Plasma. Purity 75%. ICN Cat. No. 820212
- Hemoglobin. Freeze Dried. Purity 98%. MW 64.5 kDa. ICN Cat. No. 151234
- Sodium chloride, purity DAB. Merck No. 106400
- Thrombin. From Bovine Plasma. High Purity Grade.
  Activity > 2000 NIH Units/mg Protein ICN Cat No. 154163